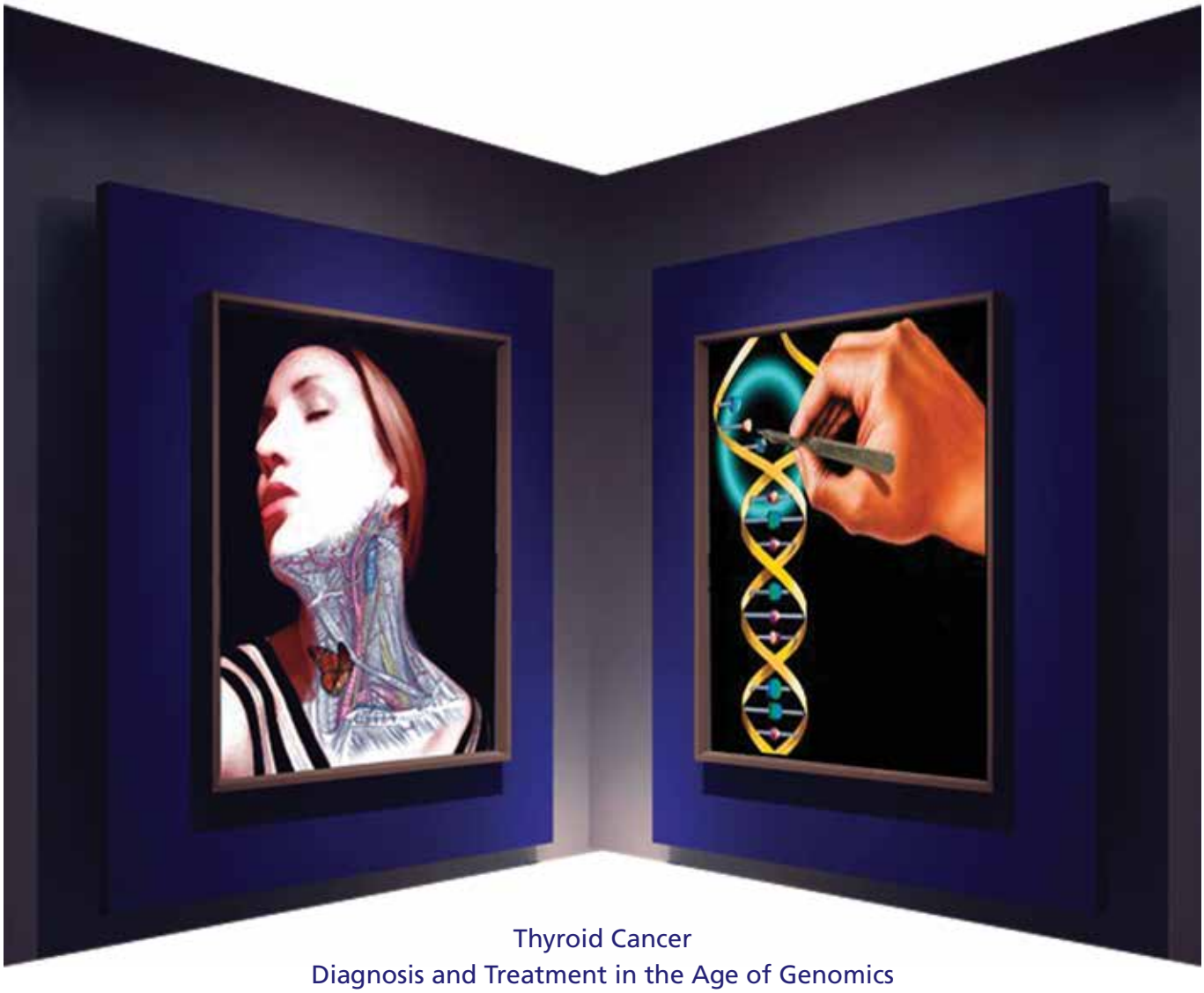
ISSN: 2146-1414

MIRT

Molecular Imaging and Radionuclide Therapy

February 2017 Volume 26 Supplement 1 www.tsnm.org



Thyroid Cancer
Diagnosis and Treatment in the Age of Genomics

"Official Journal of the Turkish Society of Nuclear Medicine"

galenos yayinevi

TURKISH SOCIETY OF NUCLEAR MEDICINE 1975

MIRT
Molecular Imaging and Radionuclide Therapy

EDITORIAL BOARD

MIRT

Molecular Imaging and Radionuclide Therapy

EDITORIAL BOARD

Turkish Society of Nuclear Medicine
Cinnah Caddesi Pilot Sokak No: 10/12 Çankaya 06650 Ankara, Turkey
Phone: +90 312 441 00 45 **Fax:** +90 312 441 12 95 **Web:** www.tsnm.org **E-mail:** dernekmerkezi@tsnm.org
"Formerly Turkish Journal of Nuclear Medicine"

♾ The paper used to print this journal conforms to ISO 9706: 1994 standard (Requirements for Permanence). The National Library of Medicine suggests that biomedical publications be printed on acid-free paper (alkaline paper). Reviewing the articles' conformity to the publishing standards of the Journal, typesetting, reviewing and editing the manuscripts and abstracts in English, creating links to source data, and publishing process are realized by Galenos.

Publisher
Erkan Mor

Publication Director
Nesrin Çolak

Publication Coordinator
Burak Sever

Project Coordinator
Ebru Boz

Web Coordinators
Eren Arsel
Turgay Akpınar

Project Assistants
Hatice Balta
Eda Kolukısa
Zeynep Altındağ

Graphics Department
Ayda Alaca
Çiğdem Birinci

Research&Development
Büşrah Toparslan

Finance Coordinator
Sevinç Çakmak

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1
34093 İstanbul, Turkey
Phone: +90 (212) 621 99 25 **Fax:** +90 (212) 621 99 27
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr

Printing at: Özgün Ofset Ticaret Ltd. Şti.
Yeşilce Mah. Aytekin Sk. No: 21 34418 4. Levent, İstanbul, Turkey
Phone: +90 (212) 280 00 09
Printing Date: January 2017
ISSN: 2146-1414 **E-ISSN:** 2147-1959
International scientific journal published quarterly.

MIRT

Molecular Imaging and Radionuclide Therapy

ABOUT US

Molecular Imaging and Radionuclide Therapy(formerly Turkish Journal of Nuclear Medicine) is the official publication of Turkish Society of Nuclear Medicine.

Focus and Scope

Molecular Imaging and Radionuclide Therapy (Mol Imaging Radionucl Ther, MIRT) is a double-blind peer-review journal published in English language. It publishes original research articles, reviews, editorials, short communications, letters, consensus statements, guidelines and case reports with a literature review on the topic, in the field of molecular imaging, multimodality imaging, nuclear medicine, radionuclide therapy, radiopharmacy, medical physics, dosimetry and radiobiology. MIRT is published three times a year (February, June, October). Audience: Nuclear medicine physicians, medical physicists, radiopharmaceutical scientists, radiobiologists.

The editorial policies are based on the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (ICMJE Recommendations)" by the International Committee of Medical Journal Editors (2013, archived at http://www.icmje.org/) rules.

Molecular Imaging and Radionuclide Therapy is indexed in PubMed, PubMed Central (PMC), Emerging Sources Citation Index (ESCI), TUBITAK-ULAKBIM, Gale/ Cengage Learning, EBSCO databases, ProQuest Health & Medical Complete, CINAHL, DOAJ, Türkiye Atıf Dizini (Türkiye Citation Index), Turk Medline.

Open Access Policy

This journal provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Open Access Policy is based on rules of Budapest Open Access Initiative (BOAI) (http://www.budapestopenaccessinitiative.org/). By "open access" to [peer-reviewed research literature], we mean its free availability on the public internet, permitting any users to read, download, copy, distribute, print, search, or link to the full texts of these articles, crawl them for indexing, pass them as data to software, or use them for any other lawful purpose, without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. The only constraint on reproduction and distribution, and the only role for copyright in this domain, should be to give authors control over the integrity of their work and the right to be properly acknowledged and cited.

Subscription Information

Manuscripts can only be submitted electronically through the Journal Agent website (http://www.journalagent.com/mirt/?plng=eng) after creating an account. This system allows online submission and review.

All published volumes in full text can be reached free of charge through the website http://mirt.tsnmjournals.org

Instructions for Authors

Instructions for authors are published in the journal and on the website http://mirt.tsnmjournals.org

Correspondence Address

Editor in Chief, Hatice Durak, MD, Professor in Nuclear Medicine
Dokuz Eylül University Faculty of Medicine, Department of Nuclear Medicine
35340 Balçova / İZMİR
Phone : +90 212 621 99 25
Fax : +90 212 621 99 27
e-mail : haticedurak@tsnmjournals.org
Web : http://mirt.tsnmjournals.org

The journal is printed on an acid-free paper.

MIRT

Molecular Imaging and Radionuclide Therapy

INSTRUCTIONS TO AUTHORS

Molecular Imaging and Radionuclide Therapy (Mol Imaging Radionucl Ther, MIRT) publishes original research articles, reviews, editorials, short communications, letters, consensus statements, guidelines and case reports with a literature review on the topic, in the field of molecular imaging, multimodality imaging, nuclear medicine, radionuclide therapy, radiopharmacy, medical physics, dosimetry and radiobiology. MIRT is published by the Turkish Society of Nuclear Medicine three times a year (February, June, October). The journal is printed on an acid-free paper.

Molecular Imaging and Radionuclide Therapy is indexed in PubMed, PubMed Central (PMC), Emerging Sources Citation Index (ESCI), TUBITAK-ULAKBIM, Gale/ Cengage Learning, EBSCO databases, ProQuest Health & Medical Complete, CINAHL, DOAJ, Türkiye Atıf Dizini (Türkiye Citation Index), Turk Medline.

Molecular Imaging and Radionuclide Therapy does not charge any article submission or processing charges.

GENERAL INFORMATION

MIRT commits to rigorous peer review, and stipulates freedom from commercial influence, and promotion of the highest ethical and scientific standards in published articles. Neither the Editor(s) nor the publisher guarantees, warrants or endorses any product or service advertised in this publication. All articles are subject to review by the editors and peer reviewers. If the article is accepted for publication, it may be subjected to editorial revisions to aid clarity and understanding without changing the data presented.

Manuscripts must be written in English and must meet the requirements of the journal. The journal is in compliance with the uniform requirements for manuscripts submitted to biomedical journals published by the International Committee of Medical Journal Editors (NEJM 1997; 336:309-315, updated 2001). Manuscripts that do not meet these requirements will be returned to the author for necessary revision before the review. Authors of manuscripts requiring modifications have a maximum of two months to resubmit the revised text. Manuscripts returned after this deadline will be treated as new submissions.

It is the authors' responsibility to prepare a manuscript that meets ethical criteria. The Journal adheres to the principles set forth in the Helsinki Declaration October 2008. (http://www.wma.net/en/30publications/10policies/b3/ index.html) and holds that all reported research involving "Human beings" conducted in accordance with such principles. Reports describing data obtained from research conducted in human participants must contain a statement in the MATERIALS AND METHODS section indicating approval by the ethical review board and affirmation that INFORMED CONSENT was obtained from each participant.

- All manuscripts reporting experiments using animals must include a statement in the MATERIALS AND METHODS section giving assurance that all animals have received humane care in compliance with the Guide for the Care and Use of Laboratory Animals (www.nap.edu) and indicating approval by the ethical review board.
- Case reports should be accompanied by INFORMED CONSENT and the identity of the patient should be hidden.
- Subjects must be identified only by number or letter, not by initials or names. Photographs of patients' faces should be included only if scientifically relevant. Authors must obtain written consent from the patient for use of such photographs.
- If the proposed publication concerns any commercial product, the author must include in the cover letter a statement indicating that the author(s) has (have) no financial or other interest with the product or explaining the nature of any relations (including consultancies) between the author(s) and editor the manufacturer or distributor of the product.

MANUSCRIPT CATEGORIES

1. Original Articles

2. Short Communications are short descriptions of focused studies with important, but very straightforward results. These manuscripts should be no longer than 2000 words, and include no more than two figures and tables and 20 references.

3. Reviews address important topics in the field. Authors considering the submission of uninvited reviews should contact the editor in advance to determine if the topic that they propose is of current potential interest to the Journal. Reviews will be considered for publication only if they are written by authors who have at least three published manuscripts in the international peer reviewed journals and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer review from qualified experts in the area.

4. Editorials are usually written by invitation of the editor by the editors on current topics or by the reviewers involved in the evaluation of a submitted manuscript and published concurrently with that manuscript.

5. Case Report and Literature Reviews are descriptions of a case or small number of cases revealing a previously undocumented disease process, a unique unreported manifestation or treatment of a known disease process, unique unreported complications of treatment regimens or novel and important insights into a condition's pathogenesis, presentation, and/or management. The journal's policy is to accept case reports only if accompanied by a review of the literature on the related topic. They should include an adequate number of images and figures.

6. Consensus Statements or Guidelines may be submitted by professional societies. All such submissions will be subjected to peer review, must be modifiable in response to criticisms, and will be published only if they meet the Journal's usual editorial standards. These manuscripts should typically be no longer than 4000 words and include no more than six figures and tables and 120 references.

7. Letters to the Editor may be submitted in response to work that has been published in the Journal. Letters should be short commentaries related to specific points of agreement or disagreement with the published work. Letters should be no longer than 500 words with no more than five complete references, and may not include any figures or tables.

Note on Prior Publication

Articles are accepted for publication on the condition that they are original, are not under consideration by another journal, or have not been previously published. Direct quotations, tables, or illustrations that have appeared in copyrighted material must be accompanied by written permission for their use from the copyright owner and authors. Materials previously published in whole or in part shall not be considered for publication. At the time of submission, authors must report that the manuscript has not been published elsewhere. Abstracts or posters displayed at scientific meetings need not be reported.

MANUSCRIPT SUBMISSION PROCEDURES

MIRT only accepts electronic manuscript submission at the web site www. http://mirt.tsnmjournals.org. After logging on to the website Click the 'online manuscript submission' icon. All corresponding authors should be provided with a password and a username after entering the information required. If you already have an account from a previous submission, enter your username and password to submit a new or revised manuscript. If you have forgotten your username and/or password, please send an e-mail to the editorial office for assistance. After logging on to the article submission system please read carefully the directions of the system to give all needed information and attach the manuscript, tables and figures and additional documents.

MIRT

Molecular Imaging and Radionuclide Therapy

INSTRUCTIONS TO AUTHORS

All Submissions Must Include:

1. Completed Copyright Assignment & Disclosure of Potential Conflict of Interest Form; This form should be downloaded from the website (provided in the author section), filled in thoroughly and uploaded to the website during the submission.

2. Informed consent (for case reports); Authors must complete the online submission forms. If you are unable to successfully upload the files please contact the editorial office by e-mail.

There is no submission fee for MIRT.

MANUSCRIPT PREPARATION

General Format

The Journal requires that all submissions be submitted according to these guidelines:

- Text should be double spaced with 2.5 cm margins on both sides using 12-point type in Times Roman font.
- All tables and figures must be placed after the text and must be labeled.
- Each section (abstract, text, references, tables, figures) should start on a separate page.
- Manuscripts should be prepared as a word document (*.doc) or rich text format (*.rtf).
- Please make the tables using the table function in Word.
- Abbreviations should be defined in parenthesis where the word is first mentioned and used consistently thereafter.
- Results should be expressed in metric units. Statistical analysis should be done accurately and with precision. Please consult a statistician if necessary.

Title Page

The title page should be a separate form from the main text and should include the following:

- Full title (in English and in Turkish) Turkish title will be provided by the editorial office for the authors who are not Turkish speakers.
- Authors' names and institutions.
- Short title of not more than 40 characters for page headings.
- At least three and maximum eight key words. (in English and in Turkish) Do not use abbreviations in the key words. Turkish key words will be provided by the editorial office for the authors who are not Turkish speakers. If you are not a native Turkish speaker, please re enter your English keywords to the area provided for the Turkish keywords. English key words should be provided from http://www.nlm.nih.gov/mesh(Medical Subject Headings) while Turkish key words should be provided from http://www.bilimterimleri.com.
- Word count (excluding abstract, figure legends and references).
- Corresponding author's e-mail and address, telephone and fax numbers.
- Name and address of person to whom reprint requests should be addressed.

Original Articles

Authors are required to state in their manuscripts that ethical approval from an appropriate committeeand informed consents of the patients were obtained. These documents may be asked by the reviewers or the editorial board during the submission process.

- **Abstract:** (in English and in Turkish) Original Articles should be submitted with a structured abstract of no more than 250 words. All information reported in the abstract must appear in the manuscript. The abstract should not include references. Please use complete sentences for all sections of the abstract. Structured abstract should include background, objective, methods, results and conclusions. Turkish abstract will be provided by the editorial office for the authors who are not Turkish speakers. If you are not a native Turkish speaker, please re enter your English abstract to the area provided for the Turkish abstract.
- Introduction
- Materials and Methods
- Results
- Discussion
- Study Limitations
- Conclusion
- **Acknowledgement:** May be given for contributors who are not listed as authors, or for grant support of the research.
- **References:** References should be cited in numerical order (in parentheses) in the text and listed in the same numerical order at the end of the manuscript on a separate page or pages. The author is responsible for the accuracy of references. Examples of the reference style are given below. Further examples will be found in the articles describing the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (Ann Intern Med.1988; 208:258-265, Br Med J. 1988; 296:401-405). The titles of journals should be abbreviated according to the style used in the Index Medicus. Journal Articles and Abstracts: Surnames and initials of author's name, title of the article, journal name, date, volume number, and pages. All authors should be listed regardless of number. The citation of unpublished papers, observations or personal communications is not permitted. Citing an abstract is not recommended. **Books:** Surnames and initials of author's names, chapter title, editor's name, book title, edition, city, publisher, date and pages.

Sample References

Journal Article: Sayit E, Söylev M, Capa G, Durak I, Ada E, Yilmaz M. The role of technetium-99m-HMPAO-labeled WBC scintigraphy in the diagnosis of orbital cellulitis. Ann Nucl Med 2001;15:41-44.

Article with DOI Number: Erselcan T, Hasbek Z, Tandogan I, Gumus C, Akkurt I. Modification of Diet in Renal Disease equation in the risk stratification of contrast induced acute kidney injury in hospital inpatients. Nefrologia 2009 doi: 10.3265/Nefrologia.2009.29.5.5449.en.full.

Article in a journal published ahead of print: Ludbrook J. Musculovenous pumps in the human lower limb. Am Heart J 2009;00:1-6. (accessed 20 February 2009).

Book Chapters: Lang TF, Duryea J. Peripheral Bone Mineral Assessment of the Axial Skeleton: Technical Aspects. In: Orwoll ES, Bliziotes M (eds). Osteoporosis: Pathophsiology and Clinical Management. New Jersey, Humana Pres Inc, 2003;83-104.

Books: Greenspan A. Orthopaedic Radiology a Pratical Approach. 3th ed. Philadelphia, Lippincott Williams Wilkins 2000, 295-330.

Website: Smith JR. 'Choosing Your Reference Style', Online Referencing 2(3), http://orj.sagepub.com (2003, accessed October 2008).

- Tables

Tables must be constructed as simply as possible. Each table must have a concise heading and should be submitted on a separate page. Tables must not simply duplicate the text or figures. Number all tables in the order of their citation in the text. Include a title for each table (a brief phrase, preferably no longer than 10 to 15 words). Include all tables in a single file following the manuscript.

- Figure Legends

Figure legends should be submitted on a separate page and should be clear and informative.

MIRT
Molecular Imaging and Radionuclide Therapy

- Figures

Number all figures (graphs, charts, photographs, and illustrations) in the order of their citation in the text. At submission, the following file formats are acceptable: AI, EMF, EPS, JPG, PDF, PPT, PSD, TIF. Figures may be embedded at the end of the manuscript text file or loaded as separate files for submission. All images MUST be at or above intended display size, with the following image resolutions: Line Art 800 dpi, Combination (Line Art + Halftone) 600 dpi, Halftone 300 dpi. Image files also must be cropped as close to the actual image as possible.

Review Articles:

- Title page (see above)
- Abstract: Maximum 250 words; without structural divisions; in English and in Turkish . Turkish abstract will be provided by the editorial office for the authors who are not Turkish speakers. If you are not a native Turkish speaker, please re enter your English abstract to the area provided for the Turkish abstract.
- Text
- Conclusion
- Acknowledgements (if any)
- References

Case Report and Literature Review:

- Title page (see above)
- Abstract: Approximately 100-150 words; without structural divisions; in English and in Turkish. Turkish abstract will be provided by the editorial office for the authors who are not Turkish speakers. If you are not a native Turkish speaker, please re-enter your English abstract to the area provided for the Turkish abstract.
- Introduction
- Case report
- Literature Review and Discussion
- References

Editorial:

- Title page (see above)
- Abstract: Maximum 250 words; without structural divisions; in English and in Turkish. Turkish abstract will be provided by the editorial office for the authors who are not Turkish speakers. If you are not a native Turkish speaker, please re enter your English abstract to the area provided for the Turkish abstract.
- Text
- References

Letters to the Editor:

- Title page (see above)
- Short comment to a published work, no longer than 500 words, no figures or tables.
- References no more than five.

Proofs and Reprints

Proofs and a reprint order are sent to the corresponding author. The author should designate by footnote on the title page of the manuscript the name and address of the person to whom reprint requests should be directed. The manuscript when published will become the property of the journal.

Archiving

The editorial office will retain all manuscripts and related documentation (correspondence, reviews, etc.) for 12 months following the date of publication or rejection.

Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. The submission has not been previously published, nor is it before another journal for consideration (or an explanation has been provided in Comments to the Editor).
2. The submission file is in Microsoft Word, RTF, or WordPerfect document file format. The text is double-spaced; uses a 12-point font; employs italics, rather than underlining (except with URL addresses); and the location for all illustrations, figures, and tables should be marked within the text at the appropriate points.
3. Where available, URLs for the references will be provided.
4. All authors should be listed in the references, regardless of the number.
5. The text adheres to the stylistic and bibliographic requirements outlined in the Author Guidelines, which is found in About the Journal.
6. English keywords should be provided from http://www.nlm.nih.gov/mesh (Medical Subject Headings), while Turkish key words should be provided from http://www.bilimterimleri.com
7. The title page should be a separate document from the main text and should be uploaded separately.
8. The "Affirmation of Originality and Assignment of Copyright/The Disclosure Form for Potential Conflicts of Interest Form" should be downloaded from the website, filled thoroughly and uploaded during the submission of the manuscript.

TO AUTHORS

Copyright Notice

The author(s) hereby affirms that the manuscript submitted is original, that all statement asserted as facts are based on author(s) careful investigation and research for accuracy, that the manuscript does not, in whole or part, infringe any copyright, that it has not been published in total or in part and is not being submitted or considered for publication in total or in part elsewhere. Completed Copyright Assignment & Affirmation of Originality Form will be uploaded during submission.

By signing this form;

1. Each author acknowledges that he/she participated in the work in a substantive way and is prepared to take public responsibility for the work.
2. Each author further affirms that he or she has read and understands the "Ethical Guidelines for Publication of Research".
3. The author(s), in consideration of the acceptance of the manuscript for publication, does hereby assign and transfer to the Molecular Imaging and Radionuclide Therapy all of the rights and interest in and the copyright of the work in its current form and in any form subsequently revised for publication and/or electronic dissemination.

Privacy Statement

The names and email addresses entered in this journal site will be used exclusively for the stated purposes of this journal and will not be made available for any other purpose or to any other party.

Peer Review Process

1. The manuscript is assigned to an editor, who reviews the manuscript and makes an initial decision based on manuscript quality and editorial priorities.
2. For those manuscripts sent for external peer review, the editor assigns at least two reviewers to the manuscript.
3. The reviewers review the manuscript.
4. The editor makes a final decision based on editorial priorities, manuscript quality, and reviewer recommendations.
5. The decision letter is sent to the author.

MIRT
Molecular Imaging and Radionuclide Therapy

CONTENTS

Reviews

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):1-9 DOI:10.4274/2017.26.suppl.01

The Art and Science of Thyroid Surgery in the Age of Genomics: 100 years after Theodor Kocher

Genomik Çağında Tiroid Cerrahi Sanat ve Bilimi: Theodor Kocher'den 100 Sene Sonra

Seza Gulec, MD, FACS
Florida International University Herbert Wertheim College of Medicine, Departments of Surgery and Nuclear Medicine, Miami, USA

Abstract

Cancer is a disorder of the genome. The thyroid cancer genome is being decoded. Recent studies have identified a mutation or a genetic alteration in 95% of thyroid cancers. The National Cancer Institute initiated the Cancer Genome Atlas project in 2006 to catalogue genetic mutations associated with cancer, using genome sequencing and bioinformatics. The project has expanded to carry out genomic characterization and sequence analysis of thyroid cancer. The concept of risk stratification based on traditional parameters will soon vacate their role for clear molecular markers of non-invasive/focal, invasive/metastatic and systemic stages/phases of neoplastic disorder. A refined classification scheme based on genomics and its phenotypic expressions will accurately reflect the biologic differences between the different morphologic definitions we use today. Tumor differentiation/de-differentiation, and clinical behavior of an individual cancer will be defined by molecular markers, in addition to standard morpho-pathology. Empiricism in science of medicine and surgery has acquired a new method for testing the appropriate treatment for individual patients; that is molecular pathology, governed by genomics. The technology is present and rapidly evolving. The surgeons will determine the extent of interventions with molecular evidence and guidance.

Keywords: Genomics, thyroid cancer, thyroidectomy, radioactive iodine, beta knife

Öz

Tiroid cerrahisinin bilimsel temelleri Theodor Kocher tarafından tanımlandı. Bu ustayı 100 yıl önce uğurladık. Tirod kanseri cerrahisi ve tiroid kanseri hastalarının tedavisi ile tartışmalar süregelmektedir. Konunun mihengi açıkçası ilk aşama cerrahi tedavinin boyutudur. Cerrahi tedaviyi izleyen kısa ve uzun dönemde uygulanabilecek tedaviler, yararlanılabilecek tetkik ve görüntüleme yöntemleri de ilk cerrahi tedavinin çerçevesiyle alakalıdır. Amerikan Tiroid Birliği'nin 2015 basımı tedavi kılavuzu dahil olmak üzere, geleneksel olarak tiroid kanserinin klinik değerlendirmesi morfolojik öğelere dayalı yapılır. Tümör büyüklüğü, histolojik varyantlar ve hasta yaşı kriterleri cerrahi ve cerrahi sonrası girişimlerin boyutunu belirler. Kanser genomu projesi kapsamında tiroid kanseri genom profili aydınlanmaya başladı. Hala birçok bilinmeyen olmakla birlikte bu konuda bilgi birikimimiz hızla artıyor. İnce iğne biyopsisi materyalinde hastaların kromozom değişiklikleri ve nokta mutasyonları tespit edilebilmekte, bu genomik değişikliklerin biyolojik anlamları araştırılabiliyor. Bu teknoloji hızla teşhis ve tedavi yönlendirilmesinde kullanıma giriyor. Bundan böyle tedavi seçimlerinde genomik kılavuz ve yönlendirme kullanılacak. Bu makale cerrahi sanatının mahir ustalarını ve cerrahi bilimindeki genomik devrimi selamlıyor.

Anahtar kelimeler: Genomik, tiroid kanseri, tiroidektomi, radyoaktif iyot, beta bıçak

Address for Correspondence: Seza Gulec, MD, FACS, Florida International University Herbert Wertheim College of Medicine, Departments of Surgery and Nuclear Medicine, Miami, USA
Phone: (786) 693 08 21 E-mail: sgulec@fiu.edu

The extirpation of the thyroid gland typifies, perhaps better than any operation, the supreme triumph of the surgeon's art. A feat which today can be accomplished by any competent operator without danger of mishap and which was conceived more than one thousand years ago might appear an unlikely competitor for a place in surgery so exalted.

William Stewart Halsted

Milestones

Theodor Kocher died 100 years ago in 1917. Kocher is considered the father of thyroid surgery (Figure 1).

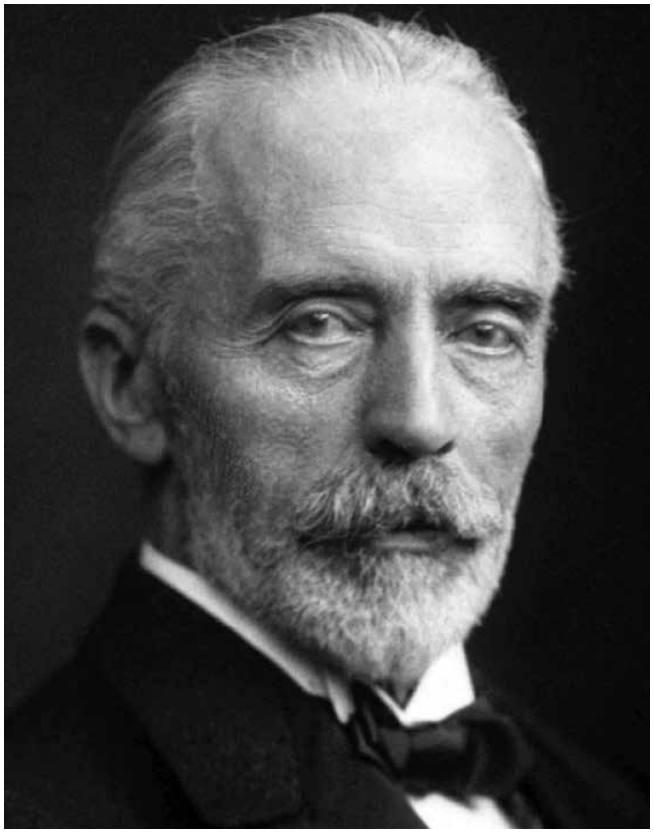


Figure 1. Theodor Kocher

He received the 1909 Nobel Prize in Physiology or Medicine for his work in the physiology, pathology and surgery of the thyroid. A few weeks before his death, at the age of 76, he made his final appearance before the Swiss Surgical Congress, reviewing his entire thyroid surgery experience. Kocher reported on approximately 5.000 operations with a mortality of about 0.5%. When he started his work in the 1870s, thyroid surgery was a high-risk procedure, with an estimated mortality of 75% in 1872. Thyroid operations were prohibited by the Academy of Medicine in France at that time. Kocher was appointed to the Chair of Surgery in Berne, Switzerland, in 1872, at the age of 31, and began his influential work in thyroid surgery and medicine. His most acclaimed achievement, his surgical technique, was marked by meticulous care in dissecting and ligating blood vessels, and precise dissection within the thyroid capsule (1). William Halsted's impression on Kocher's surgical technique was/ is quite remarkable. Halsted described Kocher's technique as "neat and precise, operating in a relatively bloodless manner, scrupulously removing the entire thyroid gland doing little damage outside its capsule" (2). Kocher was the first to describe the devastating complication(s) of total extirpation of the thyroid gland. He also recognized the underlying pathophysiologic changes in a diseased gland. This was, in a sense, a "molecular vision." He intuitively contemplated that the growth of goiter nodules was an early determined event in altered thyroid physiology, and that the abnormal thyroid tissue was the source of a goiter recurrence. He conceived the notion of autonomously growing, focally distributed clusters of follicular cells in nodular goiter. He, thus, advocated a total thyroidectomy rather than selective removal of all thyroid nodules. Kocher had realized that the so-called "subtotal" thyroidectomy, leaving behind naturally growth-prone tissue, would lead to goiter recurrence. The concept was called "Innere Chirurgie" (internal surgery), a scientific surgical philosophy based on biological considerations. This was the beginning of a new epoch. The leading minds were Kocher in Berne, Halsted in Baltimore, and Mikulicz (coined the term) in Krakau (3).

Despite impressive discoveries in surgical anatomy and physiology, the onco-biology of neoplasia and the clinico-pathologic characteristics of cancer were poorly understood at Kocher's time. Although Kocher had significant studies on malignant tumors of the thyroid gland, the modern science for cancer surgery was developed by William Halsted, who championed a radical treatment approach for breast cancer. Halsted's idea was based on the premise that cancer had a linear, step-wise growth fate and had to be be treated with total extirpation of the organ along with its lymphatic drainage network. This was believed to be necessary for cure, and was adapted by many prominent surgeons. This classic rationalistic philosophy in surgery dominated the surgical world for years. Similarly, thyroid surgery for cancer in the early post-Kocher era, called for an "en-block resection" or "conventional radical neck dissection," which usually sacrificed the sternocleidomastoid muscle, internal jugular vein, often the accessory nerve and sometimes the marginal mandibular branch of the facial nerve. This was all justified in the name of "cure. The core problem with the rationalistic logical flow is the potential/possible flaw

in the original hypothesis. The entire chain of thoughts/deductions, then, may lead to incorrect conclusions (a-priori error). The outcomes, however, need to be tested independently. (post-priori validation). This is the essence of evidence-based, data-driving scientific methodology". George "Barney" Crile Jr. should be credited as the first to challenge the radical thought process and action. He was one of the first surgeons ever to promote the idea that "the less surgery the better," and he campaigned vigorously for the abandonment of the radical operations. His ideas on thyroid cancer surgery, and later breast cancer surgery, were briskly opposed at the time, but eventually succeeded (4). The new surgical vision was reluctantly, but progressively accepted by the surgical community over time, and has since, shaped the evolution of the philosophy of surgical treatment.

Mid-century brought in a major innovation in the management of thyroid cancer, the radioactive iodine (RAI) treatment. First used by Samuel Seidlin (5), and established as a fortitude by Bierwaltes (6), RAI became an invaluable theranostic agent. The role for RAI in the management of metastatic differentiated thyroid cancer (DTC) became indisputable. Its utilization post-operatively, however, is still a matter of debate. As a guide to perplexed; RAI treatment is given in 3 distinct settings with distinct clinical indications (intents). 1) Ablation of the remnant 2) Adjuvant treatment for residual disease or occult metastatic disease 3) Therapy for known metastatic disease. The term "ablation" specifically refers to first-line RAI treatment following total surgical thyroidectomy. The specific target of this treatment is normal residual thyroid tissue- the remnant. The objectives of ablation are three-folds a) Ablation eradicates all the functioning thyroid tissue. Thereby, thyroglobulin (Tg) becomes a highly specific tumor marker. This simply facilitates the post-op long-term follow-up. b) Ablation wipes out all focal normal thyroid tissue left in-situ by the surgeon to avoid injury to laryngeal nerves and parathyroid glands. From surgical standpoint these small clusters of normal tissue remnants are inconsequential, however, they appear as focal areas of RAI uptake on future whole body imaging studies and can easily be called as metastatic disease. Eradication of these potential source of misdiagnosis, when surgeon to imager communication is still on-line, is important. c) Post-ablation whole-body scan is an excellent extent of disease evaluation tool. When the post-operative RAI treatment is contemplated with an adjuvant intent, in addition to the ablation objectives, RAI is aimed to target residual disease or occult metastatic disease. When the intent is adjuvant treatment, the risk stratification becomes important in the selection of administered activity of I-131. For remnant ablation purposes only, the risk stratification has no bearing. The therapeutic effect of I-131 works through the beta particles, thus, it should be referred as "beta-knife." Complete thyroidectomy, in the strictest sense is only possible with surgical (cold steel knife) thyroidectomy, followed by I-131 ablation (beta knife) (Table 1).

The second half of the 20th century also witnessed the birth of neo-empiricism in the acquisition and application of scientific knowledge in medicine. The established dogmas for radical surgical treatments were challenged, asking for proof of efficacy based on outcome data. The new paradigm was most palpable in the management of breast cancer and thyroid cancer. The radical versus conservative surgery argument was relatively easy to settle for breast cancer. Bernard Fisher in the US and Umberto Veronesi in Italy ran parallel trials resulting in clear demonstration of equivalency of lesser surgery over radical operations. The problem with thyroid cancer, however, remained unsettled. DTC was/is a more indolent cancer with a much more protracted course. Clinical trials with required statistical power were very difficult to perform. The lack of definitive clinical trials unleashed a never-ending controversy: Total thyroidectomy versus less than total thyroidectomy. A new, rather ambiguous, lexis for such operations entered into common use. Sub-total, near-total thyroidectomy terms subsisted. At least for the sake of clarification of the nomenclature, there should only be two standard defining operations: Total thyroidectomy and lobectomy. Total thyroidectomy is defined as the safe removal of the thyroid gland with oncologically clean margins. If/when eradication of all functioning thyroid tissue is the end point, a cold steel knife is followed by the beta-knife.

Table 1. Radioactive iodine ablation objectives

RAI treatment intent	Target	Objectives
Ablation (First-line)	Remnant	1) Increase specificity of Tg monitoring, 2) Complete post-surgical staging 3) Identify/clear ambiguous foci of uptake
Adjuvant treatment (First-line)	1) Remnant, 2) Residual disease, 3) Occult metastatic disease	1) Ablation objectives, 2) Disease control
Therapy (Second-line)	1) Recurrent disease, 2) Metastatic disease	1) Disease control

RAI: Radioactive iodine, Tg: Thyroglobulin

The Equipoise

An equipoise is a genuine disagreement among the experts as to the optimal therapeutic approach in the management of a particular condition. A true equipoise exists in the initial treatment of DTC. There are two camps, represented by two diametrically opposing philosophical thoughts. There are those aggressively favor/defend the strategy of total thyroidectomy with radioiodine ablation and periodic Tg screening for "biochemical evidence of recurrence". On the opposite direction there are those taking a conservative stand and prefer/defend performing thyroid lobectomy, when the tumor is small and limited to one lobe of the gland. This stance would automatically rule out post-surgical ablation as well as "affect" the utility of Tg screening. This approach relies more on clinical and ultrasound findings for "clinical evidence of recurrence." It is a true equipoise, as there is genuine uncertainty in the expert medical community over which approach is more beneficial. The option of lobectomy (pertaining to tumors measuring 1cm or less), was proposed in the 2009 American Thyroid Association (ATA) guidelines (7). It became the recommended option for small tumors in its 2015 edition (8). A more progressive conservatism is endorsed in the 2010 version of the Japanese guidelines for the treatment of thyroid tumors. In the Japanese guidelines, the indications for a lobectomy were extended to tumors as large as 4 cm, if they are limited to one lobe of the thyroid with little or no extra-capsular invasion and no gross lymph node involvement. As for papillary carcinoma less than 1cm, some Japanese surgeons are proposing that observation without surgical intervention may be sufficient (9).

The Japanese Guidelines, 2010

A) It is beyond dispute that patients with the following characteristics are regarded as high-risk; tumor size >5 cm, lymph node metastasis >3 cm, lymph node metastasis extending to the internal jugular vein, carotid artery, major nerves such as recurrent laryngeal nerve, and prevertebral fascia, multiple and intensely swollen lymph node metastasis, extrathyroidal extension to the trachea and esophageal mucosa and distant metastasis at diagnosis. Total thyroidectomy is recommended for patients having one or more of these characteristics (p.108)

B) Other patients are classified as a "gray-zone", but in these patients total thyroidectomy is recommended if the tumor size is >4 cm, and clinical node metastasis is detected (regardless of whether it is N1a or N1b) (p.108)

C) Although further studies with larger patient numbers and longer follow-up times are required, observation without immediate surgery for papillary microcarcinoma without metastasis or invasion can be considered a reasonable option (p.121).

It would have been easier to compose a narrative if the history of thyroid surgery was a linear progression from a radical operation towards a lesser one. The story, however, is more confounded, partly due to the particular characteristics of the disease and perhaps more so due to entrenched positions of opinionated surgeons and oncologists, the very definition of equipoise. All respectable institutions, and the thought leaders in the field have made their contributions to the controversy (10,11,12,13). Equipoise has become the standard of care in the initial surgical treatment of thyroid cancer.

Authors on both sides of the debate have pointed out that not all papillary carcinomas are equally indolent. Some grow rapidly and progress more aggressively than others often without obvious histological differences. Histological variations such as the tall cell variant and columnar cell variant have been identified, but many unusually malignant strains cannot be morphologically distinguished as being different from other examples of papillary carcinoma. Certain well-DTC will go on to have an aggressive course. They cannot be histopathologically differentiated from those with typical indolent course There appears not to have an exact way of identifying those relatively infrequent differentiated cancers that are destined to have a more malignant course. Despite the significant progress in molecular pathology, the clinical risk factors are currently the only stratification guide used in thyroid cancer diagnosis and management. Proponents of total thyroidectomy insist that the increase in complications is minimal in the hands of an experienced surgeon. Proponents of lobectomy point out that only one recurrent laryngeal nerve is at risk when only one lobe of the thyroid is being resected, thus the theoretical risk of nerve palsy is halved. Disagreement over the merits of prophylactic central node dissection has been argued in a similar context. Experience suggests that the incidence of surgical complications may not solely be dependent on the proficiency of the surgeon, but also the extent of surgical procedure performed. So the debate goes on, "equipoetically".

The American Thyroid Association Guidelines, 2015

The 2015 ATA guidelines is a 133 page document written in a dissertation format. The rationale for each recommendation was discussed, in detail, in a scholarly fashion. The section on operative approach for a biopsy diagnostic for follicular cell-derived malignancy (B7, Recommendation 35) defines three categories.

The guideline committee states that in properly selected low to intermediate risk patients, the extent of initial thyroid surgery probably has little impact on disease specific survival. While recurrence rates can be quite low in properly selected patients, it is likely that the lowest rates of recurrence during long term follow-up would be associated with a total thyroidectomy. However, since salvage therapy would be quite effective in the few patients that recur after

American Thyroid Association 2015 Guidelines

A) For patients with thyroid cancer >4 cm, or with gross extra-thyroidal extension (clinical T4), or clinically apparent metastatic disease to nodes (clinical N1) or distant sites (clinical M1), the initial surgical procedure should include a near-total or total thyroidectomy and gross removal of all primary tumor unless there are contraindications to this procedure. (Strong Recommendation, Moderate-quality evidence),

B) For patients with thyroid cancer >1 cm and <4 cm without extra-thyroidal extension, and without clinical evidence of any lymph node metastases (cN0), the initial surgical procedure can be either a bilateral procedure (near-total or total thyroidectomy) or a unilateral procedure (lobectomy). Thyroid lobectomy alone may be sufficient initial treatment for low risk papillary and follicular carcinomas; however, the treatment team may choose total thyroidectomy to enable RAI therapy or to enhance follow-up based upon disease features and/or patient preferences. (Strong Recommendation, Moderate-quality evidence),

C) If surgery is chosen for patients with thyroid cancer <1 cm without extra-thyroidal extension and cN0, the initial surgical procedure should be a thyroid lobectomy unless there are clear indications to remove the contralateral lobe. Thyroid lobectomy alone is sufficient treatment for small, unifocal, intra-thyroidal carcinomas in the absence of prior head and neck irradiation, familial thyroid carcinoma, or clinically detectable cervical nodal metastases (Strong Recommendation, Moderate-quality evidence).

thyroid lobectomy, a conservative management approach to up front surgery accepting a slightly higher risk of loco-regional recurrence is an acceptable management strategy.

The guideline committee further stated that a more selective use of RAI coupled with a greater reliance on neck ultrasound and serial serum Tg measurements for detection of recurrent disease is likely to significantly decrease the mandate for total thyroidectomies in low and intermediate risk patients done solely to facilitate RAI remnant ablation and follow-up. Near-total or total thyroidectomy is necessary if the overall strategy is to include RAI therapy postoperatively, and thus is recommended if the primary thyroid carcinoma is >4 cm, there is gross extra-thyroidal extension, or regional or distant metastases are present. For tumors that are between 1 and 4 cm in size, either a bilateral thyroidectomy (total or near-total) or a unilateral procedure (thyroid lobectomy) may serve as the surgical platform for an overall treatment plan. Older age (>45 years), contralateral thyroid nodules, a personal history of radiation therapy to the head and neck, or familial DTC may be criteria for recommending a bilateral procedure. This could help the plans for RAI therapy, facilitate follow-up strategies, and also may address suspicions of bilateral disease (8). This conclusion returns us back to square one. The accurate identification of individual patients who would benefit from a limited intervention versus those who would require the full artillery in hand remains challenged. Clearly, a statistically insignificant, but clinically very important group of outliers of risk categories defined using standard criteria does exist. Particularly for DTC, morphology (or size)-alone only partially explains the full onco-biology of the tumor.

Risk Stratification and Staging of Thyroid Cancer

All cancers present as either localized or at an advanced/metastatic stage, as does the thyroid cancer. The existing paradigm equates metastatic stage to a systemic disorder. The established staging system has become ingrained in our culture. Some of the seemingly "self-evident" concepts require revision. The extent of disease has been defined by the TNM system, and stages have been determined by outcome statistics. For DTC, patients younger than 45, are assigned to stage II despite the presence of remote metastatic disease (Table 2). A 2016 consensus report based on a review of 9484 patients from 10 institutions proposed a change in the cutoff age in the current American Joint Committee on Cancer/Union for International Cancer Control staging system from 45 years to 55 years. This change is estimated to lead to a down-staging of 12% of patients, and would improve the statistical validity of the existing model. Such a change may be clinically relevant for thousands of patients worldwide by preventing over-staging of patients with low-risk disease while providing a more realistic estimate of prognosis for those who remain high risk (14). The current paradigm has been inadequate to resolve the growing controversies. There does not appear to be a magic age cut-off that dicotomizes the

Table 2. Staging of thyroid cancer

Papillary and follicular thyroid cancer (age <45 y)			
Stage	**T**	**N**	**M**
I	Any T	Any N	M0
II	Any T	Any N	MI
Papillary and follicular thyroid cancer (age ≥45 y)			
Stage	**T**	**N**	**M**
I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
IVA	T1-3	N1a	M0
	T4a	N1b	M0
IVB	T4b	Any N	M0
IVC	Any T	Any N	M1

patients into distinct risk categories. A remote metastatic disease may not be the ultimate grave sign. There may be another "phase" in cancer, besides the T, N, and M extensions, that has not been factored-in current disease state definitions. There could be a metabolic switch converting cancer into a "systemic disorder". There may be a difference between a widespread disease and a systemic disorder, which would define/explore the cancer-host metabolic interactions beyond mere expansion of the cancer compartment. The metabolic and catabolic changes associated with this change in phase have not been identified. The road to fatality could be a phase change during any given stage of cancer expansion. The phase change could well be occurring in M0 disease or, adversely develop at a seemingly early stage. The cellular/subcellular dynamics of a true systemic disorder remain undefined. The predicative molecular signatures of the phase change has not been firmly defined. Ultimately, the molecular profiling of individual cancers would be gauging the credence of our knives. The foreseeable paradigm change awaits the maturation of the genomics revolution.

The Age of Genomics

Cancer is a disorder of the genome.

Cancer is a disorder of the genome. The complete genetic information of an organism is referred to as genome. In all-encompassing terms, the genome involves the genes (coding and the non-coding sequences of the DNA), RNA(s), the process of transcription and translation and their regulations. The concept of regulation involves the genetic constituents such as enhancer, promoter, transcription factors (TF), RNA polymerase and mechanisms such as epigenetics. Cancer is a complex expression of multiple genetic and epigenetic alterations. There is an initiating transformational mutational event, then a multitude of genetic aberrations compose a cancer phenotype. All cells throughout the body bear the same coding sequence, yet, tissues and organs have distinct morphology and functions. Normal tissue/organ differentiation is simply a function of selective reading of the genome by different cell types, most vivid in the developing embryo. Cancer is a distinct differentiation, or neo-differentiation, or dedifferentiation, in reference to the normal tissue morphology/function. As cells enter into a constitutive act of replication or repeated cycles of growth and division, the central hallmark of cancer, they also begin to assume distinct phenotypes. This is the process of neo-differentiation. The cells undergoing the neo-differentiation process become committed to form different type of tissue rather than the mature, conformal normal tissue. The genome reading has a new translation.

The gene expression is modulated by "the switches". The orchestration of gene expression by "the switches" involves multiple elements; The enhancer, a non-coding DNA sequence where the TF bind. The binding of the TF is arbitrated by a group of mediator proteins. The enhancer sequence is located some distance upstream of the promotor region, another non-coding DNA sequence, to which RNA polymerase binds. All these elements are also product of different set of coding genes (subject to mutational changes). Moreover, if a particular switch is deemed a compartment, the intra-compartmental interactions are subject to the chemical conformations altered by acetylation or methylation reactions which are not mutational, but referred to as "epigenetic" changes.

The thyroid cancer genome is being decoded. Recent studies have identified a mutation or a genetic alteration in 95% of thyroid cancers. The National Cancer Institute initiated the Cancer Genome Atlas (TCGA) project in 2006 to catalogue genetic mutations associated with cancer, using genome sequencing and bioinformatics. The project has expanded to carry out genomic characterization and sequence analysis of a multitude of cancers, including thyroid cancer. Techniques involve gene expression profiling, copy number variation profiling, single nucleotide pleomorphism genotyping, genome wide DNA methylation profiling, microRNA profiling, and exon sequencing of at least 1,200 genes. TCGA is able to sequence the entire genomes of tumors, including at least 6,000 candidate genes and microRNA sequences.

The Integrated Genomic Characterization of Papillary Thyroid Carcinoma (IGCPTC) study, the first systematic, large scale and robust genomic analysis of thyroid cancer, was published in October 2014 (15). The IGCPTC study demonstrated the role of somatic genetic "alterations." The driving somatic genetic alterations included SSNVs, indels, or fusions, in the mitogen-activated protein kinases (MAPK) and PI3K pathways in PTC. The relatively low overall density of somatic mutations was concluded to be the biological basis for the indolent clinical behavior of PTC. It is evident that the mutations and alterations are functions of time to accompany the aging process. The prognostic importance of age is a continuous variable, not determined by a cut-off value. New driver mutations were identified in PTC, either entirely novel (EIF1AX), or novel alterations of known drivers (RET, *BRAF* and ALK fusions) (Figure 1). As a result of these discoveries, the "dark matter" of the PTC genome has been reduced substantially from ~25% to less than 4%, which will have profound consequences for preoperative cancer diagnosis of thyroid nodules as well as for surgical and post-surgical treatment strategies. Molecular testing for mutation hotspots, rearrangements, and gene expression using fine-needle aspiration specimens has become an

effective diagnostic tool to more precisely select patients for an appropriate surgical procedure. Molecular testing surely would reduce the number of thyroidectomies done for benign nodules and tumors (16,17), and could guide determine the extent of initial surgical procedure (i.e., lobectomy versus total thyroidectomy). Beyond the driver mutations, it was also discovered that several individual key genes [CHEK2, ATM, and Telomerase reverse transcriptase (TERT)] and sets of functionally related genes (chromatin remodeling) with alterations or expression patterns in microRNA (miR-21 and miR-146b) define clinically-relevant subclasses and may contribute to loss of differentiation and tumor progression.

The IGCPTC study demonstrated striking signaling differences in RAS- and *BRAF*^{V600E}-driven PTCs. In particular, BVL-PTCs signal preferentially through MAPK while RL-PTCs signal through both MAPK and PI3K. The relative simplicity of the PTC genome, with dominant mutually exclusive driving events, together with the large cohort and comprehensive data analyzed in this study have led to clearly dissect these signaling differences. The overreaching conclusion of the IGCPTC study was that RL-PTCs and BVL-PTCs are fundamentally different in their genetic, epigenetic, and proteomic profiles. Based on the strength of the multidimensional genomic findings, a clinico-pathologic reclassification of follicular-derived thyroid lesions would be imperative (15).

The impact of different genomic markers on outcomes opened a new field of controversy. Conflicting results with *BRAF* (+) tumors have been reported for the past 10 years. An increasing number of studies, which include meta-analyses, demonstrated an association between the *BRAF* status and aggressive tumor behavior. Other studies, however, have failed to confirm this data, and this has resulted in uncertainty about the prognostic value of *BRAF* mutations in PTC (18,19) It has become clear that multiple genetic alterations contribute to form an individual tumor phenotype and biology. A progressive genomic instability leads to first functional, then morphologic dedifferentiation. TERT promoter mutations have been observed in 5% to 25% of DTC, and it's been reported that TERT promoter mutations contribute to aggressive behavior in DTC (20,21,22). These mutations have a significantly higher prevalence in aggressive thyroid tumor types such as widely invasive oncocytic carcinomas, poorly differentiated carcinomas, and anaplastic thyroid carcinomas (ATC) (23). Similarly, TP53 mutations are prevalent in advanced tumors with a higher recurrence (24). A co-occurrence of multiple mutations may define a more aggressive subgroup of DTC (25).

The next-generation sequencing technology allows high out-put genomic analysis. An innovative assay in thyroid cancer ThyroSeq® - was developed for targeted mutation detection by next generation sequencing technology in fine needle aspiration and tissue samples. ThyroSeq v.2 next generation sequencing panel offers simultaneous sequencing and detection in >1000 hotspots of 14 thyroid cancer-related genes and for 42 types of gene fusions known to occur in thyroid cancer (Table 3) (26). ThyroSeq is being increasingly used to further narrow the indeterminate category defined by cytology for thyroid nodules. From a surgical perspective, obviously this provides prognostic and predictive information as it relates to determination of surgical strategy. Both the genomic analysis technology and the data collection for the cancer genome atlas are rapidly developing.

Although we have sequenced the cancer genome extensively, and have identified a number of driver and passenger mutations, we are yet far from having a map of the full genomic alterations involved in the onco-pathogenesis of thyroid cancer (Figure 2). We are, though, capable of patterning the phenotypic expressions in terms of profiling. Individual tumor morphology, function and biology are compound results of mutational changes, and selective activation and/or deactivation of gene switches. Eventually, the cancer morphology, or architectural neo-differentiation will be understood and defined in terms of sets of genes that are expressed (transcribed) in neoplastic tissues. Perhaps the "papillary thyroid cancer" assignment based on morphology, its intricate variations, and perplexing functional and prognostic associations will be replaced by gene expression profiles, mathematical abstractions, with clear indications as to its biologic meaning. We will, though, continue to refer to the morphology as part of its complete profile, not a sole indicator of its prognosis and treatment options. This will also pave the road to rational treatment actions. The concept of risk stratification based on traditional parameters will soon vacate their role for clear molecular markers of non-invasive/focal, invasive/metastatic and systemic stages/phases of neoplastic disorder.

Truly local/indolent tumors could easily be addressed with less invasive techniques such as Laser ablation and

Table 3. ThyroSeq v2 panel

Gene mutations (DNA)		Gene fusions (RNA)	Gene expression (RNA)	
BRAF	*RET*	RET	PGK1	Pan-cell
NRAS	*TSHR*	PPARG	KRT7	
HRAS	*AKT1*	NTRK1	TG	Thyroid cell
KRAS	*TP53*	NTRK3	TTF1	
PIK3CA	*GNAS*	*BRAF*	NIS	
PTEN	*CTNNB1*	ALK	Calcitonin	MTC
TERT	*EIF1AX*		PTH	Parathyroid
			KRT20	Metastatic

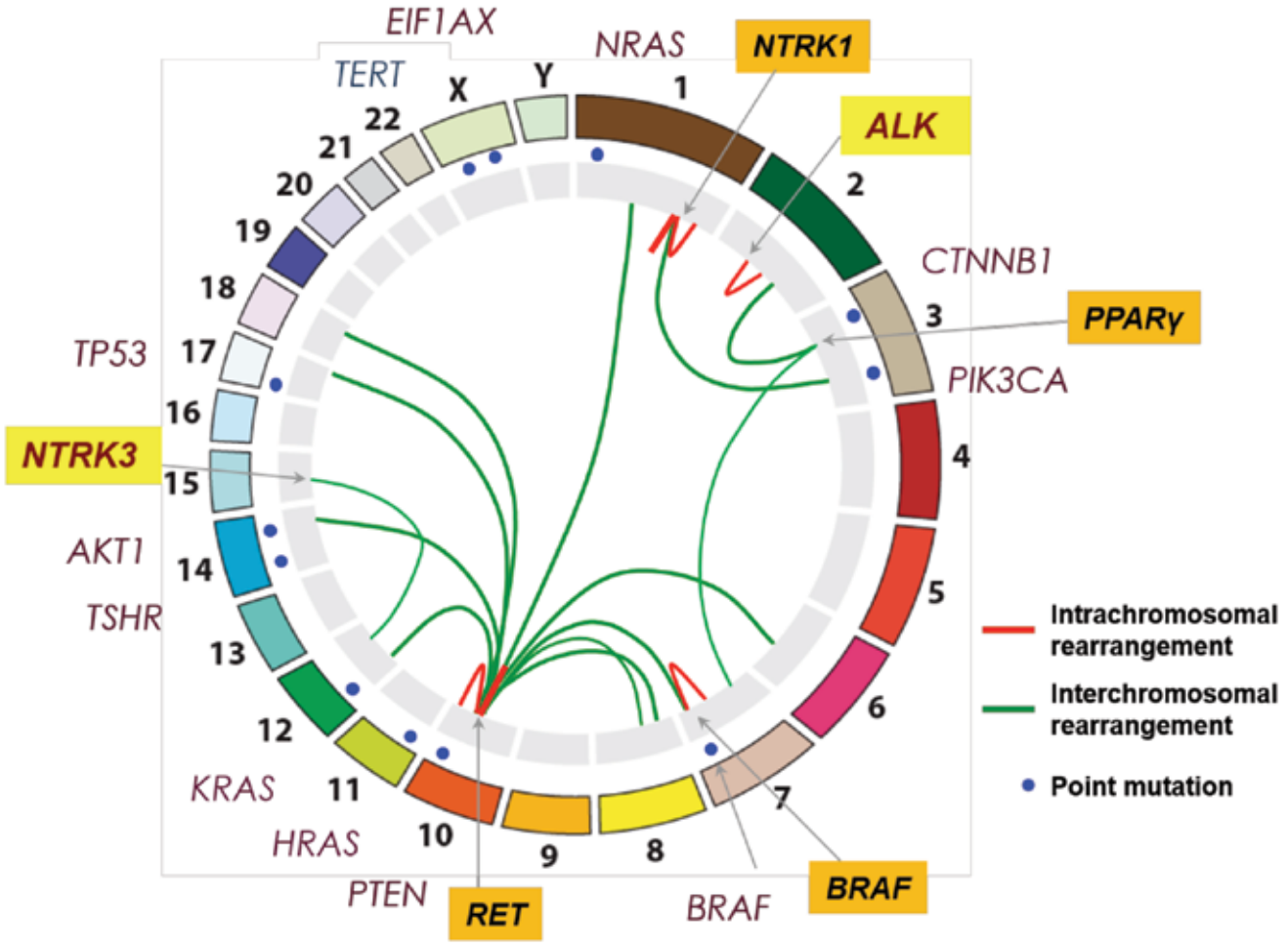


Figure 2. Main mutation mechanisms: Point mutations and chromosome rearrangements
Adapted from Nikiforov YE, Diagnostic pathology and molecular genetics of the thyroid, 2009

radiofrequency ablation, at the most by lobectomy. On the other hand when a potential for metastatic development is identified, regardless of the tumor size or patient's age, the most aggressive approach including complete thyroidectomy (surgical total thyroidectomy followed by beta-knife ablation) will be employed. We will not feel inadequate to explain to an unfortunate patient why his/her small, supposed to be indolent tumor is taking a fatal course. We will not record those cases as statistical misfits. Patient-specific treatment protocols will prevail. A refined classification scheme based on genomics and its phenotypic expressions will accurately reflect the biologic differences between the different morphologic definitions we use today. Tumor differentiation/de-differentiation, and clinical behavior of an individual cancer will be defined by molecular markers, in addition to standard morpho-pathology. Empiricism in science of medicine and surgery has acquired a new method for testing the appropriate treatment for individual patients; that is molecular pathology, governed by genomics. The technology is present and rapidly evolving. The surgeons will determine the extent of interventions with molecular evidence and guidance.

The translation of cancer genomics data into clinical insights will elevate oncology to the next level and usher in a new era in understanding, diagnosing and treating cancer. The new evidence in "evidence-based medicine" is the genomic data.

In god we trust,
All others must bring data.
William Edward Deming

References

1. Sakorafas GH. Historical evolution of thyroid surgery: from the ancient times to the dawn of the 21st century. World J Surg 2010;34:1793-1804.
2. Halsted WS. The operative story of goiter. Johns Hopkins Hosp Rev 1920;19:71-257.
3. Milestones in European Thyroidology. European Thyroid Association. http://www.eurothyroid.com/about/met/kocher.html
4. Noguchi H. Thyroid Cancer: The Evolution of Treatment Options, Updates in the Understanding and Management of Thyroid Cancer, Dr. Thomas J. Fahey (ed). 2012. http://www.intechopen.com/books/updates-in-the-understanding-and-management-of-thyroidcancer/thyroid-cancer-the-direction-of-future-treatments
5. Seidlin SM, Rossman I, et al. Radioiodine therapy of metastases from carcinoma of the thyroid; a 6-year progress report. J Clin Endocrinol Metab 1949;9:1122-1137.
6. Bierwaltes WH, Johnson PC, Solari AJ. Clinical Use of Radioisotopes. WB Saunders Co.1957. p.131
7. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009;19:1167-1214.
8. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26:1-133.
9. Takami H, Ito, Noguchi H, Yoshida A, Okamoto T. Treatment of thyroid tumor. Japanese clinical guidelines Springer Japan 2013.
10. Mazzaferri EL, Kloos RT. Clinical review 128: Current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab 2001;86:1447-1463.
11. Clark OH, Duh QY. Thyroid cancer. Med Clin North Am 1991;75:211-234.
12. Shaha AR, Shah JP, Loree TR. Low-risk differentiated thyroid cancer: the need for selective treatment. Ann Surg Oncol1997;4:328-333.
13. Hay ID, Bergstralh EJ, Grant CS, McIver B, Thompson GB, van Heerden JA, Goellner JR. Impact of primary surgery on outcome in 300 patients with pathologic tumor-node-metastasis stage III papillary thyroid carcinoma treated at one institution from 1940 through 1999;126:1173-1181.
14. Nixon IJ, Wang LY, Migliacci JC, Eskander A, Campbell MJ, Aniss A, Morris L, Vaisman F, Corbo R, Momesso D, Vaisman M, Carvalho A, Learoyd D, Leslie WD, Nason RW, Kuk D, Wreesmann V, Morris L, Palmer FL, Ganly I, Patel SG, Singh B, Tuttle RM, Shaha AR, Gönen M, Pathak KA, Shen WT, Sywak M, Kowalski L, Freeman J, Perrier N, Shah JP. An International Multi-Institutional Validation of Age 55 Years as a Cutoff for Risk Stratification in the AJCC/UICC Staging System for Well-Differentiated Thyroid Cancer. Thyroid 2016;26:373-380.
15. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676-690.
16. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, Hodak SP, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN. Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. Cancer 2014;120:3627-3634.
17. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN. Impact of the Multi-Gene ThyroSeq Next-Generation Sequencing Assay on Cancer Diagnosis in Thyroid Nodules with Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Cytology. Thyroid 2015;25:1217-1223.
18. Li J, Zhang S, Zheng S, Zhang D, Qiu X. The BRAF V600E mutation predicts poor survival outcome in patients with papillary thyroid carcinoma: a meta analysis. Int J Clin Exp Med 2015;8:22246-22253.
19. Gandolfi G, Sancisi V, Piana S, Ciarrocchi A. Time to re-consider the meaning of BRAF V600E mutation in papillary thyroid carcinoma. Int J Cancer 2015;137:1001-1011.
20. Lee SE, Hwang TS, Choi YL, Han HS, Kim WS, Jang MH, Kim SK, Yang JH. Prognostic Significance of TERT Promoter Mutations in Papillary Thyroid Carcinomas in a BRAF(V600E) Mutation-Prevalent Population. Thyroid 2016;26:901-910.
21. Jin L, Chen E, Dong S, Cai Y, Zhang X, Zhou Y, Zeng R, Yang F, Pan C, Liu Y, Wu W, Xing M, Zhang X, Wang O. BRAF and TERT promoter mutations in the aggressiveness of papillary thyroid carcinoma: a study of 653 patients. Oncotarget 2016;7:18346-18355.
22. Alzahrani AS, Alsaadi R, Murugan AK, Sadiq BB. TERT Promoter Mutations in Thyroid Cancer. Horm Cancer 2016;7:165-177.
23. Tong GX, Mody K, Wang Z, Hamele-Bena D, Nikiforova MN, Nikiforov YE. Mutations of TSHR and TP53 genes in an aggressive clear cell follicular carcinoma of the thyroid. Endocr Pathol 2015;26:315-319.
24. Kasaian K, Wiseman SM, Walker BA, Schein JE, Zhao Y, Hirst M, Moore RA, Mungall AJ, Marra MA, Jones SJ. The genomic and transcriptomic landscape of anaplastic thyroid cancer: implications for therapy. BMC Cancer 2015;15:984.
25. Song YS, Lim JA, Choi H, Won JK, Moon JH, Cho SW, Lee KE, Park YJ, Yi KH, Park do J, Seo JS. Prognostic effects of TERT promoter mutations are enhanced by coexistence with BRAF or RAS mutations and strengthen the risk prediction by the ATA or TNM staging system in differentiated thyroid cancer patients. Cancer 2016;122:1370-1379.
26. Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE. Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab 2013;98:1852-1860.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):10-15 DOI:10.4274/2017.26.suppl.02

Lymph Node Dissection for Differentiated Thyroid Cancer

Tiroid Kanseri için Lenf Nodu Diseksiyonu

Aviram Mizrachi, Ashok R. Shaha
Memorial Sloan-Kettering Cancer Center, Head and Neck Service, New York, USA

Abstract

Lymph node metastases in differentiated thyroid cancer (DTC) have a wide spectrum of clinical significance. Several variables are taken under consideration when trying to decide on the optimal management of patients with DTC. Routine prophylactic central and/or lateral lymph node dissection is not advocated with exception of central neck dissection for locally advanced tumors. When regarding recurrent disease, foundations have been laid for clinicians to make accurate decisions as to when to perform surgery and when to continue maintaining the patient's disease under observation. These complex decisions are determined based upon multiple factors, not only regarding the patient's disease but also the patient's comprehension of the procedure and apprehension levels. Nevertheless if the patient and/or clinician are emotionally keen to surgically remove the disease then the procedure should be considered.
Keywords: Thyroid cancer, lymph node metastases, neck dissection

Öz

Diferansiye tiroid kanserinde (DTK) lenf nodu metastazının geniş bir yelpazede klinik önemi vardır. DTK'lı hastaların optimal tedavi yönetimine karar verilirken birçok değişken hesaba alınır. Rutin profilaktik santral ve/veya lateral lenf nodu diseksiyonu lokal ileri tümörlerde santral boyun diseksiyonu istisnası dışında savunulmamaktadır. Rekürrens durumunda klinisyenlerin ne zaman ameliyat gerçekleştirmesi ve ne zaman takip edilmesi kararını verebilmeleri için kılavuzlar oluşturulmuştur. Bu karmaşık kararlar genellikle tek bir faktöre dayanarak değil, aksine hastanın anlama ve katılma düzeyleri ile uyum içinde birçok faktör ile dikkate alınarak verilir. Bununla birlikte, hasta ve/veya klinisyen hastalığı cerrahi olarak çıkartmaya ısrarcı ise o zaman bu seçenek düşünülmelidir.
Anahtar kelimeler: Tiroid kanseri, lenf nodu metastazı, boyun diseksiyonu

Overview

The incidence of thyroid cancer, specifically well differentiated, is rapidly increasing. The Surveillance, Epidemiology and End Results Program has estimated that there will be 62,450 new cases of thyroid cancer and an estimated 1,950 people will die of this disease in 2015. Nevertheless, the overall five-year survival rate for thyroid cancer is almost 98% and has been constant over the last three decades (1).

The majority of differentiated thyroid cancers (DTC) are usually diagnosed at an early stage, during a routine check up or more increasingly as an incidental finding of neck ultrasonography (US). In these cases, the presence of clinically apparent nodal metastases is uncommon. That being said, multiple studies have shown that the incidence of occult lymph node metastases may reach up to 60%, but this microscopic disease has no prognostic value in patients with DTC (2). Several risk factors for the presence of central lymph node metastases in DTC have been previously described and found to be primary tumor size, extra-thyroidal extension (ETE) and aggressive histological subtypes (3). The factors that are most predictive of central lymph node metastases are male sex, young and old age and primary tumor size (4). Special attention should be given to involvement of the pre-laryngeal

Address for Correspondence: Aviram Mizrachi MD, Memorial Sloan-Kettering Cancer Center, Head and Neck Service, New York, USA
Phone: +90 347-449-3137 E-mail: mizracha@mskcc.org

(Delphian) lymph node in DTC, which is often associated with ETE and increased incidence of central and lateral neck lymph node metastases. Extrapolating from that, surgeons should consider sampling the Delphian lymph node and perform a frozen section as a form of "sentinel lymph node biopsy" that if found positive may warrant further evaluation of the central and lateral nodal compartments (5). The current AJCC staging system (7th edition) makes a distinction between central nodal involvement (N1a) and lateral nodal involvement (N1b). However, in some cases it may be difficult to distinguish between levels VI and VII, which are adjacent and represent different nodal stages with the latter being N1b.

The current approach towards the role of prophylactic neck dissection precludes any survival benefit in patients with a clinically negative neck and may result in unnecessary upstaging that could subject them to radioactive iodine (RAI) treatment (6). Prophylactic central compartment neck dissection (ipsilateral or bilateral) should be considered in patients with DTC with clinically uninvolved central neck lymph nodes (cN0) who have locally advanced primary tumors (T_3 or T_4), clinically involved lateral neck nodes (cN1b), or if the information will be used to plan further steps in therapy (7). This approach has reached consensus among surgeons in relation to the lateral neck, while for the central compartment some still advocate a prophylactic lymph node dissection. One argument for this approach is that the central compartment is readily accessible while performing the thyroidectomy and that clearing this compartment during the first surgical procedure is easier and safer than in the revision setting (8). However, a fair number of studies showed increased rates of transient and permanent recurrent laryngeal nerve injury and hypoparathyroidism following prophylactic central lymph node dissection, even for low volume disease (9). The traditional paradigm assigned the same magnitude of risk for all patients with N1 disease (10). However, small-volume subclinical microscopic N1 disease clearly conveys a much smaller risk of recurrence than large-volume, macroscopic clinically apparent loco-regional metastases (Table 1). With this new information, clinicians will be better able to tailor initial treatment and follow-up recommendations. Implications of N1 stratification for DTC into small-volume microscopic disease versus clinically apparent macroscopic disease importantly relate to issues of prophylactic neck dissection utility, need for pathologic nodal size description, and suggest potential modifications to the AJCC TNM (tumor, nodal disease, and distant metastasis) and American Thyroid Association risk recurrence staging systems (11). Table 2 elaborates on the arguments for and against prophylactic central neck dissection.

Workup and Initial Management

The primary lymphatic drainage of the thyroid is to the central neck, with subsequent spread to the lower lateral (level IV and V) and then the upper lateral levels, II and III (Figure 1).

Preoperative US is extremely useful for initial staging of cervical lymph nodes. The European Thyroid Association guidelines for cervical ultrasound illustrate very eloquently the sonographic features of lymph nodes that are predictive of malignant involvement as shown in Table 3 (12). Moreover, in experienced hands US may be quite accurate in detecting sub-centimeter metastatic lymph nodes in the lateral neck and even within the central compartment (13,14).

In the presence of lateral neck disease a cross-sectional imaging is warranted. Computerized tomography (CT) provides excellent detail in regard to the local extent of the primary tumor as well as nodal disease in both lateral and central neck and should be utilized when necessary. Fine-needle aspiration (FNAB) biopsy of suspicious lymph nodes

Table 1. Risk of recurrence based on the characteristics of the cervical lymph node metastases

Pathology	Specific characteristic	Median %	Range %
Pathological N1	Clinical N0	2	0-9
	<5 metastatic nodes	4	3-8
	>5 metastatic nodes	19	7-21
	Clinical N1	22	10-42
	Clinical N1 with extranodal extension	24	15-32

Table 2. Considerations for and against prophylactic central lymph node dissection

Advantages	Disadvantages
Proper staging	Risk for permanent hypoparathyroidism
Risk stratification	Recurrent laryngeal nerve injury
Reduce the need for revision surgery	Unnecessary upstaging
	May warrant radioactive iodine
	Prolonged operative time

should be performed when the clinical and radiological findings are inconclusive and in order to determine the extent of surgery (11). Patients with evidence of nodal disease require therapeutic neck dissection. When disease is limited to the central compartment, clearance of levels VI and VII is recommended. Therapeutic central-compartment neck dissection for patients with clinically involved central nodes should accompany total thyroidectomy to provide clearance of disease from the central neck. This procedure is performed via a horizontal neck incision in a natural skin crease at the lower border of the cricoid cartilage to allow removal of all nodal tissue from the hyoid bone to the innominate artery and from one common carotid artery to the other. The recurrent laryngeal nerves should be carefully dissected and preserved, and the parathyroid glands should be identified and preserved along with their blood supply. When the parathyroid glands are devascularized, they may require auto transplantation in the sternocleidomastoid muscle.

In patients with proven lateral neck disease, therapeutic neck dissection is indicated and can be done in a somewhat selective manner. A rate of metastasis in levels I, IIb and Va are low, and in the absence of proven disease at these levels they can be spared to avoid morbidity, especially to the marginal mandibular and spinal accessory nerves. The lateral neck dissection should normally entail levels IIa through Vb and can be done simply by continuing the thyroidectomy/central neck dissection incision laterally within the same skin

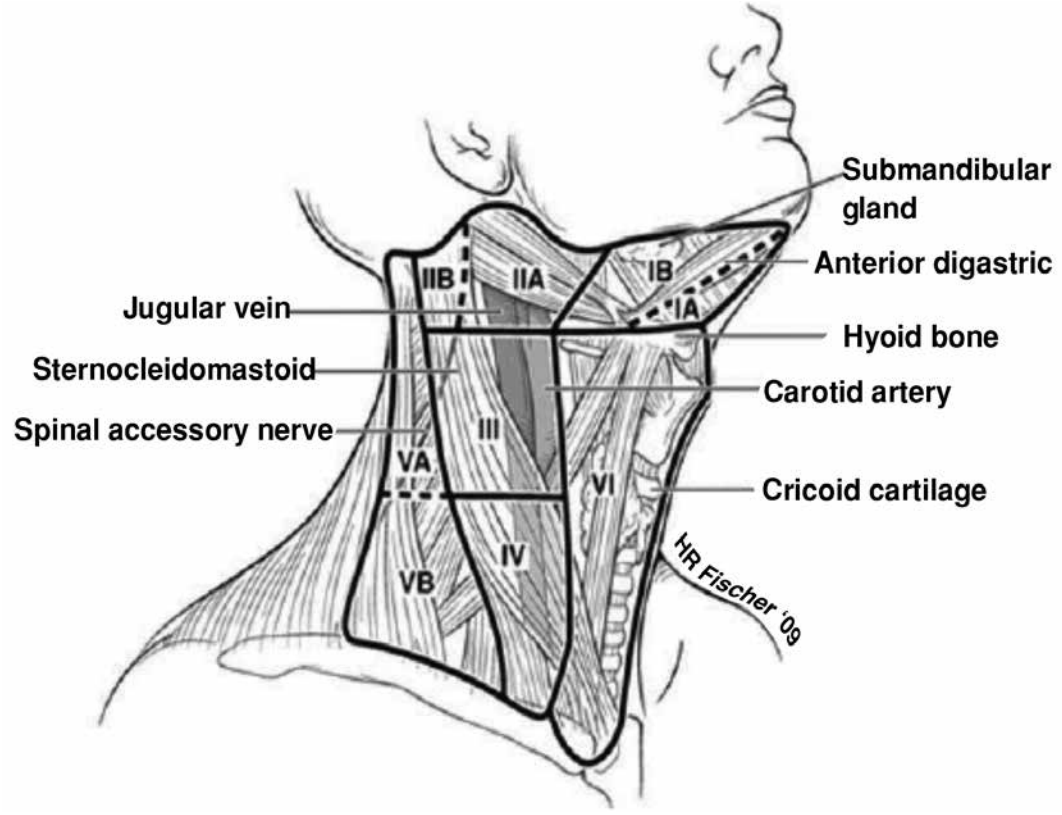


Figure 1. Lymph node compartments of the neck

Table 3. Ultrasound features of lymph nodes predictive of malignant involvement (European Thyroid Association guidelines for cervical ultrasound)

Sign	Reported sensitivity %	Reported specificity %
Microcalcifications	5-69	93-100
Cystic aspect	10-34	91-100
Peripheral vascularity	40-86	57-93
Hyperechogenicity	30-87	43-95
Round shape	37	70

crease. In thyroid cancer, special attention should be paid to nodal tissue posterior to the great vessels in level IV, as this is a common site for recurrent nodal disease. Moreover, lymph mode metastases are often found very low in the base of the neck, and dissecting this area may increase the risk for significant vascular and lymphatic injury (15).

Complications of neck dissection can be divided to intraoperative and postoperative complications. These adverse events can further be divided into minor and major. In experienced hands the risk for any complication is between 5%-7% depending on the extent of disease and the risk for major complications is less than 1%. Table 4 summarizes the different complications of lateral and central neck dissection (16).

Studies of the *BRAF*^{V600E} mutation have suggested an association between presences of the mutation and the risk of nodal disease although results across all patients with DTC are mixed. However, the presence of a *BRAF*^{V600E} mutation has a limited positive predictive value for recurrence and therefore *BRAF*^{V600E} mutation status in the primary tumor should not impact on the decision for prophylactic central neck dissection (17).

Follow up and Recurrent Nodal Disease

Neck recurrence in DTC is not an uncommon scenario with up to 30% regional recurrence reported in the literature (18). The risk factors for recurrent nodal disease in the lateral compartment are extra-nodal extension of lymph node metastases and the ratio between positive and excised lymph nodes during the initial neck dissection (19).

This requires thorough assessment of disease and patient-related factors that come into play and based on that decisions can be made. The assessment is usually best done in the context of a multidisciplinary setting. Several reports have shown that most recurrent central compartment nodules do not show clinically significant growth over several years of follow-up and can be safely followed up with serial US (20,21). This approach was subsequently reinforced by the new American Thyroid Association thyroid cancer guidelines for the management of small abnormal cervical lymph nodes (2). Recently, the Thyroid Cancer Care Collaborative (TCCC) published a decision making guide for management of recurrent nodal disease in thyroid cancer and concluded that understanding the biology of DTC allows clinicians to become more conservative in select patients as long as they comprehend the lifelong surveillance of recurrent nodal disease (22).

One of the key elements in the management of regional recurrent DTC is to identify the disease as early as possible. This involves regular surveillance for anyone who has had an operation for thyroid cancer with or without treatment of the neck. There are few modalities useful to detect recurrent disease; one is serum Tg level (23). Stimulated Tg level may be used to produce a more accurate result. However, in many cases a chemical recurrence does not necessarily translate into structural recurrence (24). Routine US is useful in detecting early nodal recurrence but is extremely operator dependent. Nevertheless, in experienced hands it allows quick evaluation of the neck with the option of obtaining FNAB to confirm recurrent nodal disease. In some cases it may be challenging to differentiate central compartment neck recurrence from local recurrence in the thyroid bed. In that setting, obtaining cross-sectional imaging may be of aid. CT scan and MRI should be considered in any case of recurrent nodal disease or in the previously dissected neck when planning a surgical intervention (25). The role of fluorodeoxyglucose positron emission tomography (FDGPET) is usually limited to less differentiated cancer subtypes, which are usually less RAI avid. These characteristics may sometimes indicate a more aggressive biological behavior of the recurrent tumor. Some tumors may undergo dedifferentiation with increased

Table 4. Intraoperative and postoperative complications of neck dissection

	Intraoperative	Postoperative
Major	Severe hemorrhage	Hematoma
	Pneumothorax	Airway obstruction
	Air embolus	Facial edema
	Phrenic nerve or Brachial plexus injury Esophageal injury Laryngotracheal injury	Carotid artery rupture Neck abscess
Minor	Hemorrhage	Hematoma
	Cranial nerve injury	Seroma
	Chyle leak Horner's syndrome	Wound infection Chyle fistula Wound dehiscence
		Electrolyte imbalance Skin anesthesia

metabolic activity. These tumors will become more highly FDG-PET avid as they display less iodine uptake. It has been suggested that this tumor behavior has a negative impact on outcome (26). A recent report concluded that the magnitude of risk for recurrence in patients with N1 disease is not uniform across the board. They found that small-volume subclinical microscopic N1 disease clearly conveys a much smaller risk of recurrence than large-volume, macroscopic clinically apparent loco-regional metastases (27). Patients undergoing revision central compartment dissection for recurrent/persistent disease are at increased risk for vocal fold dysfunction, even when the recurrent laryngeal nerve is anatomically preserved (28). In these cases it is reasonable to closely monitor low volume and sub-centimeter recurrent nodal disease, which in most patients may stay indolent and non-threatening for many years. In the case of progressive nodal disease a selective neck dissection should be performed to remove all structurally apparent disease. This approach prevents unnecessary interventions, which may result in upstaging, increased morbidity and adversely affect quality of life (19).

When gross structural disease is evident and a unilateral/bilateral paratracheal and superior mediastinal dissection is indicated, this should be done in a tertiary care center by an experienced surgeon in order to provide an ontologically safe procedure and achieve minimal morbidity, especially in a setting of prior multiple surgical procedures and/or existing vocal cord paralysis (29,30).

An alternative to surgery or observation of recurrent nodal disease is ultrasound-guided percutaneous ethanol ablation, few institutions in North America and Europe, the most prominent being the Mayo Clinic in Rochester, MN, practice this less conventional method. In their hands this approach is safe and feasible but limited by the number of neck metastases (31).

Conclusion

Lymph node metastases in DTC have a wide spectrum of clinical significance. Several variables are taken under consideration when trying to decide on the optimal management of patients with DTC. Routine prophylactic central and/or lateral lymph node dissection is not advocated with exception of central neck dissection for locally advanced tumors. When regarding recurrent disease, foundations have been laid for clinicians to make accurate decisions as to when to perform surgery and when to continue maintaining the patient's disease under observation..

These complex decisions are determined based upon multiple factors, not only regarding the patient's disease but also the patient's comprehension of the procedure and apprehension levels. Nevertheless if the patient and/or clinician are emotionally keen to surgically remove the disease then the procedure should be considered.

Authorship Contributions

Surgical and Medical Practices: Ashok R. Shaha, Aviram Mizrachi, Concept: Ashok R. Shaha, Design: Ashok R. Shaha, Aviram Mizrachi, Data Collection or Processing: Aviram Mizrachi, Analysis or Interpretation: Aviram Mizrachi, Literature Search: Aviram Mizrachi, Writing: Ashok R. Shaha, Aviram Mizrachi.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. SEER Cancer Statistics Review, 1975-2012, National Cancer Institute. Bethesda, MD. November 2014 SEER data submission, posted to the SEER web site, April 2015.
2. Haugen Bryan R, Alexander EK, Bible KC, Doherty G, Mandel SJ, Nikiforov YE, Pacini F, Randolph G, Sawka A, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward D, Tuttle RM d, Wartofsky L. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2015.
3. Iyer NG, Shaha AR. Central compartment dissection for well differentiated thyroid cancer and the band plays on. Curr Opin Otolaryngol Head Neck Surg 2011;19:106-112.
4. Thompson AM, Turner RM, Hayen A, Aniss A, Jalaty S, Learoyd DL, Sidhu S, Delbridge L, Yeh MW, Clifton-Bligh R, Sywak M. A preoperative nomogram for the prediction of ipsilateral central compartment lymph node metastases in papillary thyroid cancer. Thyroid 2014;24:675-682.
5. Iyer NG, Kumar A, Nixon IJ, Patel SG, Ganly I, Tuttle RM, Shah JP, Shaha AR. Incidence and significance of Delphian node metastasis in papillary thyroid cancer. Ann Surg 2011;253:988-991.
6. Bonnet S, Hartl D, Leboulleux S, Baudin E, Lumbroso JD, Al Ghuzlan A, Chami L, Schlumberger M, Travagli JP. Prophylactic lymph node dissection for papillary thyroid cancer less than 2 cm: implications for radioiodine treatment. J Clin Endocrinol Metab 2009;94:1162-1167.
7. Zuniga S, Sanabria A. Prophylactic central neck dissection in stage N0 papillary thyroid carcinoma. Arch Otolaryngol Head Neck Surg 2009;135:1087-1091.
8. Zetoune T, Keutgen X, Buitrago D, Aldailami H, Shao H, Mazumdar M, Fahey TJ, Zarnegar R. Prophylactic central neck dissection and local recurrence in papillary thyroid cancer: a meta-analysis. Ann Surg Oncol 2010;17:3287-3293.
9. Pereira JA, Jimeno J, Miquel J, Iglesias M, Munne A, Sancho JJ, Sitges-Serra A. Nodal yield, morbidity, and recurrence after central neck dissection for papillary thyroid carcinoma. Surgery 2005;138:1095-1100.
10. National Comprehensive Cancer Network Clinical Practice Guidelines. Available at: http://www.nccn.org/default.aspx. Accessed August 10, 2013.
11. Randolph GW, Duh QY, Heller KS, LiVolsi VA, Mandel SJ, Steward DL, Tufano RP, Tuttle RM; American Thyroid Association Surgical Affairs Committee's Taskforce on Thyroid Cancer Nodal Surgery. The prognostic significance of nodal metastases from papillary thyroid carcinoma can be stratified based on the size and number of metastatic lymph nodes, as well as the presence of extranodal extension. Thyroid 2012;22:1144-1152.
12. Leenhardt L, Erdogan MF, Hegedus L, Mandel SJ, Paschke R, Rago T, Russ G. 2013 European thyroid association guidelines for cervical ultrasound scan and ultrasound-guided techniques in the postoperative management of patients with thyroid cancer. Eur Thyroid J 2013;2:147-159.

13. Hwang HS, Orloff LA. Efficacy of preoperative neck ultrasound in the detection of cervical lymph node metastasis from thyroid cancer. Laryngoscope 2011;121:487-491.
14. Mizrachi A, Feinmesser R, Bachar G, Hilly O, Cohen M. Value of ultrasound in detecting central compartment lymph node metastases in differentiated thyroid carcinoma. Eur Arch Otorhinolaryngol 2014;271:1215-1218.
15. Ito Y, Higashiyama T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Kuma K, Miyauchi A. Risk factors for recurrence to the lymph node in papillary thyroid carcinoma patients without preoperatively detectable lateral node metastasis: validity of prophylactic modified radical neck dissection. World J Surg 2007;31:2085-2091.
16. Bier-Laning CM. Surgical complications of the neck. Cummings Otolaryngology Head and Neck Surgery. 4th ed. Elsevier Mosby, 2005;3:2646-2657.
17. Ricarte-Filho J, Ganly I, Rivera M, Katabi N, Fu W, Shaha A, Tuttle RM, Fagin JA, Ghossein R. Papillary thyroid carcinomas with cervical lymph node metastases can be stratified into clinically relevant prognostic categories using oncogenic BRAF, the number of nodal metastases, and extra-nodal extension. Thyroid 2012;22:575-584.
18. Shaha AR. Recurrent differentiated thyroid cancer. Endocr Pract 2012;18:600–603. thyroid carcinoma.
19. Ahmadi N, Grewal A, Davidson BJ. Patterns of cervical lymph node metastases in primary and recurrent papillary thyroid cancer. J Oncol 2011;2011:735678.
20. Chéreau N, Buffet C, Trésallet C, Tissier F, Leenhardt L, Menegaux F. Recurrence of papillary thyroid carcinoma with lateral cervical node metastases: Predictive factors and operative management. Surgery 2016;159:755-762.
21. Baek SK, Jung KY, Kang SM, Kwon SY, Woo JS, Cho SH, Chung EJ. Clinical risk factors associated with cervical lymph node recurrence in papillary thyroid carcinoma. Thyroid 2010;20:147-152.
22. Urken ML, Milas M, Randolph GW, Tufano R, Bergman D, Bernet V, Brett EM, Brierley JD, Cobin R, Doherty G, Klopper J, Lee S, Machac J, Mechanick JI, Orloff LA, Ross D, Smallridge RC, Terris DJ, Clain JB, Tuttle M. Management of recurrent and persistent metastatic lymph nodes in well-differentiated thyroid cancer: a multifactorial decision-making guide for the thyroid cancer care collaborative. Head Neck 2015;37:605-614.
23. Mazzaferri EL, Robbins RJ, Spencer CA, Braverman LE, Pacini F, Wartofsky L, Haugen BR, Sherman SI, Cooper DS, Braunstein GD, Lee S, Davies TF, Arafah BM, Ladenson PW, Pinchera A. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 2003;88:1433-1441.
24. Pacini F, Sabra MM, Tuttle RM. Clinical relevance of thyroglobulin doubling time in the management of patients with differentiated thyroid cancer. Thyroid 2011;21:691-692.
25. Schlüter B, Bohuslavizki KH, Beyer W, Plotkin M, Buchert R, Clausen M. Impact of FDG PET on patients with differentiated thyroid cancer who present with elevated thyroglobulin and negative 131I scan. J Nucl Med 2001;42:71-76.
26. Robenshtok E, Fish S, Bach A, Dominguez JM, Shaha A, Tuttle RM. Suspicious cervical lymph nodes detected after thyroidectomy for papillary thyroid cancer usually remain stable over years in properly selected patients. J Clin Endocrinol Metab 2012;97:2706-2713.
27. Yim JH, Kim WB, Kim EY, Kim WG, Kim TY, Ryu JS, Gong G, Hong SJ, Shong YK. The outcomes of first reoperation for locoregionally recurrent/persistent papillary thyroid carcinoma in patients who initially underwent total thyroidectomy and remnant ablation. J Clin Endocrinol Metab 2011;96:2049-2056.
28. Starmer H, Noureldine SI, Ozgursoy OB, Tufano RP. Voice outcomes following reoperative central neck dissection for recurrent/persistent thyroid cancer. Laryngoscope 2015;125:2621-2625.
29. Clayman GL, Shellenberger TD, Ginsberg LE, Edeiken BS, El-Naggar AK, Sellin RV, Waguespack SG, Roberts DB, Mishra A, Sherman SI. Approach and safety of comprehensive central compartment dissection in patients with recurrent papillary thyroid carcinoma. Head Neck 2009;31:1152-1163.
30. Yehuda MN, Freeman JL. Revision central compartment surgery: indications, management and outcomes in 60 consecutive patients. Annual Meeting of the American Head and Neck Society; San Diego, California, 2007.
31. Hay ID, Charboneau JW. The coming of age of ultrasound-guided percutaneous ethanol ablation of selected neck nodal metastases in well-differentiated thyroid carcinoma. J Clin Endocrinol Metab 2011;96:2717-2720.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):16-23 DOI:10.4274/2017.26.suppl.03

Radioactive Iodine Remnant Ablation: The Beta-knife Completion Thyroidectomy

Raydoaktif İyot Bakiye Doku Ablasyonu: Beta-knife Tamamlayıcı Tiroidektomisi

Seza Gulec, MD, FACS[1], Russ Kuker, MD[2]
[1]*Florida International University Herbert Wertheim College of Medicine, Departments of Surgery and Nuclear Medicine, Miami, USA*
[2]*University of Miami Miller School of Medicine, Department of Radiology, Miami, USA*

Abstract

The rationale and objectives for radioactive iodine (RAI) ablation remain perplexing to many. This review addresses the meaning, clinical context and the goals of "ablation": the RAI treatment after a total thyroidectomy. This article also aims to clarify the definition of a total thyroidectomy and how a thyroid remnant can introduce a confounding factor in the postoperative management of patients with differentiated thyroid cancer. The implications of an existing thyroid remnant on RAI diagnostic imaging and serum thyroglobulin levels are discussed. This review provides a rational approach validating the utility of RAI remnant ablation regardless of the patient's risk stratification.
Keywords: Radioactive iodine, ablation, thyroid cancer, thyroid remnant, I-131, I-124, dosimetry, beta knife

Öz

Radyoaktif iyot (RAİ) ablasyon kavramı mantığının ve hedeflerinin birçok kişi için hala yeterince açık olmadığını görüyoruz. Bu makalede total tiroidektomiyi takiben yapılan RAİ "ablasyon" girişiminin anlamı, hedefleri ve klinik değeri izah edilmektedir. Gerçekçi anlamda total tiroidektominin RAİ ablasyonu ile mümkün olduğu tezini savunuyor ve destekleyen verileri sistematik olarak sunuyoruz.
Anahtar kelimeler: Radyoaktif iyot, ablasyon, tiroid kanseri, bakiye tiroid dokusu, I-131, I-124, dozimetri, beta knife

Introduction

The role for radioactive iodine (RAI) in the management of metastatic differentiated thyroid cancer (DTC) is indisputable. Its utilization postoperatively as part of initial treatment, however, is still a matter of debate. The 2015 American Thyroid Association (ATA) guidelines is a 133-page document written in a dissertation format (1). The rationale for each recommendation is discussed, in detail, in a scholarly fashion, but yet, there is a sense of ambiguity in defining the indications for administration of RAI ablation. This review will discuss the ATA recommendations from a perspective that accounts surgical considerations, nuclear medicine principles, and genomic developments.

Address for Correspondence: Seza Gulec, MD, FACS, Florida International University Herbert Wertheim College of Medicine, Departments of Surgery and Nuclear Medicine, Miami, USA
Phone: (786) 693 0821 E-mail: sgulec@fiu.edu

The Role of RAI (Including Remnant Ablation, Adjuvant Therapy, or Therapy for Persistent Disease) after Thyroidectomy in the Primary Management of Differentiated Thyroid Cancer

2015 ATA Recommendation 51

A) RAI remnant ablation is not routinely recommended after thyroidectomy for ATA low-risk DTC patients. Consideration of specific features of the individual patient that could modulate recurrence risk, disease follow-up implications, and patient preferences are relevant to RAI decision-making (Weak recommendation, low-quality evidence).

B) RAI remnant ablation is not routinely recommended after lobectomy or total thyroidectomy for patients with unifocal papillary microcarcinoma, in the absence of other adverse features (Strong recommendation, moderate-quality evidence).

C) RAI remnant ablation is not routinely recommended after thyroidectomy for patients with multifocal papillary microcarcinoma in the absence of other adverse features. Consideration of specific features of the individual patient that could modulate recurrence risk, disease follow-up implications, and patient preferences are relevant to RAI decision-making (Weak recommendation, low-quality evidence).

D) RAI adjuvant therapy should be considered after total thyroidectomy in ATA intermediate-risk level DTC patients (Weak recommendation, low-quality evidence).

E) RAI adjuvant therapy is routinely recommended after total thyroidectomy for ATA high-risk DTC patients (Strong recommendation, moderate-quality evidence).

RAI treatment is given in three distinct settings with different clinical indications (intents);

1) Ablation of the remnant

2) Adjuvant treatment for residual disease or occult metastatic disease

3) Therapy for known metastatic disease.

The term "ablation" specifically refers to first-line RAI treatment after surgical total thyroidectomy.

The specific target of this treatment is normal residual thyroid tissue, i.e. the remnant, (Table 1). The confusion regarding the indications of RAI ablation stems from the underestimation of the impact of the remnant volume and patterns of uptake on imaging studies following surgical total thyroidectomy. Certain surgical details need to be explained in order to clarify the remnant concept from a clinical context.

Surgical Anatomy of Total Thyroidectomy, Sources and Locations of Thyroid Remnants

The strict definition of total thyroidectomy, as it pertains to subsequent diagnostic and therapeutic considerations, is the removal of all functioning thyroid tissue. When a clinical decision is made for a lobectomy, based on a low risk disease designation, "ablation" is not part of the equation. Ablation is, in the most simplistic terms, the eradication of all functional thyroid tissue left behind after surgical total thyroidectomy. A surgical total thyroidectomy entails removing the gland in the plane outside its "true capsule" (Figure 1). The true capsule is a very fine layer. A faithful dissection over the true capsule may occasionally pose a significant challenge to the viable preservation of the parathyroid glands and safe protection of the recurrent laryngeal nerve (RLN), as these critical structures have very intimate anatomic relations with the true capsule (Figure 2). A rational definition of total thyroidectomy is, therefore, the removal of the anatomical gland with viable and non-traumatic preservation of the parathyroid glands and RLN. It is important to emphasize that injuries to these critical structures are usually caused by ischemia resulting from devascularization, or traumatic contusion due to vigorous dissection and traction, not necessarily inadvertent transection or resection. A safe dissection, when performed in a cognizant way, may result (or even require) entering inside the true capsule and leaving a focal, thin rim of functional thyroid tissue in situ. A similar challenge may be encountered dissecting the upper pole vessels. There is an intimate relationship between the external branch of the superior laryngeal nerve and the superior thyroidal artery and vein. The dissection of the thyroid upper pole can also be challenging, particularly with a high-riding position. A safe division of the upper pole vasculature could leave behind a small remnant

Table 1. Radioactive iodine treatment objectives

RAI treatment intent	Target	Objectives
Ablation	Remnant	Increase specificity of Tg monitoring Complete post-surgical staging Identify/clear ambiguous foci of uptake
Adjuvant treatment	Residual disease Recurrent disease Metastatic disease	Ablation objectives Disease control
Therapy	Residual disease Recurrent disease Metastatic disease	Disease control

RAI: Radioactive iodine, Tg: Thyroglobulin

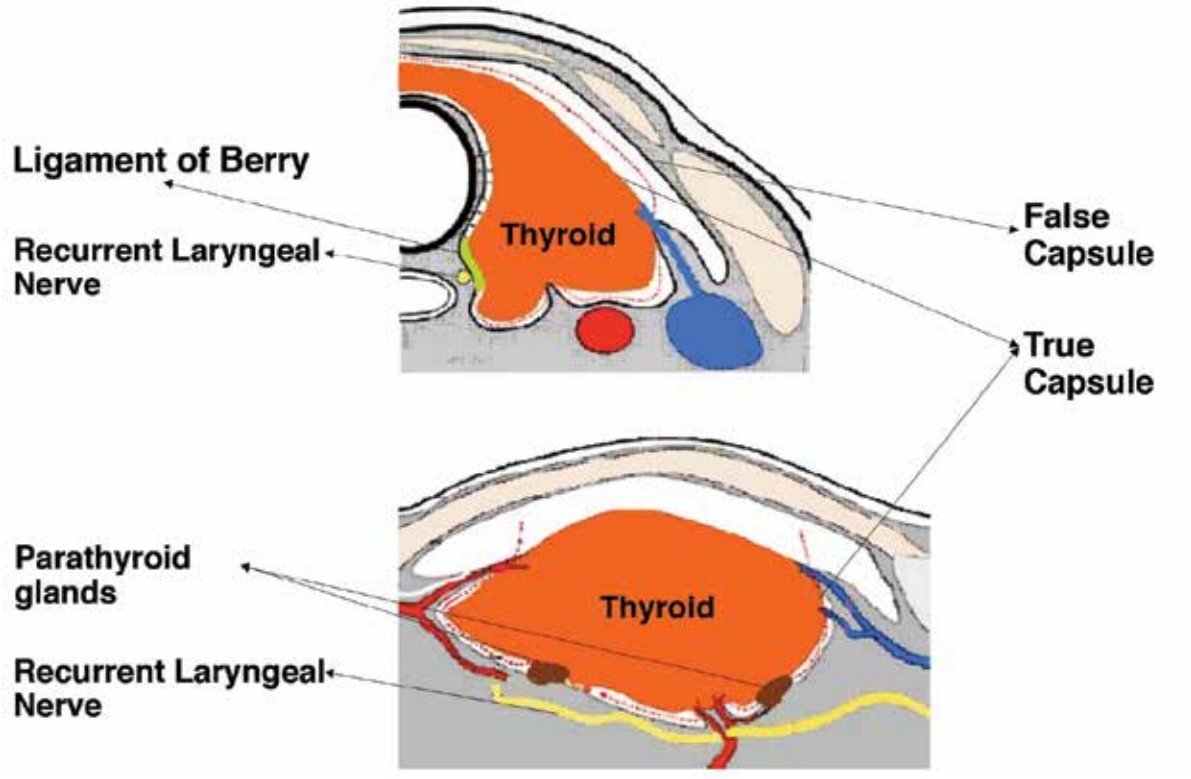


Figure 1. The surgical anatomy and technique of total thyroidectomy, remnant volume. This diagram adapted from Tan et al. (7) shows the fascial compartments in the thyroid space. The parathyroid glands are situated over the true capsule. The separation of the parathyroid glands off the true capsule can disrupt the vascular supply. The cranial end of the recurrent laryngeal nerve typically travels under the ligament of Berry and, at this position, is very close to the true capsule of the thyroid. A vigorous extracapsular dissection may traumatize these vital structures even if they are carefully identified and avoided during surgery

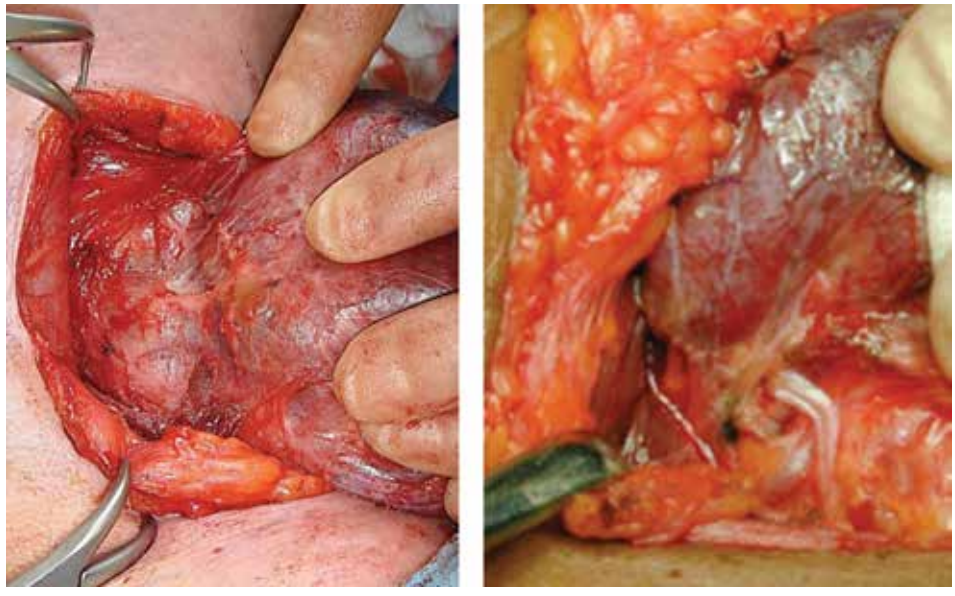


Figure 2. The image on the left shows a parathyroid gland lying on the thyroid capsule. The image on the right shows the recurrent laryngeal nerve under the ligament of Berry, and their very intimate relations with the true capsule of the thyroid gland

(Figure 3). The pyramidal lobe usually has a short extension containing functional tissue that becomes a fibrotic cord as it extends cranially. It is typically divided at a reasonable length along its tract. Occasionally this pyramidal cord may also contain functioning thyroid tissue. The totality of a total thyroidectomy is reliant on the surgeon's technique. High volume experienced surgeons can produce consistent results in terms of low remnant volume; however, the reality in community settings could vary widely. The terms near-total or sub-total thyroidectomy are disclosures of not insignificant remnant volume. Remnant thyroidectomy is accomplished by RAI ablation.

Radioactive Iodine Ablation: Beta-knife Completion Thyroidectomy

The therapeutic effect of I-131 works through beta particles, thus, it has been named a "beta-knife" (2). The objectives of ablation are three-fold a) Ablation eradicates all functioning thyroid tissue. With that goal accomplished, thyroglobulin (Tg) becomes a highly specific tumor marker. This simply facilitates the post-op long-term patient follow-up. b) Ablation wipes out all focal normal thyroid tissue left in-situ by the surgeon to avoid injury to laryngeal nerves and parathyroid glands. From a surgical standpoint, these small clusters of normal thyroid tissue remnants are inconsequential; however, they appear as focal areas of RAI uptake on future imaging studies and can easily be interpreted as metastatic disease. Eradication of these potential sources of misdiagnosis, while there is still open communication between the surgeon and the imager, is important. c) The post-ablation whole-body scan is considered the standard for extent of disease evaluation.

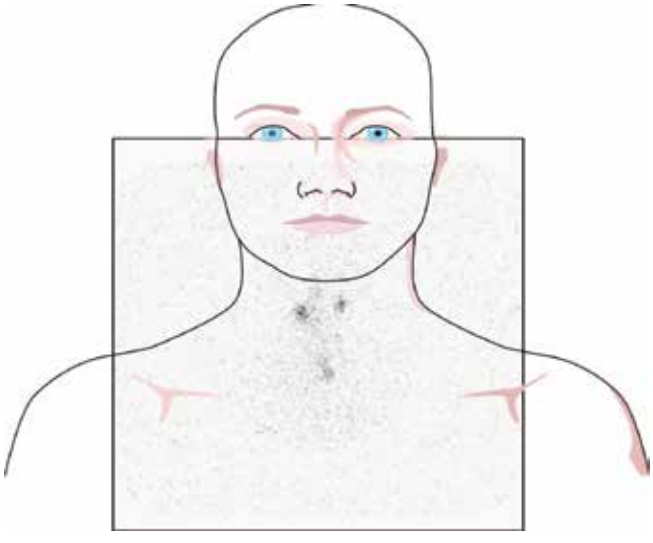


Figure 3. Normal postoperative radioactive iodine scan. This image shows a small focus of activity at the midline of the lower neck compatible with a midline remnant. There are also two small foci of radioactive iodine uptake in the upper neck, which are equivocal. Upon referring with the surgeon who performed the operation, these foci were confirmed to represent remnant activity beneath the superior parathyroid glands. Without knowledge of the operative findings these can easily be reported as metastatic nodal disease

When postoperative RAI treatment is contemplated with an adjuvant intent, in addition to the remnant ablation objectives, RAI is aimed to target residual disease or occult metastatic disease. When the intent is adjuvant treatment, risk stratification becomes important in the selection of the administered activity of I-131. For remnant ablation purposes only, the risk stratification has no bearing. Complete thyroidectomy, in the strictest sense, is only possible with surgical (cold steel knife) thyroidectomy, followed by I-131 ablation (beta knife). There exists a group of patients where surgical total thyroidectomy removes all the functional remnant tissue. Although it could be argued that RAI ablation is not necessary in this group, it is challenging to identify these patients using standard clinical evaluations.

Challenges and Potential Solutions for Accurate Assessment of Remnant Volume

The volume of the thyroid remnant is highly variable and is dependent on the surgeon's technique; it is not typically highlighted in the operative report and is usually undetectable by ultrasound (US) or other anatomic imaging modalities such as computed tomography (CT) or magnetic resonance imaging (MRI). Serum Tg can provide some information as to the presence of functioning thyroid tissue; but the marked variability in Tg levels, which can overlap with metastatic disease, makes Tg an unreliable quantitative measure of remnant volume. Occult metastatic sites may only be detected by a post-ablation scan. Typical 1-5 mCi I-131 imaging has notoriously low sensitivity for disclosing occult foci.

Postoperative/pre-ablative RAI imaging may be considered to determine the extent of residual functional thyroid tissue, or remnant volume. I-131 has become the mainstay for RAI imaging due to its availability and relatively low cost; however, one must recognize the inherent limitations of I-131 as a diagnostic imaging agent. I-131 has high energy gamma emissions with the greatest abundance of 364 keV which lead to septal penetration and limit the spatial resolution of standard gamma cameras. In addition, only low administered activities of I-131 can be given for diagnostic purposes due to concerns for stunning, especially when subsequent RAI treatment is being considered. Routine diagnostic I-131 scans are usually performed with administered activities in the range of 1-5 mCi. These low dose images are often of limited diagnostic quality and may underestimate the extent of disease evaluation. Subtle or non-visualized foci of RAI avid disease on low dose

diagnostic I-131 scans may become evident on post-treatment scans with therapeutic administered activities (Figure 4). Even with higher administered activities of I-131, it can be difficult to differentiate foci of uptake in the neck as thyroid remnants versus nodal metastatic disease. RAI avid foci in the neck can be elucidated with increased confidence only after the thyroid remnant has been completely ablated. In addition, eliminating the remnant comprised of highly avid normal thyroid tissue reduces competition for RAI at metastatic sites thereby enhancing diagnostic scan sensitivity and therapeutic efficacy (3).

Although not in routine clinical use currently, I-124 imaging has been demonstrated to be a very sensitive functional imaging agent. I-124 positron emission tomography (PET)/CT has an outstanding spatial resolution in elucidating small thyroid remnants from the right or left lobes or in the distribution of the pyramidal lobe (Figure 5). Due to its anatomic precision, I-124 PET/CT can also distinguish nodal uptake from a remnant in the thyroid bed which may not be adequately visualized with other imaging modalities. I-124 has been shown to have a similar performance in detection of RAI avid lesions as compared to post-therapeutic I-131 scans. Additionally, the risk of stunning at higher administered activities of I-131 may not be observed with I-124. Therefore, I-124 may be considered the most sensitive imaging modality for assessing remnant thyroid tissue.

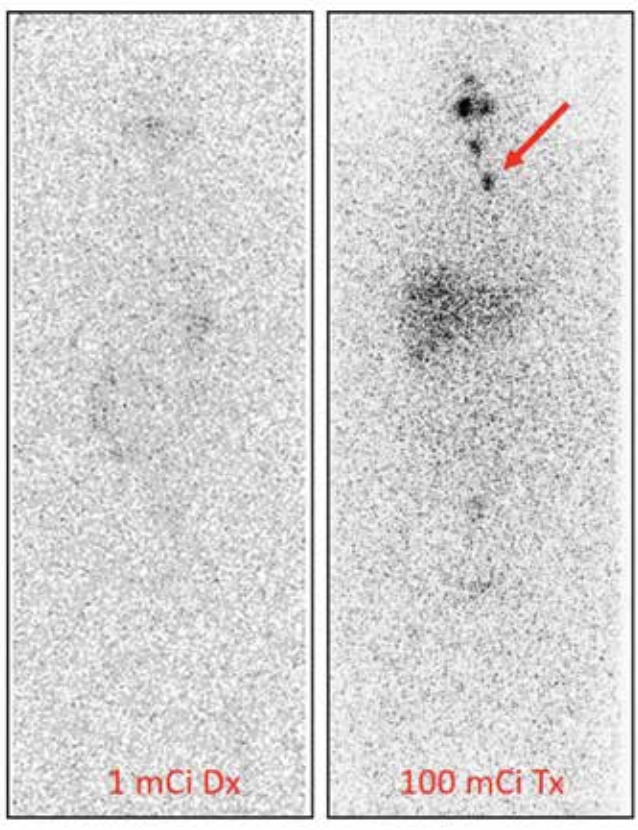


Figure 4. Radioactive iodine (RAI) scan with 1 mCi versus 100 mCi demonstrating occult remnant or nodal disease. The image on the left is a diagnostic whole body scan with 1 mCi of I-131 showing no abnormal RAI avid foci in the neck. The image on the right is a posttreatment scan for the same patient after therapy with 100 mCi of I-131. This image shows physiologic activity in the salivary glands as well as two RAI avid foci in the neck that were confirmed to represent nodal metastases

Perhaps the greatest merit of I-124 PET/CT is that it allows accurate quantitation of remnant volume and kinetics for the purposes of remnant dosimetry. Our group showed significant variations in cumulated activities and functional volumes of thyroid remnants between cohort patients. The maximum remnant activity ranged from 1.2 to 215.9 uCi with the total functional remnant volume (the total number of voxels within the remnant range of interest) ranging from 1 to 60 mL. The activity per volume of remnant tissue ranged from 0.036 to 11.265 uCi/mL. The total cumulated activity within the remnant ranged from 68 to 12757.3 uCi/hr (4). This variability highlights how a thyroid remnant may become a confounding factor in the postoperative management of DTC patients and why RAI ablation is key to maintaining a standard baseline for which future imaging and laboratory parameters can be compared.

Specificity of Thyroglobulin

Tg is a highly specific tumor marker when functioning thyroid tissue is no longer present, i.e. after total thyroidectomy and RAI ablation of the remnant. It has been estimated that 1 g of normal thyroid tissue releases about 1 ng/mL of Tg into the serum when thyroid-stimulating hormone (TSH) is normal and about 0.5 ng/mL when TSH is suppressed (<0.1 mIU/L) (5). The remnant volume, and thus remnant-produced Tg, is dependent on the surgical technique and potentially could be highly variable/unpredictable.

The factors influencing baseline and recombinant human TSH stimulated Tg levels in patients with metastatic DTC were investigated in a study by Robbins et al. (6). The authors found wide variations in Tg levels and considerable overlap between metastatic sites. The median baseline Tg level for cervical metastases was 2 ng/mL and increased to a median of 8 ng/mL after recombinant human TSH stimulation. The median baseline and stimulated Tg levels for mediastinal metastases were 4 ng/mL and 16 ng/mL, and for distant metastatic sites were 25 ng/mL and 180 ng/mL, respectively. The patients with thyroid remnants included in the study had a median baseline Tg level of 0.6 ng/mL, with the highest being 66 ng/mL. After recombinant human TSH, the median stimulated Tg in patients with thyroid remnants was 1.2 ng/mL but ranged as high as 250 ng/mL.

Following Tg levels in patients who have undergone total thyroidectomy but not RAI ablation is challenging. The Tg level is expected to increase by at least two-fold in the presence of a thyroid remnant under the influence of recombinant human TSH; however, the stimulated Tg level has been shown to increase almost four-fold in some patients. The inconsistent baseline Tg levels produced by the remnant along with the documented variability in stimulated Tg levels that overlap with metastatic disease make it very difficult to reliably detect occult metastasis. In addition, the natural progression of an un-ablated thyroid remnant under TSH suppression and its effect on Tg production are not clearly established. These issues can be avoided if the thyroid remnant is ablated with RAI thereby eradicating all functioning thyroid tissue and rendering Tg a very specific indicator of recurrent disease in post-surgical patients.

The Activity of I-131 to be Used for Remnant Ablation or Adjuvant Therapy

2015 ATA Recommendation 55

A) If RAI remnant ablation is performed after total thyroidectomy for ATA low-risk thyroid cancer or intermediate-risk disease with lower risk features (i.e., low-volume central neck nodal metastases with no other known gross residual disease or any other adverse features), a low administered activity of approximately of 30 mCi is generally favored over higher administered activities (Strong recommendation, high-quality evidence).

B) Higher administered activities may need to be considered for patients receiving less than a total or near-total thyroidectomy in which a larger remnant is suspected or in which adjuvant therapy is intended (Weak recommendation, low-quality evidence).

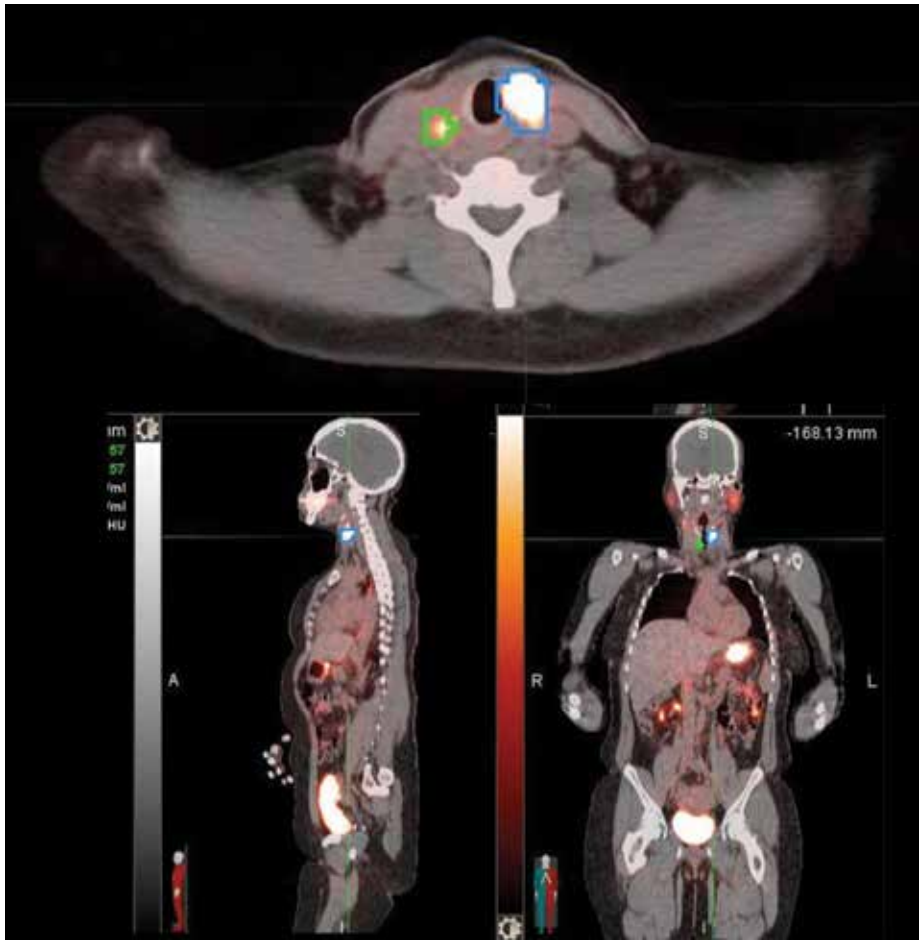


Figure 5. I-124 positron emission tomography/computed tomography image through the level of the thyroid bed shows regions of interest drawn around a thyroid remnant (blue) and a metastatic right level 3 lymph node (green). The remnant is not measurable by ultrasound or computed tomography. There is overestimation of functional volume (correctible) due to voxel saturation phenomenon

2015 ATA Recommendation 56

Administered RAI Activity (mCi) and Radiation Absorbed Dose (Rad) Relationship

When RAI is intended for initial adjuvant therapy to treat suspected microscopic residual disease, administered activities above those used for remnant ablation up to 150 mCi are generally recommended (in absence of known distant metastases). It is uncertain whether routine use of higher administered activities (>150 mCi) in this setting will reduce structural disease recurrence for T3 and N1 disease (Weak recommendation, low-quality evidence).

Currently, the most common practice in RAI ablation is to use a fixed amount of radioiodine regardless of the size of the thyroid remnant or the percentage of radioiodine uptake. Different administered activity levels have been tested over the years ranging from 30 mCi to 150 mCi. There has been a paradigm shift towards using less administered activities for RAI remnant ablation in the setting of low-risk DTC since the publication of two high-impact articles in the New England Journal of Medicine in 2012, and further propagated after release of the 2015 ATA guidelines (8,9). As paradoxical as it may seem, 30 mCi of radioiodine will completely destroy the thyroid remnant in some patients whereas 150 mCi may not be sufficient in others. The explanation is that, there is no linear relationship between the administered activity and radiation absorbed dose to the remnant. The radiation dose (in rads) depends not only on the number of millicuries administered, but also the percent uptake, the size of the remnant, and the effective half-life ($T_{1/2\ eff}$) of I-131 in the remnant. Patients with small remnants and high uptakes may receive a large radiation dose from 30 mCi whereas those with large remnants and low uptakes may receive a small and inadequate radiation dose from 150 mCi. In a 1983 dosimetric study, Becker and Hurley demonstrated a wide variation in uptake and remnant size in patients who were referred for ablation (10). There was no correlation between the number of millicuries administered and the radiation absorbed dose delivered to the remnant (Figure 6). Although this study unarguably demonstrated the unreliability of a fixed or standard amount of radioiodine for ablation, due to feasibility and logistical considerations a dosimetric approach never was adapted.

There is a clear need to develop individualized dosimetry-guided RAI ablation protocols. I-124 PET/CT offers a robust quantitative tool to achieve this goal. Active studies are being performed to determine the technical prerequisites for a time and cost-conscious standard protocol.

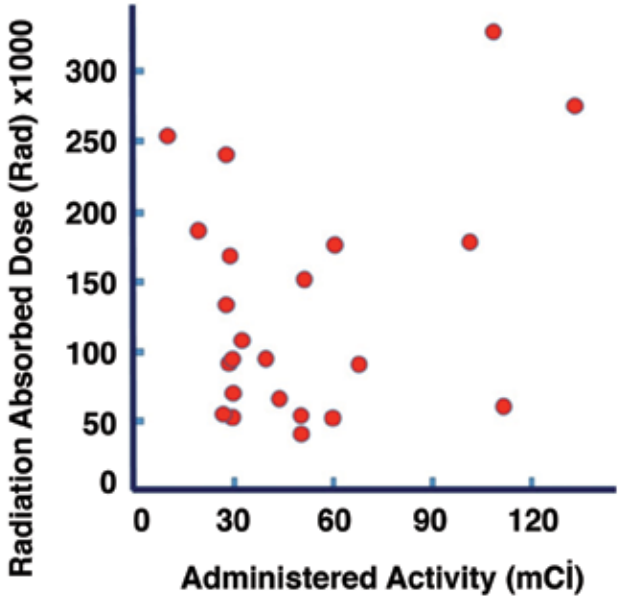


Figure 6. Lack of correlation between radiation dose to the remnant and millicuries of radioactive iodine administered. Adapted from (10) Hurley JR, Becker DV. The use of radioiodine in the management of thyroid cancer. Freeman LM, Weissman HS (eds). Nuclear Medicine Annual. New York, Raven Press 1983;329-384.

Summary and Recommendations

Although the potential side effects of RAI are real and efforts to utilize lower administered activities for ablation are commendable, this should not overlook the opportunity for achieving complete ablation. The benefits of complete remnant ablation in the future follow-up and management of DTC patients after thyroidectomy cannot be denied. From a practical standpoint, a total thyroidectomy usually leaves behind some functioning thyroid tissue. This postsurgical thyroid remnant cannot be accurately evaluated/visualized with anatomic imaging studies like CT or ultrasound, and it can be misleading on functional RAI imaging indistinguishable from RAI avid disease in the neck. Moreover, the volume of remaining thyroid tissue is dependent upon the surgical technique and invariably and unpredictably affects the Tg level at baseline and under TSH stimulation. Avoiding RAI remnant ablation because of the risk of side effects or the reliance on underpowered, short-term prospective studies or flawed retrospective analyses fails to recognize the goals of therapy and the practical approach to the postoperative follow-up of DTC patients. The therapeutic administration of RAI to ablate the thyroid remnant allows for completion of a "true" total thyroidectomy (cold steel knife + beta knife) and improves the postoperative management of DTC patients by facilitating RAI imaging and permitting the use of serum Tg as a highly specific tumor marker.

The recommendations below incorporate genomic profiling for risk stratification and dosimetric approach for selection of administered activities for RAI ablation.

Proposed Guidelines for RAI Ablation

A) RAI ablation decision should be made at the time of the surgical treatment decision. Risk stratification should include genomic profiling. RAI remnant ablation is routinely recommended after total thyroidectomy, if the preoperative intent was total elimination of anatomic and functional thyroid tissue. Main consideration is the role for Tg monitoring and RAI imaging surveillance in the long-term management of patients. RAI ablation should be administered with radiation absorbed dose to the remnant tissue optimized.

B) If micropapillary cancer was incidentally reported in the surgical pathology following a total thyroidectomy, RAI ablation may be omitted if there is no histopathologic or genomic risk factor identified.

C) RAI remnant ablation is not indicated after lobectomy. If a histopathologic or genomic risk factor is identified, a completion thyroidectomy should be performed and followed by RAI ablation.

D) If the clinical intent is adjuvant treatment for suspected/known residual/metastatic disease RAI ablation should be administered in a therapeutic context, with radiation absorbed dose to the malignant disease optimized.

E) Administered activity selection should be determined based on dosimetric considerations, for both remnant ablation and adjuvant treatment indications.

Authorship Contributions

Surgical and Medical Practices: Seza Gulec, Russ Kuker, Concept: Seza Gulec, Russ Kuker, Design: Seza Gulec, Russ Kuker, Data Collection or Processing: Seza Gulec, Russ Kuker, Analysis or Interpretation: Seza Gulec, Russ Kuker, Literature Search: Seza Gulec, Russ Kuker, Writing: Seza Gulec, Russ Kuker.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support

References

1. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26:1-133.
2. Gulec SA. The Art and Science of Thyroid Surgery 100 Years after Theodor Kocher Moving into the Age of Genomics: A Personal Insight. Mol Imaging Radionucl Ther 2016.
3. Ain KB. Radioiodine-remnant ablation in low-risk differentiated thyroid cancer: pros. Endocrine 2015;50:61-66.
4. Gulec SA, Kuker RA, Goryawala M, Fernandez C, Perez R, Khan-Ghany A, Apaza A, Harja E, Harrell M. (124)I PET/CT in Patients with Differentiated Thyroid Cancer: Clinical and Quantitative Image Analysis. Thyroid 2016;26:441-448.
5. Wartofsky L, Van Nostrand D. Thyroid Cancer: A Comprehensive Guide to Clinical Management. Totowa, NJ: Humana Press Inc 2006.
6. Robbins RJ, Srivastava S, Shaha A, Ghossein R, Larson SM, Fleisher M, Tuttle RM. Factors influencing the basal and recombinant human thyrotropin-stimulated serum thyroglobulin in patients with metastatic thyroid carcinoma. J Clin Endocrinol Metab 2004;89:6010-6016.
7. Tan YH, Du GN, Xiao YG, Guo SQ, Wu T, Chen PZ, Yan GB, Tan DX, Wang K. The false thyroid capsule: new findings. J Laryngol Otol 2013;127:897-901.
8. Schlumberger M, Catargi B, Borget I, Deandreis D, Zerdoud S, Bridji B, Bardet S, Leenhardt L, Bastie D, Schvartz C, Vera P, Morel O, Benisvy D, Bournaud C, Bonichon F, Dejax C, Toubert ME, Leboulleux S, Ricard M, Benhamou E, Tumeurs de la Thyroide Refractaires Network for the Essai Stimulation Ablation Equivalence T. Strategies of radioiodine ablation in patients with low-risk thyroid cancer. N Engl J Med 2012;366:1663-1673.
9. Mallick U, Harmer C, Yap B, Wadsley J, Clarke S, Moss L, Nicol A, Clark PM, Farnell K, McCready R, Smellie J, Franklyn JA, John R, Nutting CM, Newbold K, Lemon C, Gerrard G, Abdel-Hamid A, Hardman J, Macias E, Roques T, Whitaker S, Vijayan R, Alvarez P, Beare S, Forsyth S, Kadalayil L, Hackshaw A. Ablation with low-dose radioiodine and thyrotropin alfa in thyroid cancer. N Engl J Med 2012;366:1674-1685.
10. Hurley JR, Becker DV. The use of radioiodine in the management of thyroid cancer. Nuclear Medicine Annual. New York, Raven Press 1983;329-384.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):24-35 DOI:10.4274/2017.26.suppl.04

Genomic Profiling of Thyroid Nodules: Current Role for ThyroSeq Next-Generation Sequencing on Clinical Decision-Making

Tiroid Nodülü Genomik Profillemesi: ThyroSeq Yeni-Nesil Dizinlemenin Klinik Karar Verme Üzerinde Güncel Rolü

Atil Y. Kargi[1], Marcela Perez Bustamante[1], Seza Gulec, MD, FACS[2]
[1]*University of Miami Hospital, Clinic of Diabetes and Metabolism, Division of Endocrinology, Florida, USA*
[2]*Florida International University Herbert Wertheim College of Medicine, Departments of Surgery and Nuclear Medicine, Miami, USA*

Abstract

In recent years there has been an increased awareness of the genetic alterations underlying both benign and malignant neoplasms of the thyroid. Next-generation sequencing (NGS) is an emerging technology that allows for rapid detection of a large number of genetic mutations in thyroid fine-needle aspiration (FNA) specimens. NGS for targeted mutational analysis in thyroid tumors has been proposed as a tool to assist in the diagnosis of thyroid nodules with indeterminate FNA cytology. Results of genomic testing of thyroid nodules and thyroid cancers could also have prognostic implications and play a role in determining optimal treatment strategies including targeted therapies. We provide a critical review of existing studies assessing the performance of the ThyroSeq NGS test for the diagnosis and management of patients with thyroid nodules with indeterminate cytopathology and discuss the applicability of findings from these studies to clinical practice. While there are early indications to suggest a possible utility of data obtained from NGS to aid in prognostication and therapeutic decision-making in thyroid cancer, we recommend judicious use and cautious interpretation of such molecular testing until results of ongoing clinical trials become available. Lastly, we discuss recommendations provided from clinical practice guidelines regarding the use of mutation detection via NGS in the diagnostic evaluation of thyroid nodules.
Keywords: Thyroid cancer, thyroid nodule, genomic profiling, next-generation sequencing, ThyroSeq, molecular testing

Öz

Son yıllarda benign ve malign tiroid neoplazmlarının altında yatan genetik değişiklerle ilgili artan bir farkındalık oluşmuştur. Yeni nesil dizinleme [Next-generation sequencing (NGS)] tiroid ince iğne aspirasyon (İİA) örneklerinde çok sayıda genetik mutasyonun hızlı tespitine izin veren ve gelişmekte olan bir teknolojidir. Tiroid tümörlerinin hedeflenen mutasyon analizi için NGS, İİA ile belirsiz sitoloji saptanan tiroid nodüllerinin tanısında yardımcı olmak için bir araç olarak öne sürülmüştür. Ek olarak, tiroid nodülü ve tiroid kanserlerinin genomik test sonuçlarının prognostik etkileri olabilir ve hedefli tedaviler dahil olmak üzere uygun tedavi stratejilerinin belirlenmesinde önemli bir rol oynayabilir. Bu yazıda İİA ile belirsiz sitolojisi olan tiroid nodüllü hastaların tanı ve tedavisinde ThyroSeq NGS testinin performansını değerlendiren mevcut çalışmaların bir derlemesini sunmayı ve bu çalışmalardan elde edilen bulguların klinik pratikte uygulanabilirliğini tartışmayı amaçladık. Her ne kadar erken veriler NGS'den elde edilen verilerin tiroid kanserinde prognoz ve tedavi kararı sürecine olası yardımlarını öne sürse de, bu tür moleküler testlerin devam eden klinik çalışmaların sonuçları belirlenene kadar, akıllıca kullanılmasını ve dikkatli yorumlanmasını öneriyoruz. Son olarak, tiroid nodüllerinin tanısal değerlendirilmesinde NGS ile mutasyon tespiti ile ilgili klinik uygulama kılavuzlarından edinilmiş öneriler tartışılmıştır.
Anahtar kelimeler: Tiroid kanseri, tiroid nodülü, genomik profilleme, yeni nesil dizinleme, ThyroSeq, moleküler test

Address for Correspondence: Atil Y. Kargi MD, University of Miami Hospital, Clinic of Diabetes and Metabolism, Division of Endocrinology, Florida, USA
Phone: 305-243-3636 E-mail: akargi@med.miami.edu

Introduction

Thyroid nodules are common in the general population with higher prevalence in women and in older persons. When ultrasound is performed at random in the general population 19-68% of individuals are found to harbor one or more thyroid nodules (1). While the majority of these nodules are not clinically significant 7-15% are malignant (2). Paralleling the increased use of imaging techniques and of thyroid fine-needle aspiration (FNA) there has been dramatic increase worldwide in both the incidence of thyroid nodule diagnosis and that of thyroid cancer over the past 20-30 years (3,4).

Several clinical practice guidelines have set forth strategies to manage patients who are discovered to have thyroid nodules, yet a great deal of controversy still exists as to the optimal approach to diagnosis and treatment (5,6). The widespread use of high-resolution ultrasonography of the neck as well as thyroid FNA has significantly enhanced our ability to diagnose malignancy among thyroid nodules, however 20-30% of cytology results from thyroid FNA fall into one of three indeterminate diagnostic categories according to the Bethesda System for Reporting Thyroid Cytopathology: Atypia of undetermined significance/ Follicular lesion of undetermined significance (AUS/FLUS) (Bethesda category III), follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) (Bethesda category IV), and Suspicious for malignancy (SM) (Bethesda category V) (7). The reported frequency and risk of malignancy with each of the Bethesda reporting categories is summarized in Table 1.

Patients and physicians faced with an indeterminate cytopathology report will have to make the sometimes difficult decision of deciding on the next step in management of the thyroid nodule, which until recent years has meant choosing from one of three options: repeat FNA, observation with continued ultrasound surveillance or surgical management. Each of these strategies brings with it specific considerations and complexities; for instance in patients referred for surgery the need to decide upon the extent of thyroidectomy and the potential need for a two-step procedure of thyroid lobectomy followed by a completion thyroidectomy in the circumstance that the lobectomy results in a diagnosis of thyroid cancer.

Taking into consideration that many thyroid cancers are indolent tumors and that many patients may have an excellent prognosis even when the diagnosis and treatment has been delayed and the fact that most patients who undergo thyroidectomy for AUS/FLUS and FN/SFN cytopathology will be diagnosed with benign nodules on final surgical histopathology, clinicians and patients have been left with weighing the risks of a more conservative strategy of surveillance with that of the more aggressive approach of proceeding to thyroidectomy.

A variety of factors can predict the risk of cancer and aid in the decision on optimal management for patients presenting with nodules having indeterminate cytopathology; including patient risk factors (age, gender, family history, past exposure to ionizing radiation), serum TSH level and presence or absence of sonographic features suspicious for papillary thyroid cancer (PTC) (6,8,9). In their 2015 management guidelines pertaining to adults with thyroid nodules, the American Thyroid Association (ATA) has provided clear guidance on the criteria that should be used to determine the initial indication for FNA based on traditional risk factors and in particular a risk stratification model heavily reliant upon sonographic appearance of the nodule (6).

However, in the circumstance that FNA is performed, once patients and health care providers are faced with indeterminate cytology, it becomes much less clear from the guidelines precisely how the same criteria should be used to inform management decisions. This situation has created a need to improve on the cytological inaccuracy inherent to the diagnosis of indeterminate thyroid FNA, resulting in the development of a number of new diagnostic modalities intended for application as a "rule-in" or "rule-out" test for thyroid cancer. When discussing the performance of any of these tests it must be taken into consideration that the ideal 'rule-in' test should have a positive predictive value (PPV) similar to that of a malignant cytological diagnosis

Table 1. Bethesda system for the classification of thyroid cytopathology

Bethesda category	Cytopathologic category	Approximate expected frequency	Malignancy rate	Suggested treatment (Prior to availability of molecular testing)
I	Non-diagnostic/Inadequate	5-11%	1-4%	Repeat FNA
II	Benign	55-74%	0-3%	US follow-up
III	Atypia/follicular lesion of undetermined significance	5-15%	5-15%	Repeat FNA or US follow-up or Lobectomy
IV	Follicular neoplasm/ suspicious for FN	2-25%	15-30%	Lobectomy
V	Suspicious for malignancy	1-6%	60-75%	Lobectomy or Thyroidectomy
VI	Malignant	2-5%	97-99%	Near-total thyroidectomy

FNA: Fine-needle aspiration, FN: Follicular neoplasm, US: Ultrasonographic

(Bethesda category VI) (98.6%), while an ideal 'rule-out' test should have a negative predictive value (NPV) comparable to that of benign cytology (Bethesda category II) (96.3%) (7).

In recent years a number of diagnostic tests have been evaluated to aid in the diagnosis of indeterminate thyroid nodules, including FDG-PET and several assessments of molecular markers in FNA specimens (10,11). Molecular tests include immunohistochemistry for Galectin -3, HBME-1 and CK19; gene expression and microarray analysis; microRNA expression; and testing for mutations and gene rearrangements (6,12). Currently in the U.S.A. commercially available molecular tests include those for single or multiple mutation analysis, combination panels for mutation analysis and chromosomal rearrangements (miRInform®-Asuragen, ThyroSeq-CBLPath and University of Pittsburgh Medical Center) and a proprietary gene expression classifier (Afirma GEC®-Veracyte) (13,14,15). While initially the gene expression classifier (GEC) was proposed as the best among these tests to rule-out malignancy and mutation analysis was preferred as a "rule-in", the next-generation sequencer (NGS) ThyroSeq has recently been shown to have both a high PPV and NPV for thyroid cancer diagnosis when applied to thyroid FNA with indeterminate cytology (16,17,18). A further strength of the ThyroSeq, when compared to GEC, is that it provides detailed and specific information regarding the exact genetic alteration driving the disease, which could potentially provide prognostic and therapeutic implications including impacting upon extent of surgery, use of RAI and possible future targeted therapies.

Thyroid cancer, like all cancers, is a disease of the genome. The initiation and progression of cancer is due to the accumulation of genetic and epigenetic changes such as somatic mutations, chromosomal rearrangements, micro RNA dysregulation and alterations in gene expression (19). In differentiated thyroid cancer (DTC), the observed genetic changes frequently lead to activation of the MAPK or PI3K-AKT pathways. Approximately 70% of DTC demonstrate one of four genetic abnormalities: point mutations in the *BRAF* or *RAS* gene or either one of two chromosomal rearrangements: *RET/PTC* or *PAX8/PPARG* (19). Our knowledge of the genomic alterations explaining the remaining approximately 30% of all thyroid cancers not harboring one of the aforementioned four genetic aberrations has been greatly expanded by a number of recent discoveries, including those reported in 2014 by the National Cancer Genome Atlas Research Network, in which the genetic driver was identified in 96.5% of 496 PTC cases (20,21). The findings of this last report have led some experts to propose a reclassification of PTCs based on molecular characteristics to better reflect their underlying differentiation and signaling properties (21). While a detailed discussion of all current molecular tests in thyroid FNA is beyond the scope of this article, our review will focus on the role of NGS, a methodology which we believe may hold particular promise in diagnosis of thyroid FNA as well as future potential for use in prognostication and informing management of patients with thyroid cancer.

Next-generation Sequencing in Thyroid Fine-Needle Aspiration

NGS is a method of simultaneous sequencing of a very large number of short nucleic acid sequences that can be used to detect multiple genetic alterations in large regions of the genome (22). Compared to standard methods of sequencing, such as Sanger sequencing, NGS has the advantage of rapid simultaneous sequencing of large sections of the genome and quantitative assessment of mutated alleles. NGS can be used for whole-genome sequencing as well as in a more targeted manner directed at specific mutations in specific areas of the genome.

The ThyroSeq NGS panel provides simultaneous sequencing for detection in over a thousand hotspots of 14 thyroid cancer-related genes and for 42 types of gene fusions occurring in thyroid cancer (14,17). The genes analyzed for mutation are *AKT1, BRAF, CTTNB1, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TP53, TSHR, TERT* and *EIF1AX*. The gene list for gene fusions and expression consists of *RET, PPARG, NTRK1, NTRK3, ALK, IGF2BP3, BRAF, MET, CALCA, PTH, SLC5A5, TG, TTF1, KRT7* and *KRT20*.

The proposed uses for NGS for thyroid FNA samples include diagnosis of cytologically indeterminate thyroid nodules, prognostication in thyroid cancer and to inform selection of targeted therapies (14). The possible applications and indications of ThyroSeq include:

1. Thyroid FNA with indeterminate cytology (Bethesda categories III, IV and V),

2. Malignant thyroid cytology (Bethesda category VI), when results of the NGS are expected to affect the decision for extent of oncological surgery,

3. Benign thyroid cytology (Bethesda category II), when strong SM exists on clinical grounds such as presence of a highly suspicious sonographic pattern,

4. When the diagnosis of thyroid cancer is established cytologically or histologically and molecular profiling will effect decision regarding radioactive iodine therapy, intensity of follow up, or for selection of targeted therapies in patients with advanced cancer.

We will discuss the potential roles of NGS in thyroid FNA specimens below, with an emphasis on its role in clinical decision-making.

Potential Role of Next-Generation Sequencer in AUS/FLUS (Bethesda Category III) Cytology

The diagnosis of AUS/FLUS should be made in FNA specimens containing cells with architectural and/or

nuclear atypia more pronounced than expected for benign changes, yet not sufficient to be classified in one of the higher risk Bethesda categories (7). Although this diagnosis has an expected and recommended frequency of 7%, recent analyses have found this cytological category to be diagnosed in 1-27% of all thyroid FNA specimens (23). In studies assessing the risk of cancer in patients with Bethesda category III nodules, the rate of malignancy diagnosed in patients who went to surgery was 6-48%, with a mean risk of 16% (24).

To date only one study has assessed the performance of ThyroSeq in AUS/FLUS (17). In this study 465 FNA samples from 441 patients at a single institution diagnosed as AUS/FLUS on cytology were submitted prospectively to ThyroSeq molecular testing. In addition to the 42 gene fusions and 14 genes analyzed for point mutation, expression of eight genes were analyzed to evaluate the cell composition of the needle aspirates. Ninenty-eight of the cases (21%) had a definitive diagnosis by either surgical (n=96) or non-surgical (n=2) methods. Of all FNA samples 462 were determined to be composed of follicular cells while three samples were diagnosed as parathyroid in origin. Among the samples consisting of follicular cells 31 were positive on mutational analysis (6.7%) (Figure 1).

Of the entire group of 441 patients, 96 nodules occurring in 90 patients were surgically removed due to the finding of an additional nodule in the same gland with either Bethesda V or Bethesda VI cytology in five patients. Twenty-seven patients underwent thyroidectomy because of positive ThyroSeq results and the remaining cases were

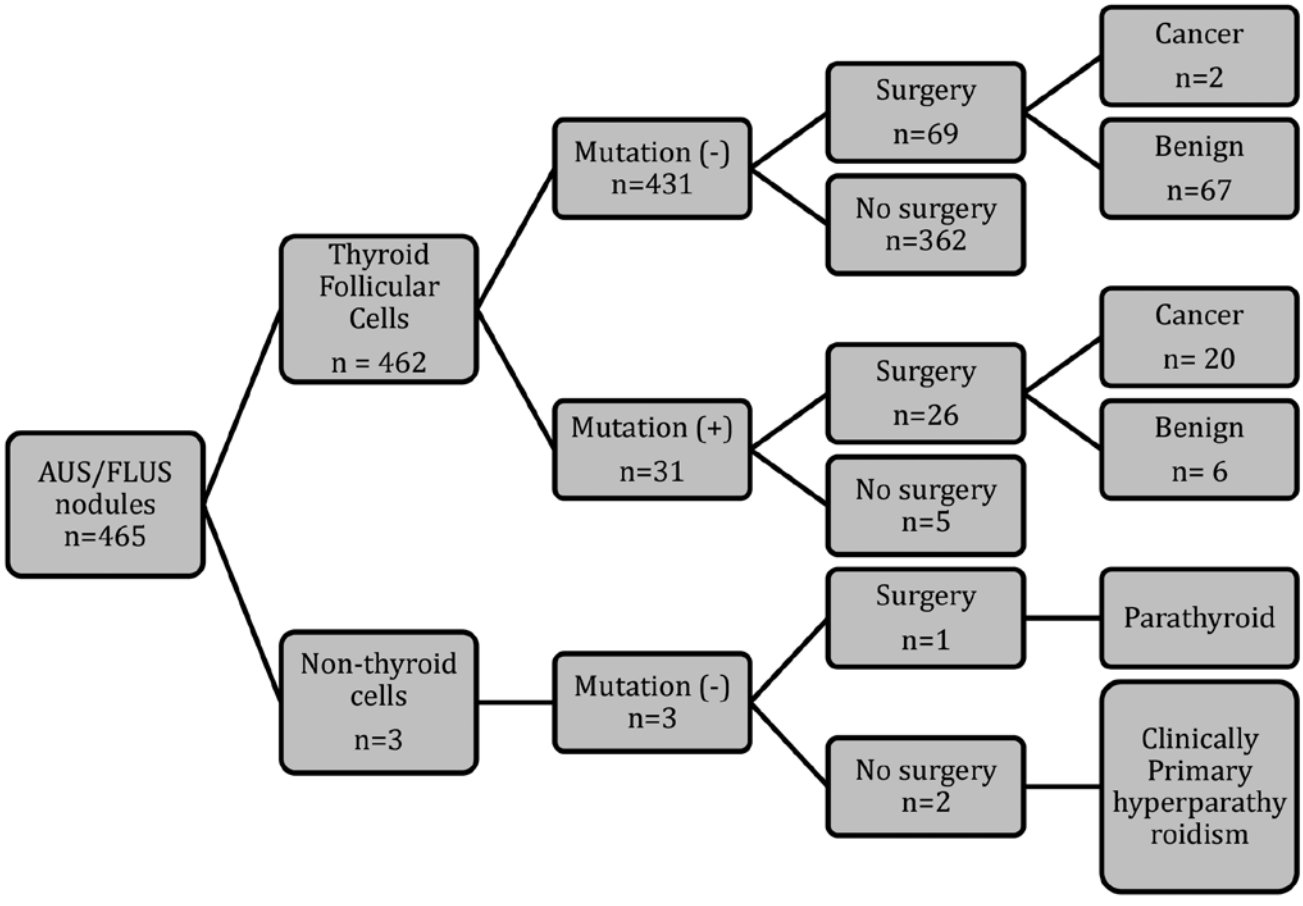


Figure 1. Schematic representation of study flow and overall performance of ThyroSeq in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance. Results showed sensitivity 90.0% [confidence interval (CI) 78.8-100], specificity 92.1% (CI 86.0-98.2), positive predictive value 76.9% (CI 60.7-93.1) and negative predictive value 97.2% (CI 78.8-100) with accuracy of 91.8% (CI 86.4-97.3). The overall prevalence of a thyroid cancer diagnosis in the study of all samples of follicular cells (n=462) that underwent molecular testing was 4.8%. (Adapted from Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN. Impact of the multi-gene ThyroSeq next-generation sequencing assay on cancer diagnosis in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology. Thyroid 2015;25:1217-1223).

reported by the authors to have been operated on based on patient preference. In all, 98 nodules from 92 patients had a definitive diagnosis, either surgical (n=96) or nonsurgical.

It is important to note that the study was conducted in a prospective manner, in that the molecular analysis was performed prior to the surgery. Therefore, the histopathologic diagnosis was provided by pathologists that were not blinded to results of the NGS test. Of all FNA samples deriving from follicular cells 31 (6.7%) were positive for mutations (n=24) or gene fusions (n=7). The most common genetic alteration encountered were mutations involving *RAS* (n=17) and only one nodule was found to be positive for the $BRAF^{V600E}$ mutation. Of the 31 nodules with positive ThyroSeq, 26 were surgically treated while 69 out of the total group of 431 mutation negative nodules, were subjected to surgical removal. Of the surgeries performed, half (n=45) were total thyroidectomies and the remaining half underwent hemi-thyroidectomy.

Among the 26 nodules with positive ThyroSeq results that underwent surgical treatment, 20 (77%) were ultimately deemed to be malignant by histopathology. Eighteen were follicular-variant papillary thyroid carcinoma and two represented the classic variant of papillary carcinoma. Of the six benign nodules that had tested positive for mutations, two had *NRAS* mutation and the others contained single mutations each in *HRAS*, *E1F1AX* or *PTEN* with one nodule harboring a *THADA* fusion. On histology, 4 out of the six benign nodules harboring mutations were classified as follicular adenomas and the other two were deemed to represent hyperplastic nodules.

Of the 69 thyroid nodules that were excised after testing negative by the next generation-gene sequencer only two were malignant on final pathology. Both tumors were papillary carcinomas, under two centimeters in diameter, confined to the thyroid and did not exhibit lymphovascular invasion.

On final analysis of test performance, ThyroSeq provided accurate classification of 91 out of 96 nodules in which a final surgical diagnosis was available as either benign (n=71) or malignant (n=20). Two false-negative and six false-positive tests were encountered in the study. Based on these findings the performance characteristics of the test were quite favorable with a 90.9% sensitivity, 92.1% specificity. The NPV was 97.2% and PPV 76.9%.

When interpreting the above performance characteristics of the ThyroSeq, it is important to note that while sensitivity and specificity are characteristics intrinsic to any test, the resulting PPV and NPV values are highly influenced by the pre-test probability of the disease, in other words the performance characteristics involving predictive value will change significantly based on the prevalence of disease in the study population. Because the prevalence of malignancy among AUS/FLUS that has been reported in the literature varies between 6% and 48%, the NPV of the molecular test would be expected to range from 99% to 92%, and the PPV between 42% and 91%.

Given the high sensitivity of the test for diagnosing thyroid cancer and the resultant NPV, which is similar to that reported for benign cytology (<5%), it has been proposed that a negative ThyroSeq in a patient with AUS/FLUS can generally be considered as a basis for observation rather than surgery (17). The exception could be a population or particular patient or nodule with a high pre-test probability for cancer.

Though the addition of several genetic markers to the previously reported seven-gene panel has resulted in a decrease of PPV from 88% to 77%, the PPV for the ThyroSeq may still be sufficient to consider it not only as a rule-out test, but also as a rule-in test for the diagnosis of thyroid cancer. A further strength of NGS is that the PPV is close to 100% in the case of certain mutations including in tumors positive for the most common *BRAF* mutations and for fusions in *PPARG*, *NTRK1*, *NTRK3* and *ALK*. One must also take into consideration that 3 out of the 6 total "false-positives" in this study were benign nodules harboring *RAS* mutations. These are clonal neoplasms and there is controversy that such tumors could represent pre-malignant lesions. In fact, several lines of evidence lend support to the hypothesis that *RAS* is an oncogene responsible for gradual progression from benign to malignant thyroid lesions (25).

Though the above described findings are encouraging, there are several limitations of the study. The study was performed at a single institution and the participants, including the patients, clinicians, surgeons and pathologists were not blinded to the results of the molecular test. In fact, the results of the test were reported to have been used as a basis to operate in at least 27 of the cases. The unblinded nature of the study could lead to an overestimation of the test accuracy, a phenomenon known as review bias or expectation bias. Given the very short-term follow-up provided and the lack of surgical definitive diagnosis for the large number of cases that had negative mutation analysis and were not operated on, we cannot know for sure the performance characteristics of the test in the entire group of patients tested, which consists mainly of patients who did not undergo surgery and of which none had long-term follow-up at the time of reporting of the study findings. Of the 462 nodules of follicular cell composition that were submitted for molecular testing only 22 were ultimately diagnosed as malignant. Given the lack of long term follow up, it is reasonable to question whether some cases of thyroid cancer remained undiagnosed among the 367 nodules that were not surgically treated.

While the authors provided data regarding the rationale to proceed to surgery in the group of patients that were submitted to thyroidectomy, details regarding the decision-making process leading to observation in the cohort not operated on and therefore not included in the analysis of test performance could also be of use in understanding the

full implications and the generalizability of the study results. What were the sonographic features of the nodules in the study? How did they correlate with results of mutation analysis and were they used in the decision making process to select nodules for surgery and on the extent of surgeries performed? What were the baseline characteristics of the patients and were other molecular tests such as GEC also performed and utilized in the decision to observe vs. proceed to surgery? The answers to these questions would be helpful in understanding how the results of the study could inform every day clinical practice.

Furthermore, while at first glance it appears to be a strength of this study that the majority of the patients who underwent thyroidectomy did so based on suspicious or malignant results of a co-existing nodule other than the nodule sampled and included in the analysis for test performance, this also may decrease the applicability of the test performance to the more common scenario in which patients undergo thyroidectomy for diagnosis of an indeterminate solitary nodule, without a co-existing nodule with a higher risk cytological diagnosis. Thyroid cancer, PTC in particular, is often multi-focal and patients harboring one malignant thyroid nodule may be more likely to have another. Whether the test would perform as well in a large cohort of patients with solitary nodules or co-existing benign nodules is a matter that demands further investigation.

To determine the true value of the NGS in clinical decision-making in this study population it would have also been helpful to know the sonographic and other traditional thyroid cancer risk factors of all the patients who had ThyroSeq testing. It is possible that in a significant number of these cases the pre-test probability of cancer may have been high (or low) enough to justify surgery or observation as the best management strategy, based on for instance very high (or low) risk sonographic nodule appearance. Also, it is possible that excluding patients who proceeded to surgery based on the mutation analysis results or due to "patient preference" from the calculations of test performance characteristics would have yielded different results.

Multi-center studies of ThyroSeq in which practitioners and participants are blinded to test results, with long-term follow-up including health outcomes data will provide even more value in assessing the performance of the ThyroSeq and its applicability and utility for "real-world" management of thyroid nodules with Bethesda III cytology. However, given the already recognized implications of the mutation analysis on diagnosis as well as emerging data suggesting its use in determining prognosis or selecting among treatment options in some cases, it may not be considered ethical, even at this early stage of inquiry into the role of molecular testing in diagnosing thyroid carcinoma, to withhold results of molecular testing from subjects enrolled in such studies.

Comparison of Utility of Next-Generation Sequencer to Other Strategies Recommended for the Management of AUS/FLUS Cytology

In regards to the strategy aimed at diagnosing and managing patients with a Bethesda category III thyroid nodule cytology result, the 2015 ATA guidelines provide the following recommendations (6):

"For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making (weak recommendation, moderate-quality evidence). If repeat FNA cytology and/or molecular testing are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference (strong recommendation, Low-quality evidence)."

Prior to the availability of molecular testing for FNAs with AUS/FLUS cytology, it was recommended to consider repeat FNA as one approach to management (26). This was based on the observation that approximately 50% of such repeat FNAs resulted in benign cytology. However, a recent report has described similar rates of malignancy in patients undergoing surgery after benign results on repeat FNA and those with repeatedly Bethesda category III cytology (27). For those patients not wanting to be subjected to a repeat FNA procedure a second-opinion review of the original FNA specimen by a high-volume cytopathologist may result in reclassification and could be a reasonable first-step in some instances (28).

Ultrasound features of the nodule with AUS/FLUS cytology may be used to aid in improving diagnostic prediction of malignity or benignity (29,30). Retrospective studies have reported a PPV of 60-100% when suspicious sonographic appearance is present. However, these studies are limited by the fact that surgical diagnosis was not available for the majority of nodules and follow-up was short-term in duration. The combination of sonographic characteristics and molecular testing in AUS/FLUS has only been reported in one study using a GEC and none using mutational analysis or NGS (31). While this study did not show any benefit in improving prediction provided by the molecular testing alone, it may have not been adequately powered.

Though not commonly performed or recommended in the evaluation of thyroid nodules, fludeoxyglucose-positron emission tomography (FDG-PET) has been reported to have a high NPV when applied to the diagnosis of cytologically indeterminate thyroid nodules. In a systematic review and meta-analysis of 6 studies FDG-PET had a low PPV (39%)

and a high NPV (96%) when performed in thyroid nodules with Bethesda category III or IV cytology (10).

While the optimal approach to the diagnosis and management of thyroid nodules with AUS/FLUS cytopathology remains controversial, molecular tests including NGS have been increasingly utilized to provide additional information to aid in the decision. In its 2015 guidelines the ATA conclude *"Further research is needed to consider the impact of considering clinical and sonographic features on the potential utility and interpretation of molecular testing of FNA specimens."*

Clinical Utility of Next-generation Sequencing in Follicular Neoplasm/Suspicious for Follicular Neoplasm (Bethesda Category IV) Cytology

According to the Bethesda system, the diagnosis of FN/SFN should be made in thyroid aspirates that have follicular cells arranged in an architectural pattern characterized by cell crowding and/or microfollicle formation and lacking nuclear features of papillary carcinoma, or are comprised

almost exclusively of oncocytic (Hurthle) cells (7). These cytological patterns are seen with follicular and Hurthle cell carcinomas and the follicular variant of papillary carcinoma, however they are commonly observed in follicular adenomas and in hyperplastic nodules as well. Since such benign lesions are

fairly common, they have a high false-positive rate on FN/SFN cytology, because only 14-34% of all nodules undergoing FNA with FN/SFN cytology are identified as malignant on the gold-standard surgical pathology (24).

In a meta-analysis of 8 studies with a total of 25,445 thyroid FNA samples, 10.1% of the results were reported as Bethesda IV with an average 26% rate of thyroid cancer diagnosed among these after surgery (32). The typical management approach has been to perform diagnostic lobectomy for such patients.

Prior to the recent introduction of ThyroSeq, available molecular tests improved either the PPV or the NPV for FN/SFN nodules, but not both at the same time. The GEC test, Afirma (Veracyte, South San Francisco, California), offers a high NPV, but its PPV is as low as 15% to 37% when applied to FN/SFN (33,34). As a result, the GEC may not be ideal to use as a basis to avoid diagnostic lobectomy in the majority of patients with this cytological diagnosis when classified as GEC suspicious, yet ultimately are found to have benign histology. The previously reported 7-oncogene panel yields a high PPV but a low NPV, which can aid in selecting patients with a higher risk of cancer and may help the surgeon decide on the appropriate extent of surgery, but does not prevent diagnostic surgeries for the majority of patients, in which the nodules are eventually determined to be benign (35). The low NPV of the 7-gene panel is due to the fact that only approximately 70% of thyroid cancer harbor a mutation in any of the 7 genes tested.

In the largest study of molecular marker testing in FN/SFN to date, Nikiforov and colleagues (16) reported findings of NGS (ThyroSeq) in 143 patients with FN/SFN cytology all of who underwent surgery for definitive diagnosis. The study included both a cohort of 91 patients in whom the molecular testing was performed retrospectively after surgery and final histopathologic diagnosis, as well as a cohort of 52 consecutive FNA samples studied prospectively in which the NGS was performed prior to thyroidectomy. While the researchers performing the molecular testing were blinded to the results of the surgical pathology, the pathologists reporting on the surgical specimens were not blinded entirely at the time of their analysis of the specimens. The ThyroSeq included testing for 13 mutant genes as well as 42 gene fusions known to occur in thyroid cancer. Expression of 8 other genes was assessed to confirm the cellular composition of the FNA sample.

Among the retrospective cohort (n=91) surgical pathology reporting was consistent with 66 benign nodules (35 follicular adenomas and 31 hyperplastic nodules) and 25 malignant nodules (Figure 2). The malignancy rate in this cohort was 27.5% with 3 FTC and 24 PTC, of which 19 were follicular variant PTC. The rate of malignancy was similar in the prospective cohort at 26.9% with 38 benign lesions and 14 malignant lesions, including 11 PTC and 3 FTC. As expected, a proportion of nodules were found to represent Hurthle cell tumors, the frequency of which was reported in detail for all groups.

The most frequent mutations identified were that of *NRAS* (n=16) and *KRAS* (n=6) in which the rate of cancer diagnosed on final histology was 81% and 83% respectively. *HRAS* mutation was discovered in two samples, both of which were malignant on final analysis. Only 1 out of 3 samples harboring a TSH-receptor gene mutation (TSHR) was malignant. Several other mutations, though encountered less frequently in the cohorts, had a much higher rate of malignancy of 100% including 4 out of 4 samples harboring *TERT* mutations and one each in samples with mutations in *BRAF*, *TP53* and *PI3K*. All of the samples identified with gene fusions (n=9), were malignant, and involved one of the three genes *PPARG*, *THADA* and *NTRK3*.

Analysis of the data revealed no differences in operating characteristics among the 2 cohorts; therefore, they were combined to assess test performance. In the entire cohort of 143 patients, the test performed at a 90% sensitivity (95% confidence interval (CI), 80%-99%), 93% specificity (95% CI, 88%-98%), an NPV of 96% (95% CI, 92%-100%), and a PPV of 83% (95% CI, 72%-95).

Because NPV and PPV are greatly affected by prevalence of disease in the test population, the variable rates of malignancy for FN/SFN cytology at different institutions would be expected to alter predictive values of any test.

In the review of 8 studies performed by Bongiovanni and colleagues (32), the cancer rate among Bethesda IV varied between 14% and 34% which would result in the ThyroSeq having a NPV between 98% and 95%, and PPV range between 68% and 87%.

Of note, of the four false-negative results representing thyroid malignancies without detected genetic abnormality, all four were intra-thyroidal and none had aggressive histopathological features. The authors speculated that the fact that no aggressive tumors were missed could be due to the fact that such tumors often have mutations in *TERT*, *BRAF* or more than one mutation. Three cancers in the series were reported to have more than one mutation. A recent case report further underscores the possible implications of the detection of multiple mutations and proposes a relationship of such a finding to aggressive tumor behavior (36).

Based on the above reported data it could be concluded that most patients with thyroid nodules with Bethesda IV cytopathology and negative NGS testing could be monitored without surgery. Notable exceptions may be in settings where the patient population has an unusually high prevalence of thyroid cancer or in individual patients in which the pre-test probability of cancer is exceptionally high due to other predictive factors such as family history, prior irradiation or high-risk sonographic characteristics of the nodule. In those unusual clinics having a high prevalence of thyroid cancer above 50% among their FN/SFN patient population, indeed the NPV of ThyroSeq would be below 90% and this could be considered too low to avoid diagnostic lobectomy.

The 2015 ATA guideline pertaining to the management of the patient with a thyroid nodule and FN/SFN cytology recommends:

"A) Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and

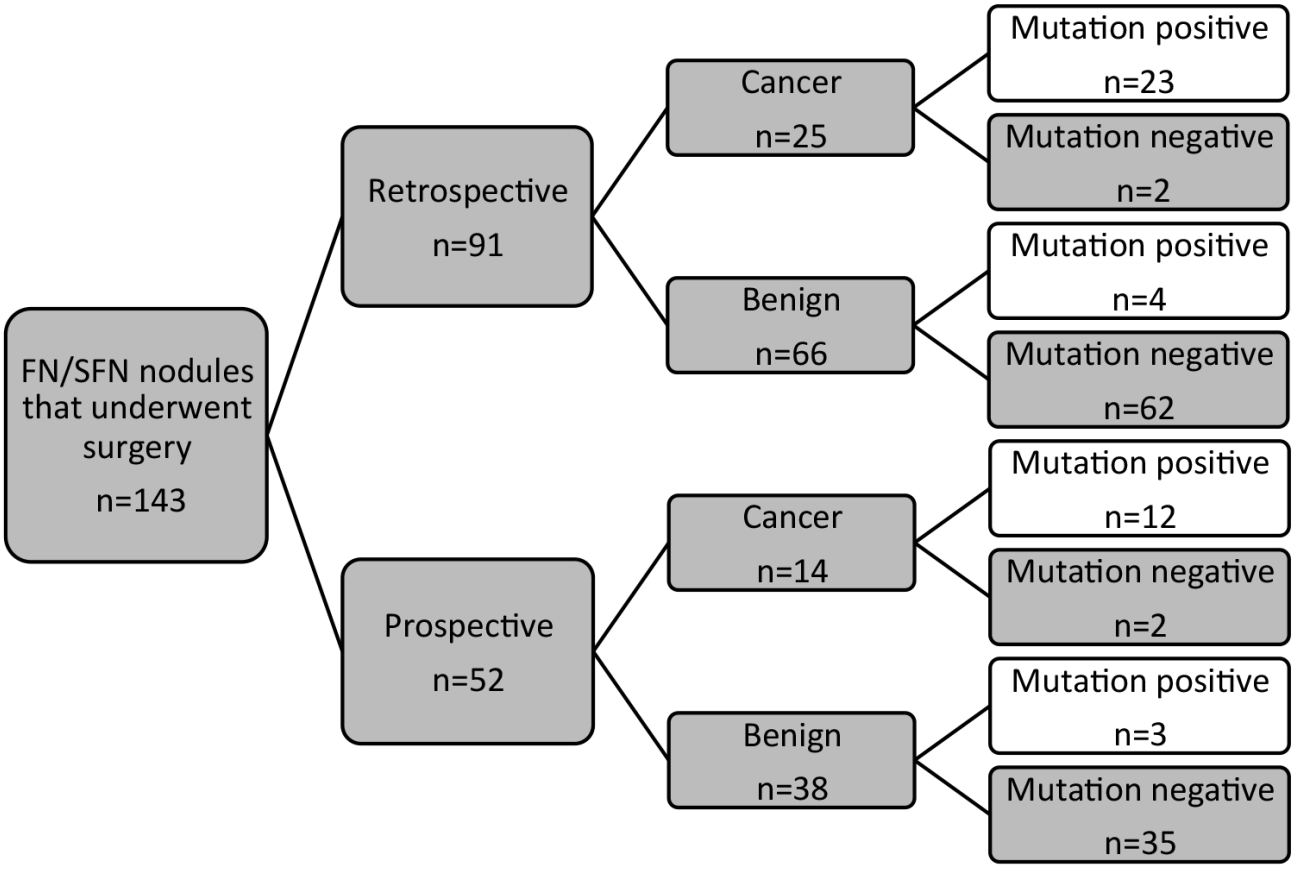


Figure 2. Schematic representation of study flow and overall performance of ThyroSeq in thyroid nodules with follicular neoplasm/suspicious for follicular neoplasm. Results showed sensitivity 90% [confidence interval (CI) 80-99], specificity 93% (CI 88-98%), positive predictive value 83% (CI 72-95) and negative predictive value 96% (CI 92-95) with accuracy of 92% (CI 88-97). (Adapted from Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, Hodak SP, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN. Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. Cancer 2014;120:3627-3634).
FN/SFN: Follicular neoplasm/suspicious for follicular neoplasm

sonographic features, molecular testing may be used to supplement malignancy risk assessment data, in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making (weak recommendation, moderate-quality evidence).

B) If molecular testing is either not performed or inconclusive, surgical excision may be considered for removal and definitive diagnosis of an FN/SFN thyroid nodule (strong recommendation, low-quality evidence)".

Recommendations on the Use of Next-generation Sequencing from Clinical Practice Guidelines

In 2015 the ATA published revised guidelines on the management of adult patients with thyroid nodules and thyroid cancer (6). These guidelines included a thoughtful and detailed discussion of the potential role of molecular testing in the diagnosis of thyroid nodules and their implications for the management of patients with thyroid nodules and cancer. It should be noted that though the guideline included discussion of the previously reported 7 gene mutational analysis panel and the published reports of ThyroSeq in FN/SFN cytology, the more recent publication regarding performance of ThyroSeq in AUS/FLUS was not available at the time of publication of the 2015 ATA guidelines.

The authors of the ATA guidelines created a framework for the use of molecular testing including a classification of such tests according to intended use as either for diagnostic purposes (for classification of a disease state), prognostic or predictive purposes (to provide predictive information regarding the probability of benefit or harm of a specific treatment) (37). The ATA authors emphasize that since there is a lack of long-term outcomes data regarding the use of molecular testing to guide therapeutic decision-making, it remains unknown whether routine use of such tests in clinical practice would result in a net benefit in the health of patients with thyroid nodules and thyroid cancer. Similarly, a National Comprehensive Cancer Network task force report has declared that the clinical utility of any molecular test must be based on strong evidence that use of the test "improves patient outcomes sufficiently to justify its incorporation into routine clinical practice" (37).

Taking into account the above principles, the ATA guidelines recommend that the current use of molecular marker testing for patients with indeterminate thyroid nodule cytology is primarily diagnostic (to rule in or rule out malignancy), with an added implication of a companion use to aid in decision-making on initial surgical management (the decision to perform surgery and to guide the extent of surgery).

The 2015 ATA guidelines also point out that while previously published guidelines, including the ATA statement on surgical application of molecular profiling of thyroid nodules, were written at a time when total or near-total thyroidectomy was recommended as the initial surgical procedure for most cases of thyroid cancer, the current guidelines suggest more conservative surgical management (i.e., hemi-thyroidectomy) be considered as an option for low-risk thyroid cancer (38). This change could affect the utility of the result of NGS to determine extent of surgery for patients with indeterminate cytology and positive mutational analysis. Furthermore, there are no long-term outcome data testing the strategy of using NGS or other molecular tests in indeterminate FNA specimens to stratify surgical approach.

In summary the 2015 guidelines of the ATA provide the following recommendation: "If molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing, and about the possible uncertainties in the therapeutic and long-term clinical implications of results".

Potential Use of Mutational Analysis for Prognostication and Targeted Therapy of Thyroid Cancer

A strength of NGS when applied as a diagnostic test to patients with thyroid nodules and thyroid cancers is the potential impact that knowledge of the underlying genetic anomaly could have on prognostication and implications for treatment decisions. Mutations involving *AKT1, TP53, PIK3CA* and *CTNNB1* are rarely present in benign thyroid nodules and common in more advanced thyroid cancers (39). *TERT* mutations in particular have been associated with increased disease specific mortality, distant metastasis and radioactive iodine refractory disease (40,41,42). $BRAF^{V600E}$ mutations are associated with higher recurrence rates and mortality in thyroid cancer (43). However, it remains controversial whether mutational status provides further prognostic information to that already provided by more traditional prognostic factors such as patient characteristics and grade and stage of disease at presentation. For thyroid papillary microcarcinoma evidence suggested that *BRAF* status together with several histopathologic features was a better predictor of extrathyroid tumor spread than either mutation or histopathologic findings alone (44).

It is likely that with increasing application of mutational analysis in thyroid nodules and cancers, and analysis of prospective studies of its use will provide data to answer this question in the future.

It must also be taken into consideration that while factors inherent to the tumor, including mutation status, have effects on prognosis; "host factors" involving the patient harboring the tumor may independently, or via complex interactions with the genomic alterations of the tumor have effects on tumor behavior and prognosis. Underscoring this point is a

recent report associating obesity with increased prevalence of *BRAF*^{V600E} mutations among patients with PTC (45).

Targeted therapies directed by results of mutational analysis are recommended for a variety of cancers. Treatment of melanoma based on *BRAF* status and assessment of *KRAS* mutational status to determine medical treatment for colorectal cancer are strategies that have been tested in clinical trials (46,47). While targeted therapy based on mutational analysis is not yet a widely accepted practice for thyroid cancer there are several clinical trials in progress to test this hypothesis. Clinical trials of MEK or *BRAF* inhibitors to increase radioiodine uptake for patients with *BRAF*-mutant, RAI-refractory thyroid carcinoma (ClinicalTrials.gov Identifier: NCT02145143) and *RAS* mutated thyroid cancer (ClinicalTrials.gov Identifier: NCT02152995) are underway. Studies of combination of *BRAF* and MEK inhibitors for patients with *BRAF* mutant anaplastic thyroid cancer (ClinicalTrials.gov Identifier: NCT02034110) are also enrolling patients. Patients with advanced thyroid cancers harboring PAX8/PPARγ fusions (ClinicalTrials.gov Identifier: NCT01655719) and NTRK alterations (ClinicalTrials.gov Identifier: NCT02122913) are now being treated in clinical trials. Previous data showed that STRN-ALK fusions occur more often with aggressive types of thyroid cancer and several reports have demonstrated that patients with advanced thyroid cancer with ALK fusions may benefit from ALK inhibitors such as Crizotinib (48). Not previously seen in thyroid cancer, a mutation in the TSC2 and in the mTOR protein was discovered in a patient with metastatic anaplastic thyroid cancer who initially achieved a near-complete response to Everolimus with posterior resistance and progression of the disease. This shows the possible benefit of sequencing a patient's cancer DNA prior to treatment and following disease recurrence, which may help guide treatment in patients with similar mutations (49).

Conclusion

As a comprehensive genome atlas of thyroid cancer is rapidly becoming a reality, and with emerging methodologies such as NGS providing detailed genetic information regarding thyroid tumors, we have now entered into the genomic age of diagnosis and treatment of thyroid nodules and thyroid cancer. Several molecular tests are now available to assist in the diagnosis of thyroid nodules among which NGS appears to be a particularly promising tool that could most accurately characterize the genetic alterations underlying these neoplasms. ThyroSeq for targeted detection of mutation has been tested for its accuracy and performance in diagnosing malignancy among thyroid nodules with indeterminate cytopathology in two single-center studies and found to have both high NPV and an improved PPV when compared to existing molecular tests. Large-scale multi-center studies are needed to validate these preliminary findings. Furthermore, future studies are needed to determine the optimal use of NGS in clinical-decision making for patients with indeterminate cytopathology after thyroid FNA. Several studies and reports point to the potential impact that knowledge of mutational status of thyroid tumors can have on prognostication and selection of targeted-therapies, though it remains to be elucidated whether strategies to treat thyroid cancer based on mutational status will improve overall outcomes. As we look ahead to the era of "personalized medicine" NGS appears to hold promise as a potentially useful tool in the detection of thyroid malignancy as well as a possible aid for the clinician in determining optimal treatment for patients with thyroid neoplasia.

Authorship Contributions

Concept: Seza Gulec, Atil Y. Kargi, Design: Atil Y. Kargi, Data Collection or Processing: Atil Y. Kargi, Marcela Perez Bustamante, Analysis or Interpretation: Atil Y. Kargi, Marcela Perez Bustamante, Seza Gulec, Literature Search: Atil Y. Kargi, Writing: Atil Y. Kargi.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Guth S, Theune U, Aberle J, Galach A, Bamberger CM. Very high prevalence of thyroid nodules detected by high frequency (13 MHz) ultrasound examination. Eur J Clin Invest 2009;39:699-706.
2. Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med 2004;351:1764-1771.
3. Ahn HS, Kim HJ, Welch HG. Korea's thyroid-cancer "epidemic" screening and overdiagnosis. N Engl J Med 2014;371:1765-1767.
4. Leenhardt L, Bernier MO, Boin-Pineau MH, Conte Devolx B, Maréchaud R, Niccoli-Sire P, Nocaudie M, Orgiazzi J, Schlumberger M, Wémeau JL, Chérie-Challine L, De Vathaire F. Advances in diagnostic practices affect thyroid cancer incidence in France. Eur J Endocrinol 2004;150:133-139.
5. Gharib H, Papini E, Paschke R, Duick DS, Valcavi R, Hegedüs L, Vitti P; AACE/AME/ETA Task Force on Thyroid Nodules. American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association medical guidelines for clinical practice for the diagnosis and management of thyroid nodules: executive summary of recommendations. J Endocrinol Invest 2010;33(Suppl 5):51-56.
6. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26:1-133.
7. Crippa S, Mazzucchelli L, Cibas ES, Ali SZ. The Bethesda System for reporting thyroid fine-needle aspiration specimens. Am J Clin Pathol 2010;134:343-344.

8. Boelaert K, Horacek J, Holder RL, Watkinson JC, Sheppard MC, Franklyn JA. Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. J Clin Endocrinol Metab 2006;91:4295-4301.
9. Brito JP, Gionfriddo MR, Al Nofal A, Boehmer KR, Leppin AL, Reading C, Callstrom M, Elraiyah TA, Prokop LJ, Stan MN, Murad MH, Morris JC, Montori VM. The accuracy of thyroid nodule ultrasound to predict thyroid cancer: systematic review and meta-analysis. J Clin Endocrinol Metab 2014;99:1253-1263.
10. Vriens D, de Wilt JH, van der Wilt GJ, Netea-Maier RT, Oyen WJ, de Geus-Oei LF. The role of [18F]-2-fluoro-2-deoxy-d-glucose-positron emission tomography in thyroid nodules with indeterminate fine-needle aspiration biopsy: systematic review and meta-analysis of the literature. Cancer 2011;117:4582-4594.
11. Xing M, Haugen BR, Schlumberger M. Progress in molecular-based management of differentiated thyroid cancer. Lancet 2013;381:1058-1069.
12. Kim MI, Alexander EK. Diagnostic use of molecular markers in the evaluation of thyroid nodules. Endocr Pract 2012;18:796-802.
13. http://www.afirma.com/physicians/. Accessed online January 8, 2016.
14. http://www.cblpath.com/products-and-services/sub-specialized-pathology/thyroid-pathology/thyroseq-next-generation-sequencing. Accessed online January 8, 2016.
15. http://asuragen.com/wp-content/uploads/2013/03/1_miRInformThyroidbroch_e.pdf. Accessed online January 8, 2016
16. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, Hodak SP, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN. Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. Cancer 2014;120:3627-3634.
17. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN. Impact of the multi-gene thyroseq next-generation sequencing assay on cancer diagnosis in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology. Thyroid 2015;25:1217-1223.
18. Nikiforov YE. Thyroid cancer in 2015: Molecular landscape of thyroid cancer continues to be deciphered. Nat Rev Endocrinol 2016;12:67-68.
19. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol 2011;7:569-580.
20. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676-690.
21. Giordano TJ. Follicular cell thyroid neoplasia: insights from genomics and The Cancer Genome Atlas research network. Curr Opin Oncol 2016;28:1-4.
22. Metzker ML. Sequencing technologies-the next generation. Nat Rev Genet 2010;11:31-46.
23. Ohori NP, Schoedel KE. Variability in the atypia of undetermined significance/follicular lesion of undetermined significance diagnosis in the Bethesda System for Reporting Thyroid Cytopathology: sources and recommendations. Acta Cytol 2011;55:492-498.
24. Bongiovanni M, Crippa S, Baloch Z, Piana S, Spitale A, Pagni F, Mazzucchelli L, Di Bella C, Faquin W. Comparison of 5-tiered and 6-tiered diagnostic systems for the reporting of thyroid cytopathology: a multi-institutional study. Cancer Cytopathol 2012;120:117-125.
25. Vitagliano D, Portella G, Troncone G, Francione A, Rossi C, Bruno A, Giorgini A, Coluzzi S, Nappi TC, Rothstein JL, Pasquinelli R, Chiappetta G, Terracciano D, Macchia V, Melillo RM, Fusco A, Santoro M. Thyroid targeting of the N-ras(Gln61Lys) oncogene in transgenic mice results in follicular tumors that progress to poorly differentiated carcinomas. Oncogene 2006;25:5467-5474.
26. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer,Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009;19:1167-1214.
27. VanderLaan PA, Marqusee E, Krane JF. Clinical outcome for atypia of undetermined significance in thyroid fine-needle aspirations: should repeated fna be the preferred initial approach? Am J Clin Pathol 2011;135:770-775.
28. Davidov T, Trooskin SZ, Shanker BA, Yip D, Eng O, Crystal J, Hu J, Chernyavsky VS, Deen MF, May M, Artymyshyn RL. Routine second-opinion cytopathology review of thyroid fine needle aspiration biopsies reduces diagnostic thyroidectomy. Surgery 2010;148:1294-1299.
29. Rosario PW. Thyroid nodules with atypia or follicular lesions of undetermined significance (Bethesda Category III): importance of ultrasonography and cytological subcategory. Thyroid 2014;24:1115-1120.
30. Yoo WS, Choi HS, Cho SW, Moon JH, Kim KW, Park HJ, Park SY, Choi SI, Choi SH, Lim S, Yi KH, Park do J, Jang HC, Park YJ. The role of ultrasound findings in the management of thyroid nodules with atypia or follicular lesions of undetermined significance. Clin Endocrinol (Oxf) 2014;80:735-742.
31. Lastra RR, Pramick MR, Crammer CJ, LiVolsi VA, Baloch ZW. Implications of a suspicious afirma test result in thyroid fine-needle aspiration cytology: an institutional experience. Cancer Cytopathol 2014;122:737-744.
32. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. Acta Cytol 2012;56:333-339.
33. Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, LiVolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med 2012;367:705-715.
34. McIver B, Castro MR, Morris JC, Bernet V, Smallridge R, Henry M, Kosok L, Reddi H. An independent study of a gene expression classifier (Afirma) in the evaluation of cytologically indeterminate thyroid nodules. J Clin Endocrinol Metab 2014;99:4069-4077.
35. Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO, Ferris RL, Yip L, Seethala RR, Tublin ME, Stang MT, Coyne C, Johnson JT, Stewart AF, Nikiforova MN. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. J Clin Endocrinol Metab 2011;96:3390-3397.
36. Shrestha RT, Karunamurthy A, Amin K, Nikiforov YE, Caramori ML. Multiple mutations detected preoperatively may predict aggressive behavior of papillary thyroid cancer and guide management-a case report. Thyroid 2015;25:1375-1378.
37. Febbo PG, Ladanyi M, Aldape KD, De Marzo AM, Hammond ME, Hayes DF, Iafrate AJ, Kelley RK, Marcucci G, Ogino S, Pao W, Sgroi DC, Birkeland ML. NCCN task force report: Evaluating the clinical utility of tumor markers in oncology. J Natl Compr Canc Netw 2011;9(Suppl 5):1-32.
38. Ferris RL, Baloch Z, Bernet V, Chen A, Fahey TJ, Ganly I, Hodak SP, Kebebew E, Patel KN, Shaha A, Steward DL, Tufano RP, Wiseman SM, Carty SE; American Thyroid Association Surgical Affairs Committee. American Thyroid Association Statement on Surgical Application of Molecular Profiling for Thyroid Nodules: Current Impact on Perioperative Decision Making. Thyroid 2015;25:760-768.
39. Ricarte-Filho JC, Ryder M, Chitale DA, Rivera M, Heguy A, Ladanyi M, Janakiraman M, Solit D, Knauf JA, Tuttle RM, Ghossein RA, Fagin JA. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. Cancer Res 2009;69:4885-4893.
40. Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, Ibrahimpasic T, Ghossein RA, Fagin JA. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab 2013;98:1562-1566.

41. Liu R, Xing M. TERT promoter mutations in thyroid cancer. Endocr Relat Cancer 2016;23:143-155.
42. Melo M, da Rocha AG, Vinagre J, Batista R, Peixoto J, Tavares C, Celestino R, Almeida A, Salgado C, Eloy C, Castro P, Prazeres H, Lima J, Amaro T, Lobo C, Martins MJ, Moura M, Cavaco B, Leite V, Cameselle-Teijeiro JM, Carrilho F, Carvalheiro M, Máximo V, Sobrinho-Simões M, Soares P. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. J Clin Endocrinol Metab 2014;99:754-765.
43. Xing M. BRAF V600E mutation and papillary thyroid cancer. JAMA 2013;310:535.
44. Niemeier LA, Kuffner Akatsu H, Song C, Carty SE, Hodak SP, Yip L, Ferris RL, Tseng GC, Seethala RR, Lebeau SO, Stang MT, Coyne C, Johnson JT, Stewart AF, Nikiforov YE. A combined molecular-pathologic score improves risk stratification of papillary micro carcinoma. Cancer 2012;118:2069-2077.
45. Lee J, Lee CR, Ku CR, Kang SW, Jeong JJ, Shin DY, Nam KH, Jung SG, Lee EJ, Chung WY, Jo YS. Association Between Obesity and BRAFV600E Mutation Status in Patients with Papillary Thyroid Cancer. Ann Surg Oncol 2015;22(Suppl 3):683-690.
46. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, Hamid O, Schuchter L, Cebon J, Ibrahim N, Kudchadkar R, Burris HA, Falchook G, Algazi A, Lewis K, Long GV, Puzanov I, Lebowitz P, Singh A, Little S, Sun P, Allred A, Ouellet D, Kim KB, Patel K, Weber J. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med 2012;367:1694-1703.
47. Lièvre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, Côté JF, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, Laurent-Puig P. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66:3992-3995.
48. Kelly LM, Barila G, Liu P, Evdokimova VN, Trivedi S, Panebianco F, Gandhi M, Carty SE, Hodak SP, Luo J, Dacic S, Yu YP, Nikiforova MN, Ferris RL, Altschuler DL, Nikiforov YE. Identification of the transforming STRN-ALK fusion as a potential therapeutic target in the aggressive forms of thyroid cancer. Proc Natl Acad Sci USA 2014;111:4233-4238.
49. Wagle N, Grabiner BC, Van Allen EM, Amin-Mansour A, Taylor-Weiner A, Rosenberg M, Gray N, Barletta JA, Guo Y, Swanson SJ, Ruan DT, Hanna GJ, Haddad RI, Getz G, Kwiatkowski DJ, Carter SL, Sabatini DM, Jänne PA, Garraway LA, Lorch JH. Response and acquired resistance to everolimus in anaplastic thyroid cancer. N Engl J Med 2014;371:1426-1433.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):36-49 DOI:10.4274/2017.26.suppl.05

Molecular Profiling of Thyroid Nodules: Current Role for the Afirma Gene Expression Classifier on Clinical Decision Making

Tiroid Nodülü Moleküler Profillemesi: Afirma Gen Ekspresyon Sınıflandırıcısının Klinik Karar Verme Üzerinde Güncel Rolü

Richard T. Kloos
Veracyte Inc., Department of Medical Affairs, Senior Medical Director-Endocrinology, California, USA

Disclosure statement: *RTK is a stock option holder and employee of Veracyte, Inc. The opinions expressed are those of the author and may not reflect the opinions of Veracyte.*

Abstract

Thyroid fine-needle aspiration biopsy results are cytologically indeterminate in 15-30% of cases. When these nodules undergo diagnostic surgery, approximately three-quarters are histologically benign. These unnecessary surgeries diminish quality of life, generate complications, and increase healthcare costs. The Afirma gene expression classifier (GEC) is validated to pre-operatively identify cytologically indeterminate nodules likely to be truly benign so that surgery can be avoided. Its performance is supported by robust multicenter prospective and blinded clinical validation studies, and supported by extensive independent clinical utility publications which show a marked reduction in surgery among patients with benign Afirma GEC results. To rule-out cancer and avoid unnecessary diagnostic surgery, Afirma's quality and depth of validation stand alone. The accuracy of a benign result is the negative predictive value (NPV). Afirma achieves an NPV ≥94% among cytologically indeterminate nodules (Bethesda III or IV). Thirteen clinical utility studies describing 1468 GEC benign patients demonstrate that few Afirma GEC benign nodules undergo surgery, including after 3 years of follow-up. With a specificity of 52%, over half of the truly benign nodules with indeterminate cytology receive a benign GEC result. High test sensitivity is critical to safely rule out cancer. The Afirma GEC's 90% sensitivity means that regardless of the pre-test risk of malignancy, 90% of all malignant nodules are GEC suspicious. The Afirma GEC has transformed patient care. Where the majority of cytologically indeterminate patients were once operated to determine if the nodule was benign or malignant, now nearly half of these surgeries can be avoided.
Keywords: Biopsy, fine-needle aspirate, gene expression, genomics, molecular diagnostic techniques, thyroid nodule

Öz

Belirsiz sitoloji tiroid ince iğne biyopsi örneklerinin %15-30'unda bulunur. Bu nodüller tanı amaçlı ameliyat edildiğinde yaklaşık dörtte üçünün histolojik olarak benign olduğu saptanmıştır. Gereksiz ameliyatlar yaşam kalitesini azaltır, komplikasyonlara neden olur ve sağlık giderlerini artırır. Afirma gen ekspresyonu sınıflandırıcı [Gene Expression Classifier (GEC)] operasyon öncesi benign olma olasılığı olan belirsiz sitolojili nodülleri tespit ederek cerrahi girişim gerekliliğini önleyebilir. Testin etkinliği güvenilir çok merkezli prospektif kör klinik doğrulama çalışmalarıyla, benign Afirma GEC sonuçları ile bu hasta grubunda cerrahinin belirgin bir azalma gösterdiği ise geniş, bağımsız klinik yarar yayınları ile desteklenmiştir. Kanseri ekarte etmek ve gereksiz tanısal cerrahiyi önlemek amacıyla Afirma, kalitesi ve doğrulama oranı ile alanında tektir. Benign bir sonucun doğruluğu testin negatif prediktif değerini (NPD) gösterir. Afirma'nın NPD'yi belirsiz sitolojili nodüller için (Bethesda III veya IV) ≥%94'e ulaşmaktadır. GEC benign 1,468 hastayı içeren on üç klinik yarar çalışması, sadece birkaç Afirma GEC benign nodülde ameliyat ihtiyacı olduğunu 3 yıllık takip süresi ile göstermektedir. Testin %52 özgüllüğü ile, gerçekten benign olan belirsiz sitolojili nodüllerin yarısından fazlasında benign GEC sonucu alınmaktadır. Kanseri güvenle ekarte etmek için yüksek

Address for Correspondence: Richard T. Kloos MD, Veracyte, Inc., Department of Medical Affairs, Senior Medical Director-Endocrinology, California, USA
Phone: +1(650)243-6300 E-mail: richard.kloos@veracyte.com

test duyarlılığı kritik öneme sahiptir. Afirma GEC'nin %90 duyarlılığı, test incesi malignite riski ne olursa olsun, tüm malign nodüllerin %90'ının GEC şüpheli olduğu anlamına gelir. Afirma GEC hasta yaklaşımını değiştirmiştir. Belirsiz sitolojili hastaların çoğunluğu nodülün iyi veya kötü huylu olup olmadığını belirlemek için ameliyat edilirken, günümüzde bu ameliyatların neredeyse yarısı önlenebilir.
Anahtar kelimeler: Biyopsi, ince iğne aspirasyon, gen ekspresyonu, genomik, moleküler tanı teknikleri, tiroid nodülü

Introduction

Prior to the adoption of thyroid nodule fine-needle aspiration biopsy (FNAB), thyroid nodules were regularly referred for diagnostic surgery because of their 5-15% risk of malignancy (ROM) (1). FNAB decreased diagnostic thyroidectomies by one-half as most FNAB results are cytologically benign and surgery is typically avoided (2). Still, 15-30% of thyroid FNABs are cytologically indeterminate, i.e. not clearly benign nor malignant (1,3). When cytologically indeterminate thyroid nodules undergo diagnostic surgery, approximately three-quarters prove to be benign on surgical histopathology (Figure 1) (4,5,6). The care of such patients is being dramatically altered by a new diagnostic strategy that pre-operatively identifies many of these benign nodules with indeterminate cytopathology [Bethesda categories III and IV (7)] as having a low risk of cancer so that diagnostic surgery can be avoided, along with its costs, complications, and inconveniences. Complications from thyroid surgery include, but are not limited to, hypothyroidism, voice changes, vocal cord dysfunction, hypocalcemia (temporary and permanent), tracheostomy, hematoma, infection, hospital readmission, and death. Complications are highest in patients older than 65 years of age, and when the procedure is performed outside of high-volume thyroidectomy hospitals (8). Among cytologically indeterminate nodules, patient clinical factors, ultrasound characteristics (9), additional cytological

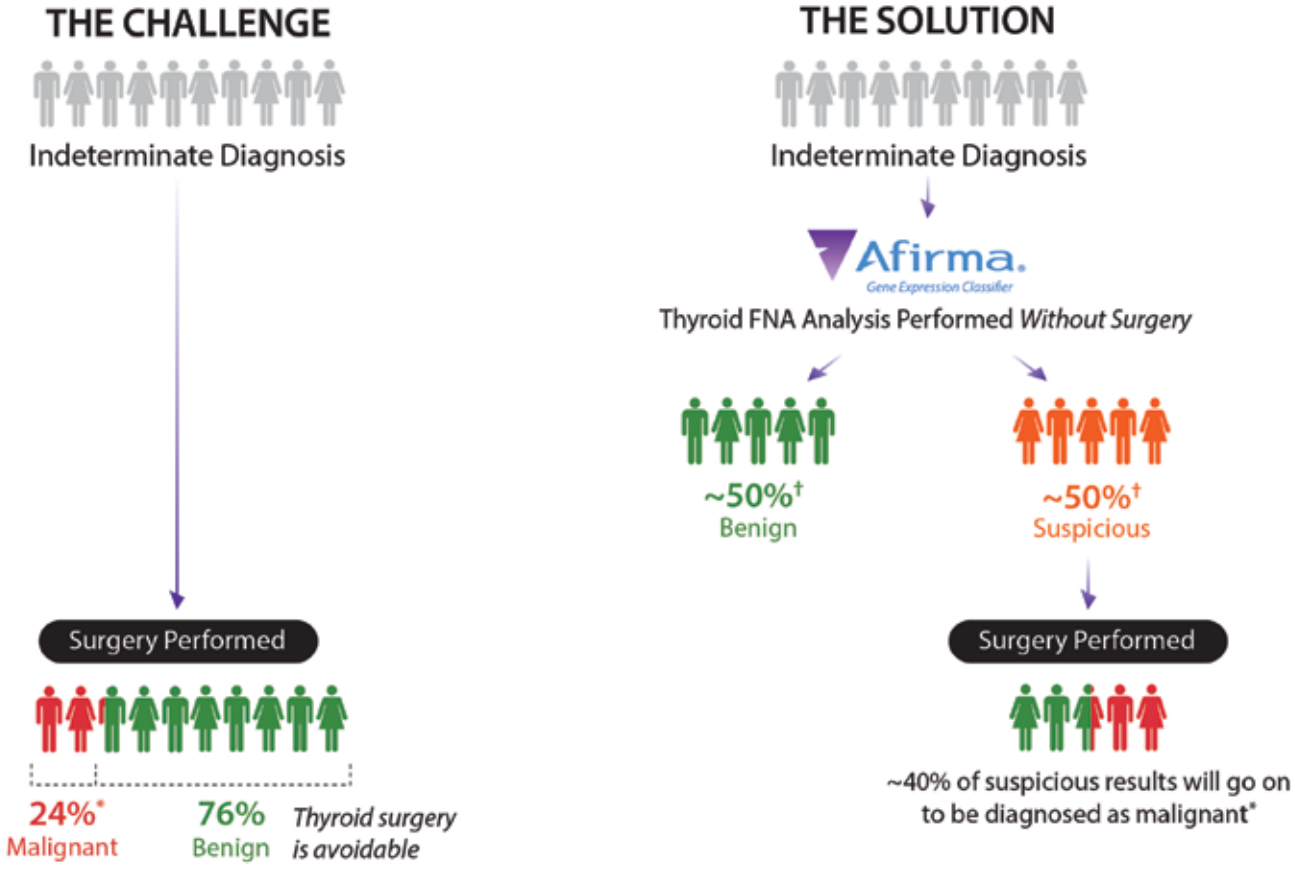


Figure 1. Afirma gene expression classifier reduces unnecessary thyroid surgeries compared to management without gene expression classifier testing
**Alexander et al. (4), †see Figure 3*

subcategorization or second opinion, and repeat FNAB have been unable to reliably identify a significant fraction of benign nodules to safely avoid surgery. For example, among Bethesda III nodules, those with any ultrasound predictive feature (solid, hypoechoic, microcalcifications, increased vascularization, or irregular margin) were found to have at least a 12% ROM, which increased further when additional features were present (10). Current excitement has focused on molecular genomics approaches. To date, only the Afirma gene expression classifier (GEC) (Veracyte Inc., South San Francisco, California) is supported by prospective, multicenter, and blinded validation studies to reclassify nodules as benign, and has been shown in multiple clinical utility studies to reduce avoidable diagnostic surgeries based on the test result.

Tests to Rule-in and Rule-out Cancer

A test with a high sensitivity and high negative predictive value (NPV) is able to rule-out cancer (11,12). Test sensitivity measures the fraction of cancers that the test identifies as "positive" (e.g. Afirma GEC suspicious). Afirma GEC test sensitivity among indeterminate nodules is 90% (4). Test NPV measures the fraction of "negative" calls by the test (e.g. Afirma GEC benign) that are correct. Afirma GEC test NPV is 94-95% amongst Bethesda III and IV nodules at a cancer prevalence of 24-25% (4). While not mutually exclusive, a test with a high specificity and high positive predictive value (PPV) is able to rule-in cancer. Test specificity measures the fraction of benign nodules that are called benign by the test. Afirma GEC test specificity is 52% (4), suggesting that just over half of the benign nodules are called GEC benign. Test PPV measures the fraction of "positive" calls by the test (e.g. Afirma GEC suspicious) that are correct. Afirma GEC test PPV is 37-38% amongst Bethesda III and IV nodules (4). Thus, the strength of the Afirma GEC is its ability to rule-out cancer (NPV), more than its ability to rule-in cancer (PPV). A rule-in test is of value when it changes clinical care, such as altering the extent of thyroid surgery from a lobectomy to a total thyroidectomy. However, the necessity of total thyroidectomy for patients with thyroid cancer less than 4 cm, without gross extra-thyroidal extension, distant metastases, or clinically apparent metastases to the lymph nodes has not been established and current guidelines do not mandate total thyroidectomy in the absence of these features (13). Thus, the utility of rule-in tests is currently questioned as patient benefit has not been established. Given the modest specificity and PPV of Afirma, it is not considered a rule-in test. While an Afirma GEC suspicious result raises the risk of cancer from 24-25% to 37-38%, it should be clear that the strength of the test is that it identifies just over one-half of all benign nodules with Bethesda III or IV cytology as genomically benign, and 90% of all cancers as genomically suspicious regardless of the cancer prevalence (Figure 1). Thus, when applied to the typical cytologically indeterminate nodule with ROM of 25% or less, the expected accuracy of a benign result (NPV) is 94% or greater. As a result, most Afirma GEC benign nodules are candidates for clinical observation in lieu of diagnostic surgery. Additional "cassettes" are tested with every Afirma GEC to identify rare neoplasms that are often difficult to accurately diagnose with cytology such as medullary thyroid cancer (MTC), parathyroid neoplasms, and metastases to the thyroid from malignant melanoma, breast, and renal cell carcinomas. Failing to trigger one of these cassettes, the GEC evaluates the expression of 142 genes that are used in a proprietary mathematical algorithm to classify indeterminate thyroid nodule samples as either GEC benign or GEC suspicious.

Rationale for the Measurement of Messenger Ribonucleic Acid Expression

The Afirma GEC is based on the measurement of messenger ribonucleic acid (mRNA) expression. There are several diagnostic advantages to using RNA instead of other approaches such as DNA mutations or microRNA expression. Unlike cancers whose cytology is Bethesda V or VI, cancers that are cytologically indeterminate (Bethesda III and IV) typically lack the most common genomic abnormality of differentiated thyroid cancer: $BRAF^{V600E}$ mutation. In its absence, the most common classic mutation amongst cytologically indeterminate cancers are RAS mutations, but these are found in the minority and are also found in benign nodules. As benign nodules outnumber malignant nodules 4:1 among nodules with indeterminate cytology, the PPV of RAS mutations is poor in a number of studies (14,15,16,17,18,19,20,21). Herein lies the challenge of mutational approaches for cytologically indeterminate nodules: many malignancies lack the known genomic abnormalities (22,23), and when present, most genomic abnormalities are not specific for cancer (22,23).

While there are only approximately 23,000 known protein-coding DNA genes (24), each of these may be transcribed into multiple alternatively-spliced variants, with more than 240.000 known mRNA isoforms. Disease-causing DNA alterations generally exert their effects, at least partially, on the transcriptome. Similarly, microRNAs impart their effects by altering transcription. Therefore, mRNA expression provides a cumulative measurement of various known (and unknown) upstream effects. Additionally, gene expression may be impacted by lifestyle and environmental factors so that mRNA expression reflects additional significant information not discernible from DNA or microRNA analysis alone.

Gene expression classifiers quantitatively evaluate the relative expression levels of multiple genes that comprise the genomic signature of the interrogated tissue. In the development of Afirma GEC, instead of discriminately relying on genes previously identified in the literature, analysis of the whole genome (transcriptome) was used

to identify candidate genes, and support vector machine learning methods were used to develop the classifier algorithm (4,25). The genes utilized in the cassettes and main Afirma GEC classifier have been published (4). This powerful methodology more fully utilizes the genomic information of the biological sample than is used by target next generation sequencing approaches.

Clinical Validation

Physicians find risk of cancer associated with a cytological benign FNAB diagnosis to be low enough to defer surgery in the vast majority of such patients. A 6-8% risk of cancer among operated cytology benign nodules has been described (4,5,26,27,28,29,30). Thus, a test that could reliably identify cytologically indeterminate nodules with a similar or lower risk of cancer (e.g. NPV ≥94%) could allow these nodules to also be considered for clinical observation instead of diagnostic surgery. Clinical validation of the Afirma GEC was initially performed on a small independent sample set of thyroid nodule FNABs within a prospective multicenter, double blind study design (25). The Afirma GEC achieved high sensitivity and NPV, including among cytologically indeterminate nodules. After further optimization, the GEC was validated in a second larger independent sample set in a prospective multicenter validation study. The second study included the largest ever prospectively collected set of thyroid FNAB biopsies from 3,789 unique patients, with a final validation set of 265 cytologically indeterminate nodules. Based on the 24% prevalence of malignancy in cytologically indeterminate samples (Bethesda III+IV), a 95% NPV for the Afirma GEC was achieved (4).

The unique and often overlooked strength of this prospective, multicenter, and blinded validation design is that it supports generalizability of the results. Prospective and multicenter study designs reduce selection bias and better represent what is likely to occur in real-world practice. The 3.789 patients were prospectively consented and enrolled in the trial before undergoing FNAB at 49 study sites across the country, including academic and community practices, which provides confidence in the external validity of the findings. Strong internal validity was demonstrated when no differences were found between the final validation cohort of 265 patients compared to the full prospective and consecutive total enrollment cohort in patient age, gender, cancer risk factors, or nodule size. As investigators were blinded to the Afirma GEC result, the test result did not influence which patients underwent surgery. These important study design elements (prospective, blinded, and multicenter) support the internal and external validity of the study, and provide confidence in the broader generalizability of the study findings to a physician's own clinical practice (13). In contrast, significant biases can be introduced when the study cohort does not mimic the intended use cohort. For example, profound bias can occur in unblinded studies where the test result influences inclusion or exclusion from the "validation" cohort (13). The Afirma GEC is the only test for cytologically indeterminate nodules demonstrated to have an accurate enough benign result (e.g. NPV ≥94%) proven in a rigorous and published prospective, blinded, and multicenter validation study to allow physicians to strongly consider clinical observation instead of surgical resection for Bethesda III and IV nodules (4).

Overall, the ROM for a thyroid nodule with Bethesda categories III and IV indeterminate cytology with an Afirma GEC benign classifier result is about 5% (1-NPV). This risk is comparable to the 6-8% cancer risk for an operated thyroid nodule with a benign cytology diagnosis (4,5,26,27,28,29,30). This demonstrates that cytologically indeterminate nodules (Bethesda categories III and IV) with an Afirma GEC benign diagnosis can be managed as would a cytologically benign nodule (4,31), as suggested by the National Comprehensive Cancer Network (NCCN) Thyroid Carcinoma Guideline (32). In contrast, others have attempted to create rule-out tests using the most common DNA point mutations, fusions, or proprietary microRNA signatures where the false negative rate may be unacceptable for routine clinical use. Asuragen reported in its prospective, multicenter, and blinded 7-gene mutation panel study that it missed as many as 53% of malignant Bethesda III and IV nodules (33), a rate significantly higher than had been seen in an earlier unblinded single center study (34). Interpace reported that its 8-gene mutation panel (ThyGenX) missed 40% of malignant nodules (35), while independent studies (22,23) have not confirmed claims of improved sensitivity and specificity with even larger mutation/fusion panels (36,37). Interpace has combined ThyGenX with a 10 microRNA classifier and in a second study reported that it missed 20% of malignancies (35). Similarly, Rosetta Genomics reported high sensitivity of its microRNA classifier when 20% of samples (1 in five cases) were excluded based on lack of histological agreement amongst 3 pathologists. In practice, physicians can't know which patients to exclude pre-operatively, so test performance is more accurately reflected amongst the entire cohort where nearly 1 in 6 cancers were missed (38).

Clinical Practice Experiences and Clinical Utility

While clinical validation demonstrates the test's ability to accurately predict the diagnosis, clinical utility measures the test's impact on real-world patient management decisions and impact on net health outcomes (39). Fourteen publications now describe the clinical experience with the Afirma GEC in routine clinical practice (9,31,40, 41,42,43,44,45,46,47,48,49,50,51). Among the Afirma GEC benign patients, only 122 of the 1211 patients (10%) were operated, demonstrating a dramatic reduction in surgery compared to the 73% historical rate of surgery (52)

when Afirma was not used (Figure 2). Five of the Afirma GEC publications were multicenter (40,41,43,46,51), two had a minimum follow-up of 1 year (45,48), and one reported patients Afirma GEC tested at least 3 years prior to study enrollment (43). In that study, 17% of Afirma GEC benign patients underwent surgery and 88% of the surgeries occurred within 2 years of the biopsy. Yang et al. (50) reported that surgery was significantly reduced in both Bethesda III and IV categories when they globally compared patient management before and after implementation of Afirma GEC testing. Overall, the findings demonstrated a durable and dramatic reduction in diagnostic surgery.

Two cohorts of 2667 (40) and 2040 GEC resulted tests (53) have reported 53% and 52% as GEC benign, respectively. Eleven independent publications report their frequencies of benign versus suspicious GEC results: 47% of 1179 tests amongst cytologically indeterminate nodules were GEC benign (Figure 3) (9,41,42,44,45,46,47,48,49,50, 51). Defining the number needed to test (NNT) as the number of tests needed to be performed to change the clinical outcome of one patient (NNT=1/(%GEC benign), and rounded to the nearest whole person), then the NNT of these series is 2. Consequently, one patient potentially avoids surgery for every two patients tested (Figure 1).

As noted above, clinical experience/clinical utility studies serve an important role in the chain of evidence regarding the effectiveness and value of a test. These allow medical centers and community practices to describe the impact the GEC has had in their management setting. As more long-term follow-up data becomes available, these important studies will allow researchers to model the overall GEC impact on reducing unnecessary surgeries nationally.

It is important to note, however that most GEC benign patients in the clinical series reported to date did not undergo surgery, consistent with the purpose of the test (9, 31,41,42,43,44,45,46,47,48,49,50,51). Thus, such clinical experience studies cannot serve as proper clinical validation studies, and clinicians should be wary when attempts are made from such studies to measure or question test performance. Operated GEC benign patients alone in a clinical experience series are insufficient to evaluate test performance, and these patients often differ from the broader group of GEC benign patients, and are more likely to include those nodules at greater risk of cancer. Therefore, findings among these patients are unlikely to be generalizable to the majority of the GEC benign case. Any attempt to measure test performance such as sensitivity and NPV requires operating on all tested patients in a contiguous intended use cohort with centralized blinded histology (e.g. clinical validation).

Additionally, clinical experience series may differ from properly performed prospective validation studies as the former may not report on a consecutive cohort of tested patients from the catchment area, but rather report only on patients who come to their attention through a variety of referral patterns. Thus, the cohort described may not reflect how the test works in the intended use population.

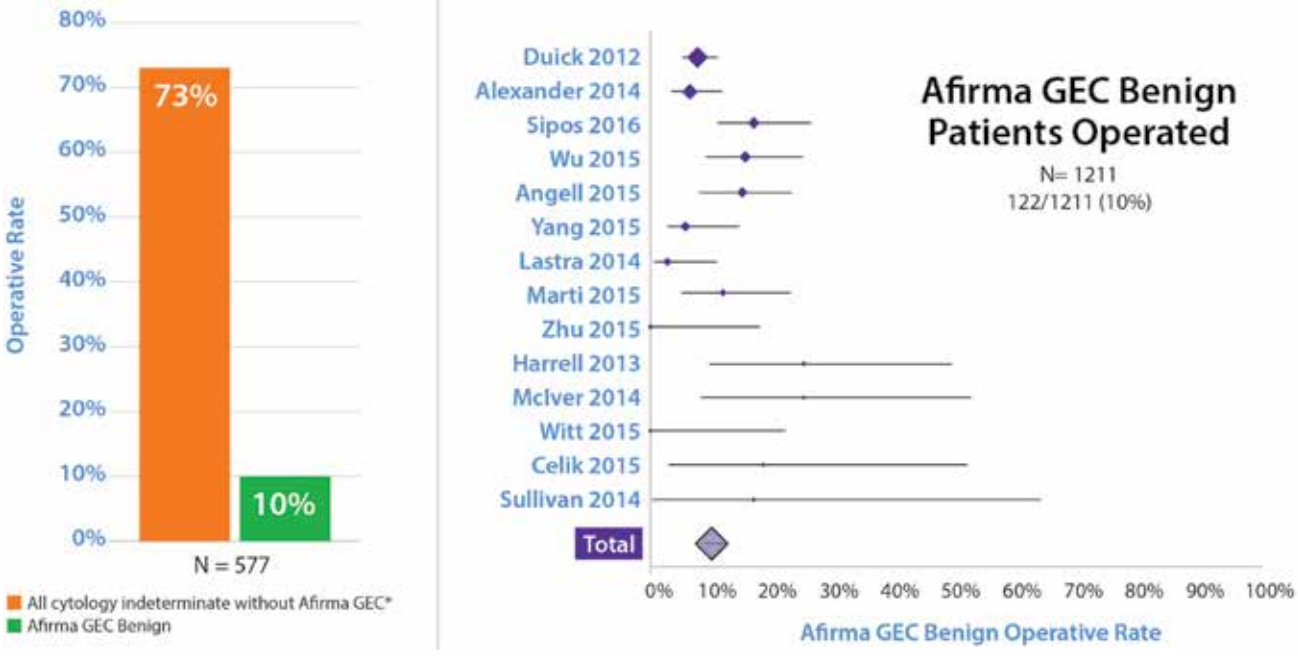
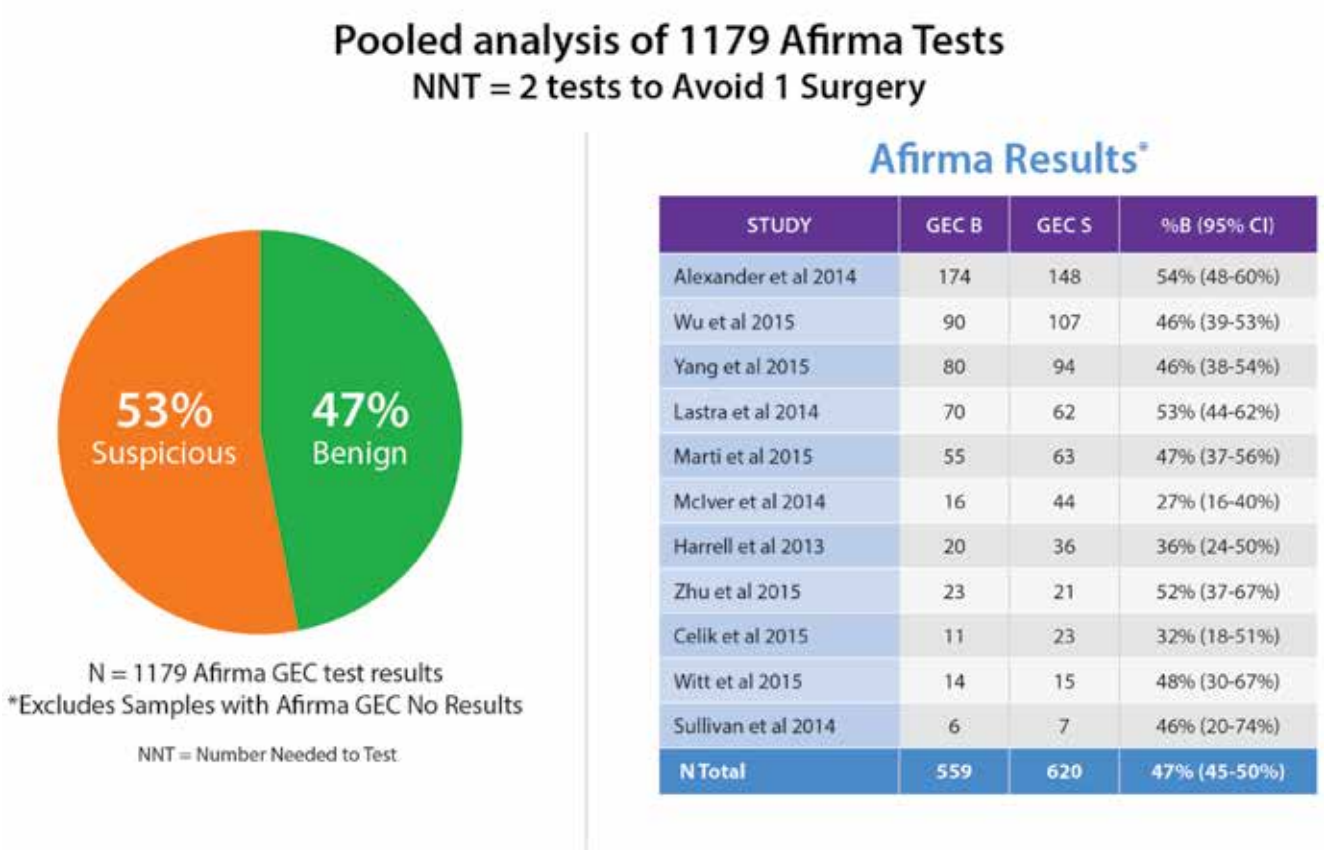


Figure 2. Real-world clinical utility

**Cibas et al. (52). Afirma gene expression classifier benign operative rate references (9,31,40,41,42,43,44,45,46,47,48,49,50,51), GEC: Gene expression classifier*

Figure 4 describes hypothetically how clinical experience studies that generate "operative NPV" results that may appear to conflict with the published 95% clinical validation NPV, but rather co-exists within the larger 95% NPV clinical validation experience. The operative NPV experience reflects the selection bias that occurs when higher ROM GEC benign patients are selected for surgery out of good clinical judgement while not operating on all continuous GEC benign patients from the entire referral base. While the operative NPV from clinical experience studies is easy to calculate, it generates great confusion for the usual reader while actually offering little clinical meaning when generated outside of a comprehensive clinical validation study. This discussion (and Figure 5) highlight the importance of study design, and the potential misinterpretations of data that can emerge from clinical experience studies.

Another limitation of clinical experience studies is that when Afirma GEC suspicious nodules are unoperated then test sensitivity among the operated cases is likely to be reduced (Figure 5). More importantly, exclusion of unoperated GEC benign nodules excludes a large number of truly benign nodules, which dramatically reduces estimates of specificity and NPV (11,42,46,50,51,54). However, as most cytologically indeterminate nodules are histologically benign, and because two clinical validation studies demonstrated a high NPV for Afirma, performance can be estimated amongst the 1468 GEC tested cytologically indeterminate patients in the published literature by pooling them together and considering GEC benign patients with malignancy found at surgery (ten patients) as malignant (false negatives), and GEC benign patients that underwent surgery and were histologically benign as benign (true negatives), or were GEC benign and not operated (704 patients). Among these GEC tested patients across multiple clinical practices, the pooled accuracy of a GEC benign result (NPV) was >98% (95% confidence interval (CI) 97-99%) (Figure 6) (4,9,31,41,42,44,45,46,47,48,49,50,51). These findings across academic and community-based practices are consistent with each other and the clinical validation of Alexander et al. (4) revealed an NPV of 94%. Two of the studies report a median follow-up of 1 year, while some patients had been followed more than 3 years. While it is true that some of the unoperated GEC benign patients may eventually be found to harbor malignancy over time, the consistently high estimated NPV seems unlikely to decline significantly. These data demonstrate a



STUDY	GEC B	GEC S	%B (95% CI)
Alexander et al 2014	174	148	54% (48-60%)
Wu et al 2015	90	107	46% (39-53%)
Yang et al 2015	80	94	46% (38-54%)
Lastra et al 2014	70	62	53% (44-62%)
Marti et al 2015	55	63	47% (37-56%)
McIver et al 2014	16	44	27% (16-40%)
Harrell et al 2013	20	36	36% (24-50%)
Zhu et al 2015	23	21	52% (37-67%)
Celik et al 2015	11	23	32% (18-51%)
Witt et al 2015	14	15	48% (30-67%)
Sullivan et al 2014	6	7	46% (20-74%)
N Total	559	620	47% (45-50%)

Figure 3. Eleven independent clinical utility studies. Afirma gene expression classifier result rate references (9,41,42,44,45,46,47,48,49,50,51)
GEC: Gene expression classifier

very low prevalence of malignancy (1-NPV) in patients with cytologically indeterminate thyroid nodules that are Afirma GEC benign, and support clinical observation in lieu of diagnostic surgery for most GEC benign patients.

The accuracy of an Afirma GEC benign call (NPV) remains high amongst Hürthle cell cytology, although the rate of benign calls is lower. Hürthle cell cytology has been a challenge for molecular diagnostics. Performance can be increased by removing these samples from clinical testing (55), but this does not help the clinician who must manage these patients. There is an overlap in the molecular profiles of benign and malignant samples. To maintain the accuracy of a benign call, the GEC can only call about half of all Bethesda III and IV samples GEC benign (Figure 3). The overlap is even greater among Hürthle cell samples. Thus, to maintain the accuracy of a benign call, the GEC calls fewer samples as GEC benign, and more samples suspicious. Among 5 cohorts of Hürthle cell samples totaling 378 nodules with an Afirma GEC benign or suspicious result, 147 (39%) were called GEC benign (42,47,53,56). Thus, three patients must be tested to avoid 1 surgery. Some observers have lamented that most Afirma results are suspicious in these cases while the prevalence of malignancy at surgery remains low within this group. However, there is no other validated method to determine which of these cases can safely avoid surgery. Brauner et al. (56) reported in a multicenter study of Massachusetts General Hospital, Brigham and Women's Hospital, and Beth Israel Deaconess Medical Center that only 3 of 26 Afirma GEC benign nodules underwent surgery (12%), and all were benign at surgical pathology, consistent with a high NPV. Including all Afirma benign and suspicious results, use of Afirma reduced the overall operative rate from 80-81% among two control groups, to 65% when the Afirma GEC was used. To date only one false negative (malignant) Hurthle cell nodule has been called benign by the Afirma

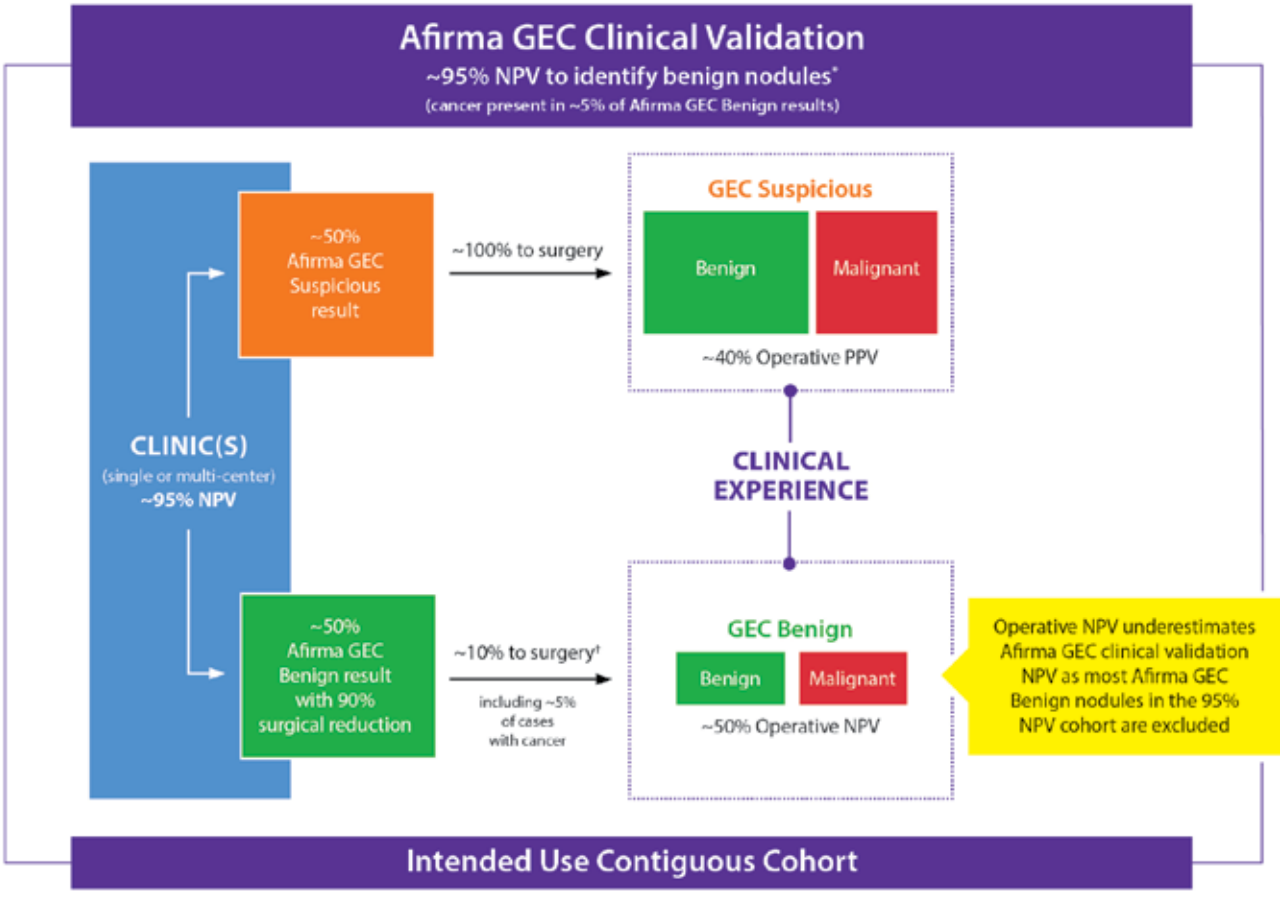


Figure 4. Afirma gene expression classifier clinical validation negative predictive value versus hypothetical "operative negative predictive value". These phenomena co-exist, rather contradict each other. Outside of a properly designed clinical validation study (Figure 5), the "operative negative predictive value" from clinical experience studies creates significant confusion while offering little clinical value

**Alexander et al. (4), †Afirma gene expression classifier benign cases referred to surgery due to ultrasound features, ultrasound changes over time, nodule growth, etc., GEC: Gene expression classifier*

GEC in a published study (4). This high degree of accuracy among Afirma GEC benign results is remarkable given the typical high degree of disagreement at surgical pathology over a benign or malignant diagnosis (52).

Implementation in Routine Clinical Practice

Physicians collect two extra FNAB passes for potential molecular testing with the Afirma GEC on every FNAB they perform, or have on-site rapid cytological assessment so that the GEC can be collected on every patient with indeterminate cytology during one patient visit (Figure 1). This patient-centric approach avoids the inconvenience, delayed diagnosis, and costs associated with repeating the FNAB should the first FNAB cytology results be indeterminate. The passed collected for Afirma are immediately placed in the Veracyte-provided RNA protective solution tube for storage and chilled box shipping (<25 °C).

It is well known that cytologically indeterminate nodules may not be categorized as indeterminate if they undergo a repeat FNAB (57). While the hope of repeating the FNAB is to re-stratify cytologically indeterminate nodules as either cytologically benign or malignant, the ability of a cytology benign result on the second FNA to safely avoid surgery is unproven. Studies on this topic are imperfect as not all patients undergo surgery to establish histological truth, however, several studies indicate a ROM amongst nodules with a Bethesda III cytology followed by a benign cytology diagnosis that is between the risk of the two categories (44,57,58), with the highest being a 29% risk of cancer (57). Some evidence suggests that the same is true when one pathologist over-reads a cytologically indeterminate

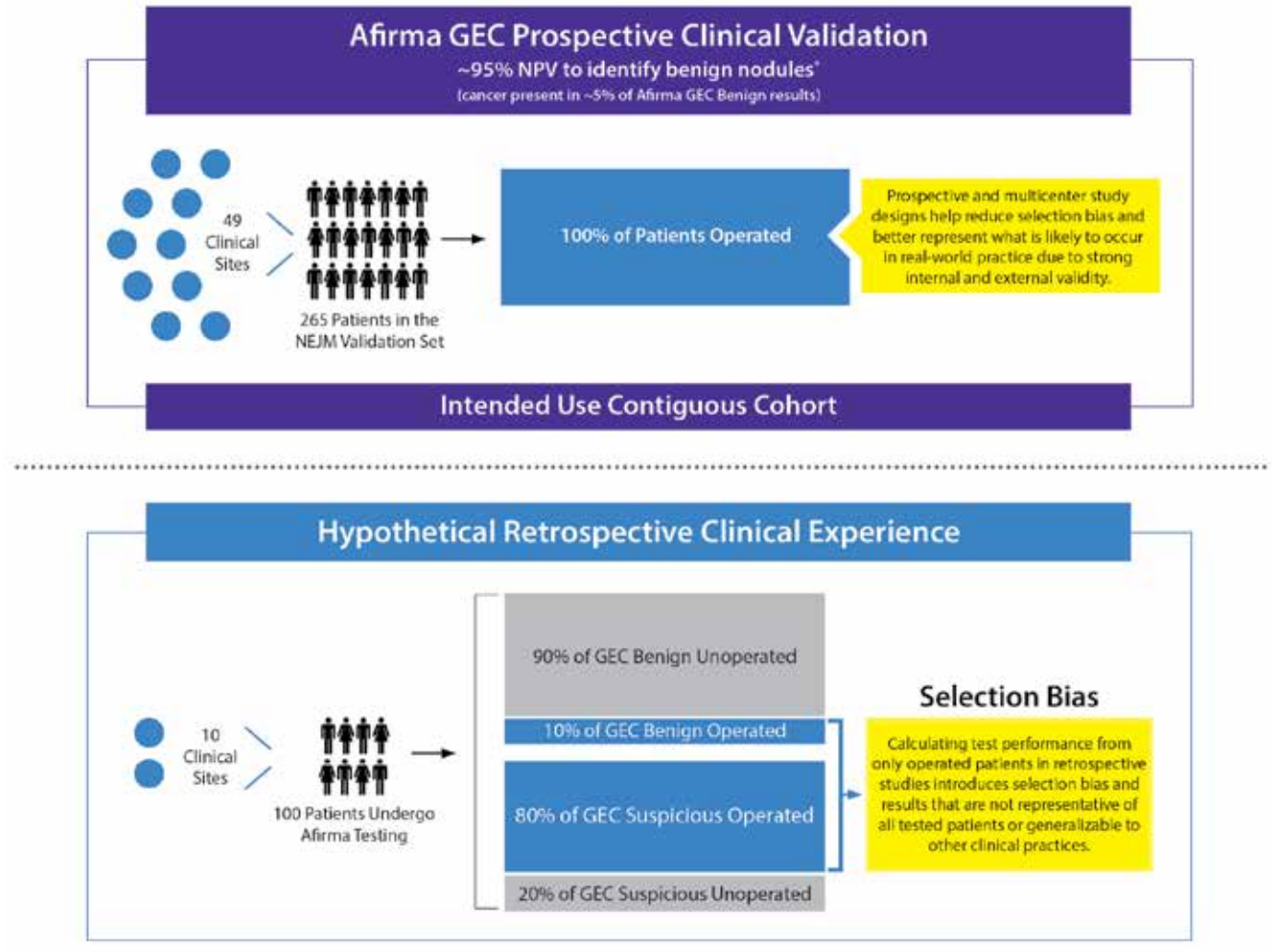


Figure 5. Clinical experience studies do not control for selection bias. Test performance is properly measured in clinical validation studies, while clinical utility is measured in clinical experience studies.

**Alexander et al. (4)*

GEC: Gene expression classifier, NPV: Negative predictive value

sample as cytologically benign (59). Indeed, investigators from Johns Hopkins University reported 7 operated patients with cytologically indeterminate FNAB findings and Afirma GEC suspicious results where their cytologists pre-operatively changed the cytology diagnosis to benign. Surgical pathology revealed malignancy in 29% of these cases (54). Thus, the risk of cancer among nodules with a benign cytology result after a repeat FNAB or after review by another cytopathologist, may exceed the ~5% or less ROM threshold of the NCCN to consider nodule observation (32,60). Similarly, the 2015 American Thyroid Association (ATA) guideline recognized these considerations against the role of repeat FNAB (13). Given the risk that a repeat FNAB may not eliminate the need for surgery, and the typical dislike of the FNAB procedure itself, some patients seek care elsewhere or elect diagnostic surgery rather than repeat FNAB. This seems like a lost opportunity as many of these patients may have benefitted from utilizing the Afirma GEC. For these reasons, it is strongly recommend that the GEC specimen be collected at the same time as the cytology sample during the first thyroid FNAB.

Clinical Decision Making

The PPV and NPV are determined by the pre-test ROM. To practice personalized medicine, it is important to consider the individual patient's pre-test risk. The patient's pre-test ROM includes their individual features (e.g. gender, history of childhood radiation treatment, ultrasound findings, serum TSH, etc.) and the interpreting cytologist's thresholds to utilize cytology indeterminate categories. Ignoring this step of personalized care and assuming that every patient at a practice or institution has the same pre-test risk ignores important medical information.

The 2015 ATA guidelines allow for either hemithyroidectomy or near-total/total thyroidectomy for thyroid malignancy 1-4 cm in size without gross extra-thyroidal extension or clinical evidence of lymph node metastases (13). Thus, multiple factors must be taken into consideration when planning surgical intervention for cytologically indeterminate nodules, such as the risks and benefits, the presence of significant contralateral nodules, long-term follow-up, the role for completion thyroidectomy with or without radioactive iodine ablation if malignancy is found, and patient preferences.

The 2015 ATA guideline emphasizes ultrasound characteristics to predict the nodule's ROM (13). Afirma is expected to identify 90% of cancers as GEC suspicious, and 52% of the benign nodules as GEC benign, regardless of the pre-test ROM. High suspicion ultrasound patterns may be associated with a >70% ROM and are found in the minority of nodules with indeterminate cytology (58,59,61,62,63,64). In nodules with such a high pre-test ROM, the NPV of Afirma is expected to be <70%, so it may not be useful to avoid surgery in such cases. If an Afirma GEC benign result is obtained in such a case, surgical

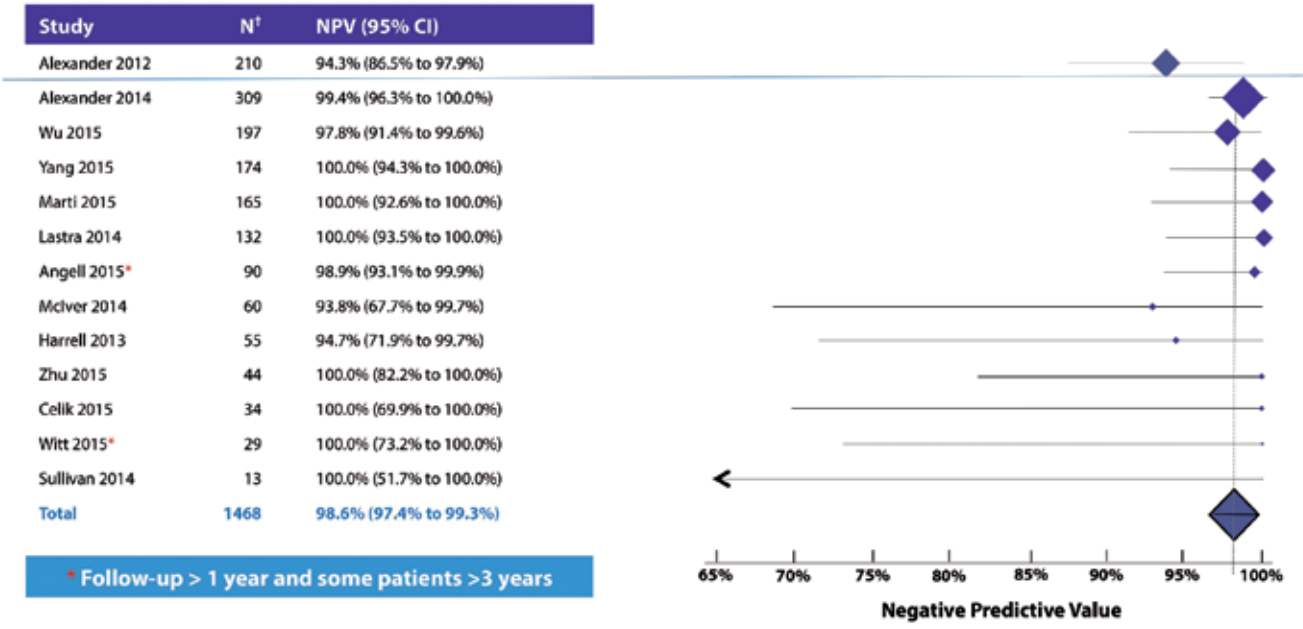


Figure 6. Consistently high estimated negative predictive value across 13 real-world studies. Negative predictive value calculated as true negatives (Afirma gene expression classifier benign and either unoperated or operated and histopathologically benign) divided by all gene expression classifier benign results *†Includes Bethesda III (atypia/follicular lesion of undetermined significance) and IV (follicular/Hürthle cell neoplasm). Figure updated from Steward and Kloos (53). References (4,9,31,41,42,44,45,46,47,48,49,50,51), NPV: Negative predictive value, CI: Confidence interval*

hemithyroidectomy might be appropriate. Alternatively, an Afirma GEC suspicious result would be expected to further increase the ROM. Bethesda III/IV nodule with high suspicious ultrasound pattern is expected to have a ROM similar to the average Bethesda V (suspicious for malignancy) nodule. The 2015 ATA guideline indicates that patients with Bethesda V cytology should be treated similar to a malignant (Bethesda VI) nodule. Alternatively, nodules with very low, low, or intermediate ultrasound suspicion are associated with a malignancy risk of 20% or less. These ultrasound findings are expected in the vast majority of cytologically indeterminate nodules. In these nodules, the Afirma GEC would be expected to have an NPV of 96% or higher, and clinical observation in lieu of surgery may be appropriate in the majority of such patients. Those with Afirma GEC suspicious results may be considered for hemithyroidectomy based on their expected <40% ROM.

Follow-up of Afirma Gene Expression Classifier Benign Patients

The 2015 ATA guidelines do not provide recommendations on the follow-up of cytologically indeterminate nodules that are Afirma GEC benign (13). Angell et al. (31) found that Afirma GEC benign nodules showed similar growth as cytopathology-benign cases, with malignancy found in only 1 Afirma GEC benign patient. The authors concluded that follow-up of Afirma GEC benign patients should be similar to that of cytology benign patients. The ATA guideline provides extensive detail and recommendations regarding the timing for follow-up for nodules with benign cytology that ranged from less than 12 months for those with high suspicion ultrasound patterns to potentially no follow-up for those with very low suspicion patterns (13). High suspicion sonographic pattern was recognized as a significantly better predictor of malignancy than nodule growth alone. Routine repeat FNAB was recommended only among cytologically benign nodules with high suspicion ultrasound patterns. For nodules with low or intermediate suspicion ultrasound patterns, only those that demonstrated growth or new suspicious sonographic features met criteria for repeat FNAB. The role of ultrasound follow-up for nodules with very low suspicion ultrasound patterns was less certain. For nodules found to be stable during follow-up the value of additional imaging was reported as low. The guideline suggested a diminishing frequency of additional ultrasound examinations for stable and asymptomatic nodules.

Cost-Effectiveness

An independent cost-effectiveness study found no difference in the number of missed cancers between paradigms with and without the Afirma GEC in a Markov model employing 10,000 Monte Carlo simulations of the expected range of probabilities for different potential outcomes (65). However, they did find that the Afirma paradigm reduced direct healthcare costs by $4,953 per five year episode of care, allowing $1,453 in direct savings using the then current Medicare reimbursement rates for surgery and the Afirma test, while modestly improving quality of life by 0.07 quality-adjusted life-year (QALY) (65). One criticism of this study has been the assumed test specificity of 75%, compared to the specificities of Afirma of 52% in Alexander et al. (4), as opposed to the specificity of 76% (95% CI 50-92%) in Chudova et al. (25). Still, cost savings/QALY was demonstrated in univariate analysis for specificity at the lowest value tested (60%) with cost savings and cost-effectiveness appearing likely at even lower specificities.

Lee et al. (66) modeled cost-effectiveness of the Afirma GEC and a 7-gene panel alone, and in combination, for Bethesda III nodules in the US and Canadian healthcare setting. In the US, the most cost-effective strategy was the Afirma GEC followed by the 7-gene panel in GEC suspicious cases, while in Canada management without molecular testing was most cost-effective. Wu et al. (48) compared routine Afirma GEC testing to conventional management in a decision tree model and found routine Afirma GEC testing more effective and most costly with an incremental cost-effectiveness ratio of $119,700/QALY, and found greater cost-effectiveness when either the prevalence of malignancy or the cost of the test were lowered. In Monte Carlo simulations, conventional management was the preferred strategy just over half the time. Base-case limitations of the both studies included that all Afirma GEC suspicious cases were directed to diagnostic hemithyroidectomy, and when malignant all cases then underwent completion thyroidectomy and added this significant cost. In practice, some patients may have elected total thyroidectomy and therefore avoided the added cost of completion thyroidectomy. In the model of Wu et al. (48), if more than just 3.1% of patients elected a total thyroidectomy instead of lobectomy in the absence of Afirma GEC testing then routine GEC testing became cost-effective. In a series of 165 Bethesda III/IV nodules operated without Afirma GEC testing, we reported that the use of total thyroidectomy was as low as 39% for Bethesda III nodules in academic centers to as high as 60% in Bethesda IV nodules in community practice settings [(67) supplemental data]. These data support the cost-effectiveness of the Afirma GEC as it can replace not only hemithyroidectomy, but can also significantly replace usage of the even more expensive total thyroidectomy with clinical observation. In addition, the mandated second (completion) surgery among malignant cases in the Lee (66) and Wu (48) models is not consistent with ATA and NCCN guidelines which suggest that thyroid lobectomy may be adequate treatment for most of these patients (13,60). Further, Lee et al. (66) added substantial penalties for delayed diagnosis when Afirma GEC benign patients were found to have cancer,

including penalties for increased risk of cancer recurrence and death. These added costs are not consistent with the excellent outcome of known papillary thyroid cancer confined to the thyroid despite delayed treatment (68), or the excellent outcome of the few Afirma GEC false negative cases reported in the literature (31). Finally, the Lee et al. (66) model included significant costs for a yearly follow-up ultrasound examination of unoperated nodules, whereas the ATA guideline advocates for diminishing frequency of ultrasound follow-up over time (13).

Additional limitations of the 3 cost-effectiveness studies described above include that none consider indirect costs due to time lost from work and impacted responsibilities of daily living as a result of surgery and its recovery. Neither Lee et al. (66) nor Wu et al. (48) include costs for potential perioperative death, occurring in up to three in 1000 patients (8,69,70,71,72,73,74). The study methodologies may underestimate the impact of complications on the patient, including voice outcomes (75,76) and hypoparathyroidism (77). Additionally, all of the studies measure quality-adjusted life expectancies by multiplying the time spent in the health state by the utilities assigned to those states. The base-case utilities assigned to uncomplicated surgery are quite high and leave little room to improve quality of life by avoiding unnecessary diagnostic surgery. It does not seem correct that quality of life is diminished from surgery only when a complication occurs. Li et al. (65) assigned a higher base-case utility to an uncomplicated hemithyroidectomy than to observation, and the lower limit of the estimated utility range for observation was lower than the lower utility range of total thyroidectomy, suggesting that quality of life from observation could be worse than quality of life from an uncomplicated total thyroidectomy. These utility estimates (and those for complications) were derived from the opinions of people who have not undergone these procedures or experienced these complications. It seems likely that the value of avoiding diagnostic surgery may be greatly under-appreciated by those who have not actually experienced the event than those that have, a finding shown to be true for hypoparathyroidism (77). Future research is needed to better quantify relevant utility values so that changes in quality of life resulting from changes in patient care can be better measured.

Malignancy Classifiers

While the current greatest value of molecular diagnostics among cytologically indeterminate nodules is to identify nodules that do not require surgery (a rule-out test), there is value to a test that can identify malignancy (a rule-in test) only when it alters clinical care to the benefit of the patient. Clinical care can be altered by enhancing the rationale for surgery, and more directly by altering the extent of surgical care (11). The Afirma Malignancy Classifiers include a *BRAF*^{V600E} point mutation classifier, and a cassette for MTC. Additional cassettes automatically run with every Afirma GEC test screen for parathyroid tissue (benign and malignancy) (78), and metastases to the thyroid from malignant melanoma, breast, and renal cell carcinomas.

MTC is frequently a cytological challenge to diagnose, and the field has had attention recently drawn to the low sensitivity of FNAB for the specific diagnosis of MTC (79,80). MTC cases are found among all 6 Bethesda cytological categories. When MTC is not recognized pre-operatively then delayed diagnosis may result (79), and those that undergo surgery may not be pre-operatively evaluated for MEN2 associated hyperparathyroidism, or concomitant pheochromocytoma (81). Surgery on a patient with an unrecognized pheochromocytoma may result in death. MTC that is not specifically recognized pre-operatively as MTC is unlikely to undergo the optimal initial surgery, typically considered to be a total thyroidectomy and central neck dissection at a minimum (81). In a recently study, only 18.7% of MTC patients underwent surgery for an accurate diagnosis (79). The Afirma MTC classifier has been evaluated in patients and tissue, and has exceptionally high sensitivity (96%), specificity (>99%), PPV (98%), and NPV (>99%) (82,83). With more than 40,000 Afirma GEC tests performed, Veracyte is aware of only one MTC case that was GEC suspicious, but not identified by the classifier as MTC, and 1 false positive case, an intra-parathyroidal, intra-thyroidal paraganglioma (82,84). The MTC classifier is routinely run and reported with every GEC test globally. In the US, the MTC classifier may be obtained without the Afirma GEC on Bethesda V or VI nodules.

Point mutations in *BRAF* are by far the most common genomic abnormality associated with papillary thyroid carcinomas, and nearly all are *BRAF*^{V600E} mutations (85). The Afirma *BRAF*^{V600E} classifier is based on the mRNA molecular signature of 128 genes (86). Compared to a sensitive quantitative PCR assay, high positive and negative percent agreement was demonstrated (PPA 90.4% and NPA 99.0%). Establishing appropriate cut-off points to separate positive from negative tests is critical to avoid false positive results than can drive inappropriate treatment (87). When cut-offs are correctly established, *BRAF*^{V600E} mutation is uncommon among Bethesda III and IV nodules, while it is more common among Bethesda V and VI nodules (67,86). Unlike RAS mutations (14,15,16,17,18,19,20,21), *BRAF*^{V600E} mutations are almost exclusively found in malignant nodules (e.g. PPV ~100%). In a consecutive cohort of 7,066 de-identified FNABs, 3,187 samples were benign by Afirma GEC, of which none were Afirma *BRAF* positive (88). Thus, testing only Afirma GEC suspicious samples increases the rate of positive tests and decreases healthcare costs. The Afirma *BRAF*^{V600E} classifier is accurate among samples that constitute up to 60% blood. Interestingly, a double-mutant that resulted in the V600E amino acid change but was negative by qPCR but was identified by the Afirma *BRAF* classifier. The non-diagnostic

rates were lower (7.6%) for Afirma *BRAF* than for qPCR (24.5%), a further advantage of using RNA in FNAB small sample biopsies. In the US, the *BRAF*^{V600E} classifier is an option on Afirma GEC suspicious nodules, and Bethesda V or VI nodules without Afirma GEC.

Conclusion

Cytologically indeterminate nodules have historically been referred for surgery given that their ROM was above the typical threshold of ~5% for physicians to consider clinical observation in lieu of diagnostic surgery. Molecular diagnostic testing of these nodules has rapidly become accepted. Current guidelines include that molecular testing may be used among Bethesda III/IV nodules to add additional information about the nodule's ROM. The 2015 ATA guideline reviews the molecular testing landscape, and voices caution over tests supported only by single center and unblinded validation data, and those with no published clinical utility data to demonstrate a change in clinical care and patient benefit as a result of the test. The Afirma GEC is the only molecular test supported by multicenter, prospective, and blinded validation data, and the only test supported by published clinical utility data demonstrating a dramatic reduction in diagnostic surgery for patients with benign Afirma GEC results. Nearly 1 out of every 2 Afirma GEC tests performed yields a molecularly benign result, and >80% of patients with a benign GEC result remain unoperated 3 years after the biopsy in real-world experience. Reducing unnecessary diagnostic surgery improves patient safety, reduces healthcare costs, and improves patient quality of life.

References

1. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009;19:1167-1214.
2. Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med 2004;351:1764-1771.
3. Melillo RM, Santoro M. Molecular biomarkers in thyroid FNA samples. J Clin Endocrinol Metab 2012;97:4370-4373.
4. Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, LiVolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med 2012;367:705-715.
5. Wang CC, Friedman L, Kennedy GC, Wang H, Kebebew E, Steward DL, Zeiger MA, Westra WH, Wang Y, Khanafshar E, Fellegara G, Rosai J, Livolsi V, Lanman RB. A large multicenter correlation study of thyroid nodule cytopathology and histopathology. Thyroid 2011;21:243-251.
6. Bryson PC, Shores CG, Hart C, Thorne L, Patel MR, Richey L, Farag A, Zanation AM. Immunohistochemical distinction of follicular thyroid adenomas and follicular carcinomas. Arch Otolaryngol Head Neck Surg 2008;134:581-586.
7. Cibas ES, Ali SZ. The Bethesda system for reporting thyroid cytopathology. Thyroid 2009;19:1159-1165.
8. Weiss A, Parina RP, Tang JA, Brumund KT, Chang DC, Bouvet M. Outcomes of thyroidectomy from a large California state database. Am J Surg 2015;210:1170-1176.
9. Zhu QL, Faquin WC, Samir AE. Relationship between sonographic characteristics and afirma gene expression classifier results in thyroid nodules with indeterminate fine-needle aspiration cytopathology. AJR Am J Roentgenol 2015;205:861-865.
10. Kuru B, Atmaca A, Tarim IA, Kefeli M, Topgul K, Yoruker S, Elmali M, Danaci M. Risk factors associated with malignancy and with triage to surgery in thyroid nodules classified as Bethesda category III (AUS/FLUS). Eur J Surg Oncol 2015;42:87-93.
11. Nishino M. Molecular cytopathology for thyroid nodules: A review of methodology and test performance. Cancer Cytopathol 2016;124:14-27.
12. Xing M, Haugen BR, Schlumberger M. Progress in molecular-based management of differentiated thyroid cancer. Lancet 2013;381:1058-1069.
13. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26:1-133.
14. Cantara S, Capezzone M, Marchisotta S, Capuano S, Busonero G, Toti P, Di Santo A, Caruso G, Carli AF, Brilli L, Montanaro A, Pacini F. Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. J Clin Endocrinol Metab 2010;95:1365-1369.
15. Eszlinger M, Krogdahl A, Münz S, Rehfeld C, Precht Jensen EM, Ferraz C, Bösenberg E, Drieschner N, Scholz M, Hegedüs L, Paschke R. Impact of molecular screening for point mutations and rearrangements in routine air-dried fine-needle aspiration samples of thyroid nodules. Thyroid 2014;24:305-313.
16. Eszlinger M, Piana S, Moll A, Bösenberg E, Bisagni A, Ciarrocchi A, Ragazzi M, Paschke R. Molecular testing of thyroid fine-needle aspirations improves presurgical diagnosis and supports the histologic identification of minimally invasive follicular thyroid carcinomas. Thyroid 2015;25:401-409.
17. Rossi M, Buratto M, Tagliati F, Rossi R, Lupo S, Trasforini G, Lanza G, Franceschetti P, Bruni S, Degli Uberti E, Zatelli MC. Relevance of BRAF(V600E) mutation testing versus RAS point mutations and RET/PTC rearrangements evaluation in the diagnosis of thyroid cancer. Thyroid 2015;25:221-228.
18. Moses W, Weng J, Sansano I, Peng M, Khanafshar E, Ljung BM, Duh QY, Clark OH, Kebebew E. Molecular testing for somatic mutations improves the accuracy of thyroid fine-needle aspiration biopsy. World J Surg 2010;34:2589-2594.
19. Krane JF, Cibas ES, Alexander EK, Paschke R, Eszlinger M. Molecular analysis of residual ThinPrep material from thyroid FNAs increases diagnostic sensitivity. Cancer Cytopathol. 2015;123:356-361.
20. Medici M, Kwong N, Angell TE, Marqusee E, Kim MI, Frates MC, Benson CB, Cibas ES, Barletta JA, Krane JF, Ruan DT, Cho NL, Gawande AA, Moore FD Jr, Alexander EK. The variable phenotype and low-risk nature of RAS-positive thyroid nodules. BMC Med 2015;13:184.
21. Gill MS, Nayan S, Kocovski L, Cutz JC, Archibald SD, Jackson BS, Young JE, Gupta MK. Local molecular analysis of indeterminate thyroid nodules. J Otolaryngol Head Neck Surg 2015;44:52.
22. Kloos R, Walsh P, Pagan M, Matsuzaki H, Huang J, Travers K, Tom E, Wong M, Mathison J, Kim S, Kennedy G. Detection of increasing numbers of point mutations and fusions in benign thyroid nodules using next-generation sequencing dramatically reduces mutation marker panel specificity (abstract). Endocrine Practice 2015;21(Suppl 2):235.
23. Pagan M, Kloos RT, Lin CF, Travers KJ, Matsuzaki H, Tom EY, Kim SY, Wong MG, Stewart AC, Huang J, Walsh PS, Monroe RJ, Kennedy GC. The Diagnostic application of RNA sequencing in patients with thyroid cancer: an analysis of 851 variants and 133 fusions in 524 genes. BMC Bioinformatics 2016;17(Suppl 1):6.

24. Grody WW, Thompson BH, Hudgins L. Whole-exome/genome sequencing and genomics. Pediatrics 2013;132(Suppl 3):211-215.
25. Chudova D, Wilde JI, Wang ET, Wang H, Rabbee N, Egidio CM, Reynolds J, Tom E, Pagan M, Rigl CT, Friedman L, Wang CC, Lanman RB, Zeiger M, Kebebew E, Rosai J, Fellegara G, LiVolsi VA, Kennedy GC. Molecular classification of thyroid nodules using high-dimensionality genomic data. J Clin Endocrinol Metab 2010;95:5296-5304.
26. Shrestha M, Crothers BA, Burch HB. The impact of thyroid nodule size on the risk of malignancy and accuracy of fine-needle aspiration: a 10-year study from a single institution. Thyroid 2012;22:1251-1256.
27. Lewis CM, Chang KP, Pitman M, Faquin WC, Randolph GW. Thyroid fine-needle aspiration biopsy: variability in reporting. Thyroid 2009;19:717-723.
28. Borget I, Vielh P, Leboulleux S, Allyn M, Iacobelli S, Schlumberger M, de Pouvourville G. Assessment of the cost of fine-needle aspiration cytology as a diagnostic tool in patients with thyroid nodules. Am J Clin Pathol 2008;129:763-771.
29. Renshaw A. An estimate of risk of malignancy for a benign diagnosis in thyroid fine-needle aspirates. Cancer Cytopathol 2010;118:190-195.
30. Marchevsky AM, Walts AE, Bose S, Gupta R, Fan X, Frishberg D, Scharre K, Zhai J. Evidence-based evaluation of the risks of malignancy predicted by thyroid fine-needle aspiration biopsies. Diagn Cytopathol 2010;38:252-259.
31. Angell TE, Frates MC, Medici M, Liu X, Kwong N, Cibas ES, Kim MI, Marqusee E. Afirma benign thyroid nodules show similar growth to cytologically benign nodules during follow-up. J Clin Endocrinol Metab 2015;100:1477-1483.
32. NCCN Clinical Practice Guidelines in Oncology. Thyroid Carcinoma. Available: http://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf. Accessed December 10, 2015.
33. Beaudenon-Huibregtse S, Alexander EK, Guttler RB, Hershman JM, Babu V, Blevins TC, Moore P, Andruss B, Labourier E. Centralized molecular testing for oncogenic gene mutations complements the local cytopathologic diagnosis of thyroid nodules. Thyroid 2014;24:1479-1487.
34. Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO, Ferris RL, Yip L, Seethala RR, Tublin ME, Stang MT,Coyne C, Johnson JT, Stewart AF, Nikiforova MN. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. J Clin Endocrinol Metab 2011;96:3390-3397.
35. Kumar G, Song-Yang J, Timmaraju VA, Taylor S, Mireskandari A, Finkelstein S, Hosono S. Thyroid mirna classifier (ThyraMir) complements mutation detection (ThyGenX) NGS tests for improved molecular diagnosis of indeterminate thyroid nodule needle aspirates. Thyroid. 2015;25:271-272. (abstract).
36. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN. Impact of the multi-gene thyroseq next-generation sequencing assay on cancer diagnosis in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology. Thyroid 2015;25:1217-1223.
37. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, Hodak SP, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN. Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. Cancer 2014;120:3627-3634.
38. Bar D, Yania GL, Goren Y, Shtabsky A, Zubkov A, Morgenstern S, Strenov Y, Feinmesser M, Kravtsov V, Leon M, Granstrem O, Vorobyov S, Hajduch M, VandenBussche C, Ashkenazi K, Sanden M, Mitchell H, Noller M, Dromi N, Tabak S, Kadosh E, Meiri E. A first-of-its-kind, microRNA-based diagnostic assay for accurate thyroid nodule classification. Thyroid 2015;25:A382 (abstract).
39. Teutsch SM, Bradley LA, Palomaki GE, Haddow JE, Piper M, Calonge N, Dotson WD, Douglas MP, Berg AO; EGAPP Working Group. The evaluation of genomic applications in practice and prevention (EGAPP) initiative: methods of the EGAPP Working Group. Genet Med 2009;11:3-14.
40. Duick DS, Klopper JP, Diggans JC, Friedman L, Kennedy GC, Lanman RB, McIver B. The impact of benign gene expression classifier test results on the endocrinologist-patient decision to operate on patients with thyroid nodules with indeterminate fine-needle aspiration cytopathology. Thyroid 2012;22:996-1001.
41. Alexander EK, Schorr M, Klopper J, Kim C, Sipos J, Nabhan F, Parker C, Steward DL, Mandel SJ, Haugen BR. Multicenter clinical experience with the Afirma gene expression classifier. J Clin Endocrinol Metab 2014;99:119-125.
42. Harrell RM, Bimston DN. Surgical utility of Afirma: effects of high cancer prevalence and oncocytic cell types in patients with indeterminate thyroid cytology. Endocr Pract 2014;20:364-369.
43. Sipos JA, Blevins TC, Shea HC, Duick DS, Lakhian SK, Michael BE, Thomas MJ, Sosa JA. Long-term non-operative rate of thyroid nodules with benign results on the afirma gene expression classifier. Endocrine Pract 2016.
44. Sullivan PS, Hirschowitz SL, Fung PC, Apple SK. The impact of atypia/follicular lesion of undetermined significance and repeat fine-needle aspiration: 5 years before and after implementation of the Bethesda System. Cancer Cytopathol 2014;122:866-872.
45. Witt RL. Outcome of thyroid gene expression classifier testing in clinical practice. Laryngoscope 2016;126:524-527.
46. Marti JL, Avadhani V, Donatelli LA, Niyogi S, Wang B, Wong RJ, Shaha AR, Ghossein RA, Lin O, Morris LG, Ho AS. Wide Inter-institutional variation in performance of a molecular classifier for indeterminate thyroid nodules. Ann Surg Oncol 2015;22:3996-4001.
47. Lastra RR, Pramick MR, Crammer CJ, LiVolsi VA, Baloch ZW. Implications of a suspicious afirma test result in thyroid fine-needle aspiration cytology: an institutional experience. Cancer Cytopathol 2014;122:737-744.
48. Wu JX, Lam R, Levin M, Rao J, Sullivan PS, Yeh MW. Effect of malignancy rates on cost-effectiveness of routine gene expression classifier testing for indeterminate thyroid nodules. Surgery 2016;159:118-126.
49. Celik B, Whetsell CR, Nassar A. Afirma GEC and thyroid lesions: An institutional experience. Diagn Cytopathol 2015;43:966-970.
50. Yang SE, Sullivan PS, Zhang J, Govind R, Levin MR, Rao JY, Moatamed NA. Has Afirma gene expression classifier testing refined the indeterminate thyroid category in cytology? Cancer Cytopathol 2016;124:100-109.
51. McIver B, Castro MR, Morris JC, Bernet V, Smallridge R, Henry M, Kosok L, Reddi H. An independent study of a gene expression classifier (Afirma) in the evaluation of cytologically indeterminate thyroid nodules. J Clin Endocrinol Metab 2014;99:4069-4077.
52. Cibas ES, Baloch ZW, Fellegara G, LiVolsi VA, Raab SS, Rosai J, Diggans J, Friedman L, Kennedy GC, Kloos RT, Lanman RB, Mandel SJ, Sindy N, Steward DL, Zeiger MA, Haugen BR, Alexander EK. A prospective assessment defining the limitations of thyroid nodule pathologic evaluation. Ann Intern Med 2013;159:325-332.
53. Steward DL, Kloos RT. Clinical diagnostic gene expression thyroid testing. Otolaryngol Clin North Am 2014;47:573-593.
54. Noureldine SI, Olson MT, Agrawal N, Prescott JD, Zeiger MA, Tufano RP. Effect of gene expression classifier molecular testing on the surgical decision-making process for patients with thyroid nodules. JAMA Otolaryngol Head Neck Surg 2015;141:1082-1088.
55. Keutgen XM, Filicori F, Crowley MJ, Wang Y, Scognamiglio T, Hoda R, Buitrago D, Cooper D, Zeiger MA, Zarnegar R, Elemento O, Fahey TJ. A panel of four miRNAs accurately differentiates malignant from benign indeterminate thyroid lesions on fine needle aspiration. Clin Cancer Res 2012;18:2032-2038.
56. Brauner E, Holmes BJ, Krane JF, Nishino M, Zurakowski D, Hennessey JV, Faquin WC, Parangi S. Performance of the afirma gene expression classifier in hurthle cell thyroid nodules differs from other indeterminate thyroid nodules. Thyroid 2015;25:789-796.
57. VanderLaan PA, Marqusee E, Krane JF. Clinical outcome for atypia of undetermined significance in thyroid fine-needle aspirations: should repeated fna be the preferred initial approach? Am J Clin Pathol 2011;135:770-775.

58. Rosario PW. Thyroid nodules with atypia or follicular lesions of undetermined significance (Bethesda Category III): importance of ultrasonography and cytological subcategory. Thyroid 2014;24:1115-1120.
59. Maia FF, Matos PS, Pavin EJ, Vassallo J, Zantut-Wittmann DE. Value of ultrasound and cytological classification system to predict the malignancy of thyroid nodules with indeterminate cytology. Endocr Pathol 2011;22:66-73.
60. Tuttle RM, Haddad RI, Ball DW, Byrd D, Dickson P, Duh QY, Ehya H, Haymart M, Hoh C, Hunt JP, Iagaru A, Kandeel F, Kopp P, Lamonica DM, Lydiatt WM, McCaffrey J, Moley JF, Parks L, Raeburn CD, Ridge JA, Ringel MD, Scheri RP, Shah JP, Sherman SI, Sturgeon C, Waguespack SG, Wang TN, Wirth LJ, Hoffmann KG, Hughes M. Thyroid carcinoma, version 2.2014. J Natl Compr Canc Netw 2014;12:1671-1680.
61. Rosario PW, Salles DS, Bessa B, Purisch S. Contribution of scintigraphy and ultrasonography to the prediction of malignancy in thyroid nodules with indeterminate cytology. Arq Bras Endocrinol Metabol 2010;54:56-59.
62. Kim DW, Lee EJ, Jung SJ, Ryu JH, Kim YM. Role of sonographic diagnosis in managing Bethesda class III nodules. AJNR Am J Neuroradiol 2011;32:2136-2141.
63. Chng CL, Kurzawinski TR, Beale T. Value of sonographic features in predicting malignancy in thyroid nodules diagnosed as follicular neoplasm on cytology. Clin Endocrinol (Oxf) 2015;83:711-716.
64. Russ G, Royer B, Bigorgne C, Rouxel A, Bienvenu-Perrard M, Leenhardt L. Prospective evaluation of thyroid imaging reporting and data system on 4550 nodules with and without elastography. Eur J Endocrinol 2013;168:649-655.
65. Li H, Robinson KA, Anton B, Saldanha IJ, Ladenson PW. Cost-effectiveness of a novel molecular test for cytologically indeterminate thyroid nodules. J Clin Endocrinol Metab 2011;96:1719-1726.
66. Lee L, How J, Tabah RJ, Mitmaker EJ. Cost-effectiveness of molecular testing for thyroid nodules with atypia of undetermined significance cytology. J Clin Endocrinol Metab 2014;99:2674-2682.
67. Kloos RT, Reynolds JD, Walsh PS, Wilde JI, Tom EY, Pagan M, Barbacioru C, Chudova DI, Wong M, Friedman L, LiVolsi VA, Rosai J, Lanman RB, Kennedy GC. Does addition of BRAF V600E mutation testing modify sensitivity or specificity of the afirma gene expression classifier in cytologically indeterminate thyroid nodules? J Clin Endocrinol Metab 2013;98:761-768.
68. Davies L, Welch HG. Thyroid cancer survival in the United States: observational data from 1973 to 2005. Arch Otolaryngol Head Neck Surg 2010;136:440-444.
69. Shrime MG, Goldstein DP, Seaberg RM, Sawka AM, Rotstein L, Freeman JL, Gullane PJ. Cost-effective management of low-risk papillary thyroid carcinoma. Arch Otolaryngol Head Neck Surg 2007;133:1245-1253.
70. Hundahl SA, Cady B, Cunningham MP, Mazzaferri E, McKee RF, Rosai J, Shah JP, Fremgen AM, Stewart AK, Hölzer S. Initial results from a prospective cohort study of 5583 cases of thyroid carcinoma treated in the united states during 1996. U.S. and German Thyroid Cancer Study Group. An American College of Surgeons Commission on Cancer Patient Care Evaluation study. Cancer 2000;89:202-217.
71. Bergenfelz A, Jansson S, Kristoffersson A, Martensson H, Reihner E, Wallin G, Lausen I. Complications to thyroid surgery: results as reported in a database from a multicenter audit comprising 3,660 patients. Langenbecks Arch Surg 2008;393:667-673.
72. Sosa JA, Bowman HM, Tielsch JM, Powe NR, Gordon TA, Udelsman R. The importance of surgeon experience for clinical and economic outcomes from thyroidectomy. Ann Surg 1998;228:320-330.
73. Hauch A, Al-Qurayshi Z, Randolph G, Kandil E. Total thyroidectomy is associated with increased risk of complications for low- and high-volume surgeons. Ann Surg Oncol 2014;21:3844-3852.
74. Weiss A, Lee KC, Brumund KT, Chang DC, Bouvet M. Risk factors for hematoma after thyroidectomy: results from the nationwide inpatient sample. Surgery 2014;156:399-404.
75. Vicente DA, Solomon NP, Avital I, Henry LR, Howard RS, Helou LB, Coppit GL, Shriver CD, Buckenmaier CC, Libutti SK, Shaha AR, Stojadinovic A. Voice outcomes after total thyroidectomy, partial thyroidectomy, or non-neck surgery using a prospective multifactorial assessment. J Am Coll Surg 2014;219:152-163.
76. Kuhn MA, Bloom G, Myssiorek D. Patient perspectives on dysphonia after thyroidectomy for thyroid cancer. J Voice 2013;27:111-114.
77. Cho NL, Moalem J, Chen L, Lubitz CC, Moore FD, Ruan DT. Surgeons and patients disagree on the potential consequences from hypoparathyroidism. Endocr Pract 2014;20:427-446.
78. Kloos R, Harrell RM, Kennedy GC, Monroe RJ, Traweek ST, Lanman RB. Preoperative identification of parathyroid tissue by an mRNA classifier on prospectively collected thyroid nodule fine-needle aspiration biopsies. American Association of Clinical Endocrinologists Annual Meeting. Las Vegas, NV. 2014 (abstract).
79. Ha EJ, Baek JH, Na DG, Kim JH, Kim JK, Min HS, Song DE, Lee KE, Shong YK. The Role of core needle biopsy and Its impact on surgical management in patients with medullary thyroid cancer: clinical experience at 3 medical institutions. AJNR Am J Neuroradiol 2015;36:1512-1517.
80. Trimboli P, Treglia G, Guidobaldi L, Romanelli F, Nigri G, Valabrega S, Sadeghi R, Crescenzi A, Faquin WC, Bongiovanni M, Giovanella L. Detection rate of FNA cytology in medullary thyroid carcinoma: a meta-analysis. Clin Endocrinol (Oxf) 2015;82:280-285.
81. Wells SA Jr, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moley JF, Pacini F, Raue F, Frank-Raue K, Robinson B, Rosenthal MS, Santoro M, Schlumberger M, Shah M, Waguespack SG; American Thyroid Association Guidelines Task Force on Medullary Thyroid Carcinoma. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid 2015;25:567-610.
82. Kloos RT, Kennedy G, Monroe R, Traweek ST, Lanman R. Validation of a molecular classifier for preoperative identification of medullary thyroid cancer in thyroid nodule fine-needle aspiration biopsies. American Association of Clinical Endocrinologists Annual Meeting. Las Vegas, NV. 2014 (abstract).
83. Pankratz D, Hu Z, Kim SY, Monroe R, Wong M, Diggans J, Traweek T, Kumm J, Lanman R, Kloos R, Walsh S, Kennedy G. Analytical validation of a gene expression classifier for medullary thyroid carcinoma. American Associate of Clinical Endocrinologists Annual Meeting. Las Vegas, NV. 2014 (abstract).
84. Shvets L, Lubitz S. A unique case of intrathyroidal intraparathyroidal paraganglioma. American Association of Clinical Endocrinologists Annual Meeting. Las Vegas, NV. 2014 (abstract).
85. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676-690.
86. Diggans J, Kim SY, Hu Z, Pankratz D, Wong M, Reynolds J, Tom E, Pagan M, Monroe R, Rosai J, Livolsi VA, Lanman RB, Kloos RT, Walsh PS, Kennedy GC. Machine learning from concept to clinic: reliable detection of BRAF V600e DNA mutations in thyroid nodules using high-dimensional RNA expression data. Pac Symp Biocomput 2015:371-382.
87. DiLorenzo MM, Miller JL, Tuluc M, Wang ZX, Savarese VW, Pribitkin EA. False-positive FNA due to highly sensitive BRAF assay. Endocr Pract 2014;20:8-10.
88. Walsh P, Diggans J, Hu Z, Kim SY, Pankratz D, Tom E, Wilde J, Reynolds J, Velichko S, Wong M, Mathison J, Kloos R, Lanman R, Kennedy G. Pre-operative identification of BRAF V600E mutational status in thyroid nodules using a novel gene expression classifier. American Association of Clinical Endocrinologists Annual Meeting. Las Vegas, NV. 2014 (abstract).

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):50-65 DOI:10.4274/2017.26.suppl.06

Nuclear Molecular and Theranostic Imaging for Differentiated Thyroid Cancer

Diferansiye Tiroid Kanserinde Nükleer Moleküler ve Teranostik Görüntüleme

Arif Sheikh[1], Berna Polack[2], Yvette Rodriguez[3], Russ Kuker[4]
[1]*Columbia University Medical Center, Clinic of Radiology, New York, USA*
[2]*Dokuz Eylül University Faculty of Medicine, Department of Nuclear Medicine, İzmir, Turkey*
[3]*Florida International University, Herbert Wertheim College of Medicine, Department of Surgery, Miami, USA*
[4]*University of Miami Miller School of Medicine, Department of Radiology, Miami, USA*

Abstract

Traditional nuclear medicine is rapidly being transformed by the evolving concepts in molecular imaging and theranostics. The utility of new approaches in differentiated thyroid cancer (DTC) diagnostics and therapy has not been fully appreciated. The clinical information, relevant to disease management and patient care, obtained by scintigraphy is still being underestimated. There has been a trend towards moving away from the use of radioactive iodine (RAI) imaging in the management of the disease. This paradigm shift is supported by the 2015 American Thyroid Association Guidelines (1). A more systematic and comprehensive understanding of disease pathophysiology and imaging methodologies is needed for optimal utilization of different imaging modalities in the management of DTC. There have been significant developments in radiotracer and imaging technology, clinically proven to contribute to the understanding of tumor biology and the clinical assessment of patients with DTC. The research and development in the field continues to evolve, with expected emergence of many novel diagnostic and therapeutic techniques. The role for nuclear imaging applications will continue to evolve and be reconfigured in the changing paradigm. This article aims to review the clinical uses and controversies surrounding the use of scintigraphy, and the information it can provide in assisting in the management and treatment of DTC.

Keywords: Nuclear medicine, RAI, I-131, I-123, I-124, FDG-PET/CT, thyroid carcinoma, differentiated thyroid cancer, theranostics

Öz

Geleneksel nükleer tıp, moleküler görüntüleme ve teranostik kavramları ile hızla değişmektedir. Ancak bu prosedürlerin değeri diferansiye tiroid kanserleri (DTK) alanında küçümsenmektedir. Bu durum kısmen hastalığa klinik yaklaşımdaki gelişmelere kısmen de klinisyen ve nükleer görüntüleyicilerin bu görüntüleme ile ilgili bilgilerinin eksikliği nedeniyle değerinin anlaşılamamasına bağlıdır. Sonuç olarak, DTK'da sintigrafinin kullanımı açısından veriler ancak sınırlı bir yarar göstermiş, ve yakın zamanda yayımlanan Amerikan Tiroid Birliği yönergesinde (1) de ifade edildiği gibi hastalığın tedavisinde görüntüleme kullanımından uzaklaşma eğilimi doğmuştur. Her ne kadar görüntülemenin değeri tartışmalı olsa da, görüntülemeye ve bunların DTK yönetiminde uygulanmasına daha sofistike bir yaklaşımın gerekliliği ve yararı konusunda evrensel görüş birliği vardır. Radyo-farmasötikler ve teknolojide, DTK nükleer görüntülemesinde yararı kanıtlanan birçok gelişme olmuştur ve bu gelişmeler devam etmektedir. Gelecekte yeni terapötik ve görüntüleme seçeneklerinin ortaya çıkmasıyla birlikte, görüntülemenin yararı yeniden revize edilebilir. Bu makale sintigrafinin kullanımının klinik faydaları ve etrafındaki tartışmalarla birlikte, sağladığı bilgilerin DTK yönetimi ve tedavisindeki etkilerini araştırmaktadır.

Anahtar kelimeler: Nükleer tıp, radyoaktif iyot, I-131, I-123, I-124, FDG-PET/BT, tiroid kanseri, diferansiye tiroid kanseri, theranostics

Address for Correspondence: Arif Sheikh MD, Columbia University Medical Center, Clinic of Radiology, New York, USA
Phone: +1 212 305 9335 E-mail: as5010@cumc.columbia.edu

Introduction

Clinical Radioiodine Imaging

Over the years, several options for imaging differentiated thyroid cancer (DTC) in nuclear medicine have been introduced, allowing for the progression in both the tracers and technology used. The standard imaging for DTC has been radioactive iodine (RAI), in particular iodine-131 (I-131). In fact, this can be considered the first truly theranostic agent as it provides both diagnostic capabilities and therapeutic abilities. It is a high energy tracer with a half-life of about 8 days, obtained from byproducts of nuclear reactors, and thus is the cheapest form of RAI. It can be used in pre-therapy imaging, but is also the sole radiopharmaceutical for post-therapy imaging, making it an ideal theranostic agent. The drawbacks against I-131 imaging are its lower resolution and higher radiation exposure profile as compared to other radiopharmaceuticals. In pre-therapy imaging, this leads to lower accuracy of disease detection.

Iodine-123 (I-123), since its introduction in nuclear medicine, has gained popularity in pre-therapy imaging. Its lower imaging energy peak allows for improved resolution as well as lower radiation exposure to the patient. This, in turn, leads to improved sensitivity for disease detection as compared to pre-therapy I-131 imaging as well as better predictive value of the post-therapy scan than the pre-therapy I-131 scan (2). Unlike I-131, it is cyclotron produced and has a shorter half-life of approximately 13 hours. The cyclotron production makes I-123 much more difficult to produce and distribute, and as such, a more expensive tracer. Also, due to the lack of cyclotron facilities, especially in third world countries, its availability may be severely limited. Its shorter half-life realistically limits imaging to within 48 hours; therefore, lesions with low grade uptake may not show up on delayed images. Thus, performing extended dosimetry is practically not feasible with I-123.

There are certain advantages of I-131 over I-123. The usage of I-131 may be more effective in obese patients due to better tissue penetration. Its long half-life allows for delayed imaging, which could improve image contrast over time and increase tumor accumulation, resulting in better visualization of the disease including portions that might have been missed on images taken at earlier time points. This could lead to better therapy planning assessment. Moreover, I-131 is well suited for dosimetry should one wish to perform the procedure. Although I-123 is considered superior in many instances in the pre-therapy evaluation, I-131 still remains the standard and currently the only possible modality for post-therapy imaging.

SPECT and CT Imaging

On the technological side, advancements in RAI imaging relate to the development of single photon emission computed tomography-computed tomography (SPECT-CT), which have dramatically improved the capabilities for disease evaluation and management. Generally, RAI imaging is performed as a whole body planar scan. Additional static dedicated views of the neck and chest regions are usually recommended in order to evaluate for potential artifacts, as well as to increase disease detection sensitivity. Pinhole imaging is sometimes used with these views as it may improve resolution and sensitivity, but at the cost of geometrically distorting the detected lesions. Following the introduction of SPECT, it was recognized that the 3-dimensional views it provided could further enhance disease evaluation. RAI images inherently have several artifacts and thus lesions can be difficult to localize without further more definitive anatomic information being available (3).

SPECT-CT is a device that sequentially combines the ability of a camera to perform RAI imaging by SPECT, as well as a clinical CT scan, all performed within one imaging session. The two images acquired can then be fused, resulting in a sound anatomic correlation of uptake localizations detected by the SPECT study. Nevertheless, its routine use is often hindered by the perception that the additional CT component significantly increases the radiation burden to the patient. What is often overlooked by nuclear medicine physicians and referring clinicians is that the CT portion of the study is actually first for image correction (attenuation, scatter, etc.) that enhances the SPECT portion of the study, and that this procedure is not simply an overlay of the two studies. This combination of SPECT enhancement and definitive anatomic localization leads to improvements in disease detection and demonstrable changes in the management of pre-therapy, post-therapy and follow-up scans (4,5). Another misunderstanding amongst referring physicians is that the CT portion of the study is usually a low quality study since its primary purpose is for image correction and anatomic localization, rather than a full diagnostic capability. Whereas many current SPECT-CT devices have multidetector CT capabilities that can perform clinical diagnostic imaging, they are usually only used within limited capability. Thus, any extra radiation burden to the patient from SPECT-CT diagnostic imaging is less significant than perceived, while markedly enhancing management capabilities of the patient's disease.

Post-Therapy Imaging: The 'True' Diagnostic Scan

The post-therapy scan is performed after administration of therapeutic activities of I-131, generally >1.22 GBq. It is well recognized that the post-therapy scan is the most sensitive scan for the detection of RAI avid disease, and thus can be considered as the gold standard for disease detection (Figure 1). In that sense, the post-therapy scan is

the optimal single scan for disease detection and staging, and therefore, a paramount diagnostic study. The post-therapy scan provides a wealth of information on patient management when performed correctly, and this data is prudently integrated into the patient's overall clinical information. The scan provides the greatest information about the extent of disease as well as guidance on predicting treatment response (6), and can lay out the kind of follow-up to be performed after treatment even though it may not alter subsequent therapy administration. Since the count rate is often higher than the rate found in pre-therapy scans, this is accepted as an appropriate time to consider performing additional SPECT-CT views to better understand the location of the disease, especially of lesions not detected on planar whole body images. The recently released American Thyroid Association (ATA) 2015 guidelines "strongly recommend" to perform SPECT-CT scanning after administration of any therapeutic RAI (1).

Pre-Therapy Imaging

In contrast to post-therapy scanning, the use of the pre-therapy scan remains controversial for several reasons. Although usually referred to as the diagnostic scan, the most often cited reason against its routine use is precisely its limited diagnostic value and poor ability to localize disease, which is clearly inferior to post-therapy scanning. Futhermore, it has little impact on changing management, and the issue of stunning is of great concern as it could actually lead to worse outcomes. As a result, the use of pre-therapy scintigraphy has been declining and not found to be useful by many. Nevertheless, the value that the diagnostic scan does provide is often overlooked. In the correct setting, it could provide a wealth of information that could assist in the approach to RAI therapy, as well as in the overall management of the patient.

Theranostic Value and Impact on Disease Management

The information provided by the pre-therapy scan has a much broader role in managing patients than simply its staging and diagnostic capabilities alone. Some studies have noted that if attention is paid to the technical aspects when performing a pre-therapy scan, it improves the diagnostic capability of the scan. In particular, performing a scan at a slower speed, spending time to do spot views, and performing extra pinhole images

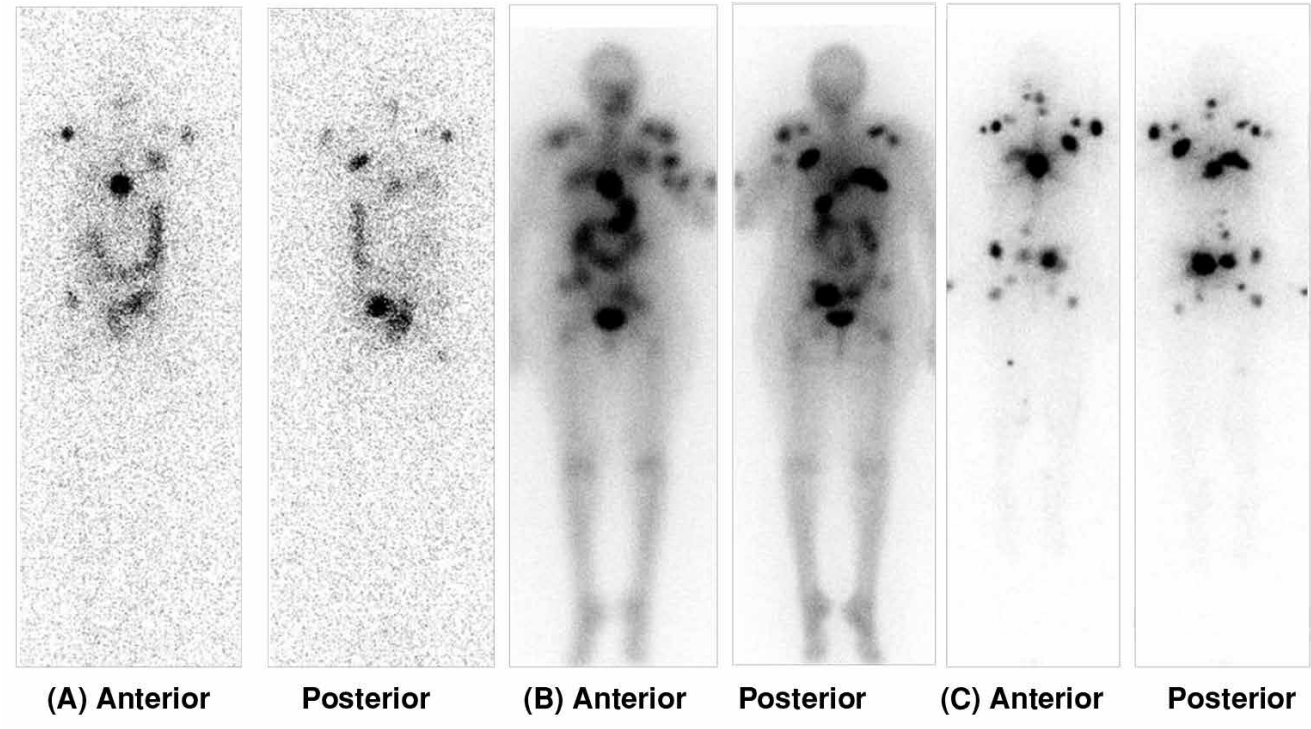


Figure 1. Images show (A) Whole Body Pre-therapy (dosimetry) scan 3 days following administration of 148 MBq I-131. Post-therapy scan 1 day (B) and 8 days (C) following administration of a therapeutic amount of I-131. There are clearly more lesions seen in the post-therapy scan than the pre-therapy one, but the timing of the scan is also important, as (C) shows more lesions than does (B). As noted by Sabra, et al. (6) the appearance of lesions not seen in the pre-therapy scan portends a lower therapeutic potential for those seen only in the post-therapy scan, and thus an early identifier of potential treatment failure, well before the first post-treatment Thyroglobulin levels are drawn. This could not have been known if the Pre-therapy scan was not performed

can markedly improve diagnostic capability. As noted earlier, performing SPECT-CT improves diagnostic ability compared to planar imaging alone. Hence, despite the perception that there is little data to support doing pre-therapy scans, there has actually been a number of studies over the years supporting its use (7,8).

The next question is whether these scans alter management. This question is particularly posed for the low or moderate risk patients. Because of the ability to upstage patients, it is important to answer if the frequency of these changes justifies the cost and effort of performing a scan. The supporting studies show management changes in a significant number of patients ranging between 22-42% of the time (9,10). The alterations in management include increasing RAI administered activities due to upstaging patients, increasing or decreasing RAI administered activities based on the residual tissue volume and/or number of lesions seen and warrant additional surgical management not otherwise anticipated. Although less controversial in higher risk patients, one may be able to manage patients in that setting as well if it is thought that therapeutic approaches will not change. In some of these patients, dosimetry could be considered as a pre-therapy evaluation to guide treatment and disease management, but there is little data to support its use. Nevertheless, the information provided by such an evaluation and its impact could outweigh any negative effect the procedure may have due to stunning or cost.

The final question then arises as to whether these differences in management lead to improvement in outcomes. This last issue is more difficult to answer because the kind of management changes proposed by studies have become less relevant in more updated guidelines, which have become more conservative in their approaches to surgery, RAI administration, as well as follow-up management. Thus previous studies which reported changes in management based on pre-therapy scans may have less impact according to more recent guidelines where the resultant change is no longer found to have a clinical impact. These newer management strategies themselves remain contentious and old data has to be continuously re-visited or re-studied in order to support the ongoing relevance of RAI pre-therapy scans in the era of novel management guidelines. Nevertheless, recent studies continue to show an impact, still lending support that the procedure is useful when executed correctly (11).

Additional Utility of Pre-Therapy Imaging

The pre-therapy scan may provide additional information that could be helpful in clinical care and treatment decisions for patients. In a study focused on the overall utility of pretreatment imaging, Van Nostrand et al. (12), investigated not only increased diagnostic utility, but also other clinical management issues. These included regulating salivary gland RAI uptake, to reduce sialadentis by administration of sailogogues, and minimizing the absorbed dose to the colon by administration of laxatives if excessive bowel retention was seen, or changing post-treatment radiation safety precautions if overall clearance was found to be significantly different than what was empirically calculated (Figure 2). They noted that the

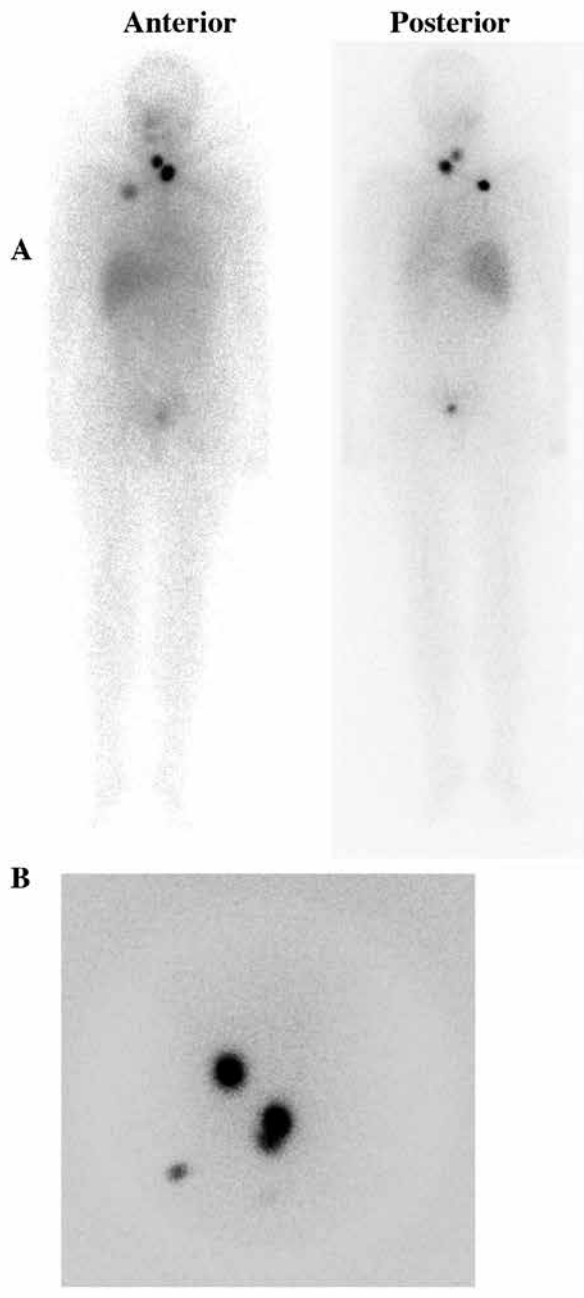


Figure 2. (A) Post-treatment whole body scan showing residual neck thyroid tissue and lung metastatic disease, (B) Pinhole neck images show an additional cervical lymph node not appreciated on the whole body scan.

scans could provide clinically relevant information in 53% of patients (Table 1). Additional studies have also shown that visualization on a pre-therapy scan, or the visualization of lesions as compared to the post-therapy scans, are prognostic in the ability of a lesion to respond to therapy (13). The pattern of lesional uptake is perhaps not so detailed to propose the scan as a true diagnostic agent in this setting. Therefore the term "diagnostic scan" is more appropriate for the post-therapy setting. The pre-therapy RAI scan should be more appropriately referred to as a "management planning scan".

The Stunning Phenomenon

DTC is a unique disease in that the use of imaging itself can impact therapeutic efficacy, and thus must be used judiciously. The use of intravenous CT contrast prior to RAI imaging and subsequent therapy could blunt I-131 uptake and therapeutic outcomes; therefore, it is recommended to wait at least a month between the contrast administration and the RAI procedures (1). In addition, the use of RAI scanning itself is subject to the stunning phenomenon. Stunning is the event in which the uptake seen in a post-therapy scan in certain tissues of thyroid origin appears to have less uptake than predicted by the pre-therapy scan. Although the phenomenon of stunning has been universally observed, its subsequent clinical impact remains controversial. Some studies have shown that pre-therapy scans subsequently lead to a decrease in the success of ablations (14), although several other studies have not shown such results (15,16) including those in high risk patients (17). Studies at the cellular level demonstrate that stunning is a real problem, particularly with I-131 (18), although the reason is likely to be multifactorial (19). Due to the stunning concern, I-123 has gained interest, potentially to replace I-131, both for its apparent increased accuracy at disease detection, and to avoid stunning. Interestingly, some studies have shown that even I-123 can result in stunning, albeit with lower frequency than I-131 (20) or in similar amounts. Other reports may suggest that stunning may not be a clinically relevant phenomenon (21). It has been recommended that RAI therapy be administered within two days of the pre-therapy scan to minimize the clinical impact of the stunning phenomenon. Standard dosimetry is performed using I-131, thus, the issue of stunning has to be taken into account.

Table 1. Pre-therapy scanning

Findings	Percent of scans with findings
Neck foci	
0	6%
≥6	12%
Intra- or extrathyroidal bed foci	14%
Excess thyroid bed percent uptake	8%
Foci >1 thyroid lobe	1%
Largest focus	4%
Non-thyroidal uptake/altered biodistibution	17%

Adapted from Van Nostrand D, et al. (21)

To minimize the impact of stunning on subsequent treatment, several other strategies can be utilized. Traditionally, pre-therapy scans have been done with a standard administered activity of I-131. However, factually, post-thyroidectomy patients may have variable amounts of remnant volume. The absorbed dose in the remnants, thus, could be variable. In remnants receiving higher absorbed doses, stunning may be a true clinical issue. When a large remnant volume is suspected or anticipated in a patient with recent thyroidectomy, a screening pertechnetate scan or neck ultrasound could be utilized to determine the volume of the remnant tissue. The administered activity of I-131 can be modified based on the residual amount of tissue to decrease stunning effect. If a significantly large remnant volume is identified, a completion thyroidectomy may be considered.

In the final analysis, it should be noted that the stunning phenomenon has been demonstrated to be a physiologic event based on studies which have evaluated the efficacy of thyroid tissue ablation as an endpoint. However, studies that have looked at the overall disease response or long term outcomes have not found significant impact of this phenomenon (22).

F-18 FDG PET/CT Imaging in Well-Differentiated Thyroid Cancer

Although RAI imaging is the standard for DTC, and can be optimized for higher diagnostic yield and clinical utility, there is clearly a group of tumors with poor differentiation or classified as "differentiated" by morphology, which are not RAI avid. These tumors cannot be imaged by RAI. There have been attempts to image these lesions with Thallium-201, Tc-99m Sestamibi and others; however, with the advent of F-18 flurodeoxyglucose (FDG) and positron emission tomography (PET), a strong imaging tool was added to our armamentarium for disease management. F-18 FDG PET/CT has gained wide acceptance for the detection, staging, and management of a multitude of malignant tumors. The role of F-18 FDG PET/CT in well-differentiated thyroid cancer (WDTC) is limited since increased FDG uptake is mostly restricted to aggressive and undifferentiated, high grade tumors, with no significant FDG uptake in well- differentiated thyroid tumors (23). In the 2015 ATA guidelines, F-18 FDG PET/CT is strongly recommended in high risk WDTC patients with elevated serum thyroglobulin (Tg)

(generally >10 ng/ml), and negative radioiodine imaging (1). According to the ATA guidelines, F-18 FDG PET/CT may also be considered a) as part of initial staging in poorly differentiated thyroid cancers and invasive Hurtle cell carcinoma, especially those with other evidence of disease, found on imaging or due to elevated serum Tg levels, b) as a prognostic tool in patients with metastatic disease in order to identify lesions as well as patients at highest risk of rapid disease progression and disease-specific mortality, and c) as an evaluation of post-treatment response, following systemic or local therapy of either metastatic or locally invasive disease. Although the utility of F-18 FDG PET/CT is limited in WDTC, the indications for FDG PET/CT depend on the clinical setting and thyroid cancer histology.

F-18 FDG PET/CT in Thyroid Incidentalomas

A discrete focus of uptake in the thyroid gland by F-18 FDG PET/CT in a patient studied for non-thyroidal purposes is an incidentaloma. The normal thyroid tissue shows no or minimal uptake of FDG. While most thyroid incidentalomas are benign, some are malignant, and can either be primary thyroid tumors or secondary lesions from other tumors. Diffuse FDG uptake generally indicates chronic or acute thyroiditis, however, focal FDG uptake in the thyroid gland indicates a significant risk of malignancy. In a meta-analysis study, Treglia et al. (24), reported that the pooled prevalence and malignancy risk of focal incidental FDG uptake in the thyroid were 1.92% [95% confidence interval (CI): 1.87-1.99%] and 36.2% (95% CI: 33.8-38.6%) respectively, without significant differences among various geographic areas. The most frequent malignant histological type responsible for FDG positive thyroid incidentalomas was papillary thyroid cancer. There was no safe, definite cutoff SUVmax value reported for differentiating malignant incidentalomas from benign ones. However, it was noted that a higher SUVmax value increased the likelihood of malignancy (25). Further ultrasonography and histological investigation is recommended in FDG positive incidentalomas since approximately one third of focal FDG avid thyroid lesions are malignant. ATA guidelines strongly recommend fine needle aspiration cytology (FNAC) for the thyroid nodules which are sonographically confirmed and have demonstrated focal FDG uptake.

F-18 FDG PET/CT in Thyroid Nodules with Indeterminate Fine-Needle Aspiration Cytology

An indeterminate fine-needle aspiration cytology (FNAC) result of a thyroid nodule is a clinical problem, because 20 to 30% of patients suffer from malignancy. The role of F-18 FDG PET/CT in thyroid nodules with indeterminate FNAC, has been studied to identify the patients who would benefit from lobectomy or thyroidectomy. In a meta-analysis of six studies which investigated the diagnostic role of FDG PET scans in thyroid nodules with indeterminate FNAC, Vriens et al. (26), reported that the pooled prevalence of malignancy was 26%. Pooled sensitivity, specificity, positive predictive value, negative predictive value (NPV), and accuracy of FDG PET were 95% (95% CI, 86%-99%), 48% (95% CI, 40%-56%), 39% (95% CI, 31%-47%), 96% (95% CI, 90%-99%), and 60% (95% CI, 53%-67%), respectively. Sensitivity increased to 100% for the 164 lesions that measured >15 mm in greatest dimension. A negative FDG PET scan ruled out thyroid cancer in patients with >15 mm thyroid nodules and indeterminate FNAC in a pooled population of 225 patients. However, a positive FDG PET did not necessarily indicate malignancy. In another meta-analysis, Wang et al. (27), analyzed seven studies using 267 patients and they reported that the pooled sensitivity and specificity of FDG PET or PET/CT for cancer detection in thyroid nodules with indeterminate FNAC results were 89% and 55%, respectively. These studies clearly demonstrate that the sensitivity of FDG PET or PET/CT is high, but specificity and positive predictive values are very low in differentiating cancer in indeterminate thyroid nodules. In the ATA guidelines, FDG PET/CT is not routinely recommended for the evaluation of thyroid nodules with indeterminate FNAC.

F-18 FDG PET/CT in Patients with Elevated Thyroglobulin Levels, But Negative Neck Ultrasound and Radioiodine Whole Body Scan

A major clinical challenge in the management of WDTC, is the particular patient that displays elevated Tg but both negative neck ultrasound (US) and I-131 whole body scan (WBS) after initial I-131 treatment. In many cases, the initial high Tg levels gradually decrease over time after I-131 treatment. The decrease in Tg to the lowest level may take months to years when left untreated. However, if Tg antibody is negative and there is no evidence of residual thyroid tissue, a rising level of stimulated or un-stimulated serum Tg over time may indicate which patients have residual or recurrent disease. In this particular group of patients, the most common finding would be local recurrence in the thyroid bed as well as either cervical or mediastinal lymph node metastasis. If the US and I-131 WBS are negative for recurrence and metastasis but Tg is high and rising, two possible reasons can be considered:

1) The tumor or metastatic tissue is too small to be detected with both imaging modalities, or;

2) The tumor might not be able to concentrate iodine but is still able to produce Tg (28).

Cervical US is a very sensitive imaging modality used to detect recurrence in the thyroid bed as well as nodal

metastasis in the neck, but it has low specificity in patients with altered anatomy after surgery because of difficulty in differentiating between scar tissue and local recurrence, as well as differentiating between nonspecific nodal growth and nodal metastases. Cervical US also cannot show distant metastases (29,30). If cervical US is negative or equivocal, CT and magnetic resonance imaging may be other useful tools for detecting recurrence and metastases in the neck or mediastinum. If cervical US and I-131 WBS are not able to show recurrence or metastatic disease, F-18 FDG PET/CT can be used. In a recently performed meta-analysis of 20 studies, Caetano et al. (31), reported both the sensitivity and specificity of conventional PET and PET/CT in detecting recurrence of WDTC in patients with high Tg but negative I-131 WBS as 84% and 84% respectively and 93% and 81% respectively. The overall accuracies were 91% and 93% for PET and PET/CT, respectively. Another meta-analysis of 25 studies with 789 patients showed that FDG PET/CT is a more sensitive imaging method than FDG PET in the follow-up of WDTC recurrence or metastases in patients with negative I-131 WBS (32). The increased FDG uptake in iodine-negative recurrent and metastatic lesions could be due to the growth of more aggressive tumor cells that have lost the expression of the membrane glycoprotein sodium-iodide symporter, which is responsible for the active transport of iodine in the thyroid tissue, with increased expression of the glucose transporter-1 (GLUT-1) (31). The F-18 FDG PET/CT may lead to a change in treatment plan in 9 to 54% of cases; especially by detecting unexpected distant metastases.

In the ATA guidelines (1), FDG PET/CT imaging is strongly recommended in patients with negative I-131 WBS and serum Tg >10 ng/ml. Although serum Tg levels correlate with tumor load, and the sensitivity of FDG PET/CT in detecting lesions increases as Tg levels increase, several studies report that FDG PET/CT could also be helpful in selected cases with Tg levels below 10 ng/ml. Shammas et al. (33), reported that the sensitivity of FDG PET/CT in detecting recurrent and metastatic lesions in patients with elevated Tg but negative I-131 WBS changed according to the serum Tg levels of the patients. It was reported that the overall sensitivity, specificity, and accuracy of FDG PET/CT were 68.4%, 82.4% and 73.8%, respectively within the study. The sensitivity of FDG PET/CT at serum Tg levels of less than 5, 5-10, and greater than 10 ng/ml were 60%, 63%, and 72%, respectively. FDG PET/CT changed clinical management in 44% of patients in this study. Another study by Giovanella et al. (34), showed that FDG PET/CT scans were true-positive in 46% of patients who had Tg serum levels <10 ng/ml.

Another issue to be addressed is the effect of TSH stimulation on the sensitivity of FDG PET/CT in detecting recurrent and metastatic disease in patients with negative cervical US and I-131 WBS but high Tg serum levels. Studies have shown that TSH stimulates the glucose transport system by enhancing the number of functional GLUT transporters in the plasma membrane (35). A meta-analysis by Ma et al. (36), revealed the results of 7 prospectively controlled clinical trials which were aimed to establish the effects of TSH stimulation on the uptake of FDG in 168 patients who had elevated serum Tg levels but negative I-131 WBS. The number of patients with FDG true-positive lesions, FDG-detected lesions, and the tumor-to-background ratios significantly increased after TSH stimulation. FDG PET/CT undertaken with TSH stimulation altered clinical management in 9% of patients within five paired studies. TSH stimulation can be done by either T4 withdrawal or recombinant human TSH (rhTSH) administration. In rhTSH stimulation, which avoids the adverse effect of hypothyroidism on quality of life, FDG PET/CT scans can be performed either 24 or 48 hours after rhTSH administration. Leboulleux et al. (37), analyzed 108 lesions and reported that rhTSH stimulated FDG PET/CT was significantly more sensitive than basal PET/CT for lesion detection and tended to be more sensitive in the detection of involved organs. Basal FDG PET/CT altered clinical management in 19% of patients, whereas lesions found only by rhTSH stimulated FDG PET/CT contributed to an altered therapeutic plan in eight patients among whom only 6% were true positive on pathology. According to their results, rhTSH stimulated FDG PET/CT significantly increased the number of lesions detected, but the numbers of patients in whom any lesion was detected did not differ between basal and rhTSH stimulated PET/CT scans.

Figure 3 shows a 65-year-old patient with papillary thyroid cancer follicular variant. The patient had undergone a total thyroidectomy. A multifocal papillary cancer, the largest tumor being 2 cm in diameter and one lymph node metastasis, was found on histopathological examination. Nine months after 150 mCi radioiodine ablation therapy, I-131 WBS and neck US were normal. Stimulated Tg was 10.1 ng/ml and Anti-Tg was normal. FDG PET/CT imaging was performed to search for recurrent/metastatic disease. Cervical metastatic lymph nodes were detected with FDG PET/CT in multiple levels of the patient's right neck, which were confirmed by histological examination.

The Value of F-18 FDG PET/CT in Determining Prognosis

FDG PET/CT can be used as a prognostic tool for patients with DTC. A poor prognosis and reduced survival can be predicted in patients who have metastatic DTC and high FDG uptake, which suggests dedifferentiated, aggressive, and metabolically active tumor cells. Wang et al. (38), investigated the prognostic value of FDG PET/CT in 125 patients with high Tg and negative I-131

WBS. In this study, univariate analysis demonstrated that survival was reduced in patients over 45 years of age, those with distant metastases, PET positivity, high rates of FDG uptake, and high volume of FDG-avid disease (>125 mL); multivariate analysis demonstrated that the single strongest predictor of survival was the volume of FDG-avid disease. Masson-Deshayes et al. (39), evaluated quantitative FDG PET/CT parameters such as the number of FDG-avid lesions, the SUVmax, the SULpeak of the lesion with the highest FDG uptake, the overall metabolic tumor volume (MTV), and the total lesion glycolysis (TLG), in predicting progression-free and overall survival in 35 patients with metastatic DTC. Progression-free survival was better in patients with less than 10 FDG-avid lesions, a SUVmax less than 10, a SULpeak less than 5, and a TLG less than 154. In their study, cox analysis displayed that only the PET scan result was predictive of survival (age, tumor node metastasis (TNM) stage, histology, and the I-131 WBS were not associated with prognosis). In the univariate analysis, prognostic factors for progression-free survival and overall survival were the SUVmax, the SULpeak, and the TLG. The number of FDG-avid lesions was significantly associated with progression-free survival, but not the MTV. In the multivariate analysis, the number of FDG-avid lesions and the SULpeak were independent prognostic factors. These studies showed that high FDG uptake in lesions may be a strong indicator of patient prognosis, and that management can be altered according to the results of FDG PET/CT findings.

F-18 FDG PET/CT in Initial Staging or Follow-up in High Risk Patients with Aggressive Histological Subtypes

Hurtle cell carcinoma (HTCC), poorly differentiated thyroid carcinoma, and anaplastic thyroid carcinoma are not common thyroidal malignancies. These tumors usually do not concentrate a significant amount of radioiodine and their prognosis is worse than WDTC. FDG PET/CT can be used in the initial diagnosis to evaluate the extent of the disease and to obtain prognostic information (40). HTCC,

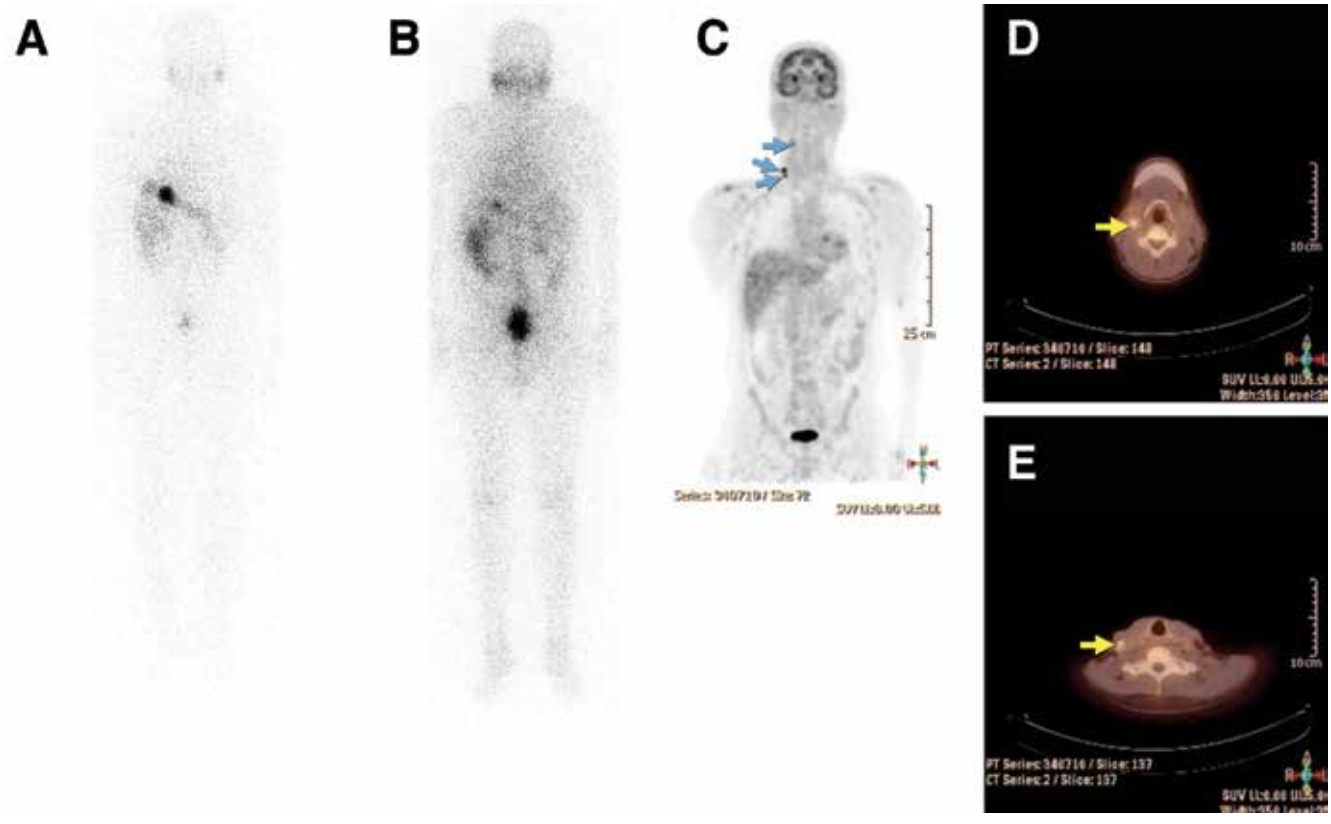


Figure 3. A 65 year-old patient with papillary thyroid cancer with follicular variant. Nine months after initial I-131 treatment, a diagnostic I-131 whole body scan was performed (A, anterior and B, posterior) with no evidence of residual or recurrent/metastatic disease. Neck ultrasound did not show any pathological findings. However, the patient had a high and rising serum Tg level suspicious for metastatic disease. FDG PET/CT imaging showed multiple lymph node metastases (white and blue arrows) in the right neck (C). Cervical lymph node dissection was performed and metastatic lymph nodes were resected (D and E)

about 3.6% of thyroid cancers, used to be considered as a subtype of WDTC, but because of its biological behavior, has been accepted as an aggressive histologic subtype. Few studies have been published so far on the role of FDG PET in the management of HTCC patients. The sensitivity and specificity of FDG PET in HTCC have been reported as 92 to 95% and 80 to 95%, respectively. According to the ATA guidelines (1) FDG PET/CT may be considered in the initial staging of poorly differentiated thyroid cancers or HTCC, especially those with evidence of disease on other imaging modalities or with elevated serum Tg levels.

Nascimento et al. (41), found that FDG PET/CT was more sensitive than I-131 post-ablation WBS in detecting individual lesions in patients with aggressive histology thyroid cancer, mostly tall cell papillary carcinoma and poorly differentiated carcinoma. Both FDG PET/CT and post-ablation WBS were complementary with 41% of the lesions detected only by FDG-PET/CT and 31% only by post-ablation WBS. The authors reported that the only risk factor for abnormal FDG-PET/CT was a stimulated Tg level measured at ablation >10 ng/mL, with persistent disease showing FDG uptake in 72% of the patients with a stimulated Tg >10 ng/mL and in 10% of the patients with a stimulated Tg ≤10 ng/mL.

Rosenbaum-Krumme et al. (42), analyzed the benefit of FDG PET/CT at initial diagnosis in 90 patients with high-risk differentiated thyroid carcinoma and determined whether the FDG PET/CT results led to a deviation from the standard procedure. TNM staging changed due to the FDG PET/CT results in 8 patients, and clinical management changed in 19 of the 90 patients (21%), including all patients with only FDG-positive lesions and all patients with both FDG-positive and iodine-positive lesions. The same authors recently published three year follow-up results of their initial series (43). In their analysis of 109 patients with high risk DTC, the NPV of FDG PET/CT scans at initial staging, regarding full remission on follow-up, were 85% and 91% for patients without FDG-positive lesions and for patients without any lesions, respectively. They concluded that the change in management in patients with iodine-negative lesions can lead to a higher rate of full remissions during follow-up after additional surgery. Therefore, FDG-PET/CT can be considered in all high-risk DTC patients in the context of the first radioiodine therapy to improve patient management and risk stratification.

Figure 4.1 shows FDG PET/CT findings in the initial staging of a high risk patient with papillary thryoid cancer with follicular variant. The patient had a large mass in the right

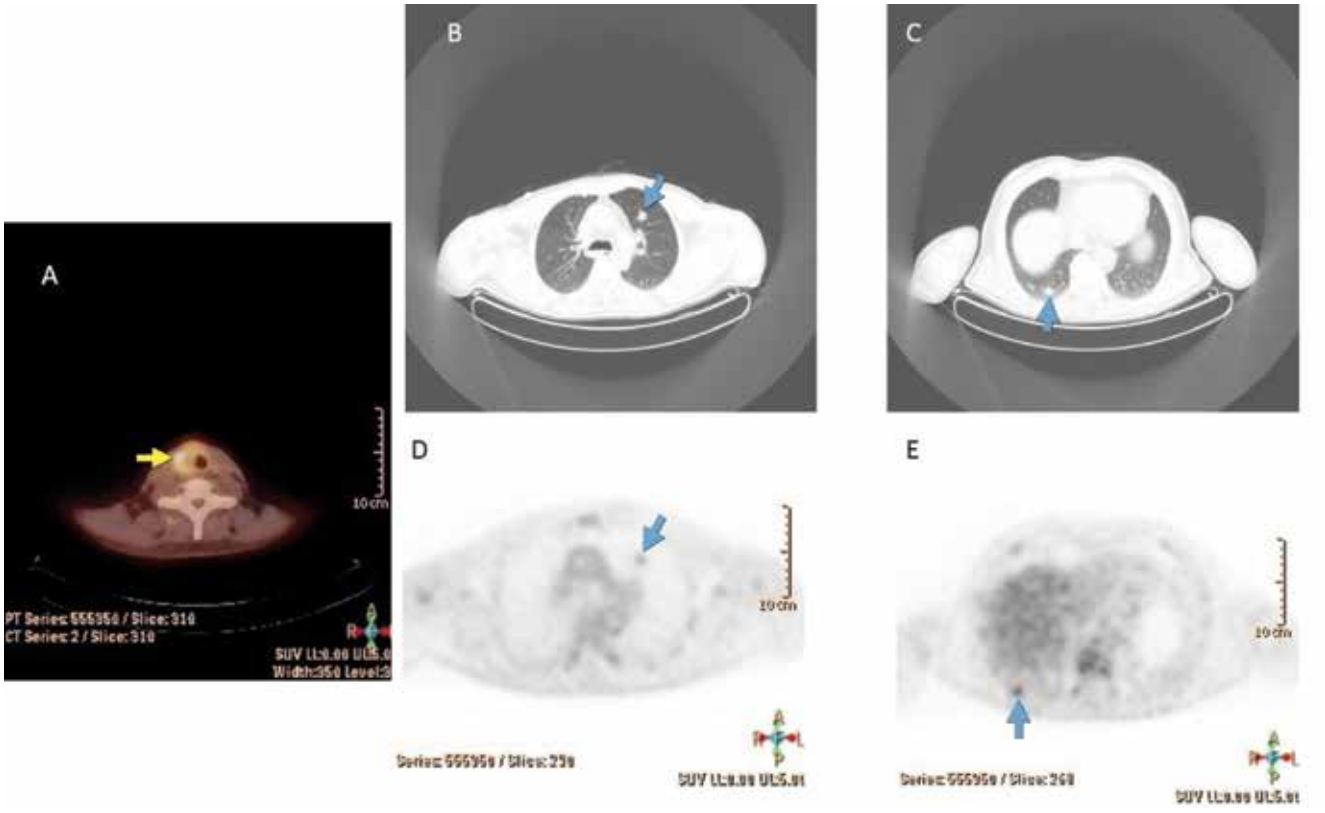


Figure 4.1. F-18 FDG PET/CT images of a high risk patient with papillary thyroid cancer with folicular variant. Increased FDG uptake was seen in a right thyroid lobe mass invading adjacent soft tissue structures (A, yellow arrow). There are also left and right small lung nodules which show increased FDG uptake (B, C, D, E, blue arrows)

thyroid lobe invading peripheral soft tissues and two lung nodules (one on the right, one on the left) with the greatest diameter of 1 cm which were suspicious for metastases. FDG PET/CT showed remarkably increased FDG uptake in the primary thyroid tumor and mild to moderate uptake in both lung nodules which were consistent with metastases. One month after FDG PET/CT, total thyroidectomy, with laryngectomy and right lateral neck dissection was performed and histopathological examination demonstrated thyroid papillary carcinoma with follicular variant, laryngeal involvement and metastatic lymph nodes at level 4. Two months after the surgery, the patient received 200 mCi of I-131. Post-treatment WBS (stimulated Tg: 42 ng/ml) did not reveal any residual or metastatic I-131 uptake in the whole body images (Figure 4.2). The lung nodules did not show radioiodine uptake. On follow-up, both Tg and the number of metastatic nodules in the lungs increased which confirmed the FDG PET/CT findings. The patient was accepted as having radioiodine refractory disease.

F-18 FDG PET/CT in Management and Therapy Evaluation of Patients with Radioiodine Refractory Disease

Most patients with RAI-refractory disease (disease which is not responsive to RAI treatment) can be categorized in 4 groups:

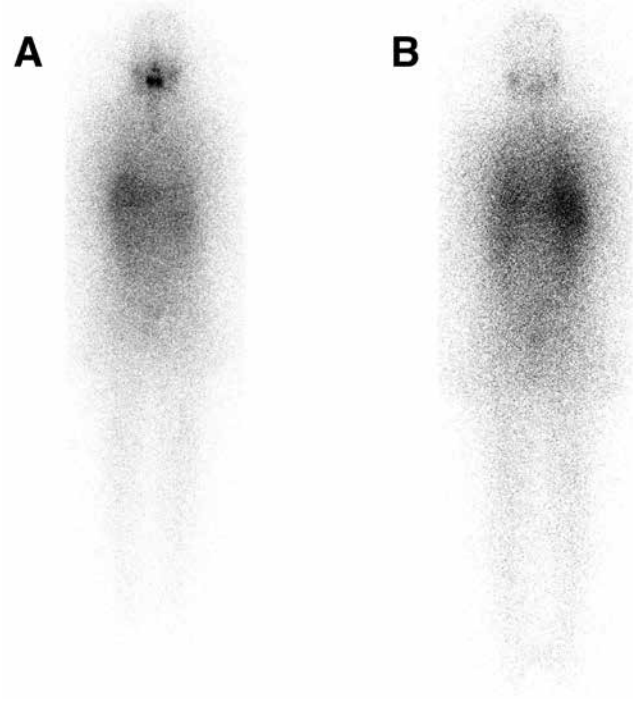


Figure 4.2. Post-therapy I-131 anterior (A) and posterior (B) whole body images of the same patient which show no evidence of RAI avid metastatic disease.

1) Patients with metastatic disease that does not show RAI uptake at the time of initial treatment,

2) Patients whose tumors lose the ability to uptake RAI after previous evidence of uptake,

3) Patients with retained RAI uptake in some lesions but not all and,

4) Patients with metastatic disease that progresses despite RAI uptake in the lesions (44).

Prognosis and survival in patients with RAI-refractory disease are variable; some patients show rapid progression of the disease, while some show indolent disease. Currently, novel treatment options for these patients are being clinically evaluated. Multi-targeted thyrosine kinase inhibitors (TKIs), MAPK pathway inhibitors, and angiogenesis inhibitors, are some of these treatments. TKIs are limited to a highly select group of metastatic patients, because their toxicities are sometimes serious and can be fatal (45). FDG PET/CT may have a role in selecting these patients and evaluating the early response to TKI agents. There are studies investigating the efficacy of FDG PET or PET/CT in the early treatment response evaluation of lesions that were FDG positive at baseline (46,47). Carr et al. (47), conducted a phase II study to assess the efficacy of sunitinib in patients with RAI-refractory disease. They designed their study using at least one FDG PET/CT avid lesion with uptake clearly above the blood-pool background, an objective criterion for trial entry, and evaluated the response per FDG PET/CT after 7 days of treatment as an early indicator of response. The disease control was in 78% of patients with a significant average SUV percent change in Response Evaluation Criteria In Solid Tumors (RECIST) response. The average SUVs significantly decreased in patients with partial/complete response and stable disease as compared to the values in patients with progressive disease. However, Kloos et al. (48), did not find any clear correlation between FDG PET response (the % change in SUV_{max} and metabolic volume as compared to pretherapy values) and objective tumor response. They also did not report a consistent change in SUV_{max} and MTV pattern. It is clear that more studies are needed to investigate the specific role of PET/CT in patients with RAI-refractory disease.

F-18 FDG PET/CT for Radioguided Surgery

Radioguided surgery (RGS) with F-18 FDG has been used in malignancies like colorectal carcinoma, malignant melanoma, breast carcinoma and metastatic head and neck carcinoma. In this technique, a handheld PET probe has been used to locate FDG-positive loco-regional metastases. A limited number of studies have been published on RGS with FDG in thyroid cancer patients with FDG positive and I-131 negative lesions (49,50,51). It has been reported that a hand-held probe could be

complementary to FDG PET/CT imaging, in intraoperative detection of FDG positive lesions. In these studies, the intraoperative PET probe did not identify any new foci during surgery, however it localized all the lesions which were visualized on PET/CT images and thus enabled the surgeon to verify that all the detected foci had been completely resected.

F-18 FDG PET/CT for External-Beam Radiotherapy Planning

External-beam radiotherapy (EBRT) is a rarely used therapy modality in WDTC patients. However, EBRT could be useful in high risk patients who have gross residual non-RAI-avid locoregional disease remaining after surgical resection and/or metastatic lesions. These tumors are more likely to be more aggressive and FDG positive on PET/CT imaging. EBRT can be considered as an adjuvant therapy for mostly older patients (>45 to 50 years) who have undergone complete surgical resection of all visible non-RAI-avid tumor in the setting of gross extrathyroidal extension into surrounding major structures. ERBT is not routinely recommended as an adjuvant therapy for low risk disease, for patients with microscopic extrathyroidal extension detected only on histological examination, or patients with locoregional lymph node involvement in the absence of other very high risk features (52).

The main objective of EBRT treatment is to deliver the highest dose of radiation possible to the tumor without causing damage to the surrounding normal tissues. Image-guided-intensity modulated radiation therapy [IG-IMRT or image-guided radiotherapy (IGRT)] can be a valuable radiotherapy technique in order to achieve this objective. FDG PET/CT guided IMRT can be useful as a radiotherapy method in patients who have aggressive and non-RAI-avid but FDG-avid tumors. Further studies on this subject are needed.

Imaging and Management with I-124 and PET

Despite the success of RAI in imaging and treatment, there are still issues in optimization of its use. The diagnostic limitations of I-131 have been described earlier. Although I-123 improves upon these drawbacks, it has limited ability to perform dosimetry, whether on a whole body or lesional basis. This, in turn, limits its ability to provide a more personalized determination of aministered activitiy for a given patient as compared to I-131. Even as a SPECT imaging agent, I-123 imaging is still limited. FDG PET imaging has superior resolution, but provides very different functional information than RAI imaging, and thus cannot supplant the latter.

Iodine-124 (I-124) is a positron emitting isotope with a half-life of approximately 4 days. This provides a high resolution RAI scan utilizing PET imaging, and its half-life enables performing dosimetry. The tracer is used for diagnostic purposes in the pre-therapy setting, however drawbacks include the complex decay schema and stunning which theoretically could be comparable to that of I-131. I-124 is more costly than I-123, and currently, the clinical availability of I-124 is limited. Nevertheless, it has been advocated as a modality that could assist in answering some critical questions regarding RAI treatment that have not been adequately addressed with other RAI agents. I-124 also has the potential to provide invaluable information in managing select individuals with high risk disease.

The superior performance of I-124 PET imaging has been validated by studies that displayed increased sensitivity compared to SPECT tracers, seeing as many as 50% more lesions in 32% more patients in the pre-therapy setting (53). A recent study by Gulec et al. (54), showed over 22% more lesions with I-124 PET/CT as compared to planar I-131 post-therapy scans. Moreover, they were able to show different iodine kinetic profiles of salivary glands, remnant thyroid tissue, and variability between various metastatic tissues.

Imaging with I-124 PET also provides an opportunity to study kinetics of in vivo processes. Freudenberg et al. (55), reported that the radiation absorbed doses using rhTSH vs. withdrawal protocols were not significantly different when patients were given the same administered activities for treatment. This finding was important because there has been a concern amongst many, when using rhTSH in the therapy of higher risk patients. Another clinically relevant use is in the protection of salivary glands from excess radiation absorbed doses. The use of agents that induce salivary secretions have been traditionally recommended. Jentzen et al. (56), proposed that the use of lemon juice immediately after I-131 therapy could increase salivary gland damage. Rosenbaum-Krumme et al. (57), showed the positive effects of rosiglitazone in a cohort of patients to improve RAI uptake in tumors with negligible or absent uptake. In a similar fashion, Ho et al. (58), showed a clinically meaningful increase in uptake in a subset of patients who underwent selumetinib therapy. The use of rhTSH in the patient preparation for RAI imaging has also been a topic of discussion.

The greatest impact of I-124 is the ability to do accurate image based lesional dosimetry to an extent that has not been possible until now. This has shown to have an impact on the management of more advanced DTC, and other issues related to RAI administration. An earlier study by Freudenberg et al. (59), showed that I-124 PET imaging had an impact on 28% of patients. The imaging not only accurately localized small lesions that were previously unsuspected, but also increased the administered I-131 activity due to lower predicted radiation doses to lesions using empiric amounts, avoided therapy administration altogether due to insufficient predicted RAI uptake, and thus led to earlier surgical management of some patients.

Despite the higher diagnostic accuracy of I-124 imaging, it is obvious that tumor biology limits even this modality. As pointed out previously, FDG is the modality that is used in diseases that have lost iodine avidity and/or in aggressive tumors. By combining the information from CT and both PET modalities (I-124 and FDG), Freudenberg et al achieved sensitivity rates of >95% for both locoregional and metastatic disease, and positive predictive values of 95-100% from just the PET information. Furthermore, they also noticed the complex behavior of tumors by noting the mixed uptake of I-124 vs. FDG (60). This has been correlated more closely with GLUT1 and Ki-67 expression by Grabellus et al (61). They demonstrated an inverse relationship between glucose and radioiodine uptake. This inverse relationship becomes more prominent as the tumors undergo dedifferentiation process.

Although the information obtained from I-124 imaging is clearly valuable in answering some of the critical questions regarding the use of RAI, it is still unclear whether this modality is of practical use in the routine management of DTC, and the identification of patients who would benefit most from I-124 imaging remains to be further clarified. Clearly, the patients who would most likely benefit, are those with high risk disease and/or complicated medical issues. It is of no doubt that this modality can potentially contribute substantially to the understanding of the radiobiology of RAI therapy (Figure 5).

The Follow-up Scan: The Surveillance Scan

Another controversial issue is whether to obtain a follow-up scan after a treatment cycle has been completed, during a patient's surveillance period. This scan is typically done approximately 6-12 months after the first course of RAI treatment. The need for a surveillance scan depends upon many factors, mainly the evaluation of response to initial treatment. This can best be assessed by following thyroglobulin (Tg) levels at regular intervals on appropriate thyroxine suppression, and based on the risk stratification of the disease. Follow-up neck ultrasound results could also help guide the need for surveillance scintigraphy. If there is clinical, serologic or imaging suspicion for residual or recurrent disease in a patient initially staged as intermediate-high risk, or accurate assessment of disease status is blunted [e.g. the presence of Tg Antibodies (TgAb)] then a follow-up scan is definitely useful. In many cases, this scan may in fact serve as a pre-therapy scan as a prelude to another round of RAI treatment. The ATA guidelines give a Strong Recommendation for performing a surveillance scan in this setting (1).

The role of the surveillance scan is less clear in the low risk group, or others with no evidence of residual or recurrent disease. In the low risk group, follow-up scans confirm whether there has been successful ablation of residual thyroid tissue, or if there still is residual disease. This information is helpful in the consideration of additional RAI treatment. Even when the Tg level has not completely normalized, various groups have shown that stimulated Tg levels without imaging, or with neck ultrasounds are more sensitive in disease detection than RAI scans in low risk patients (62). It was recommended that these patients can be followed by ultrasound and Tg/TgAb in the surveillance. RAI scintigraphy should be performed only if findings warrant potential additional RAI treatment. While there is general consensus that RAI scans provide limited benefit in a low risk population, we believe that it remains important to establish a baseline. Thyroid cancer is a disease that is followed over many years, and often by several different physicians over the

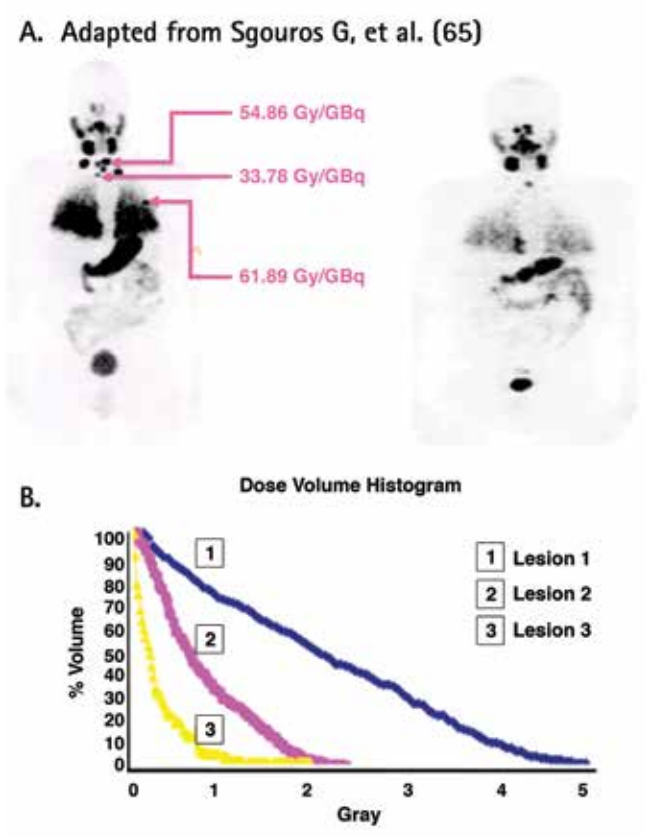


Figure 5. I-124 PET Pre-therapy scan (A). Serial imaging can be done to calculate lesional dosimetry as demonstrated. Even though lesional dosimetry would appear as an ideal solution, some issues remain, such as how to accurately calculate dosimetry for cases of diffuse lung uptake. Additionally, analysis of the Dose Volume Histograms of individual lesions (B) show that the uptake is quite heterogeneous so that a deeper understanding of tumor radiobiology is needed to comprehend its response, and thus pick an appropriate dosage prior to treatment.
The patient was subsequently treated with 4.96 GBq I-131. A follow-up I-124 PET scan done approximately 5 months later showed a response to the degree predicted by dosimetry, but clearly disease remained despite apparent robust uptake on the prior scan. Nevertheless, the overall uptake was also decreased, suggestive of an interim therapeutic response

course of a patient's lifetime. Thus, establishment of a baseline is helpful for long-term surveillance. The patient follow-up may be assumed by another physician and the conditions at the initial therapy could be forgotten. Additionally, in the setting of recurrence, the identification of new sites of disease could be made easier.

Another issue is that once a surveillance scan is done and is found to be negative, how often should subsequent scans be performed? As alluded to above, it may not be necessary to do more than one surveillance study in a low risk population. In fact, Caballero-Calabuig et al. (63), showed that in 10-20% of cases the RAI scan may be positive even though serum Tg levels are low or negative. This could again justify obtaining at least one RAI scan after therapy completion to document the presence of residual tissue, if any is present. However, these patients can subsequently be easily followed, with other modalities as long as there is no clinical evidence of recurrence.

Intermediate-higher risk patients have traditionally been evaluated in increasing intervals between sequential negative scans, as long as there is no evidence of disease. However, de Meer et al. (64), showed that the risk is decreased with a completely negative scan along with serologic and clinical absence of disease, and that frequent follow-up surveillance scans may not be needed as these patients appear to have similar outcomes as low risk patients. Still, the long term data, clarifying how far along the survival curve is to be extended, in the follow-up of low risk patients is not conclusive. In these patients, surveillance imaging could still be considered every 5-10 years provided that the disease status remains stable throughout the follow-up period.

Using FDG PET/CT in the follow-up and surveillance setting would generally not be indicated unless there is evidence of recurrence potentially with RAI resistant disease. It can be used for follow-up in patients with known residual intermediate-high risk disease, again in whom RAI scans fail to show the disease, and in whom FDG scans have detected a lesion. There are no recommendations in how frequently these should be done, and they should be performed based on the stability of the patient's clinical course (Figure 6).

Future Goals and Directions

In the final analysis, the use of all diagnostic tools can only be as good as the user who understands their value, and the tools should not be restricted but rather balanced between their cost and impact on improving overall patient care. The understanding of disease biology as well as the strengths and limitations of the diagnostic tools continue to evolve. More comprehensive information will help refine the treatment of DTC, and may actually set a balance between limiting potentially unnecessary radiation and delivering appropriate radiation doses to achieve a result that is more therapeutic than futile.

The use of RAI and FDG also evolve parallel to the increased sophistication in treatment methods. As discussed, I-124 can be used to refine the utilization of RAI therapy in cases when it could be avoided, or pursuing aggressive treatment when the study shows it to be feasible. Since the limitations associated with RAI are becoming better understood, it is likely that future treatments will involve combining RAI with other modalities, allowing for more thorough evaluations. FDG may be used to identify patients who are RAI refractory prior to starting treatment, and thus both RAI and targeted therapy could be used to treat different aspects of the same disease. The enhanced information provided by I-124 PET/CT can be coordinated with external beam treatments in advanced disease to target radiation to certain areas of the tumor, whereas FDG could be used to provide an idea where the external beam radiation should be intensified.

Conclusion

The use of theranostic molecular imaging is very valuable if used to its full capacity. It is important to realize that the sophisticated techniques work best when used in the proper clinical context, after evaluating the advantages vs. disadvantages of any intervention. As stated earlier, the utility of imaging will likely evolve, and the recommended protocols will require adaption to changing paradigms. Some applications may only be accessible to skilled surgeons in high volume centers, endocrinologists experienced in the follow-up of patients, or oncologists well versed with the treatment of advanced disease. Hence, the use of these techniques and technologies could vary between high volume academic centers, where more advanced disease tends to be seen, versus other practices where technical and expert personnel resources may be limited. Additionally, it is important to use all the information available to determine when and how to perform imaging. The clinical management decisions should definitely incorporate Tg, neck ultrasounds, as well as clinical assessment of the patient, including staging and subsequent clinical and molecular risk stratification.

Authorship Contributions

Concept: Arif Sheikh, Berna Polack, Yvette Rodriguez, Russ Kuker, Design: Arif Sheikh, Berna Polack, Yvette Rodriguez, Russ Kuker, Data Collection or Processing: Arif Sheikh, Berna Polack, Yvette Rodriguez, Russ Kuker, Analysis or Interpretation: Arif Sheikh, Berna Polack, Yvette Rodriguez, Russ Kuker, Literature Search: Arif Sheikh, Berna Polack, Yvette Rodriguez, Russ Kuker, Writing: Arif Sheikh, Berna Polack, Yvette Rodriguez, Russ Kuker.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

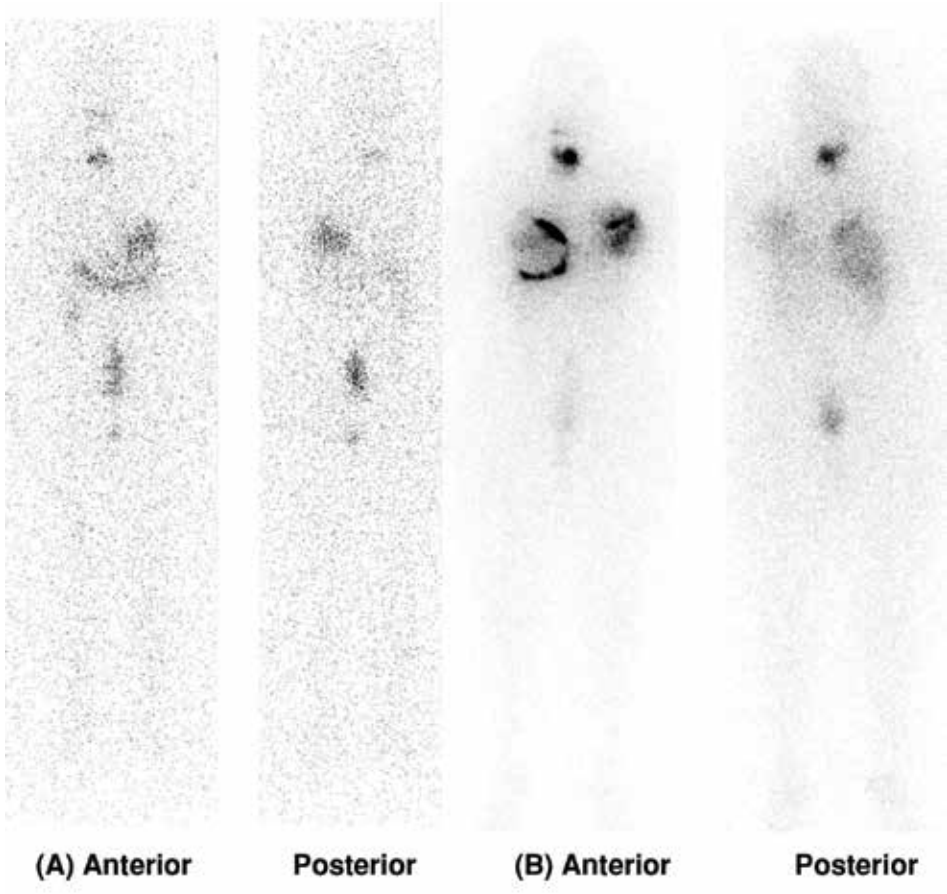


Figure 6. I-131 Follow-up diagnostic scan (A) 1 year after treatment with I-131 for a Low-moderate risk patient, in which a Post-therapy scan was done (B), but no pre-therapy scan. The patient had been breast feeding prior to the treatment, but was clinically thought to have stopped lactating. The post-therapy scan (B) showed bilateral diffuse breast uptake, indicating the patient's breasts had still not returned to non-lactating state. A pre-therapy scan could have indicated this, and changed management by deferring treatment until later when lactation would have completely subsided.
This is also an example of where a Follow-up scan was done after ablation. Clearly, some tissue remains in the thyroid bed, but this would not warrant further RAI treatment and could be followed. This scan can be used as a baseline. Had a scan not been done in the initial follow-up period, these findings could lead to confusion, or worse, misdiagnosis for recurrence if discovered years after the initial therapy

References

1. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM,Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26:1-133.
2. Anderson GS, Fish S, Nakhoda K, Zhuang H, Alavi A, Mandel SJ. Comparison of I-123 and I-131 for whole-body imaging after stimulation by recombinant human thyrotropin: a preliminary report. Clin Nucl Med 2003;28:93-96.
3. Yamamoto Y, Nishiyama Y, Monden T, Matsumura Y, Satoh K, Ohkawa M. Clinical usefulness of fusion of 131I SPECT and CT images in patients with differentiated thyroid carcinoma. J Nucl Med 2003;44:1905-1910.
4. Mustafa M, Kuwert T, Weber K, Knesewitsch P, Negele T, Haug A, Linke R, Bartenstein P, Schmidt D. Regional lymph node involvement in T1 papillary thyroid carcinoma: a bicentric prospective SPECT/CT study. Eur J Nucl Med Mol Imaging 2010;37:1462-1466.
5. Grewal RK, Tuttle RM, Fox J, Borkar S, Chou JF, Gonen M, Strauss HW, Larson SM, Schöder H. The Effect of Posttherapy 131I SPECT/CT on risk classification and management of patients with differentiated thyroid cancer. J Nucl Med 2010;51:1361-1367.
6. Sabra MM, Grewal RK, Tala H,Larson SM, Tuttle RM. Clinical outcomes following empiric radioiodine therapy in patients with structurally identifiable metastatic follicular cell-derived thyroid carcinoma with negative diagnostic but positive post-therapy 131I whole-body scans. Thyroid 2012;22:877-883.
7. Ruf J, Lehmkuhl L, Bertram H, Sandrock D, Amthauer H, Humplik B, Ludwig Munz D, Felix R. Impact of SPECT and integrated low-dose CT after radioiodine therapy on the management of patients with thyroid carcinoma. Nucl Med Commun 2004;25:1177-1182.
8. Barwick T, Murray I, Megadmi H, Drake WM, Plowman PN, Akker SA, Chew SL, Grossman AB, Avril N. Single photon emission computed tomography (SPECT)/computed tomography using Iodine-123 in patients with differentiated thyroid cancer: additional value over whole body planar imaging and SPECT. Eur J Endocrinol 2010;162:1131-1139.

9. Maruoka Y, Abe K, Baba S, Isoda T, Sawamoto H, Tanabe Y, Sasaki M, Honda H. Incremental diagnostic value of SPECT/CT with 131I scintigraphy after radioiodine therapy in patients with well-differentiated thyroid carcinoma. Radiology 2012;265:902-909.
10. Spanu A, Solinas ME, Chessa F, Sanna D, Nuvoli S, Madeddu G. 131I SPECT/CT in the follow-up of differentiated thyroid carcinoma: incremental value versus planar imaging. J Nucl Med 2009;50:184-190.
11. Avram AM, Esfandiari NH, Wong KK. Preablation 131-I scans with SPECT/CT contribute to thyroid cancer risk stratification and 131-I therapy planning. J Clin Endocrinol Metab 2015;100:1895-1902.
12. Van Nostrand D, Aiken M, Atkins F, Moreau S, Garcia C, Acio E, Burman K, Wartofsky L. The utility of radioiodine scans prior to iodine 131 ablation in patients with well-differentiated thyroid cancer. Thyroid 2009;19:849-855.
13. Sabra MM, Grewal RK, Tala H, Larson SM, Tuttle RM. Clinical outcomes following empiric radioiodine therapy in patients with structurally identifiable metastatic follicular Cell-derived thyroid carcinoma with negative diagnostic but positive post-therapy 131I whole-body scans. Thyroid 2012;22:877-883.
14. Hu YH, Wang PW, Wang ST, Lee CH, Chen HY, Chou FF, Huang YE, Huang HH. Influence of 131I diagnostic dose on subsequent ablation in patients with differentiated thyroid carcinoma: discrepancy between the presence of visually apparent stunning and the impairment of successful ablation. Nucl Med Commun 2004;25:793-797.
15. Dam HQ, Kim SM, Lin HC, Intenzo CM. 131I Therapeutic Efficacy İs Not influenced by stunning after diagnostic whole-body scanning. Radiology 2004;232:527-533.
16. Karam M, Gianoukakis A, Feustel PJ, Cheema A, Postal ES, Cooper JA. Influence of diagnostic and therapeutic doses on thyroid remnant ablation rates. Nucl Med Commun 2003;24:489-495.
17. Yap BK and Murby B. No adverse affect in clinical outcome using low preablation diagnostic (131)I activity in differentiated thyroid cancer: refuting thyroid-stunning effect. J Clin Endocrinol Metab 2014;99:2433-2440.
18. Lundh C, Lindencrona U, Postgård P, Carlsson T, Nilsson M, Forssell-Aronsson E. Radiation-induced thyroid stunning: differential effects of (123)I, (131)I, (99m)Tc, and (211)At on iodide transport and NIS mRNA expression in cultured thyroid cells. J Nucl Med 2009;50:1161-1167.
19. Sisson JC, Avram AM, Lawson SA, Gauger PG, Doherty GM. The so-called stunning of thyroid tissue. J Nucl Med 2006;47:1406-1412.
20. Hilditch TE, Dempsey MF, Bolster AA, McMenemin RM, Reed NS. Self-stunning in thyroid ablation: evidence from comparative studies of diagnostic 131I and 123I. Eur J Nucl Med 2002;29:783-788.
21. Silberstein EB. Comparison of outcomes after (123)I versus 131I pre-ablation imaging before radioiodine ablation in differentiated thyroid carcinoma. J Nucl Med 2007;48:1043-1046.
22. Yap BK, Murby B. No adverse affect in clinical outcome using low preablation diagnostic (131)I activity in differentiated thyroid cancer: refuting thyroid stunning effect. J Clin Endocrinol Metab 2014;99:2433-2440.
23. Grabellus F, Nagarajah J, Bockisch A, Schmid KW, Sheu SY. Glucose transporter 1 expression, tumor proliferation, and iodine/glucose uptake in thyroid cancer with emphasis on poorly differentiated thyroid carcinoma. Clin Nucl Med 2012;37:121-127.
24. Treglia G, Bertagna F, Sadeghi R, Verburg FA, Ceriani L, Giovanella L. Focal thyroid incidental uptake detected by 18F-fluorodeoxyglucose positron emission tomography. Meta-analysis on prevalence and malignancy risk. Nuklearmedizin 2013;52:130-136.
25. Bertagna F, Treglia G, Piccardo A, Giovannini E, Bosio G, Biasiotto G, Bahij el K, Maroldi R, Giubbini R. F18-FDG-PET/CT thyroid incidentalomas: a wide retrospective analysis in three Italian centres on the significance of focal uptake and SUV value. Endocrine 2013;43:678-685.
26. Vriens D, de Wilt JH, van der Wilt GJ, Netea-Maier RT, Oyen WJ, de Geus-Oei LF. The role of [18F]-2-fluoro-2-deoxy-d-glucose-positron emission tomography in thyroid nodules with indeterminate fine-needle aspiration biopsy: systematic review and meta-analysis of the literature. Cancer 2011;117:4582-4594.
27. Wang N, Zhai H, Lu Y. Is fluorine-18 fluorodeoxyglucose positron emission tomography useful for the thyroid nodules with indeterminate fine needle aspiration biopsy? A meta-analysis of the literature. J Otolaryngol Head Neck Surg 2013;1;42:38.
28. Ma C, Kuang A, Xie J, Ma T. Possible explanations for patients with discordant findings of serum thyroglobulin and 131I whole-body scanning. J Nucl Med 2005;46:1473-1480.
29. Alzahrani AS, Alsuhaibani H, Salam SA, Al Sifri SN, Mohamed G, Al Sobhi S, Sulaiman O, Akhtar M. Diagnostic accuracy of high-resolution neck ultrasonography in the follow-up of differentiated thyroid cancer: a prospective study. Endocr Pract 2005;11:165-171.
30. Galloway RJ, Smallridge RC. Imaging in thyroid cancer. Endocrinol Metab Clin North Am 1996;25:93-113.
31. Caetano R, Bastos CR, de Oliveira IA, da Silva RM, Fortes CP, Pepe VL, Reis LG, Braga JU. Accuracy of positron emission tomography and positron emission tomography-CT in the detection of differentiated thyroid cancer recurrence with negative (131) I whole-body scan results: A meta-analysis. Head Neck 2016;38:316-327.
32. Dong MJ, Liu ZF, Zhao K, Ruan LX, Wang GL, Yang SY, Sun F, Luo XG. Value of 18F-FDG-PET/PET-CT in differentiated thyroid carcinoma with radioiodine-negative whole-body scan: a meta-analysis. Nucl Med Commun 2009;30:639-650.
33. Shammas A, Degirmenci B, Mountz JM, McCook BM, Branstetter B, Bencherif B, Joyce JM, Carty SE, Kuffner HA, Avril N. 18F-FDG PET/CT in patients with suspected recurrent or metastatic well-differentiated thyroid cancer. J Nucl Med 2007;48:221-226.
34. Giovanella L, Ceriani L, De Palma D, Suriano S, Castellani M, Verburg FA. Relationship between serum thyroglobulin and 18FDG-PET/CT in 131I-negative differentiated thyroid carcinomas. Head Neck 2012;34:626-631.
35. Filetti S, Damante G, Foti D. Thyrotropin stimulates glucose transport in cultured rat thyroid cells. Endocrinology 1987;120:2576-2581.
36. Ma C, Xie J, Lou Y, Gao Y, Zuo S, Wang X. The role of TSH for 18F-FDG-PET in the diagnosis of recurrence and metastases of differentiated thyroid carcinoma with elevated thyroglobulin and negative scan: a meta-analysis. Eur J Endocrinol 2010;163:177-183.
37. Leboulleux S, Schroeder PR, Busaidy NL, Auperin A, Corone C, Jacene HA, Ewertz ME, Bournaud C, Wahl RL, Sherman SI, Ladenson PW, Schlumberger M. Assessment of the incremental value of recombinant thyrotropin stimulation before 2-[18F]-Fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography imaging to localize residual differentiated thyroid cancer. J Clin Endocrinol Metab 2009;94:1310-1316.
38. Wang W, Larson SM, Fazzari M, Tickoo SK, Kolbert K, Sgouros G, Yeung H, Macapinlac H, Rosai J, Robbins RJ. Prognostic value of [18F]fluorodeoxyglucose positron emission tomographic scanning in patients with thyroid cancer. J Clin Endocrinol Metab 2000;85:1107-1113.
39. Masson-Deshayes S, Schvartz C, Dalban C, Guendouzen S, Pochart JM, Dalac A, Fieffe S, Bruna-Muraille C, Dabakuyo-Yonli TS, Papathanassiou D. Prognostic value of (18)F-FDG PET/CT metabolic parameters in metastatic differentiated thyroid cancers. Clin Nucl Med 2015;40:469-475.
40. Treglia G, Annunziata S, Muoio B, Salvatori M, Ceriani L, Giovanella L. The role of fluorine-18-fluorodeoxyglucose positron emission tomography in aggressive histological subtypes of thyroid cancer: an overview. Int J Endocrinol 2013;2013:856189.
41. Nascimento C, Borget I, Al Ghuzlan A, Deandreis D, Hartl D, Lumbroso J, Berdelou A, Lepoutre-Lussey C, Mirghani H,Baudin E, Schlumberger M, Leboulleux S. Postoperative fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography: an important imaging modality in patients with aggressive histology of differentiated thyroid cancer. Thyroid 2015;25:437-444.
42. Rosenbaum-Krumme SJ, Görges R, Bockisch A, Binse I. 18F-FDG PET/CT changes therapy management in high-risk DTC after first radioiodine therapy. Eur J Nucl Med Mol Imaging 2012;39:1373-1380.

43. Ruhlmann M, Binse I, Bockisch A, Rosenbaum-Krumme SJ. Initial [18F]FDG PET/CT in high-risk DTC patients. A three-year follow-up. Nuklearmedizin 2016;55:99-103.
44. Schlumberger M, Brose M, Elisei R, Leboulleux S, Luster M, Pitoia F, Pacini F. Definition and management of radioactive iodine-refractory differentiated thyroid cancer. Lancet Diabetes Endocrinol 2014;2:356-358.
45. Haugen BR, Sherman SI. Evolving approaches to patients with advanced differentiated thyroid cancer. Endocr Rev 2013;34:439-455.
46. Marotta V, Ramundo V, Camera L, Del Prete M, Fonti R, Esposito R, Palmieri G, Salvatore M, Vitale M, Colao A, Faggiano A. Sorafenib in advanced iodine-refractory differentiated thyroid cancer: efficacy, safety and exploratory analysis of role of serum thyroglobulin and FDG-PET. Clin Endocrinol (Oxf) 2013;78:760-767.
47. Carr LL, Mankoff DA, Goulart BH, Eaton KD, Capell PT, Kell EM, Bauman JE, Martins RG. Phase II study of daily sunitinib in FDG-PET-positive, iodine-refractory differentiated thyroid cancer and metastatic medullary carcinoma of the thyroid with functional imaging correlation. Clin Cancer Res 2010;16:5260-5268.
48. Kloos RT, Ringel MD, Knopp MV, Hall NC, King M, Stevens R, Liang J, Wakely PE Jr, Vasko VV, Saji M, Rittenberry J,Wei L, Arbogast D, Collamore M, Wright JJ, Grever M, Shah MH. Phase II trial of sorafenib in metastatic thyroid cancer. J Clin Oncol 2009;27:1675-1684.
49. Kraeber-Bodéré F, Cariou B, Curtet C, Bridji B, Rousseau C, Dravet F, Charbonnel B, Carnaille B, Le Néel JC, Mirallié E. Feasibility and benefit of fluorine 18-fluoro-2-deoxyglucose-guided surgery in the management of radioiodine-negative differentiated thyroid carcinoma metastases. Surgery 2005;138:1176-1182.
50. Kim WW, Kim JS, Hur SM, Kim SH, Lee SK, Choi JH, Kim S, Choi JY, Lee JE, Kim JH, Nam SJ, Yang JH, Choe JH. Radioguided surgery using an intraoperative PET probe for tumor localization and verification of complete resection in differentiated thyroid cancer: a pilot study. Surgery 2011;149:416-424.
51. Francis CL, Nalley C, Fan C, Bodenner D, Stack BC Jr. 18F-fluorodeoxyglucose and 131I radioguided surgical management of thyroid cancer. Otolaryngol Head Neck Surg 2012;146:26-32.
52. Tuttle RM, Rondeau G, Lee NY. A risk-adapted approach to the use of radioactive iodine and external beam radiation in the treatment of well-differentiated thyroid cancer. Cancer Control 2011;18:89-95.
53. Van Nostrand D, Moreau S, Bandaru VV, Atkins F, Chennupati S, Mete M, Burman K, Wartofsky L. (124)I positron emission tomography versus (131)I planar imaging in the identification of residual thyroid tissue and/or metastasis in patients who have well-differentiated thyroid cancer. Thyroid 2010;20:879-883.
54. Gulec SA, Kuker RA, Goryawala M, Fernandez C, Perez R, Khan-Ghany A, Apaza A, Harja E, Harrell M. (124)I PET/CT in Patients with Differentiated Thyroid Cancer: Clinical and Quantitative Image Analysis. Thyroid 2016;26:441-448.
55. Freudenberg LS, Jentzen W, Petrich T, Frömke C, Marlowe RJ, Heusner T, Brandau W, Knapp WH, Bockisch A. Lesion dose in differentiated thyroid carcinoma metastases after rhTSH or thyroid hormone withdrawal: 124I PET/CT dosimetric comparisons. Eur J Nucl Med Mol Imaging 2010;37:2267-2276.
56. Jentzen W, Balschuweit D, Schmitz J, Freudenberg L, Eising E, Hilbel T, Bockisch A, Stahl A. The influence of saliva flow stimulation on the absorbed radiation dose to the salivary glands during radioiodine therapy of thyroid cancer using 124I PET(/CT) imaging. Eur J Nucl Med Mol Imaging 2010;37:2298-2306.
57. Rosenbaum-Krumme SJ, Freudenberg LS, Jentzen W, Nagarajah J. Effects of Rosiglitazone on Radioiodine Negative and Progressive Differentiated Thyroid Carcinoma as Assessed by 124I PET/CT Imaging. Clin Nucl Med 2012;37:47-52.
58. Ho AL, Grewal RK, Leboeuf R, Sherman EJ, Pfister DG, Deandreis D, Pentlow KS, Zanzonico PB, Haque S, Gavane S,Ghossein RA, Ricarte-Filho JC, Domínguez JM, Shen R, Tuttle RM, Larson SM, Fagin JA. Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. N Engl J Med 2013;368:623-632.
59. Freudenberg LS, Jentzen W, Görges R, Petrich T, Marlowe RJ, Knust J, Bockisch A. 124I-PET dosimetry in advanced differentiated thyroid cancer: Therapeutic impact. Nuklearmedizin 2007;46:121-128.
60. Freudenberg LS, Antoch G, Frilling A, Jentzen W, Rosenbaum SJ, Kühl H, Bockisch A, Görges R. Combined metabolic and morphologic imaging in thyroid carcinoma patients with elevated serum thyroglobulin and negative cervical ultrasonography: role of 124I-PET/CT and FDG-PET. Eur J Nucl Med Mol Imaging 2008;35:950-957.
61. Grabellus F, Nagarajah J, Bockisch A, Schmid KW, Sheu SY. Glucose transporter 1 expression, tumor proliferation, and Iodine/glucose uptake in thyroid cancer with emphasis on poorly differentiated thyroid carcinoma. Clin Nucl Med 2012;37:121-127.
62. Schlumberger M, Berg G, Cohen O, Duntas L, Jamar F, Jarzab B, Limbert E, Lind P, Pacini F, Reiners C, Sánchez Franco F, Toft A, Wiersinga WM. Follow-up of low-risk patients with differentiated thyroid carcinoma: a European perspective. Eur J Endocrinol 2004;150:105-112.
63. Caballero-Calabuig E, Cano-Terol C, Sopena-Monforte R, Reyes-Ojeda D, Abreu-Sánchez P, Ferrer-Rebolleda J, Sopena-Novales P, Plancha-Mansanet C, Félix-Fontestad J. Influence of the thyroid remnant in the elevation of the serum thyroglobulin after thyroidectomy in differentiated thyroid carcinoma. Importance of the diagnostic iodine total-body scanning. Eur J Nucl Med Mol Imaging 2008;35:1449-1456.
64. de Meer SG, Vriens MR, Zelissen PM, Borel Rinkes IH, de Keizer B. The role of routine diagnostic radioiodine whole-body scintigraphy in patients with high-risk differentiated thyroid cancer. J Nucl Med 2011;52:56-59.
65. Sgouros G, Kolbert KS, Sheikh A, Pentlow KS, Mun EF, Barth A, Robbins RJ, Larson SM. Patient-specific dosimetry for 131I thyroid cancer therapy using 124I PET and 3-dimensional-internal dosimetry (3D–ID) software. J Nucl Med 2004;45:1366-1372.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):66-73 DOI:10.4274/2017.26.suppl.07

I-124 Imaging and Dosimetry

I-124 Görüntüleme ve Dozimetri

Russ Kuker[1], Manuel Sztejnberg[2], Seza Gulec, MD, FACS[3]
[1]*University of Miami Miller School of Medicine, Department of Radiology, Miami, USA*
[2]*National Atomic Energy Commission of Argentina, Division of Instrumentation and Dosimetry, Buenos Aires, Argentina*
[3]*Florida International University Herbert Wertheim College of Medicine, Departments of Surgery and Nuclear Medicine, Miami, USA*

Abstract

Although radioactive iodine imaging and therapy are one of the earliest applications of theranostics, there still remain a number of unresolved clinical questions as to the optimization of diagnostic techniques and dosimetry protocols. I-124 as a positron emission tomography (PET) radiotracer has the potential to improve the current clinical practice in the diagnosis and treatment of differentiated thyroid cancer. The higher sensitivity and spatial resolution of PET/computed tomography (CT) compared to standard gamma scintigraphy can aid in the detection of recurrent or metastatic disease and provide more accurate measurements of metabolic tumor volumes. However the complex decay schema of I-124 poses challenges to quantitative PET imaging. More prospective studies are needed to define optimal dosimetry protocols and to improve patient-specific treatment planning strategies, taking into account not only the absorbed dose to tumors but also methods to avoid toxicity to normal organs. A historical perspective of I-124 imaging and dosimetry as well as future concepts are discussed.
Keywords: I-124 positron emission tomography/computed tomography, dosimetry, thyroid cancer

Öz

Radyoaktif iyot görüntüleme ve tedavisi teranostiğin ilk uygulamalarından biri olmasına rağmen, halen tanı teknikleri ve dozimetri protokollerinin optimizasyonu ile ilgili çözümlenmemiş klinik sorular bulunmaktadır. Pozitron emisyon tomografi (PET) radyofarmasötiği olarak I-124, mevcut klinik uygulamada diferansiye tiroid kanserlerinin tanı ve tedavisini geliştirme potansiyeline sahiptir. Standart gamma sintigrafisi ile karşılaştırıldığında PET/bilgisayarlı tomografinin yüksek duyarlılık ve uzaysal çözünürlüğü nüks veya metastatik hastalığın saptanmasında yardımcı olabilir ve metabolik tümör hacimlerinin daha doğru ölçülmesini sağlayabilir. I-124'ün karmaşık bozunma süreci kantitatif PET görüntüleme için zorluklar teşkil etmektedir. Optimum dozimetre protokollerini tanımlamak ve hastaya özgü tedavi planlama stratejilerini sadece tümör tarafından abzorbe edilen doza değil normal organlara toksisiteyi önleyecek metotlara dayanarak iyileştirecek prospektif çalışmalara ihtiyaç vardır. I-124 görüntüleme ve doz ölçümünün tarihsel perspektifi ile gelecekteki kavramlar tartışılmıştır.
Anahtar kelimeler: I-124 pozitron emisyon tomografi/bilgisayarlı tomografi, dozimetri, tiroid kanseri

Address for Correspondence: Russ Kuker MD, University of Miami Miller School of Medicine, Department of Radiology, Miami, USA
Phone: (305) 585-7955 E-mail: rkuker2@med.miami.edu

Introduction

The mainstay of current clinical radioactive iodine (RAI) imaging and dosimetry utilizes the radioisotope I-131. The physical half life of I-131 is 8.02 days that allows imaging and data acquisition at sequential time points over a period of several days. The high energy gamma emissions of 364 keV, however, are a drawback due to poor image quality resulting in less than optimal evaluation of extent of disease and quantitation. Another factor contributing to poor image quality is the relatively small administered activities in the range of 2-5 mCi. The clinical safety/efficacy of higher administered activities of I-131 has been questioned due to concern for the "stunning effect" when it is used prior to RAI treatment. I-131 remains the standard treatment for differentiated thyroid cancer (DTC) because of its beta emissions with a maximum energy of 606 keV and tissue penetration of 0.6-2 mm.

I-123 is predominantly a gamma emitter with an energy of 159 keV, which is near the optimal range for standard gamma camera imaging. In addition, higher administered activities can be used as compared to I-131 because of less concern for stunning. Although the physical properties of I-123 contribute to improved image quality, there are also several drawbacks. I-123 is is more expensive and is not as widely available as I-131. The absence of beta decay does not permit the use of I-123 for therapeutic applications, and the relatively short half life of 13 hours limits its utility for dosimetry protocols.

I-124 is a PET radiopharmaceutical with a 4.2-day half life. It offers superior imaging characteristics with enhanced spatial resolution and image sensitivity due to coincidence detection on PET cameras. I-124 also has a favorable half life that permits the evaluation of in-vivo iodine kinetics. However, I-124 has a rather complex decay schema, which creates challenges in optimal imaging. There exist a number of reports attesting the potential clinical benefits of I-124 imaging in patients with DTC, however, a uniform clinical protocol for imaging, image analysis and quantitation has not yet been firmly established.

History of I-124 in Clinical Practice

The use of I-124 in clinical practice in a patient with DTC was first described in 1960 (1), and the first PET-based dosimetry study based on serial measurements in DTC patients was published in 1999 (2) after phantom studies demonstrated that quantification was feasible (3). Since then, there have been several key articles demonstrating the clinical utility of I-124 and its role in the detection of residual thyroid tissue and/or metastasis in patients with DTC (4,5,6,7,8); and the following are some more recent highlights.

In 2010, Van Nostrand et al. (6) compared the ability of I-124 PET versus I-131 planar whole body imaging in detecting residual thyroid tissue and/or metastatic DTC. Twenty-five patients were included in the study. Eight of the 25 patients showed more positive foci of uptake on I-124 images than on I-131. I-124 demonstrated the same number of foci as on I-131 in 16 patients. One patient had one additional positive focus on I-131 not seen on I-124, which could not be confirmed as a metastasis. Out of 97 positive foci, I-124 identified 49 that were not seen with I-131, and I-131 identified one positive focus not seen with I-124. They concluded that relative to I-131 planar whole body imaging, I-124 PET identified as many as 50% more foci of radioiodine uptake suggestive of residual thyroid tissue and/or metastases in as many as 32% more patients who had DTC.

In 2015, as part of the THYROPET study, Kist et al. (7) focused on a subset of patients with suspected recurrence from DTC, based on an increased thyroglobulin level and negative neck ultrasound. All patients underwent I-124 PET/CT after rhTSH stimulation. Subsequently, after 4-6 weeks of thyroid hormone withdrawal, the patients were treated with 150-200 mCi of I-131 and underwent a planar whole body scan (WBS) one week later. The study was terminated preliminarily after inclusion of 17 patients. Eight post-therapy WBS were negative (47%), all of which were correctly predicted by a negative I-124 PET/CT. Nine post-therapy WBS showed RAI avid tumor, of which only four also had positive I-124 PET/CT findings. The authors observed that 29% of patients (5/17) would have been denied potentially effective RAI therapy if I-124 PET/CT was implemented. Of the false negative lesions with anatomical correlates on CT, the majority were located in the lungs. They concluded that the high false negative rate of rhTSH stimulated I-124 PET/CT precludes its use as a scouting procedure in this cohort of patients. The authors postulate that their observed false negative rate may have been influenced by the 2 mCi dose of I-124 as compared to the higher doses of I-131 and the use of rhTSH instead of thyroid hormone withdrawal.

In 2016, our group conducted a phase I/II study to determine the imaging characteristics and clinical feasibility of I-124 PET/CT for the determination of disease extent and evaluation of RAI kinetics in its physiologic and neoplastic distribution in patients with DTC (8). Fifteen patients were included in the study. All patients who had I-124 imaging subsequently underwent RAI treatment with administered activities of I-131 in the range of 100 to 300 mCi. Post-treatment planar whole body and static images were obtained 5 to 7 days after RAI treatment and used as a reference to compare with the pre-treatment I-124 PET/CT scans. Forty-six distinct lesions were identified in these 15 patients on I-124 PET/CT images with a sensitivity of 92.5%. I-124 identified 22.5% more foci of RAI avid lesions as compared to the planar I-131 post-treatment scans. In addition, the I-124 images provided discriminating details in terms of location and laterality of remnant thyroid tissue even when no remnant tissue was appreciated on

anatomical imaging. Our group concluded that I-124 PET/CT is a valuable clinical imaging tool/agent, in both extent of disease evaluation in the setting of metastatic DTC and the functional volumetric and kinetic evaluation of target lesions.

I-124 Properties and Decay Schema

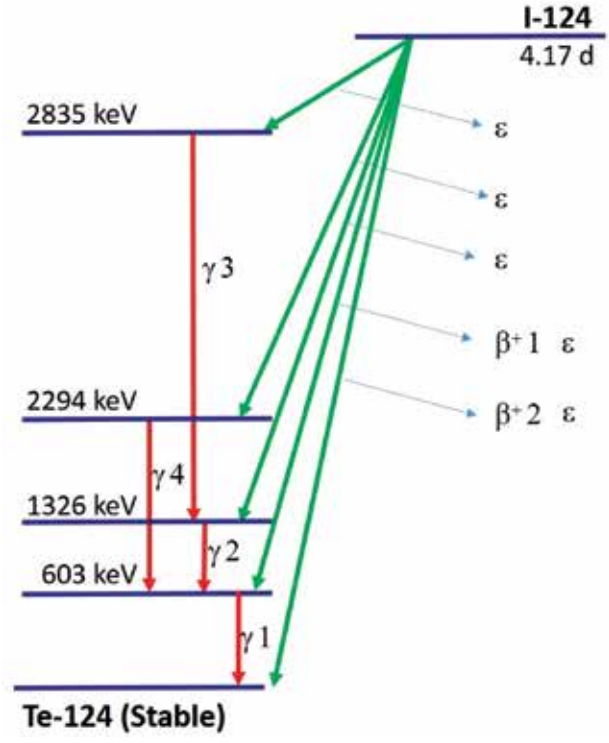
I-124 is cyclotron produced and because of its 4.2-day half life can be easily transported for clinical use. I-124 has a positron abundance (number of positrons emitted per decay) of only 23% with maximum and mean positron energies of 2138 and 819 keV, respectively. In contrast, the most common PET radiotracer, F-18, has a positron abundance of 97% with maximum and mean positron energies of 634 and 250 keV, respectively. It is estimated that this high positron emission energy results in a spatial resolution loss of about 1 mm compared to F-18 FDG which has a spatial resolution on the order of 5-10 mm depending on the scanner and clinical reconstruction parameters (3).

In addition to positron emissions, I-124 emits a rather large portion of gamma rays during its decay, over half of which have an energy of 603 keV (Table 1). Coincidences of this 604 keV photon and a 511 keV annihilation photon cannot be distinguished from the true coincidences involving two 511 keV annihilation photons. These gamma coincidences result in background activity on the PET images which has also been described for other PET radiotracers with longer half lives such as Y-86 and Br-76 (9,10). Multiple correction methods have been suggested to address this background activity but their effectiveness is limited in the setting of the low count rates observed on clinical I-124 scans. In addition, the large amount of gamma emissions leads to higher random coincidence rates, increased dead time and inaccurate dead time correction (10). Improved gamma coincidence correction and dead time correction methods are necessary to ensure accurate quantitative imaging of I-124 (11).

Background of Radioactive Iodine Dosimetry

The general principle of RAI dosimetry relies on the premise that administered activities for diagnostic or therapeutic purposes should be as low as reasonably achievable to limit the radiation burden to the individual as well as the general population. RAI treatment is one of the earliest applications of theranostics and is certainly a strong therapeutic intervention, along with surgical treatment, in the management of DTC. The term "ablation" is used when RAI is given to destroy residual thyroid tissue in the absence of functioning thyroid cancer. The term "therapy" refers to administration of RAI for residual, recurrent or metastatic DTC. Although RAI therapy is generally well tolerated, serious side effects can occur and may be deterministic

Table 1. I-124 simplified decay schema and main emissions



Decay Type	Energy (keV)	Probability (%)
β+ 1	1535	12
β+ 2	2138	11
γ 1	603	63
γ 2	723	10
γ 3	1510	3
γ 4	1691	11
X	27.2	17
X	27.5	31
ε	866	11
ε	2557	25
ε	3160	24

(severity increases with dose and threshold dependent) or stochastic (occur by chance and non-threshold dependent). Deterministic effects of RAI therapy can be early and usually transient (i.e. gastritis, sialadenitis, hypospermia or amenorrhea) or late (i.e. sicca syndrome, lung fibrosis or bone marrow depression). Secondary malignancy is the main stochastic effect of RAI therapy although the incidence is relatively low and comparable to other modalities such as external radiotherapy.

The activity to be used for RAI therapy remains a subject of discussion. Although an empiric fixed-dose RAI treatment protocol is still in common use, the therapeutic profile of RAI is best achieved by application of dosimetry-guided, patient-specific treatment planning. Two approaches for RAI dosimetry have been applied in clinical practice: the maximum safe dose approach which was first introduced by Benua and Leeper and is aimed at administering the highest amount of I-131 which does not result in bone marrow suppression (<2 Gy absorbed dose to the bone marrow with a whole body retention <4.44 GBq at 48 hours) (12,13) and the lesion-based dosimetry method based on the data by Maxon et al. (14,15) in which a target dose (at least 80 Gy to metastatic sites and 300 Gy to thyroid remnants) is calculated on the basis of planar diagnostic I-131 scanning.

The maximum safe dose approach focuses on the safety of RAI treatment. Blood is used as a surrogate of the red bone marrow, which is considered as the critical organ in this approach. Over the years, the original Benua method was refined with improved patient specificity using Medical Internal Radiation Dose (MIRD) methodology. Strengths of the blood-based dosimetry approach include determination of the maximum safe activity of RAI for each individual patient, identification of patients for whom empiric fixed activities are not considered safe, and the potential to administer higher activities at once instead of multiple fractions of lower activity in order to avoid changes in tumor/lesion biokinetics. Although there is a long history of using this treatment strategy at multiple institutions, there is a paucity of clinical data to show improved response or outcome rates. In addition, the absorbed dose to the tumor is not known in this approach and although controversial, there is a risk of stunning with dosimetric administrations of I-131 that may alter tumor/lesion biokinetics during subsequent treatment (16).

The objective of lesion-based dosimetry is to determine the RAI activity that delivers the recommended absorbed dose of radiation to ablate thyroid remnants or treat metastatic disease while minimizing the risk to patients. The calculation of lesion dose is generally based on MIRD methodology and for smaller lesions the spherical model of OLINDA/EXM can be employed. In lesion-based dosimetry, not only is it important to determine how much activity is contained within the lesion, it is also necessary to calculate the mass of the lesion. This can be challenging especially for ablation purposes as measuring the size of thyroid remnants is often not reliable using anatomical imaging such as ultrasound or computed tomography (CT). Some disadvantages of a lesion-based approach to RAI treatment include the wide range of absorbed doses to lesions within a given patient and the inhomogeneous absorbed dose distributions and lack of accurate models to reflect RAI kinetics within individual lesions (16). Since the accuracy of all dosimetric approaches relies on the accurate quantitation of RAI kinetics, I-124 being a PET radiotracer with enhanced spatial resolution may play a pivotal role in this area.

I-124 Dosimetry Protocols and Clinical Applications

Why is I-124 well-suited for RAI dosimetry? Of the available radio-iodine isotopes, I-123 would be the preferred agent for dosimetry given its photopeak that is close to the optimal range for gamma camera imaging; however, its relatively short half life precludes imaging at sequential time points in a practical manner. I-131 is relatively inexpensive, is widely available and has become the mainstay of RAI dosimetry. However, only small administered activities can be given because of the risk of stunning. Additionally the high energy gamma emissions result in poor image quality. I-124 behaves biochemically similar to I-131 and its physical half life of 4.2-days makes it suitable for sequential time point imaging and absorbed dose calculations. With I-131 planar imaging, there is uncertainty regarding RAI avid lesion dimensions, and with single-photon emission computed tomography (SPECT), the counting rate at diagnostic activity levels is inadequate for accurate quantification. Given the added benefit of PET/CT with I-124, not only can fine details be discriminated that are not visible on low-dose planar imaging but also areas of radiotracer uptake can be detected that do not have a measurable anatomical correlation on CT (such as with thyroid remnants as observed in our study). However, there are drawbacks of using I-124 for RAI dosimetry including its relatively high cost and complex decay schema, which may lead to background noise and voxel oversaturation even at low administered activities.

Several investigational studies have successfully used I-124 PET alone and with CT to guide postsurgical treatment and, in particular, RAI therapy in patients with DTC. The number of measurement time points varies for each I-124 dosimetry protocol described in the literature (17,18,19,20,21,22,23). An important observation that should be highlighted is the high intra- and inter-lesional variability in RAI avid tumors (18) as well as in normal tissues such as salivary glands (24,25).

Our group demonstrated different kinetic profiles for normal thyroid remnants, salivary glands, and metastatic lesions as well as individual variations in functional volumes, and thus cumulated activities (8). The sequential

I-124 PET/CT images consistently demonstrated the maximum activity within thyroid remnant tissue to occur at 24 hours. After the peak activity was reached, the clearance was mono-exponential. The maximum remnant activity ranged from 0.044 to 7.988 MBq with the total functional remnant volume (the total number of voxels within the remnant ROI) ranging from 1 to 60 ml. Physiologic activity within the salivary glands reached a peak at 4 hours after radioiodine administration. The salivary gland clearance was bi-exponential with an average of 81% of the activity being cleared from the salivary glands by 24 hours. In contrast, metastatic disease to lymph nodes demonstrated an uptake pattern that was significantly different than the thyroid remnant or physiologic salivary gland activity. A progressive increase in activity with protracted retention was identified as a characteristic pattern for metastatic nodal disease. The remnant data is particularly interesting as it again highlights the potential value of individualized patient-specific dosimetric assessment even for purposes of thyroid remnant ablation rather than an empiric or "one dose fits all" approach. However, a larger scale remnant dosimetry study is required to address this issue.

Why do we need RAI dosimetry? Although many centers have adapted a fixed-dose of I-131 in the range of 100-200 mCi for the treatment of metastatic DTC, there are several drawbacks to using this empiric technique. Without knowledge of the rate of radiobiological clearance as well as the degree of intra-lesion variability in absorbed dose, a proportion of patients will be over- or under-treated using an empiric dose of I-131. Benua et al. (12) observed that repeated sub-therapeutic doses of RAI might induce dedifferentiation and loss of iodine-concentrating ability of tumors. The rationale of using the highest possible dose is based on the radiobiologic fact that radiation treatment efficacy is directly related to the radiation dose delivered. Therefore, the goal of dosimetry-guided RAI therapy is to derive a patient-specific dose that will deliver the highest possible tumoricidal effect to metastatic sites while minimizing toxicity to normal tissues. Another potential application of RAI dosimetry involves the ablation of thyroid remnants where the goal is to deliver the lowest possible ablative dose taking into account patient-specific factors such as the functional volume of thyroid tissue remaining as well as the variability in dose distribution.

Technical Considerations of I-124 Dosimetry

Traditional I-131 dosimetry using MIRD methodology involves sequential time point whole body imaging and blood sampling to obtain time activity curves and residence times for the whole body and bone marrow compartments. The classic formula $D=\tilde{A}xS$, takes into account the cumulated activities derived from both compartments incorporating gamma emissions from the whole body to the bone marrow and beta emissions from the bone marrow to the bone marrow. The S values are obtained from anthropomorphic phantoms and assume uniform distribution of activity within a source organ of defined geometry.

Our group applied the MIRD schema to define a protocol for I-124 dosimetry. The protocol involves acquiring whole body PET/CT images and blood samples at 4 different time points, i.e. 4, 24, 48 and 72 hours following the administration of a 2 mCi dose of I-124. Regions of interest were drawn around the whole body at each time point in order to generate a time activity curve for the whole body compartment. Similarly, I-124 counts were measured from the blood samples at each sequential time point to generate a time activity curve for the bone marrow compartment (assuming a uniform distribution of blood within the bone marrow). Cumulated activities and residence times for the whole body and bone marrow compartments were then obtained by calculating the area under each respective time activity curve. The target organ in this case is the bone marrow since the goal of dosimetry-guided RAI treatment is to deliver the maximum permissible dose to the patient without permanent damage to the bone marrow. The source organs are the whole primarily (primary in the form of gamma emissions) and the bone marrow (primarily in the form of beta emissions). The absorbed dose to the target organ is the sum of the cumulated activities from the source organs multiplied by their respective S values.

What are some drawbacks of this method? One drawback of the traditional MIRD schema is that the absorbed dose to the tumor is not routinely calculated. Given the wide range of dose variability within a tumor, one cannot be confident (based on bone marrow dosimetry alone) that a lesion will receive a sufficient dose to produce the desired tumoricidal effect. Another limitation revolves around the use of the S values which are derived from anthropomorphic phantoms with uniform activity distribution and defined geometry. Although this is generally acceptable for bone marrow dosimetry (with only two source organs), it is not applicable for lesion-based dosimetry where commonly lesions are encountered with complex shapes and non-uniform distributions of activity. A distinct advantage of I-124 dosimetry lies in its application for lesion-based dosimetry where the higher resolution PET/CT images allow for improved lesion contouring and quantification. Our group demonstrated that there is a significant variation in cumulated activities within individual lesions, whether the lesions depict normal physiologic uptake (as in the salivary glands or thyroid remnants) versus metastatic foci (8). These variations in cumulated activities clearly alter the dosimetric input and cannot be distinguished using traditional I-131 techniques.

For complex lesions which cannot be evaluated using standard anthropomorphic phantoms, voxel-based can be employed, which utilizes or not Monte Carlo based codes. In the simplest voxel-based dosimetry, a lesion (whether it

be normal tissue, an individual tumor or a region within a tumor) is divided into voxels of the same 3-dimensional geometry. MIRD formalism is then applied so that the cumulated activity is calculated for each individual voxel comprising the lesion. Each voxel has an assigned S value based on the radioisotope and its location within the lesion, which is assumed to be a homogeneous tissue medium. The absorbed dose to a target lesion is then calculated by summing the products of the cumulated activities and S values of the individual source voxels in a 3-dimensional array (34). The S values are precalculated standarized parameters which do not consider patient-specific tissue heterogeneities. In this case they represent point-dose or voxel-dose kernels. They can be calculated through MC methods (in this case the dosimetry might be considered a type of MC based dosimetry) or through deterministic ones.

MC transport codes can take lesion-based dosimetry to another level by addressing tissue inhomogeneity and assessing their influence on the resulting dose distributions. Target lesions are again divided into iso-volumetric voxels and the cumulated activities are calculated for each individual voxel. However, instead of utilizing S values kernels from standarized uniform tissues, MC based fully developed dosimetries utilize a more complex approach. MC codes can incorporate the probabilistic interactions of radiation with matter including multiple different particles (beta, gamma, photoelectric absorption, Compton scatter and electron-positron pair production) contributing to the absorbed target dose from multiple different pathways that are modeled by computer program computations. MC codes together with appropriate physical data and modeling can provide the most realistic numerical evaluations. Amongst the most evolved, complete and reliable codes of this type are MCNP6 (26), GEANT4 (27,28), and FLUKA (29,30).

Future I-124 Dosimetry Concepts

Areas of future investigation for I-124 dosimetry involve issues related to data collection and data processing. It is widely accepted that 4 time points are required for accurate curve fitting models in order to calculate the cumulated activity. Can this method be streamlined so that fewer time points are needed to generate similar time activity curves with minimal effect on the cumulated activity? This may be plausible given the shorter half life of I-124 as compared to I-131. Another area that our group is focusing on involves the identification of a surrogate for bone marrow activity on anatomical imaging rather than relying on blood sampling. Can a region of interest be drawn on PET/CT (for example around the aorta, vertebral bodies or soft tissues) to simulate the activity within the bone marrow compartment? Our preliminary data indicates that this is likely feasible but a larger patient population is needed for statistical power.

Applying MC codes in I-124 dosimetry is another area of future investigation. MC codes for particle transport pursue the calculation of the random walk, or the track, of (numerical) particles analogous to what would occur in reality. I-124 imaging intrinsically gives information of iodine position and concentration and, therefore, can be used as a surrogate for other iodine isotopes if isotopical correlation and decay correction coefficients are known. Consequently, having iodine distribution defined from I-124 imaging, one can feed the model with decay information of I-124 as well as from other iodine isotopes (considering the mentioned corrections) and obtain dose distributions for isotopes such as I-131.

The combination of MC codes and data from I-124 PET/CT studies can help develop patient- and compound-specific numerical models for 3D dosimetry evaluations (31,32). This type of dosimetry can involve triple voxelized models with resolutions as good as imaging studies allow: voxelized geometry utilizing CT data, voxelized emission distribution utilizing PET data, and voxelized distributions of dose estimators. This means that one can reach optimized resolutions in three aspects: geometry of the regions of interest, source distribution, and detection.

Utilizing this modality, one can assess more realistic particle transport and more representative dose values for large and small regions. The voxelized dose estimators are an important tool for defining hot and cold spots and they provide data for construction of dose-volume histograms or other dosimetry analysis tools. A great advantage of this modality is that it allows for accurate estimation of volume-representative dose values. This means one can determine the volume of influence of a certain dose. For example, if bone marrow is considered, one can determine the maximum, mean, and minimum dose and what fraction of the marrow would receive more than the tolerance dose.

Great contrast appears if this dosimetry modality is compared to previous methods such as those based on the utilization of generic numerical phantoms, e.g. OLINDA/EXM (33), where geometry is not patient specific, organs are considered uniform, emitters are assumed homogeneously distributed in tissues, and doses are whole organ averages. This is also in contrast with more recent modalities based on patient-specific PET/CT images that do not perform the particle transport for each case but utilize standardized fixed point-dose kernels, voxel-dose kernels or other approximations (34). In some cases, the differences are not important but, when heterogeneities are present, large differences might appear. This can be the case for regions with tissue changes or with large activity gradients such as in thyroid remnants.

Conclusions

There are a number of ways in which I-124 PET/CT may contribute to current clinical practice in the management of patients with DTC. Studies have shown that I-124 PET/CT is

a superior imaging agent as compared to diagnostic I-131 planar whole body scintigraphy with lesion detectability similar to post-treatment I-131 scans. In addition, the potential applications of quantitative I-124 PET/CT imaging for RAI treatment planning have been described that show similar benefits over traditional I-131 dosimetry techniques. However, due to the physical properties of I-124 and its complex decay schema, there remains a need for improved correction methods to ensure the accuracy of diagnostic images and quantitative analysis. Also, there is a need for larger prospective trials to address the number and timing of scans needed for optimal dosimetry protocols. Despite its benefits in lesion detection and measurement of metabolic tumor volumes, for I-124 to be used mainstream, it needs to be more commercially available and at a lower cost.

Authorship Contributions

Surgical and Medical Practices: Russ Kuker, Seza Gulec, Concept: Russ Kuker, Seza Gulec, Manuel Sztejnberg, Design: Russ Kuker, Seza Gulec, Data Collection or Processing: Russ Kuker, Seza Gulec, Analysis or Interpretation: Russ Kuker, Seza Gulec, Literature Search: Russ Kuker, Seza Gulec, Writing: Russ Kuker, Seza Gulec, Manuel Sztejnberg.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Phillips AF, Haybittle JL, Newberry GR. Use of iodine-124 for the treatment of carcinoma of the thyroid. Acta Unio Int Contra Cancrum 1960;16:1434-1428.
2. Erdi YE, Macapinlac H, Larson SM, Erdi AK, Yeung H, Furhang EE, Humm JL. Radiation dose assessment for I-131 therapy of thyroid cancer using I-124 PET imaging. Clin Positron Imaging 1999;2:41-46.
3. Pentlow KS, Graham MC, Lambrecht RM, Daghighian F, Bacharach SL, Bendriem B, Finn RD, Jordan K, Kalaigian H,Karp JS, Robeson WR, Larson SM. Quantitative imaging of iodine-124 with PET. J Nucl Med 1996;37:1557-1562.
4. Freudenberg LS, Antoch G, Jentzen W, Pink R, Knust J, Görges R, Müller SP, Bockisch A, Debatin JF, Brandau W. Value of 124I-PET/CT in staging of patients with differentiated thyroid cancer. Eur Radiol. 2004;14:2092-2098.
5. Phan HT, Jager PL, Paans AM, Plukker JT, Sturkenboom MG, Sluiter WJ, Wolffenbuttel BH, Dierckx RA, Links TP. The diagnostic value of I124I- PET in patients with differentiated thyroid cancer. Eur J Nucl Med Mol Imaging 2008;35:958-965.
6. Van Nostrand D, Moreau S, Bandaru VV, Atkins F, Chennupati S, Mete M, Burman K, Wartofsky L. (124)I positron emission tomography versus (131)I planar imaging in the identification of residual thyroid tissue and/or metastasis in patients who have well-differentiated thyroid cancer. Thyroid 2010;20:879-883.
7. Kist JW, de Keizer B, van der Vlies M, Brouwers AH, Huysmans DA, van der Zant FM, Hermsen R, Stokkel MP, Hoekstra OS, Vogel WV; THYROPET Study Group; other members of the THYROPET Study group are John M.H. de Klerk. 124I PET/CT to predict the outcome of blind 131I treatment in patients with biochemical recurrence of differentiated thyroid cancer; results of a multicenter diagnostic cohort study (THYROPET). J Nucl Med 2016;57:701-707.
8. Gulec SA, Kuker RA, Goryawala M, Fernandez C, Perez R, Khan-Ghany A, Apaza A, Harja E, Harrell M. (124)I PET/CT in patients with differentiated thyroid cancer: clinical and quantitative image analysis. Thyroid 2016;26:441-448.
9. Pentlow KS, Finn RD, Larson SM, Erdi YE, Beattie BJ, Humm JL. Quantitative imaging of Yttrium-86 with PET. The occurrence and correction of anomalous apparent activity in high density regions. Clin Positron Imaging 2000;3:85-90.
10. Lubberink M, Schneider H, Bergstrom M, Lundqvist H. Quantitative imaging and correction for cascade gamma radiation of Br-76 with 2D and 3D PET. Phys Med Biol 2002;47:3519-3534.
11. Lubberink M, Abdul Fatah S, Brans B, Hoekstra OS, Teule GJ. The role of (124)I PET in diagnosis and treatment of thyroid carcinoma. Q J Nucl Med Mol Imaging 2008;52:30-36.
12. Benua RS, Cicale NR, Sonenberg M, Rawson RW. The relation of radioiodine dosimetry to results and complications in the treatment of metastatic thyroid cancer. AJR 1962;87:171-182.
13. Benua RS, Leeper RD. A method and rationale for treating thyroid carcinoma with the largest safe dose of I-131. In: Meideros-Neto GA, Gaitan E (eds). Frontiers of Thyroidology Vol. II. New York, NY: Plenum, 1986:1317-1321.
14. Maxon HR, Thomas SR, Hertzberg VS, Kereiakes JG, Chen IW, Sperling MI, Saenger EL. Relation between effective radiation dose and outcome of radioiodine therapy for thyroid cancer. N Engl J Med 1983;309:937-941.
15. Maxon HR, Englaro EE, Thomas SR, Hertzberg VS, Hinnefeld JD, Chen LS, Smith H, Cummings D, Aden MD. Radioiodine-131 therapy for well differentiated thyroid cancer–a quantitative radiation dosimetric approach: outcome and validation in 85 patients. J Nucl Med 1992;33:1132-1136.
16. Lassmann M, Reiners C, Luster M. Dosimetry and thyroid cancer: the individual dosage of radioiodine. Endocr Relat Cancer 2010;17:161-172.
17. Eschmann SM, Reischl G, Bilger K, Kupferschlager J, Thelen MH, Dohmen BM, Besenfelder H, Bares R. Evaluation of dosimetry of radioiodine therapy in benign and malignant thyroid disorders by means of iodine-124 and PET. Eur J Nucl Med Mol Imaging 2002;29:760-767.
18. Sgouros G, Kolbert KS, Sheikh A, Pentlow KS, Mun EF, Barth A, Robbins RJ, Larson SM. Patient-specific dosimetry for 131I thyroid cancer therapy using 124I PET and 3-dimensional-internal dosimetry (3D-ID) software. J Nucl Med 2004;45:1366-1372.
19. Freudenberg LS, Jentzen W, Görges R, Petrich T, Marlowe RJ, Knust J, Bockisch A. 124I-PET dosimetry in advanced differentiated thyroid cancer: therapeutic impact. Nuklearmedizin 2007;46:121-128.
20. Jentzen W, Freudenberg L, Eising EG, Sonnenschein W, Knust J, Bockisch A. Optimized 124I PET dosimetry protocol for radioiodine therapy of differentiated thyroid cancer. J Nucl Med 2008;49:1017-1023.
21. Freudenberg LS, Frömke C, Petrich T, Marlowe RJ, Koska WW, Brandau W, Eising EG, Knust EJ, Bockisch A, Jentzen W. Thyroid remnant dose: 124I-PET/CT dosimetric comparison of rh TSH versus thyroid hormone withholding before radioiodine remnant ablation in differentiated thyroid cancer. Exp Clin Endocrinol Diabetes 2010;118:393-399.
22. Freudenberg LS, Jentzen W, Petrich T, Frömke C, Marlowe RJ, Heusner T, Brandau W, Knapp WH, Bockisch A. Lesion dose in differentiated thyroid carcinoma metastases after rh TSH or thyroid hormone withdrawal: 124I PET/CT dosimetric comparisons. Eur J Nucl Med Mol Imaging 2010;37:2267-2276.
23. Plyku D, Hobbs R, Huang K, Atkins F, Garcia C, Sgouros G, Van Nostrand D. 124I-PET/CT based tumor dosimetry for 131I therapy of metastatic differentiated thyroid cancer (DTC)-a comparison of recombinant human thyroid-stimulating hormone vs thyroid hormone withdrawal patient preparation methods. J Nucl Med 2015;56(Suppl 3):394.
24. Kolbert KS, Pentlow KS, Pearson JR, Sheikh A, Finn RD, Humm JL, Larson SM. Prediction of absorbed dose to normal organs in thyroid cancer patients treated with 131I by use of 124I PET and 3-dimensional internal dosimetry software. J Nucl Med 2007;48:143-149.

25. Hobbs RF, Jentzen W, Bockisch A, Sgouros G. Monte Carlo-based 3-dimensional dosimetry of salivary glands in radioiodine treatment of differentiated thyroid cancer estimated using 124I PET. Q J Nucl Med Mol Imaging 2013;57:79-91.
26. Goorley T, James M, Booth T. Initial MCNP6 Release Overview LA-UR-11-07082. Los Alamos National Laboratory. Nucl Technol 2012;80:298-315.
27. Allison J, Amako K, Apostolakis J, Araujo H. Geant4 developments and applications. IEEE Trans Nucl Sci 2006;53:270-278.
28. Agostinelli S, Allison J, Amako K, Apostolakis J, Araujo H, Arce P, Asa M, Axen D, Banerjee S, Barrand G, Behner F, Bellagamba L, Boudreau J, Broglia L, Brunengo A, Burkhardt H, Chauvie S, Chuma J, Chytracek R, Cooperman G, Cosmo G, Degtyarenko P, Dell'Acqua A, Depaola G, Dietrich D, Enami R, Feliciello A, Ferguson C, Fesefeldt H, Folger G, Foppiano F, Forti A, Garelli S, Giani S, Giannitrapani R, Gibin D. Geant4–a simulation toolkit. Nucl Instr Meth Phys Res A 2003;506:250-303.
29. Ferrari A, Sala PR, Fassò A, Ranft J. FLUKA: a multi-particle transport code. CERN-2005-10 (2005), INFN/TC_05/11, SLAC-R-773.
30. Battistoni G, Muraro S, Sala PR, et al. The FLUKA code: Description and benchmarking. Proc. Hadronic Shower Simulation Workshop 2006, Fermilab 6-8 September 2006, Albrow M and Raja R eds. AIP Conf Proc 2007;896:31-49.
31. Sgouros G, Hobbs RF, Atkins FB, Van Nostrand D, Ladenson PW, Wahl RL. Three-dimensional radiobiological dosimetry (3D-RD) with 124I PET for 131I therapy of thyroid cancer. Eur J Nucl Med Mol Imaging 2011;38(Suppl 1):41-47.
32. Botta F, Mairani A, Hobbs RF, Vergara Gil A, Pacilio M, Parodi K, Cremonesi M, Coca Pérez MA, Di Dia A, Ferrari M, Guerriero F, Battistoni G, Pedroli G, Paganelli G, Torres Aroche LA, Sgouros G. Use of the FLUKA Monte Carlo code for 3D patient-specific dosimetry on PET-CT and SPECT-CT images. Phys Med Biol 2013;58:8099-8120.
33. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second generation personal computer software for internal dose assessment in nuclear medicine. J Nucl Med 2005;46:1023-1027.
34. Bolch WE, Bouchet LG, Robertson JS, Wessels BW, Siegel JA, Howell RW, Erdi AK, Aydogan B, Costes S, Watson EE, Brill AB, Charkes ND, Fisher DR, Hays MT, Thomas SR. MIRD Pamphlet No. 17: The dosimetry of nonuniform activity distributions–radionuclide S values at the voxel level. Medical Internal Radiation Dose Committee. J Nucl Med 1999;40:11-36.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):74-79 DOI:10.4274/2017.26.suppl.08

Radioactive Iodine Therapy of Differentiated Thyroid Carcinoma: Redesigning the Paradigm

Diferansiye Tiroid Kanserinde Radyoaktif İyot Tedavisi: Paradigmanın Yeniden Dizaynı

Stanley J. Goldsmith

Weill Cornell Medical College, Clinic of Radiology; New York-Presbyterian Hospital, Clinic of Radiology; Weill Medical College of Cornell University, Departments of Radiology and Medicine, New York, USA

Abstract

Radioactive iodine therapy has evolved over the past 70 years from treatment of known metastatic thyroid carcinoma to include adjuvant use to decrease the incidence of recurrent disease and to ablation of normal remnant tissue following thyroidectomy, even for minimal tumor involvement. Advances in laboratory testing, development of drugs useful in radioiodine treatment, as well as advances in radiation detection and imaging instrumentation, have progressively improved the utility of radioiodine therapy of differentiated thyroid carcinoma. Guidelines have proliferated and they have become more detailed and complex. This trend is likely to continue as the science and technology involved increases in sophistication and efficacy.

Keywords: Radioactive iodine, differentiated thyroid carcinoma

Öz

Son 70 yıl içinde radyoaktif iyot tedavisi metastatik tiroid kanseri tedavisinde kullanımdan minimal tümör tutulumunda bile nüks insidansını azaltmak ve tiroidektomi sonrasında kalan normal dokunun ablasyonunu sağlamak için adjuvan amaçlı kullanılmaya başlamıştır. Laboratuvar testlerindeki ilerlemeler, radyoaktif iyot tedavisinde yararlı ilaçların geliştirilmesi ve radyasyon algılama ve görüntüleme cihazlarındaki gelişmeler diferansiye tiroid kanserinde radyoaktif iyot tedavisinin yararını arttırmıştır. Bu konudaki kılavuz sayısı artmış ve daha detaylı ve karmaşık hale gelmiştir. Bu eğilim ilgili bilim ve teknoloji alanlarında kapsam ve etkinliğin artması ile birlikte gelecekte de devam edecektir.

Anahtar kelimeler: Radyoaktif iyot, diferansiye tiroid karsinomu

Introduction

Following the initial production of radioactive isotopes of iodine in 1937, physicians and physicists from the Massachusetts General Hospital and the Massachusetts Institute of Technology used these tracers, initially in animals, for the study of iodine physiology. In their initial publication based on studies in 48 rabbits, they concluded that *"it is therefore logical to suppose that when strongly active materials are available, the concentrating power of hyperplastic and neoplastic thyroid for radioactive iodine may be of clinical or therapeutic significance"*. Subsequently, the Massachusetts team studied the biodistribution and kinetics of iodine radiotracers in humans, particularly in patients with hyperfunctioning thyroid glands. They observed increased accumulation of the administered radioiodine in the thyroids of animals and patients with increased thyroid gland function (1). Shortly thereafter, in March 1941, Saul Hertz, MD, the Chief of the Endocrine Clinic at the Massachusetts General Hospital, and Arthur Roberts, a physicist at the Massachusetts Institute of Technology, administered multiple doses of radioactive iodine to a patient who had a hyper-functioning thyroid gland. The radioiodine

Address for Correspondence: Stanley J. Goldsmith MD, Weill Cornell Medical College, Clinic of Radiology; New York-Presbyterian Hospital, Clinic of Radiology; Weill Medical College of Cornell University, Departments of Radiology and Medicine, New York, USA
E-mail: sjg2002@med.cornell.edu

was likely a mixture of the 12-hour half-life Iodine-130 and the 8 day half-life ^{131}iodine (^{131}I). Their initial results on the treatment of hyperthyroidism with radioactive iodine, the first application of targeted radionuclide therapy, were presented at a scientific meeting in Atlantic City in 1942 but were not published until after the conclusion of World War II (2). Nevertheless, the news spread that radioactive iodine, in sufficient quantity, could retard thyroid function. In 1942, Samuel Seidlin, the Chief of Endocrinology at the Montefiore Hospital in the Bronx, New York began to evaluate the distribution of radioiodine in a patient who was exhibiting features of hyperthyroidism despite previously having had his thyroid gland removed for the diagnosis of thyroid carcinoma. Seidlin found no localization in the region of the thyroid but definite evidence of radioactivity accumulation in the right parietal region of the patient's skull at the site of a soft tissue swelling that was demonstrated on subsequent x-ray examination to coincide with a lytic osseous lesion. He concluded that this mass was metastatic differentiated thyroid carcinoma. Following repeated administration of increased amounts of radioactive iodine preparations, less and less localization of radioactivity was noted in the sites of previous accumulation and the patient's clinical symptoms of hyperthyroidism cleared with a notable gain in body weight and an overall improvement in his quality of life (2). At the time, this experience was unusual as other patients with malignant thyroid tumors did not demonstrate vigorous radioiodine uptake. It was also in 1942 that Albert Keston and colleagues at the College of Physicians and Surgeons of Columbia University observed vigorous radioiodine uptake in boney metastases following at least sub-total thyroidectomy, leading to the conclusion that metastatic differentiated thyroid carcinoma concentrated radioiodine better if the bulk of the thyroid gland had been removed previously (3). Following the conclusion of World War II, the embargo on publication of research on nuclear materials was lifted and medical reports of these experiences with radioactive iodine as a therapeutic agent were published. Thus, targeted radionuclide therapy was born. The term "atomic cocktail" was coined to describe treatment of thyroid disease (both thyroid cancer and hyperthyroidism). The "Atoms for Peace" program was initiated, stimulating public interest and government support for the production of radionuclides for use in medicine.

A Paradigm Develops

It was soon realized, however, that treatment of thyroid cancer was not as simple as simply administering radioactive iodine orally or parenterally.

- There was the issue of patient preparation. In 1948, Seidlin reported his several year experiences with the use of radioiodine in patients with differentiated thyroid malignancy, confirming Keston's findings and concluding that it was necessary to eliminate the normal thyroid tissue to optimize radioiodine uptake by thyroid metastases. This recommendation was reaffirmed by Drs. William Blahd and William Bierwaltes, clinical thyroidologists in Los Angeles and Ann Arbor (4,5). In 1984, Bierwaltes concluded "there is no question... we should ablate normal thyroid as part of the treatment of differentiated thyroid carcinoma" (5).

- Over time, the protocol involving patient preparation prior to ^{131}I therapy became more complex. After 1947, the radionuclide used was likely always ^{131}I. It was recognized that ^{131}I uptake of remnant tissue and differentiated tumor was augmented when in addition to removal of the bulk of the normal thyroid tissue, ^{131}I uptake of residual tissue and tumor was stimulated when the patient was hypothyroid; that is deprived of thyroid hormone producing elevation of serum throid-stimulating hormone (TSH)-although this assay was not routinely available for many years. The practice arose to discontinue replacement thyroid hormone-usually desiccated thyroid hormone, prepared from bovine or porcine thyroid tissue that had became available from the meat industry. Subsequently, animal-origin TSH also became available by extracting TSH from the pituitary gland of slaughtered animals. Although the use of animal-derived TSH was more convenient than discontinuing thyroid hormone replacement and allowing hypothyroidism to develop over 4-6 weeks, the practice was discontinued in the early 1970s because of urticarial and other allergic reactions to the foreign protein. Because the TSH assay was not as conveniently available as it is today, there was a delay in obtaining TSH levels. Nevertheless, the practitioner had the option of determining that there was clinical evidence of hypothyroidism. It was considered good practice to at least draw a blood sample to retrospectively confirm that the serum TSH was indeed elevated to 30 uU/ml or more.

- The practice of radioiodine therapy of differentiated thyroid carcinoma was becoming more complex. The patient was initially evaluated by the clinician and advised to discontinue thyroid hormone. After 3-4 weeks, a low iodine diet was initiated and at week 5 or 6, the patient was evaluated clinically for evidence of a thyroid deficient state, followed by administration of a dose of ^{131}I either to assess if there was evidence of functioning metastases, or as ^{131}I therapy.

- Until the development of mechanical scanning equipment, the involved clinician performed "hand scanning" several days after the ablative or therapy ^{131}I dose, or at follow-up evaluations; that is, the clinician held a radiation detecting instrument over various body parts, particularly the neck, anterior mediastinum, spine and the long bones. The instrumentation evolved from simple Geiger-Muller type devices to shielded probes

with scintillation crystals and a photomultiplier tube. With sufficient shielding, the device provided more specific localization of the radioiodine accumulation. The clinician relied on an audio output proportional to the count rate. Hand-scanning was supplemented by collection of the entire urinary output for 24-48 hours. An aliquot of the total volume was counted and the percent of the total excretion of the administered radioactivity was determined. If all of the activity could be accounted for based on counting an aliquot of the collected urine, the patient was deemed to be free of functioning metastases. Of course, the urine collections were rarely complete, necessitating a decision by the clinician whether or not the discrepancy represented simply incomplete collection or retention by tissue metastases which could be confirmed by the radiation detecting probe. Although the hand-held probe and lead shielding weighed perhaps 25-30 lbs, this practice continued through the 1960s when it was replaced by rectilinear scanners that produced life size images of the scanned area (6).

- In the United States, the practice developed that requiring hospitalization and relative isolation of patients receiving >30 mCi of ^{131}I until external detecting instruments indicated that the whole body burden had decreased below that level.

- Subsequently, Mazzaferri (7) reported on the clinical outcome of 1,004 patients with differentiated thyroid carcinoma who were more than 45 years old and had tumors greater than 1.5 cm, either given no post-operative medical therapy, received thyroid hormone replacement or remnant ablation with 1.1-7.2 GBq (30-200 mCi) of ^{131}I. Median follow-up was 21.3 years, 18.7 years and 14.7 years, respectively. There was no difference identified between patients receiving a low or high dose of ^{131}I. In the group who received ^{131}I ablation, regardless of the dose, tumor recurrence was significantly lower, fewer patients developed distant metastases and there were fewer cancer deaths than in the other groups.

- Hence, the practice of radioactive iodine therapy as treatment for metastatic disease evolved toward a paradigm that advised radioactive iodine (^{131}I) for virtually all patients with the diagnosis of differentiated thyroid cancer (DTC) to include treating patients with:

- Metastatic disease (the original indication for radioiodine therapy), treatment of patients with probable residual tumor based on histopathology evidence of positive margins, extra-thyroidal extension or tumor involvement in resected lymph nodes,

- Adjuvant therapy based on the assumption that there might be occult tumor based on assessment of risk factors,

- Ablation of remnant tissue to improve the sensitivity of radioactive iodine to detect and destroy recurrent disease.

As a practical matter, there is little difference between adjuvant therapy (radioiodine administration based on the assumption that there is residual disease even though it has not been confirmed) and ablative therapy, radioiodine administration to eliminate residual normal tissue so that it would not compete for administered radioiodine in the event that there was tumor recurrence although, in general, practitioners often administered 30 mCi doses (1.11 GBq) as an ablative dose whereas 75-150 mCi (2.75-5.50 GBq) doses were recommended for adjuvant therapy (8,9).

Existing Paradigms

The present practice of radioiodine therapy including ablation has grown increasingly complex. Professional and scientific organizations responded to these complexities by the creation and publication of Guidelines setting forth a "paradigm" for the utilization of ^{131}I in the management of patients with differentiated carcinoma arising from the follicular cells of the thyroid. Paradigms are generally assumed to provide useful guidance for patient management of complex clinical situations (Boxed Text 1).

Definition of "Paradigm"

In the on-line Urban Dictionary, there are multiple definitions of the word "paradigm":

1. "An example the majority of people follow; an established set of values or ways" (Miso)

2. "... a closed set of scientific theories that is coherent and is well accepted by the larger scientific community" (Nathan Leichoz)

3. "The most annoying and misused word in the English language; used intentionally by stupid people to sound smart or by smart people to sound unintentionally stupid" (Jambone)

In 2002, the Society of Nuclear Medicine published Version 2 of a Guideline entitled "Procedure Guideline for Therapy of Thyroid Disease with ^{131}I" (Boxed Text 2) (9).

Version 2 of the Society of Nuclear Medicine Guideline in the use of ^{131}I for the Treatment of Thyroid Disease

Part I: Purpose

Part II: Background information and definitions

Part III: Common indications

Part IV: Procedure

Part V: Issues requiring further clarification

Part VI: Concise bibliography

Part VII: Disclaimer

This document provided clinical practice guidelines for the use of ^{131}I for the treatment of hyperthyroid, non-toxic goiter and thyroid carcinoma. The document was 5 pages in length as published in the Journal of Nuclear Medicine. The Guideline consisted of 6 parts (Boxed Text 2). Part V of this Procedure Guideline is entitled "Issues Requiring Further Clarification" and identifies the following:

- The use of ^{131}I whole-body imaging before ^{131}I therapy for thyroid cancer and whether "stunning" of the thyroid remnant occurs.

- The role of alternative imaging agents such as ^{123}I to avoid stunning.

- The necessity of treating small (<1.0 cm) papillary cancers with ^{131}I.

- Treatment of ^{131}I-scan-negative, thyroglobulin positive patients.

- The role of recombinant human TSH in therapy.

Part VI is a Concise Bibliography containing 15 references. The Guideline concludes with a Part VII: Disclaimer which is 1 paragraph in length concluding with the following sentence: "Advances in medicine occur at a rapid rate. The date of a guideline should always be considered in determining its current applicability." In 2012, Version 3 entitled "The Society of Nuclear Medicine Practice Guideline for Therapy of Thyroid Disease with ^{131}I 3.0" was published; it is 19 pages in length (Boxed Text 3) (10).

Version 3 of "The Society of Nuclear Medicine Practice Guideline for Therapy of Thyroid Disease with ^{131}I 3.0"

Preamble

Introduction: patient management, licensure

Goals

Definitions: risk levels

Common clinical indications

Qualifications and responsibilities of personnel (in the United States)

Procedure/specifications of the examination

Documentation/reporting

Equipment specification

Quality control and improvements

Safety, infection control, and patient education concerns

Radiation dosimetry

Acknowledgements

References

Approval

The portion specific to ^{131}I therapy of thyroid cancer alone is 8 pages (Boxed Text 4).

"The Society of Nuclear Medicine Practice Guideline for Therapy of Thyroid Disease with ^{131}I 3.0"

VI. Procedure/specifications of the examination

A. Therapy of Graves disease, toxic nodules, and nontoxic nodular goiter

B. ^{131}I therapy of thyroid cancer to ablate post-thyroidectomy remnants and destroy residual or recurrent tumor

1. Indications for treatment with ^{131}I: relationship to staging

2. Patient preparation and information the patient needs: diet, thyroid-stimulating hormone level, informed consent, side effects

3. Information required by the physician performing the procedure: blood tests

4. Selection of activity

5. Therapeutic procedure for administration of ^{131}I

6. Follow-up

C. Radiation Safety issues, patient discharge, home instructions

D. Interactions of ^{131}I with other forms of diagnosis or treatment

E. Radiopharmaceuticals

F. Issues requiring further clarification

Within this Section, there is a sub-section entitled "Issues requiring further clarification".

1. Utility of routine use of ^{123}I or ^{131}I whole-body imaging, especially SPECT/CT in patients after total thyroidectomy before initial ^{131}I ablation therapy for thyroid cancer,

2. Pathologic and prognostic significance of stunning of the thyroid remnant and metastatic deposits,

3. Diagnostic role of alternative imaging agents for thyroid cancer such as ^{123}I, ^{124}I and ^{99m}Tc,

4. The role of ^{124}I in thyroid dosimetry, and the efficacy of lesion dosimetric planning,

5. The necessity of therapy for low risk papillary cancers less than 1.0 cm in diameter if there is an unfavorable molecular assessment [e.g. *BRAF* expression (a proto-oncogene encoding a serine/threonine protein kinase called B-RAF)], unfavorable histology, and no evidence of distant metastases,

6. Equivalence between recombinant human TSH... and endogenous TSH elevation from thyroid hormone withdrawal,

7. Frequency and length of long term follow-up after ^{131}I therapy... in a variety of clinical situations,

8. Prediction of the time required for the TSH to rise sufficiently... after thyroid hormone withdrawal... before ^{131}I therapy,

9. The need to attain a serum TSH level of at least 30 uU/ml,

10. Standardization of ^{131}I dosimetry to deliver... ablative radiation doses to remnants,

11. Benefits and risks of empiric high-activity ^{131}I therapy (e.g. >9.25 GBq [250 mCi]) for patients with serum thyroglobulin elevation but negative iodine scintigraphy,

12. Therapeutic benefit of administered activities in excess of, for example 9.25 GBq in iodine-avid metastatic disease, relative to lower activities of ^{131}I,

13. Determination of whether external-beam radiotherapy delivered to the neck metastases before therapeutic ^{131}I decreases the subsequent ^{131}I therapeutic effect,

14. Prevention of radiation sialadenitis and oral mucositis.

In 2008, the European Association of Nuclear Medicine (EANM) published a 19 page Guideline in the European Journal of Nuclear Medicine and Molecular Imaging (11). This guideline is quite complete. There are no substantial differences between it and the Society of Nuclear Medicine and Molecular Imaging (SNMMI) Guideline. The Abstract and abstracted Discussion of the publication reads as follows".

"**Abstract:** The purpose of the present guidelines on the radioiodine therapy of DTC formulated by the EANM Therapy Committee is to provide advice to nuclear medicine clinicians and other members of the DTC-treating community on how to ablate thyroid remnant or treat inoperable advanced DTC or both employing large ^{131}I activities. Discussion: For this purpose, recommendations have been formulated based on recent literature and expert opinion regarding the rationale, indications and contraindications for these procedures, as well as the radioiodine activities and the administration and patient preparation techniques to be used."

The International Atomic Energy Agency has published a 271-page Technical Document entitled "Nuclear Medicine in Thyroid Cancer Management: A Practical Approach" that covers essentially the same material, providing greater detail in the description of procedures (12). There are still some differences in radiation safety requirements among several European countries. In this regard, the United States is currently less restrictive in terms of the need to isolate patients following ^{131}I administration, basing the requirement of exposure of others and taking into account the term "occupancy factor". Since, except for overnight sharing of a bed, most individuals will not spend an extended period in close proximity to another individual, the exposure that any member of the public or even immediate family would receive is only a fraction of the total radiation flux emitted from the treated individual. The exposure another individual would receive is a product of the residual activity in the treated patient (a value that in the case of ^{131}I decreases rapidly based on renal excretion), the proximity to the patient as a source (with the radiation flux falling off rapidly based on the inverse square of the distance; as a practical matter, at one meter, the value is less than 10% of the flux detected at the source) and the duration of time at that location-a value that is reflected in the occupancy factor rather than assuming total exposure of another individual from all of the source activity. Other than that element of radioiodine administration, there is general agreement about the issues and the degree of certainty or uncertainty in the benefits associated with any component of the procedures and decisions involved.

Finally, in 2015, the American Thyroid Association (ATA) published in it's original form as a 411-page document entitled "Management Guidelines for Adult Procedures with Thyroid Nodules and Differentiated Thyroid Cancer". Seventy-two pages deal with the diagnosis, procedures and decisions involved in the assessment of thyroid nodules. Nevertheless, of the remaining 339 pages, 278 are devoted to various aspects of DTC and management. There are 94 pages of references and 1078 references are cited. This document has been reformatted and published in the journal Thyroid in January 2016 (13). The ATA Guidelines provide recommendations about what procedure or action is reasonable given the evidence available in addition to assessing the quality of the research or data upon which published conclusions are based (and providing a grade for the quality of that data). "As of February 2014, the SNMMI Guidelines will now be referred to as Procedure Standards. This change was initiated in an effort to better reflect the terminology being used by external organizations. Any previous Practice Guideline that describes how to perform a procedure is now considered as SNMMI Procedure Standard" (Quoted from the SNMMI website under the heading Procedure Standards). Collectively, these Guidelines are quite comprehensive. They present a thoughtful review of the state of knowledge at the time the Guideline was released and published. In addition, they identify areas that require further clarification (research). Nevertheless, the diagnosis and management of patients with differentiated thyroid carcinoma continues to be the subject of a great deal of clinical and when possible, basic research. Recently, in fact, a remarkable observation has been reported: the potential use of a class on pharmaceuticals, tyrosine kinase inhibitors, that suppress or counteract the genome defect that produced a proto-oncogene involved in the failure of differentiated thyroid carcinoma cells to express the Sodium Iodide transporter, thus restoring the ability of these cells to trap and organify (thus retaining) iodide ion, in general, and ^{131}I in particular into metastatic sites that had not demonstrated radioiodine ^{131}I uptake. The potential for this exciting therapeutic intervention was initially observed in a subset of patients in whom the potential for effective ^{131}I therapy was predicted based on ^{124}I positron emission tomography imaging and dosimetry which was subsequently confirmed (14).

Redesigning the Paradigm

This review has identified existing Guidelines or Procedure Standards (the current preferred nomenclature) for Radioactive Iodine Therapy of Differentiated Thyroid Carcinoma. They are comprehensive and lengthy. Upgrades will be necessary from time to time as new basic research identifies opportunities for clinical innovations and clinical research validates opinions and recommendations of the "cognoscenti". At this time, however, other than enriching what already exists with appropriate upgrades, there does not appear to be a need to "redesign the paradigm".

References

1. Hertz S, Roberts A, Evans RD, Jackson H. Radioactive iodine as an indicator in the study of thyroid physiology. Proc Soc Exper Med Biol 1938;38:510-513.
2. Hertz S, Roberts A. Radioactive iodine in the study of thyroid physiology VII. The use of radioactive iodine therapy in hyperthyroidism. JAMA 1946;131:86-90.
3. Keston AS, Ball RP, Frantz VK, Palmer WW. Storage of radioactive iodine in a metastasis from thyroid carcinoma. Science 1942;95:362-363.
4. Blahd WH, Nordyke RA, Bauer FK. Radioactive iodine (I-131) in the postoperative treatment of thyroid cancer. Cancer 1960;13:745-752.
5. Beierwaltes WH, Rabbani R, Dmuchowski C, Lloyd RV, Eyre P, Mallette S. An analysis of 'ablation of thyroid remnants' with I-131 in 511 patients from 1947-1984: experience at University of Michigan. J Nucl Med 1984;25:1287-1293.
6. Cassen B. Theory of the performance characteristics of radioisotope distribution imaging systems. J Nucl Med 1964;5:95-100.
7. Mazzaferri EL. Thyroid remnant 131I ablation for papillary and follicular thyroid carcinoma. Thyroid 1997;7:265-271.
8. Hurley JR, Becker DV. The use of radioiodine in the management of thyroid cancer. In: Freeman LM, Weissman HS (eds). Nuclear Medicine Annual 1983;329-384.
9. Meier DA, Brill DR, Becker DV, Clarke SE, Silberstein EB, Royal HD, Balon HR; Society of Nuclear Medicine: Procedure guideline for therapy of thyroid disease with 131-iodine. J Nucl Med 2002;43:856-861.
10. Silberstein EB, Alavi A, Balon HR, Clarke SE, Divgi C, Gelfand MJ, Goldsmith SJ, Jadvar H, Marcus CS, Martin WH,Parker JA, Royal HD, Sarkar SD, Stabin M, Waxman AD. The SNMMI practice guideline for therapy of thyroid disease with 131I 3.0. J Nucl Med 2012;53:1633-1651.
11. Luster M, Clarke SE, Dietlein M, Lassmann M, Lind P, Oyen WJ, Tennvall J, Bombardieri E; European Association of Nuclear Medicine (EANM). Guidelines for radioiodine therapy of differentiated thyroid cancer. Eur J Nucl Med Mol Imaging 2008;35:1941-1959.
12. Nuclear Medicine in Thyroid Cancer Management: A Practical Approach. 2009;TECDOC 1608 [IAEA, Vienna]
13. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid 2016;26:1-133.
14. Ho AL, Grewal RK, Leboeuf R, Sherman EJ, Pfister DG, Deandreis D, Pentlow KS, Zanzonico PB, Haque S, Gavane S, Ghossein RA, Ricarte-Filho JC, Domínguez JM, Shen R, Tuttle RM, Larson SM, Fagin JA. Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. N Eng J Med 2013;368:623-632.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):80-86 DOI:10.4274/2017.26.suppl.09

Redifferentiating Thyroid Cancer: Selumetinib-enhanced Radioiodine Uptake in Thyroid Cancer

Tiroid Kanser Re-diferansiyasyonu: Tiroid Kanserinde Selumetinib ile Uyarılmış Radyoaktif İyot Tutulumu

Steven M. Larson, Joseph R. Osborne, Ravinder K. Grewal, R. Michael Tuttle
Memorial Sloan Kettering Cancer Center, New York, USA

''All happy families are alike; every unhappy family is unhappy in its own way''. L. Tolstoy, from Anna Karenina, quoted by John Kuniyan (U.C. Berkeley), while discussing early TKI inhibitor drugs.

Abstract

In a recent article, we reported a restorative therapeutic intervention that turned individual thyroid cancer lesions into more efficient tissues for taking up radioactive iodine (RAI), resulting in clinically significant and durable responses. A group of Iodine-131 refractory thyroid cancer patients were treated with the MEK tyrosine kinase inhibitor (TKI) selumetinib, and RAI uptake was restored in a subset of patients. We employed Iodine-124 positron emission tomography to measure radiation absorbed dose, on a lesion by lesion basis. The process can be thought of as a re-differentiation of the cancer toward a more nearly normal state most like the tissue from which the cancer arose. Remarkably, in its own way, a change was detected within a few weeks of treatment, restoring uptake with therapeutically effective levels of RAI and in some patients, previously completely refractory to radioiodine treatment. In this article, we summarize the basic work that led to this seminal study, and make the case for lesional dosimetry in thyroid cancer with Iodine-124 as a new optimal radiotracer for precision medicine in patients with well differentiated thyroid cancer.
Keywords: Redifferentiation, thyroid cancer, selumetinib, radioiodine

Öz

Yakın tarihli bir yayında bireysel tiroid kanseri lezyonlarını radyoaktif iyot (RAİ) tutulumu açısından daha etkin dokulara çeviren ve klinik olarak anlamlı ve kalıcı cevaba yol açan restoratif terapötik bir girişim bildirdik. Bir grup İyot-131 refrakter tiroid kanseri hastası MEK tirozin kinaz inhibitörü selumetinib ile tedavi edildi ve bir kısım hastada RAI tutulumu sağlandı. Lezyon-lezyona temelinde İyot-124 pozitron emisyon tomografisi ile abzorbe edilen radyasyon dozunu ölçtük. Bu işlem kanserin, ilk çıktığı dokudaki normal duruma yakın bir hale re-diferansiyasyonu olarak düşünülebilir. İlgi çekici bir şekilde birkaç hafta tedavi ile öncesinde tamamen RAİ tedavisine refrakter olan bazı hastalarda terapötik olarak efektif dozlarda RAİ tutulumu sağlandı. Bu makalede, bu çığır açıcı temel çalışma özetlenmekte ve iyi diferansiye tiroid kanser hastalarında tedavi için yeni bir optimal radyofarmasötik olarak tiroid kanserinde lezyonel dozimetri için İyot-124 kullanımı sunulmaktadır.
Anahtar kelimeler: Re-diferansiyasyon, tiroid kanseri, selumetinib, radyoaktif iyot

Address for Correspondence: Steven M. Larson MD, Memorial Sloan Kettering Cancer Center, New York, USA
Phone: 646888359 E-mail: larsons@mskcc.org

Introduction

If we think of a tissue with cancer as an unhappy family of interacting cells (in contrast to the "happy" well differentiated normal tissue), we begin to understand that the sad fact of malignant change can truly occur in many ways. Often, the culprits are diverse changes in the genetic code, which are now understood to be the basis for a significant number of common cancers in man. These cancer-causing genes, "oncogenes," may transform cells from normal to malignant by simple alterations in base sequences that are, in turn, transformed into alterations in the protein product of the oncogene. When a mutation is the cause of the malignant transformation, this genetic change is called a "driver oncogene." The tissue affected is said to be "oncogene-addicted" and is found when growth and even viability are dependent on the presence and continuous activity of the oncoprotein product of the specific mutation. Specific oncogenes have been identified in lung cancer, breast cancer, lymphomas, leukemias, and pediatric cancers. With regard to thyroid cancer, knowledge about the genetic lesions associated with papillary thyroid cancer has increased enormously, in the last few years. More than 95% of the driver oncogene mutations are known, and 75% of these mutations occur in the mitogen-activated protein (MAP) kinase signal transduction pathway (Figure 1).

Integrated Genomic Characterization of Papillary Thyroid Carcinoma

Effector molecules such as MEK, which in turn signal ERK, indirectly promote cellular proliferation and growth and accompany the de-differentiation of the thyroid cell, driving it to take on the properties of neoplasia. For thyroid cancer, this means a down regulation of key mature tissue functions such as iodine uptake and the formation of thyroid hormone, as the tumor cell "goes primitive" and develops a new phenotype of sustained growth and metastasis.

Pre-clinical Observations Setting the Stage for Re-induction Therapy

The genetic mutation in the *BRAF* gene encodes the serine/threonine signal transduction molecule *BRAF* (v-raf murine sarcoma viral oncogene homolog B1), resulting in amino acid transformation at the 600 locus in the *BRAF* protein. This transformation occurs as the "driver mutation" in about 40% of thyroid cancers, and about 70% of melanomas. *BRAF* is actually an enzyme member of a signal transduction pathway, and it is this enzymatic function that transmits a message by activating through phosphorylation, the next protein in sequence, MEK [Text Box 1] (Figure 2).

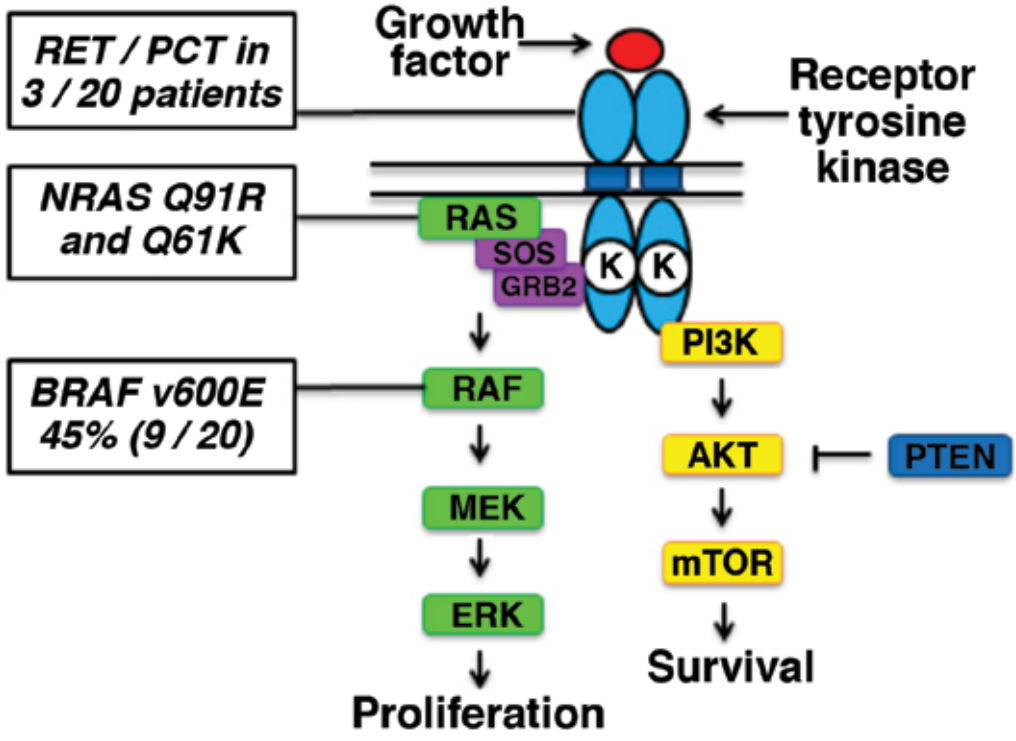


Figure 1. Mitogen-activated protein kinase signaling in papillary thyroid carcinoma is depicted along with statistical information regarding mutations and specifications at distinct points on the pathway. (Courtesy of James A. Fagin and Alan L. Ho, Memorial Sloan Kettering Cancer Center.)

My colleagues at Memorial Sloan Kettering Cancer Center developed a mouse model for thyroid cancer in order to study the effect of the BRAF driver oncogene on a molecular basis and its role in creating the tumor phenotype. The mouse model was transgenic, in which thyroid follicular cells were induced to carry a BRAF (V600E) mutation that could be activated by treatment with doxycycline. Under treatment of these special mice with doxycycline, the thyroid of the mice transformed to take on the characteristics of a poorly differentiated thyroid cancer, for example, losing the ability to take up radioactive iodine. In their seminal paper, the authors noted the following: "Strikingly, treatment with the MAPK pathway inhibitors rendered the tumor cells susceptible to a therapeutic dose of radioactive iodine. Our data show that thyroid tumors carrying *BRAF*^{V600E} mutations are exquisitely dependent on the oncoprotein for viability and that genetic or pharmacological inhibition of its expression or activity is associated with tumor regression and restoration of radioactive iodine uptake in vivo in mice. These findings have potentially significant clinical ramifications" (Figure 2) (2).

Inhibitor drugs that block the activity of this "driver oncogene," particularly in melanoma, have been developed and widely used, greatly benefiting select cases of advanced melanoma (3). This class of medications, called tyrosine kinase inhibitor drugs, or TKIs, act by blocking the enzymatic activity of a signal transduction protein. Examples include vemurafinib to block *B-RAF* directly and selumetinib as a blocker of the downstream MEK activity. Using this logic, the team of Chakravarty et al. (2) demonstrated proof of principle that inhibition of the MAPK pathway in the transgenic model of mutant *BRAF*-induced thyroid cancer partially restored radioactive iodine (RAI) uptake mechanisms within the cancer cell, sufficient to both increase RAI uptake and prolong retention in the cell. The effects were sufficient to achieve a major treatment effect on mouse thyroid cancer. This finding led to the extension of this approach to man, described in more detail below.

One feature of the preclinical paper described above was the rapid effect of the mutant *BRAF* gene induction on transformation of the thyroid cell into a de-differentiated state. Similarly, pharmacologic effects were also diffused and non-focal when restoring features of the differentiated state. This

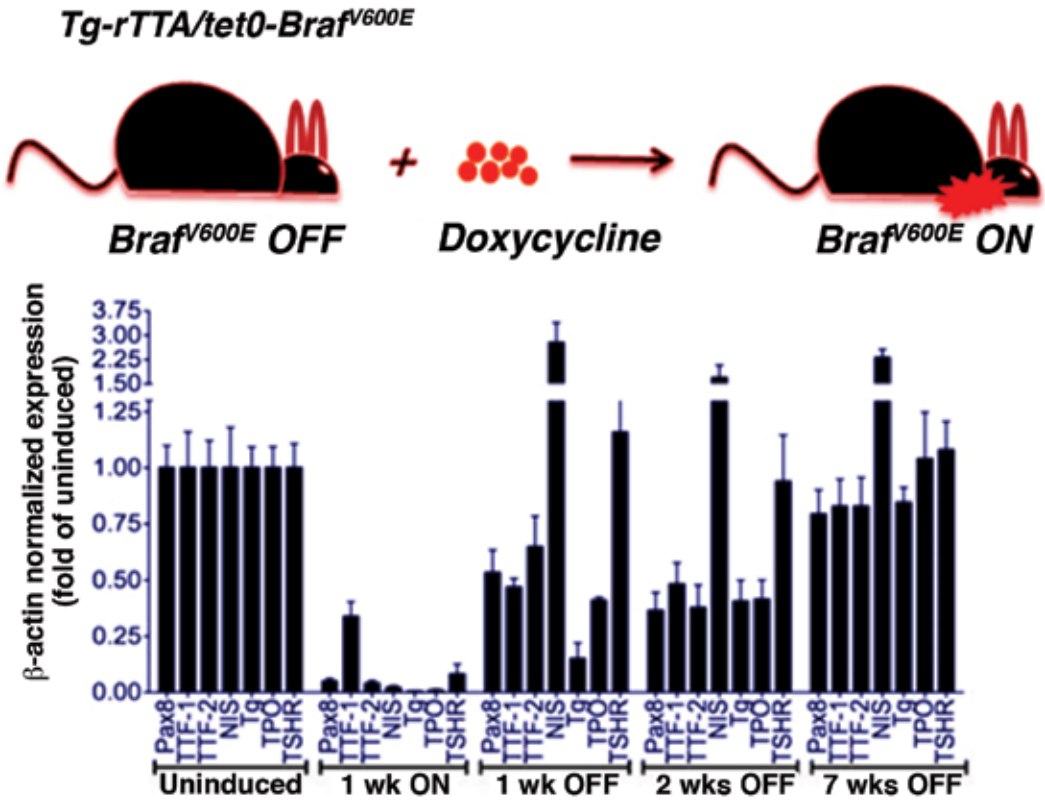


Figure 2. Transgenic mouse model demonstrating the effect of doxycline induction of mutant B-Raft in thyroid cells. Note that within a week of induction of the driver oncogene, the cadre of proteins responsible for uptake of radioiodine are rapidly down regulated, as the effect of the driver oncogene takes hold. If the doxycline is stopped, this effect can be ameliorated, and it is the sodium iodine symporter protein, of Na^+/I^- symporter, that responds most quickly (from reference 1).

is consistent with direct cellular effect, which is not related to clonal selection of sensitive cells, because the maturation occurs too fast and indeed throughout the tumor mass.

There is an increasing awareness that cancers growing in the human body are capable of continuously transforming themselves, often under the pressure of specific treatments, in both fundamental and meaningful ways. This biologic transformation is especially common for patients undergoing treatment with targeted kinase inhibitor drugs (TKIs), whereby during treatment, further evolution of genetic changes may result in resistance to specific anti-tumor drugs. For example, one of the earliest targeted therapies, imatinib (trade name: Gleevec), was found to be highly effective against chronic forms of myeloid leukemia, due to specific effectiveness through binding to BCR-ABL kinase. The mode of action of imatinib was based on blocking the enzymatic kinase action of this protein. However, resistance developed in many patients through changes in the genetic makeup of the genes encoding BCR-ABL, resulting in alterations at multiple points in the protein, which in turn nullified the binding action of imatinib. The same process could take place during inhibition of mutant *BRAF* in thyroid cancer, by drug treatment, and the patient may ultimately escape the effects of therapy.

Iodine-124 at Memorial Sloan Kettering Cancer Center

At MSKCC, we developed positron emission tomography (PET) imaging, as a quantitative molecular imaging tool in the study of thyroid cancer, and one of our earliest efforts was methodology for iodine-124 (^{124}I) production, supported by the Department of Energy (DOE) (Figure 3) (4). We used ^{124}I as a tool for characterization of targeting and dosimetry of radioantibodies, such as 3F8, an anti-GD2 antibody (5), and A33, an anti-A33 antibody (colon cancer) (6). We and others have also found this radionuclide of special benefit in defining the lesional dosimetry of iodine-131 (^{131}I) therapy in man (7,8). Although ^{131}I can also be imaged with cross-sectional imaging, using single-photon emission computed tomography in the post-therapy setting (9) indicates there is little doubt that ^{124}I and PET imaging can detect more lesions and also quantitate more readily, especially lesions in the 1-2 cm. in diameter range, a size commonly seen in pulmonary metastases. An example of ^{124}I imaging in man is shown in Figure 3, which shows a reprojection image in a patient with documented thyroid cancer metastatic to the lung, neck nodes as well as to bone.

Re-induction of Therapeutic Levels of Radioiodine Uptake in Metastatic Thyroid Cancer in Man

In a 20-patient clinical pilot trial, we effectively used quantitative ^{124}Iodine-NaI PET imaging to estimate individual lesion dosimetry before and after a four-week course of the serine-threonine kinase MEK inhibitor selumetinib (AZD6244) to identify patients who would benefit from additional RAI therapy and to individualize therapeutic dosing recommendations. We demonstrated that selumetinib re-induced iodine uptake in a significant

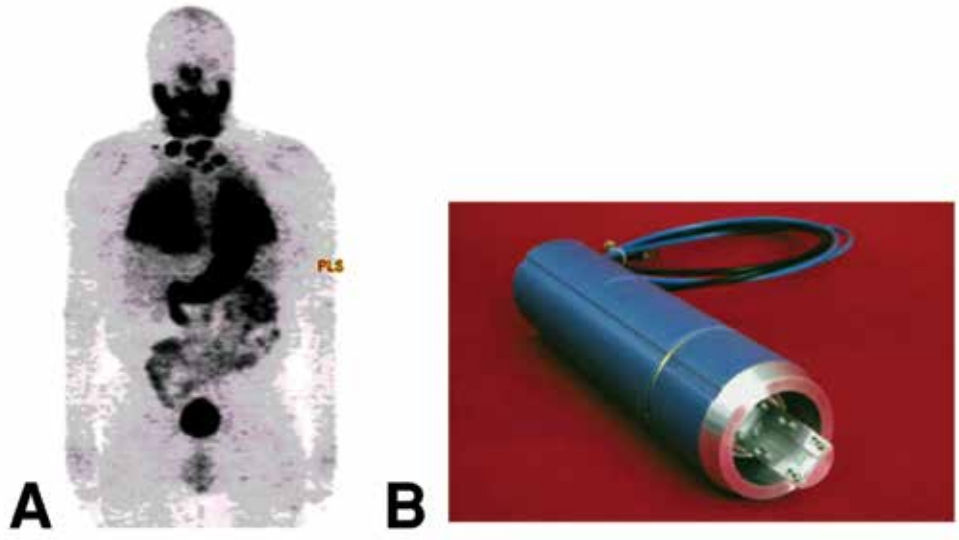


Figure 3. A) Reprojection positron emission tomography imaging in a patient with well differentiated thyroid cancer, metastatic to the lungs, regional neck nodes and also suspected metastases in the scapula and T-12 vertebrae. At the time of imaging, thyroglobulin levels >11,000 ng/mL; estimated dose from 250 mCi of ^{131}I estimated at >50,000 cGy; follow-up at 13 years post-multiple ^{131}I treatments (1500 mCi total), TG <0.2, complete clinical response. B) MSKCC (Finn) target system, used to manufacture ^{124}I. Tellurium-124.) 15 MeV protons. ^{124}I can be produced in significant quantities by modern hospital and radiopharmacy based cyclotrons

subset of patients with radioiodine refractory metastatic thyroid cancer (1): 12/20 RAI-refractory patients achieved improved lesional radioiodine uptake with selumetinib therapy. Of those 12 patients, 8 had at least one tumor with ^{124}I uptake that predicted delivery of ≥2,000 cGy with ≤300 mCi therapeutic ^{131}I, and went on to experience tumor reductions with therapeutic ^{131}I given concomitantly with selumetinib (5 Response Evaluation Criteria in Solid Tumors (RECIST) partial responses, 3 RECIST stable disease). Equally important, the lesional dosimetry also identified a subset of patients in whom high-dose RAI would not be effective, thereby avoiding unnecessary radioiodine exposure. These findings illustrated the potential role of quantitative PET imaging as a tool for documenting drug effects in vivo, and supported the concept that ^{124}I PET imaging can be used to measure a threshold radiation absorbed tumor dose that will identify patients who will respond to ^{131}I (Figure 4).

The Clinical Problem of Radioactive Iodine Treatment of Thyroid Cancer: A Rationale for Lesional Dosimetry

^{131}I therapy is widely used in thyroid cancer for thyroid ablation and treatment of metastatic disease. However, the standard approach of using empirical administered activities of ^{131}I to treat metastatic disease without reliable measurements of lesional dosimetry leads to multiple ineffective RAI treatments in patients subsequently proven to have RAI refractory disease, as well as to sub-optimal dosing even in patients with RAI-avid disease. Since ^{131}I therapy can be associated with significant toxicity (salivary gland damage, lacrimal duct obstruction, and at higher cumulative doses, myelodysplastic syndrome and leukemia), an accurate dosimetry test that predicts whether or not patients will respond to a therapeutic dose of ^{131}I is sorely needed.

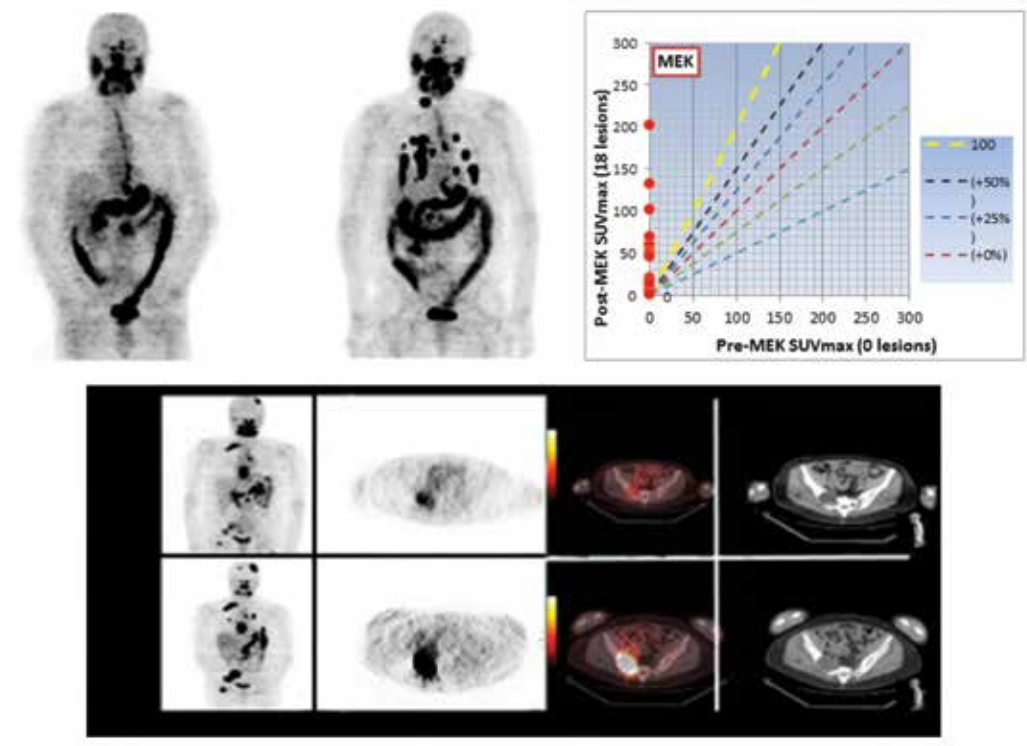


Figure 4. A thyroid cancer patient with widely metastatic neck nodes, pulmonary nodules, mediastinum, and osseous sites. The upper row is the baseline study before seluemetanib treatment (left), and the post four week MEK treatment image showing a number of pulmonary lesions now taking up the radioactive iodine. The graph (top, right) compares the standardized uptake values (SUV) before (abscissa) and after (ordinate) treating in quantitative terms. No lesions were detectable in terms of ^{124}I prior to treatment and subsequently, the uptake that developed was seen. There is a different patient showing baseline (upper row) and the re-induction effects of treatment on radioiodine uptake (bottom row). There are new lesions in the lungs and the osseous lesions have greater uptake (left panel coronal image baseline, above and post-treatment below); cross-sectional imaging ^{124}I imaged on positron emission tomography, baseline (above) and post-treatment (below). There is marked increase in the intensity of uptake in the R. pelvis, and the relationship to computed tomography findings is dramatically displayed in the fusion positron emission tomography/computed tomography, which shows a space occupying lesion in the R. ileum, now with therapeutic levels of uptake. As part of the selumetinib pilot trial, we have been able to document dose-response relationships between ^{124}I lesional dosimetry and subsequent structural disease response as determined by Response Evaluation Criteria in Solid Tumors criteria in eight differentiated thyroid cancer patients (1).

Distant metastases are identified in 10-15% of patients with differentiated thyroid cancer at some point in the course of their disease. For more than 50 years, RAI has been the mainstay of therapy in these patients (10). However, even in RAI-avid metastatic disease, multiple administrations of RAI are usually required to control the disease. Furthermore, RAI therapy rarely results in clinical remission except in young patients with very small volume, RAI-avid, miliary metastases. The majority of patients with structurally evident RAI-avid distant metastases will have a clinical response marked by either disease stabilization or partial regression of macroscopic disease. Then again, no prospective randomized placebo-controlled studies have established efficacy on progression-free survival or overall survival. Survival rates are less than ideal, with five-year survival rates of 80% and ten-year survival rates of 55%, even when the distant metastases are RAI-avid (11). Thus, whereas RAI may be an effective treatment in a subset of patients with "RAI-avid" distant metastases, it is seldom curative, and the tools to predict who may benefit and who may not have not been definitively established.

Compared with older series that evaluated responses to therapy on the basis of post-therapy RAI scans and chest radiographs, recent retrospective studies demonstrate that few patients with macroscopic distant metastases have clear-cut RECIST responses when the effectiveness of RAI therapy is assessed with computed tomography or magnetic resonance imaging, or with other modalities such as fludeoxyglucose-PET or the plasma biomarker thyroglobulin (12,13,14,15,16). Furthermore, it is unclear whether the "disease stabilization" seen after RAI is a treatment effect or due to the natural history of insidious growth of well differentiated. RAI-avid metastatic disease (17). It is critically important to determine if inadequate lesional dose is the reason for the relative ineffectiveness of RAI therapy. Recent indications suggest that this may indeed be the case; i.e., pre-treatment with the MEK inhibitor selumetinib prior to RAI therapy dramatically increased the lesional dose in patients with RAI-refractory metastatic thyroid cancer and resulted in a high rate of responses as determined by RECIST criteria (1). Thus, compelling evidence now indicates that inadequate lesional dose is a key limitation to the effectiveness of RAI therapy. Studies designed to quantify, enhance, and optimize lesional dose have become even more critical.

Several approaches to determining lesional dosimetry have been published (18,19) but none of them has gained acceptance into clinical practice outside of a few specialized centers. More recently, ^{124}I-PET has emerged as a potentially clinically applicable methodology for scanning and quantifying lesional dosimetry in differentiated thyroid cancer (7,20,21). While it is clear that whole-body scanning and lesional dosimetry measurements with ^{124}I are feasible, studies correlating ^{124}I lesional dosimetry with the effectiveness of RAI as measured by cross-sectional imaging (which are needed to define dose-response relationships) have not been conducted.

While lesional dosimetry is a key component of optimal RAI dosing, it is also important to define the maximal tolerable dose that can be administered without complications. Optimal RAI dosing should be based on precise lesional dosimetry (to define an appropriate therapeutic dose), coupled with whole-body dosimetry studies to ensure that the proposed activity can be safely administered without unacceptable side effects. Unfortunately, in clinical practice, the selection of the RAI-administered activity is based far more on its potential toxicity than on the dose likely to be required to achieve a tumoricidal effect.

Over the last 50 years, several empiric dosing regimens that called for fixed doses of RAI at specific intervals have been shown in retrospective studies to be generally well tolerated and often effective (10,12,22). However, recent studies suggest that these empirical dosing recommendations may exceed the maximum safe radiation dose in a significant proportion of older patients with RAI-avid metastatic disease (23,24). In fact, concerns over the frequency and severity of bone marrow and pulmonary toxicity associated with repeated high-dose RAI treatments were expressed in the 1950s and 1960s, which led to the development of dosimetry models to calculate the maximal tolerable activity that can be safely administered without causing excessive radiation exposure to the lungs or bone marrow (25). The value of this approach was recently discussed in a retrospective study demonstrating superior clinical outcomes when RAI therapy was based on whole-body dosimetry rather than empirical RAI dosing (13,14).

The inability to precisely define an optimal treatment activity of RAI means that clinicians cannot tailor treatment recommendations to individual patients. Therefore, some patients are treated with suboptimal doses and do not receive the complete potential therapeutic benefit of RAI. Conversely, some patients receive repeated RAI doses that are ineffective, which unnecessarily expose them to potential side effects. Salivary gland dysfunction, nasolacrimal obstruction, reproductive disturbances, conjunctivitis, and hematological abnormalities can be observed after RAI treatment (26). Although the majority of these side effects are minor and often transient, several studies have demonstrated that high cumulative administered activities of RAI given after traditional thyroid hormone withdrawal can be associated with leukopenia, thrombocytopenia, anemia, and even an increased risk of leukemia with cumulative doses >300-500 mCi (27,28).

Conclusion

In conclusion, there is an unmet need for the development of accurate quantitative lesional and whole-body dosimetry that cannot be derived from diagnostic studies with the radioiodine isotopes currently used in practice. ^{124}I dosimetry is the appropriate modality to achieve this, thus

helping select patients likely to benefit from RAI therapy and sparing all others from unnecessary radiation exposure. Not only is ^{124}I and PET imaging an exquisite methodology for research project such as ours, there is good reason to believe that ^{124}I can be made into a practical clinical reagent to replace ^{131}I for treatment dosimetry.

References

1. Ho AL, Grewal RK, Leboeuf R, Sherman EJ, Pfister DG, Deandreis D, Pentlow KS, Zanzonico PB, Haque S, Gavane S, Ghossein RA, Ricarte-Filho JC, Domínguez JM, Shen R, Tuttle RM, Larson SM, Fagin JA. Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. N Engl J Med 2013;368:623-632.
2. Chakravarty D, Santos E, Ryder M, Knauf JA, Liao XH, West BL, Bollag G, Kolesnick R, Thin TH, Rosen N, Zanzonico P, Larson SM, Refetoff S, Ghossein R, Fagin JA. Small-molecule MAPK inhibitors restore radioiodine incorporation in mouse thyroid cancers with conditional BRAF activation. J Clin Invest 2011;121:4700-4711.
3. Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA; BRIM-3 Study Group. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 2011;364:2507-2516.
4. Pentlow KS, Graham MC, Lambrecht RM, Daghighian F, Bacharach SL, Bendriem B, Finn RD, Jordan K, Kalaigian H, Karp JS, Robeson WR, Larson SM. Quantitative imaging of iodine-124 with PET. J Nucl Med 1996;37:1557-1562.
5. Larson SM, Pentlow KS, Volkow ND, Wolf AP, Finn RD, Lambrecht RM, Graham MC, Di Resta G, Bendriem B, Daghighian F, et al. PET scanning of iodine-124-3F9 as an approach to tumor dosimetry during treatment planning for radioimmunotherapy in a child with neuroblastoma. J Nucl Med 1992;33:2020-2023.
6. O'Donoghue JA, Smith-Jones PM, Humm JL, Ruan S, Pryma DA, Jungbluth AA, Divgi CR, Carrasquillo JA, Pandit-Taskar N, Fong Y, Strong VE, Kemeny NE, Old LJ, Larson SM. 124I-huA33 antibody uptake is driven by A33 antigen concentration in tissues from colorectal cancer patients imaged by immuno-PET. J Nucl Med 2011;52:1878-1885.
7. Sgouros G, Kolbert KS, Sheikh A, Pentlow KS, Mun EF, Barth A, Robbins RJ, Larson SM. Patient-specific dosimetry for 131I thyroid cancer therapy using 124I PET and 3-dimensional-internal dosimetry (3D-ID) software. J Nucl Med 2004;45:1366-1372.
8. Larson SM, Robbins R. Positron emission tomography in thyroid cancer management. Semin Roentgenol 2002;37:169-174.
9. Grewal RK, Tuttle RM, Fox J, Borkar S, Chou JF, Gonen M, Strauss HW, Larson SM, Schöder H. The effect of posttherapy 131I SPECT/CT on risk classification and management of patients with differentiated thyroid cancer. J Nucl Med 2010;51:1361-1367.
10. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009;19:1167-1214.
11. Durante C, Puxeddu E, Ferretti E, Morisi R, Moretti S, Bruno R, Barbi F, Avenia N, Scipioni A, Verrienti A, Tosi E, Cavaliere A, Gulino A, Filetti S, Russo D. BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. J Clin Endocrinol Metab 2007;92:2840-2843.
12. Durante C, Haddy N, Baudin E, Leboulleux S, Hartl D, Travagli JP, Caillou B, Ricard M, Lumbroso JD, De Vathaire F, Schlumberger M. Long-term outcome of 444 patients with distant metastases from papillary and follicular thyroid carcinoma: benefits and limits of radioiodine therapy. J Clin Endocrinol Metab 2006;91:2892-2899.
13. Klubo-Gwiezdzinska J, Burman KD, Van Nostrand D, Mete M, Jonklaas J, Wartofsky L. Radioiodine treatment of metastatic thyroid cancer: relative efficacy and side effect profile of preparation by thyroid hormone withdrawal versus recombinant human thyrotropin. Thyroid 2012;22:310-317.
14. Klubo-Gwiezdzinska J, Burman KD, Van Nostrand D, Mete M, Jonklaas J, Wartofsky L. Potential use of recombinant human thyrotropin in the treatment of distant metastases in patients with differentiated thyroid cancer. Endocr Pract 2013;19:139-148.
15. Sabra M, Tuttle RM. Recombinant human thyroid-stimulating hormone to stimulate 131-I uptake for remnant ablation and adjuvant therapy. Endocrine Prac 2013;19:149-156.
16. Sabra MM, Dominguez JM, Grewal RK, Larson SM, Ghossein RA, Tuttle RM, Fagin JA. Clinical outcomes and molecular profile of differentiated thyroid cancers with radioiodine-avid distant metastases. J Clin Endocrinol Metab 2013;98:829-836.
17. Sabra MM, Grewal RK, Tala H, Larson SM, Tuttle RM. Clinical outcomes following empiric radioiodine therapy in patients with structurally identifiable metastatic follicular cell-derived thyroid carcinoma with negative diagnostic but positive post-therapy 131I whole-body scans. Thyroid 2012;22:877-883.
18. Maxon HR, Thomas SR, Hertzberg VS, Kereiakes JG, Chen IW, Sperling MI, Saenger EL. Relation between effective radiation dose and outcome of radioiodine therapy for thyroid cancer. N Engl J Med 1983;309:937-941.
19. Van Nostrand D, Atkins F, Yeganeh F, Acio E, Bursaw R, Wartofsky L. Dosimetrically determined doses of radioiodine for the treatment of metastatic thyroid carcinoma. Thyroid 2002;12:121-134.
20. Erdi YE, Macapinlac H, Larson SM, Erdi AK, Yeung H, Furhang EE, Humm JL. Radiation Dose Assessment for I-131 Therapy of Thyroid Cancer Using I-124 PET Imaging. Clinical Positron Imaging 1999;2:41-46.
21. Freudenberg LS, Jentzen W, Petrich T, Frömke C, Marlowe RJ, Heusner T, Brandau W, Knapp WH, Bockisch A. Lesion dose in differentiated thyroid carcinoma metastases after rhTSH or thyroid hormone withdrawal: 124I PET/CT dosimetric comparisons. Eur J Nucl Med Mol Imaging 2010;37:2267-2276.
22. Tuttle RM, Ball DW, Byrd D, Dilawari RA, Doherty GM, Duh QY, Ehya H, Farrar WB, Haddad RI, Kandeel F, Kloos RT, Kopp P, Lamonica DM, Loree TR, Lydiatt WM, McCaffrey JC, Olson JA Jr, Parks L, Ridge JA, Shah JP, Sherman SI, Sturgeon C, Waguespack SG, Wang TN, Wirth LJ; National Comprehensive Cancer Network. J Natl Compr Canc Netw 2010;8:1228-1274.
23. Kulkarni K, Van Nostrand D, Atkins F, Aiken M, Burman K, Wartofsky L. The relative frequency in which empiric dosages of radioiodine would potentially overtreat or undertreat patients who have metastatic well-differentiated thyroid cancer. Thyroid 2006;16:1019-1023.
24. Tuttle RM, Leboeuf R, Robbins RJ, Qualey R, Pentlow K, Larson SM, Chan CY. Empiric radioactive iodine dosing regimens frequently exceed maximum tolerated activity levels in elderly patients with thyroid cancer. J Nucl Med 2006;47:1587-1591.
25. Benua RS, Cicale NR, Sonenberg M, Rawson RW. The relation of radioiodine dosimetry to results and complications in the treatment of metastatic thyroid cancer. Am J Roentgenol Radium Ther Nucl Med 1962;87:171-182.
26. Lee SL. Complications of radioactive iodine treatment of thyroid carcinoma. J Natl Compr Canc Netw 2010;8:1277-1286.
27. Brown AP, Chen J, Hitchcock YJ, Szabo A, Shrieve DC, Tward JD. The risk of second primary malignancies up to three decades after the treatment of differentiated thyroid cancer. J Clin Endocrinol Metab 2008;93:504-515.
28. Rubino C, de Vathaire F, Dottorini ME, Hall P, Schvartz C, Couette JE, Dondon MG, Abbas MT, Langlois C, Schlumberger M. Second primary malignancies in thyroid cancer patients. Br J Cancer 2003;89:1638-1644.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):87-91 DOI:10.4274/2017.26.suppl.10

Thyroid Stimulating Hormone Receptor

Tiroid Stimulan Hormon Reseptörü

Murat Tuncel
Hacettepe University Faculty of Medicine, Department of Nuclear Medicine, Ankara, Turkey

Abstract

Thyroid stimulating hormone receptor (TSHR) plays a pivotal role in thyroid hormone metabolism. It is a major controller of thyroid cell function and growth. Mutations in TSHR may lead to several thyroid diseases, most commonly hyperthyroidism. Although its genetic and epigenetic alterations do not directly lead to carcinogenesis, it has a crucial role in tumor growth, which is initiated by several oncogenes. This article will provide a brief review of TSHR and related diseases.
Keywords: Thyroid stimulating hormone receptor, genetic, epigenetic alteration, thyroid cancer, adenoma

Öz

Tiroid stimulan hormon reseptörü (TSHR) tiroid hormon metabolizmasında merkezi bir rol oynar. Tiroid hücrelerinin fonksiyonlarının ve büyümesinin kontrolünde yardımcıdır. TSHR mutasyonları bir dizi tiroid hastalığına, en çok hipertiroidizme neden olmaktadır. Her ne kadar bu reseptörün genetik ve epigenetik değişiklikleri direkt olarak karsinogeneze yol açmasa da, farklı onkogenler tarafından başlatılmış tümörün büyümesinde ciddi bir rol oynar. Bu makalede TSHR ve ilişkili hastalıklar özetlenmektedir.
Anahtar kelimeler: Tiroid stimulan hormon reseptörü, genetik, epigenetik değişiklikler, tiroid kanseri, adenom

Introduction

Thyroid stimulating hormone receptor (TSHR) has been first cloned in 1989. It is located on chromosome 14q and contains 10 exons (1). It encodes the synthesis of a protein with 764 amino acids, and has a molecular weight of 87 kDa. The first 9 exons of the gene encode a large amino-terminal ectodomain, while exon 10 encodes seven transmembrane segments and a intracytoplasmic domain with a carboxyl-terminal segment. The long amino-terminal segment of the receptor creates high affinity for TSH binding. TSHR is divided into two subunits (α and β) by post-translational proteolysis and each subunit is linked to each other via disulfide bonds. The receptor than undergoes post-translational glycosylation and palmitoylation for full functionality (2).

TSHR belongs to a group of G-protein-coupled seven-transmembrane receptors and is located at the basolateral membrane of thyroid follicular cells (2). Studies suggest that several G protein subtypes are involved in the signal transfer, but Gαs and Gαq have been shown to be the major subtypes that mediate TSHR signals (3). TSHR activation results in intracellular signaling via G proteins that modulate the effector molecule activity. Among these; Gs protein leads to activation of the cyclic adenosine monophosphate (AMP) cascade, and the Gq protein activates the phospholipase C (PLC) cascade. At higher TSH concentrations, cAMP binds to protein kinase A (PKA), which phosphorylates different effectors with its enhanced catalytic activity. Inositol 1,4,5-triphosphate and diacylglycerol are generated by activated PLC. These molecules stimulate the release of Ca^{2+} into the cytoplasm and activate the protein kinase C (PKC) pathway. Increased levels of intracellular Ca^{2+} and PLC activity play a major role in the regulation of H_2O_2 production, thyroglobulin (Tg) iodination and iodide efflux, while adenylate cyclase and

Address for Correspondence: Murat Tuncel MD, Hacettepe University Faculty of Medicine, Department of Nuclear Medicine, Ankara, Turkey
Phone: +90 536 213 03 41 E-mail: muratmtx@yahoo.com

cAMP regulate transcription of sodium-iodide symporter (NIS), Tg and thyroid peroxidase (TPO), as well as iodide uptake (4,5).

TSH levels positively modulate TSHR in normal cells up to a certain limit, while down regulating TSHR at high concentrations (6). Over-activation of the cAMP pathway by chronic TSHR stimulation causes excess hormone secretion and thyroid hyperplasia, which results in clinical hyperthyroidism. Increased secretion of the thyroid hormone than leads to negative feedback at the hypothalamic-pituitary level resulting in suppressed TSH secretion. This mechanism may be clearly detected by scintigraphic methods in a hyperthyroid patient with suppressed TSH levels. Images show decreased radiotracer uptake in normal parts of the thyroid gland due hot toxic adenoma with high radiotracer uptake. The uptake by the normal thyroid gland returns to normal levels following ablation of toxic adenoma, while the TSH levels also return to normal (7).

Thyroid Stimulating Hormone Receptor Mutations in Benign Diseases

Several mutations may occur in the TSHR gene that influence either the protein component or post-translational modifications of the receptor. The mutations may be activating (constitutive) or deactivating. These are dominant mutations and modification in one allele is sufficient for generating the abnormal phenotype. TSHR mutations are defined in several diseases like familial gestational hyperthyroidism, autonomous toxic adenomas, hereditary or sporadic toxic thyroid hyperplasia, familial non-autoimmune hyperthyroidism, Graves' disease and autoimmune hypothyroidism (8,9). Autosomal dominant non-autoimmune hyperthyroidism may be caused by germ-line TSHR mutations, and de novo mutations may lead to sporadic non-autoimmune hyperthyroidism.

Somatic activating mutations of the TSHR or Gsα proteins constitutively activate the cAMP pathway. This activation causes clonal autonomous growth and hyper-functioning of the thyroid follicular cells which results in a toxic adenoma. Cells with activating mutation may have an increased expression of the NIS, which is seen as a high uptake or 'hot nodule' image on scintigraphy (10). The prevalence of TSH receptor mutations in toxic adenomas varies in different studies, but is reported to be as high as 80%. Differences in iodine intake, sampling technique, and methodological approaches might explain this variance (11). Activating mutations are mainly located in the β subunit of the TSHR. However, Kopp et al. (12) reported constitutive activation to the receptor, caused by substitutions at serine 281 (S281I/N/T), which is a residue located in the extracellular α subunit. The exact mechanisms that result in function gain is not clearly understood. It has been suggested that mutations may alter the configuration of the transmembrane segments, mimicking the structural changes occurred after binding of ligand or alternatively some mutations may change the structure of the domains that inhibit receptor coupling to G proteins in the absence of TSH (8,13).

In contrast to hyper-functioning nodules, cold hypo-functioning nodules have a low incidence of mutations. Mutations in Gs protein were detected in 27% of nonfunctioning adenomas in one series, however, this was not verified by others. These nodules are believed to have mutations of genes linked with de-differentiation. Activating mutations in the RAS proto-oncogene pathway have been detected in 20% of thyroid adenomas with frequencies similar to those found in follicular thyroid (FTC) and papillary thyroid carcinomas (PTC) (11).

Thyroid Stimulating Hormone Receptor and Gs Gene Mutations in Thyroid Carcinomas

Mutations of Gsa subunit and the TSHR gene rarely occur in well-differentiated thyroid cancers. Although activated cAMP pathway results in enhanced growth, it is not sufficient for malignant transformation of normal thyrocytes. Based on available data, TSHR and Gs gene mutations are not involved in carcinogenesis, except in a small proportion (<6%) of cases (14,15). However, in thyroid carcinomas with a poor response to TSH and high basal adenylate cyclase activity, mutations in TSHR and Gs were reported in 12% of FTC and in 13% of PTC (16).

TSHR mutations were also reported in malignant hot nodules at scintigraphy. Niepomniszcze et al. (17) reported a case of FTC presenting as a hot nodule. Sequence analysis revealed a constitutive mutation at codon 620 of the TSHR gene and a G12C Ki-RAS mutation. It has been reported that RAS mutation could be the driver for transformation, since hot nodules only rarely progress to carcinoma. Gozu et al. (18) described a TSHR mutation in a PTC presenting as a hot nodule, and a similar finding was observed by Camacho et al. (19) in a FTC. Finally, Russo and colleagues described an autonomously functioning Hurthle cell carcinoma with a TSHR mutation and absence of either RAS or TP53 mutations (20). According to these observations, screening of mutations in different oncogenes related to thyroid cancer and the role of TSHR mutations in transformation was not well established. From the limited data available, it seems that activation of the cAMP pathway does not a major role in cell transformation. Most hyper-functioning tumors harbor both TSHR mutations and proto-oncogene mutations; this coexistence suggests that carcinomas arise from the activity of classical oncogenes, such as RAS and RET/PTC, and that the TSHR and Gs mutations contribute to the hyper-functioning features of the neoplasms.

Thyroid Stimulating Hormone Receptor Pathway, Relations with Other Genetic and Epigenetic Alterations

Several pathways are responsible for tumor carcinogenesis in thyroid cancer. Multiple genetic and epigenetic alterations that lead to activation of the mitogen-activated protein kinases and phosphatidylinositol-3-kinase-AKT signaling pathways are required for the development and progression of thyroid cancer. Common genetic alterations found in thyroid cancer include point mutation of the *BRAF* and RAS genes (seen up to 45% of patients) as well as RET/PTC and PAX8/PPARγ chromosomal rearrangements (21). Ionizing radiation, chemical mutagenesis and dietary iodine excess were suggested as etiologic factors of these chromosomal rearrangements and mutations (22). As described earlier, TSH-TSHR signaling pathway plays a critical role for thyroid cell growth and proliferation. It acts via common pathways as other oncogenes and has a role in controlling cell growth and carcinogenesis. Several animal studies were performed to evaluate the role of TSHR signaling pathway and its relation with other oncogenes in thyroid cancer. Lu et al. (23) used a special mouse model ($TR\beta^{PV/PV}$ mice) that has a negative mutation (PV) of the thyroid hormone-β receptor (TRβ). These mice have elevated TSH and serum thyroid hormone levels, and they spontaneously develop FTC. The authors observed that when these mice were crossed with TSH receptor gene knockout $TSHR^{-/-}$ mice, and these mice did not develop thyroid cancer. This study demonstrated the requirement of TSH-TSHR signaling pathway in thyroid carcinogenesis in this mouse model. Similarly, in thyroid-specific knock-in of $BRAF^{V600E}$ LSL-*Braf(V600E)*/TPO-Cre) mouse model in which mice develop aggressive PTC, crossing of these mice with $TSHR^{-/-}$ mice blocked the development of thyroid cancer (24). However, it is not clearly demonstrated if THSH-TSHR signaling is essential for the initiation of thyroid cancer or if it is required for the TSHR-dependent generation and growth of oncogene-stimulated thyroid cancer cells. Over-activation of TSH-TSHR pathway through activating mutations in TSHR or Gs_α is known to cause benign hyper-functional FT; however, these tumors almost never undergo malignant transformation. This suggests that TSHR signaling may be protective against malignant transformation of thyroid cells. TSHR may avoid malignant transformation of thyroid cells and suppress the occurrence of thyroid cancer, but it may promote the growth and progression of thyroid cancer once it has been initiated by oncogenic modifications. TSHR expression is also related with other thyroid specific genes. Presence of TSHR gene expression effects other thyroid specific genes. In their study, Feng et al. (25) showed that after transfection of recombinant plasmid pcDNA3.1-hTSHR into dedifferentiated FTC-133 cells, the ^{125}I uptake, TSHR, NIS, TPO and Tg mRNAs were significantly increased by 2, 9, 1.7, 4, 1.5 and 2.2 times, respectively, as compared to control levels. The authors concluded that decreased TSHR expression correlated with FTC-133 dedifferentiation, and TSHR transfection contributed to the re-differentiation of these FTC cells. Based on these studies, it can be suggested that TSHR is needed in early progression of the disease and that it is not required after de-differantiation (contrary it re-induce cell differentiation).

The most common and well recognized genetic alteration in thyroid cancer is *BRAF(V600E)* mutation, which is present in up to 45% of thyroid malignancies and in up to 62% of radioactive iodine-resistant thyroid tumors. This mutation is associated with down regulation of several thyroid specific genes. Kleiman et al. (26) evaluated the effect of *BRAF* inhibition and TSH supplementation on (131)I uptake in *BRAF(V600E)*-mutant (WRO) human thyroid cancer cells. Transfection of WRO cells with small interfering RNA targeting *BRAF* causes an increase in expression of the NIS gene by 5.5-fold and the TSHR gene by 2.8-fold (p=0.02). This increase was also noted in NIS and TSHR protein levels. The effect of *BRAF* inhibition was also TSH dependent and not detected in case of TSH depletion. In their study Durante et al. (27) characterized the expression of thyroid-specific genes associated with *BRAF* mutation. mRNA levels for NIS, apical iodide transporter (AIT-B), Tg, TPO, TSHR, the transcription factor PAX8, and glucose transporter type 1 (Glut1) were measured, and these levels for all thyroid-specific genes were reported to be reduced in all PTCs vs. normal thyroid tissues. NIS, AIT-B, Tg, and TPO expression was significantly lower in *BRAF*-mut tumors than in the *BRAF*-wt group. However, in this study, TSHR expression was not significantly effected by *BRAF* mutation status. Glut-1 transcript levels were increased in all PTCs, and additional increases were noted in *BRAF*-mut tumors. Authors stated that $BRAF^{V600E}$ mutation in PTCs was associated with reduced expression of key genes involved in iodine metabolism and that this may suggest a more aggressive tumor as can be predicted by an increase in Glut-1 transcript levels.

In addition to mutations, the age of the patient was also important for thyroid specific gene expression in thyroid tumors. Espadinha et al. (28) have found that among PTCs, the mean expression of Pendred syndrome gene (PDS), TPO and TSH-R was significantly lower in the elderly. The finding of higher PDS, TPO and TSH-R mRNA expression in pediatric vs. adult primary tumor tissues supports the hypothesis that this might contribute to the increased functional activity of metastases in the pediatric group.

Several epigenetic alterations like DNA methylation and histone modification may also occur in thyroid specific

genes. Among these changes, methylation of TSHR is a common form of epigenetic alteration in thyroid cancers and correlates with the presence of other oncogenes. Khan et al. (29) determined methylation of the promoter region of TSHR gene in 25% (15 of 60) of thyroid cancer patients. These patients also had higher TSH levels than the non-methylated patients, suggesting a loss in function of TSHR after methylation. In this study group, *BRAF*^{V600E} mutation was found in 25 % (15 of 60) patients and within this sub-group the TSHR promoter was methylated in 73.3 % (11 of 15). This study showed the importance of TSHR gene methylation and its significant association with *BRAF*^{V600E} mutation in thyroid tumors, depicting a positive correlation between TSHR pathway and MAP Kinase pathway. The methylation of TSHR was also confirmed by our group; we showed that after application of DNA methylation inhibitor 5-Azacytidine, TSH-R mRNA expression was increased in both normal thyroid and BCPAP papillary thyroid cancer cell lines. Unfortunately, 5-Azacytidine did not increase radioiodine uptake in the cancer cell line, which suggests that multiple genetic and post-translational alterations are involved in the expression of thyroid specific genes into protein and functional levels (30).

Conclusion

TSHR and its genetic & epigenetic alterations is a stimulating research area that needs further evaluation. It has important correlations with thyroid specific genes, and with several oncogenic pathways in thyroid cancer. Future studies focusing on the modification of genetic and epigenetic alterations of TSHR and the related genes will help better understand the disease process and may lead to a potential cure.

References

1. Parmentier M, Libert F, Maenhaut C, Lefort A, Gerard C, Perret J, Van Sande J, Dumont JE, Vassart G. Molecular cloning of the thyrotropin receptor. Science 1989;246:1620-1622.
2. Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM. The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. Endocr Rev 1998;19:673-716.
3. Huber GK, Weinstein SP, Graves PN, Davies TF. The positive regulation of human thyrotropin (TSH) receptor messenger ribonucleic acid by recombinant human TSH is at the intranuclear level. Endocrinology 1992;130:2858-2864.
4. Field JB, Ealey PA, Marshall NJ, Cockcroft S. Thyroid-stimulating hormone stimulates increases in inositol phosphates as well as cyclic AMP in the FRTL-5 rat thyroid cell line. Biochem J 1987;247:519-524.
5. Riedel C, Levy O, Carrasco N. Post-transcriptional regulation of the sodium/iodide symporter by thyrotropin. J Biol Chem 2001;276:21458-21463.
6. Akamizu T, Ikuyama S, Saji M, Kosugi S, Kozak C, McBride OW, Kohn LD. Cloning, chromosomal assignment, and regulation of the rat thyrotropin receptor: expression of the gene is regulated by thyrotropin, agents that increase cAMP levels, and thyroid autoantibodies. Proc Natl Acad Sci U S A 1990;87:5677-5681.
7. Vassart G, Dumont JE. The thyrotropin receptor and the regulation of thyrocyte function and growth. Endocr Rev 1992;13:596-611.
8. Kopp P. The TSH receptor and its role in thyroid disease. Cell Mol Life Sci 2001;58:1301-1322.
9. Vassart G, Kleinau G. TSH Receptor Mutations and Diseases. In: Endotext. edn. Edited by De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R et al. South Dartmouth (MA); 2000.
10. Dumont JE, Lamy F, Roger P, Maenhaut C. Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiol Rev 1992;72:667-697.
11. Sobrinho-Simoes M, Maximo V, Rocha AS, Trovisco V, Castro P, Preto A, Lima J, Soares P. Intragenic mutations in thyroid cancer. Endocrinol Metab Clin North Am 2008;37:333-362.
12. Kopp P, Muirhead S, Jourdain N, Gu WX, Jameson JL, Rodd C. Congenital hyperthyroidism caused by a solitary toxic adenoma harboring a novel somatic mutation (serine281–>isoleucine) in the extracellular domain of the thyrotropin receptor. J Clin Invest 1997;100:1634-1639.
13. Paschke R, Tonacchera M, Van Sande J, Parma J, Vassart G. Identification and functional characterization of two new somatic mutations causing constitutive activation of the thyrotropin receptor in hyperfunctioning autonomous adenomas of the thyroid. J Clin Endocrinol Metab 1994;79:1785-1789.
14. Cetani F, Tonacchera M, Pinchera A, Barsacchi R, Basolo F, Miccoli P, Pacini F. Genetic analysis of the TSH receptor gene in differentiated human thyroid carcinomas. J Endocrinol Invest 1999;22:273-278.
15. Spambalg D, Sharifi N, Elisei R, Gross JL, Medeiros-Neto G, Fagin JA. Structural studies of the thyrotropin receptor and Gs alpha in human thyroid cancers: low prevalence of mutations predicts infrequent involvement in malignant transformation. J Clin Endocrinol Metab 1996;81:3898-3901.
16. Said S, Schlumberger M, Suarez HG. Oncogenes and anti-oncogenes in human epithelial thyroid tumors. J Endocrinol Invest 1994;17:371-379.
17. Niepomniszcze H, Suarez H, Pitoia F, Pignatta A, Danilowicz K, Manavela M, Elsner B, Bruno OD. Follicular carcinoma presenting as autonomous functioning thyroid nodule and containing an activating mutation of the TSH receptor (T620I) and a mutation of the Ki-RAS (G12C) genes. Thyroid 2006;16:497-503.
18. Gozu H, Avsar M, Bircan R, Sahin S, Ahiskanali R, Gulluoglu B, Deyneli O, Ones T, Narin Y, Akalin S, Cirakoglu B. Does a Leu 512 Arg thyrotropin receptor mutation cause an autonomously functioning papillary carcinoma? Thyroid 2004;14:975-980.
19. Camacho P, Gordon D, Chiefari E, Yong S, DeJong S, Pitale S, Russo D, Filetti S. A Phe 486 thyrotropin receptor mutation in an autonomously functioning follicular carcinoma that was causing hyperthyroidism. Thyroid 2000;10:1009-1012.
20. Russo D, Wong MG, Costante G, Chiefari E, Treseler PA, Arturi F, Filetti S, Clark OH. A Val 677 activating mutation of the thyrotropin receptor in a Hurthle cell thyroid carcinoma associated with thyrotoxicosis. Thyroid 1999;9:13-17.
21. Catalano MG, Fortunati N, Boccuzzi G. Epigenetics modifications and therapeutic prospects in human thyroid cancer. Front Endocrinol (Lausanne) 2012;3:40.
22. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 2013;13:184-199.
23. Lu C, Zhao L, Ying H, Willingham MC, Cheng SY. Growth activation alone is not sufficient to cause metastatic thyroid cancer in a mouse model of follicular thyroid carcinoma. Endocrinology 2010;151:1929-1939.
24. Franco AT, Malaguarnera R, Refetoff S, Liao XH, Lundsmith E, Kimura S, Pritchard C, Marais R, Davies TF, Weinstein LS, Chen M, Rosen N, Ghossein R, Knauf JA, Fagin JA. Thyrotrophin receptor signaling dependence of Braf-induced thyroid tumor initiation in mice. Proc Natl Acad Sci U S A 2011;108:1615-1620.

25. Feng F, Wang H, Hou S, Fu H. Re-induction of cell differentiation and (131)I uptake in dedifferentiated FTC-133 cell line by TSHR gene transfection. Nucl Med Biol 2012;39:1261-1265.
26. Kleiman DA, Buitrago D, Crowley MJ, Beninato T, Veach AJ, Zanzonico PB, Jin M, Fahey TJ, Zarnegar R. Thyroid stimulating hormone increases iodine uptake by thyroid cancer cells during BRAF silencing. J Surg Res 2013;182:85-93.
27. Durante C, Puxeddu E, Ferretti E, Morisi R, Moretti S, Bruno R, Barbi F, Avenia N, Scipioni A, Verrienti A, Tosi E, Cavaliere A, Gulino A, Filetti S, Russo D. BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. J Clin Endocrinol Metab 2007;92:2840-2843.
28. Espadinha C, Santos JR, Sobrinho LG, Bugalho MJ. Expression of iodine metabolism genes in human thyroid tissues: evidence for age and BRAFV600E mutation dependency. Clin Endocrinol (Oxf) 2009;70:629-635.
29. Khan MS, Pandith AA, Masoodi SR, Wani KA, Ul Hussain M, Mudassar S. Epigenetic silencing of TSHR gene in thyroid cancer patients in relation to their BRAF V600E mutation status. Endocrine 2014;47:449-455.
30. Tuncel M, Aydin D, Yaman E, Tazebay UH, Güç D, Doğan AL, Taşbasan B, Uğur O. The comparative effects of gene modulators on thyroid-specific genes and radioiodine uptake. Cancer Biother Radiopharm 2007;22:443-449.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):92-101 DOI:10.4274/2017.26.suppl.11

The Thyroid Na^+/I^- Symporter: Molecular Characterization and Genomic Regulation

Tiroid Na^+/I^- Simporter: Moleküler Tanımlaması ve Genomik Kontrolü

Hani Alotaibi[1], Merve Tuzlakoğlu-Öztürk[2], Uygar Halis Tazebay[2]
[1]Dokuz Eylül University, Biomedicine and Genome Center (IBG-İzmir), İzmir, Turkey
[2]Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey

Abstract

Iodide (I^-) is an essential constituent of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4), and the iodide concentrating mechanism of the thyroid gland is essential for the synthesis of these hormones. In addition, differential uptake of iodine isotopes (radioiodine) is a key modality for the diagnosis and therapy of thyroid cancer. The sodium dependent iodide transport activity of the thyroid gland is mainly attributed to the functional expression of the Na^+/I^- Symporter (NIS) localized at the basolateral membrane of thyrocytes. In this paper, we review and summarize current data on molecular characterization, on structure and function of NIS protein, as well as on the transcriptional regulation of NIS encoding gene in the thyroid gland. We also propose that a better and more precise understanding of NIS gene regulation at the molecular level in both healthy and malignant thyroid cells may lead to the identification of small molecule candidates. These could then be translated into clinical practice for better induction and more effective modulation of radioiodine uptake in dedifferentiated thyroid cancer cells and in their distant metastatic lesions.

Keywords: Iodide, thyroid, Na^+/I^- symporter, radiotherapy, thyroid cancer, gene regulation

Öz

İyot (I^-), tiroid bezi kaynaklı hormonlar olan triiodotironin (T_3) ve tiroksinin (T_4) temel bileşenlerindendir, ve tiroid bezinde iyodun derişimini artıran moleküler mekanizmalar aynı zamanda bu hormonların sentezlenmesinde de anahtar rol oynarlar. Bunun yanı sıra, radyoaktif özellikler gösteren iyot izotoplarının normal ve neoplastik dokulara taşınma kinetiklerindeki farklılıklar, bu radyoizotopların tiroid kanserinin teşhis ve tedavisinde etkili olarak kullanılmalarına yol açmıştır. Tiroid bezinde bulunan bu sodium gradyanına eşlenmiş iyot taşıma aktivitesi, temel olarak tiroid folikül hücrelerinin bazolateral hücre zarında bulunan Na^+/I^- simporter (NIS) proteininin işlevsel ifadesine bağlıdır. Bu derlemede, NIS proteininin yapısı, işlevi ve transkripsiyon ve translasyon sonrası çeşitli moleküler mekanizmalar ile düzenlenmesinin moleküler belirleyicileri özetlenmiştir. Sağlıklı ve kanserleşme özelliği taşıyan tiroid dokularında NIS düzenlenmesinin moleküler detayları daha iyi ve daha detaylı şekilde anlaşıldıkça, hem genetik ve hem de protein düzeyinde NIS'in radyoiyot taşıma etkinliğini düzenleyecek yeni küçük moleküllerin tanımlanabileceğini, bunların klinik uygulamalara aktarılmasının ise özellikle dedifferansiye olmuş tiroid kanseri ve metastazlarının teşhis ve tedavisinde yenilikçi ve etkili uygulamalara yol açacağını öne sürüyoruz.

Anahtar kelimeler: İyot, tiroid, Na^+/I^- simporter, radyoterapi, tiroid kanseri, gen düzenlenmesi

Address for Correspondence: Uygar Halis Tazebay MD, Gebze Technical University, Department of Molecular Biology and Genetics, Kocaeli, Turkey
Phone: +90 262 605 25 22 E-mail: tazebay@gtu.edu.tr

Introduction

Biological Significance of Iodide Transport

Iodine (^{127}I) is the heaviest element metabolized in biological material. It is also a limiting element for the synthesis of covalently bound iodine-containing thyroid hormones, which are essential for proper growth and development of many organs in vertebrates (1,2). Aside from the thyroid gland, which has a remarkable mechanism for collecting and organifying this rare element from nutrients (3), evidence for the presence of iodide (I-) concentrating mechanisms in other vertebrate tissues date back to as early as 1856 when Claude Bernard described the presence of iodine in the salivary gland, in the milk of nursing mothers, as well as in the hair, skin, ovaries, placenta, kidney, stomach and intestines in mammals (4). The availability of radioisotopes by the 1940s improved the techniques in which lower amounts of iodide could be measured, and thus led to precise identification of tissues where iodide transport takes place (4). Also, radioiodide was first used as a therapeutic modality for Graves' disease, in 1942 at the Massachusetts Institute of Technology (5).

The existence of a mRNA capable of encoding a functional iodide transporter in thyroid cells was illustrated by the expression of a specific mRNA isolated from the thyroid cell line FRTL-5 in the oocytes of *X. laevis* (6). This study eventually led to cloning of the cDNA of the rat sodium iodide symporter [Na^+/I^- Symporter, or NIS; (7)]. The cloning of the NIS gene provided an invaluable tool for the analysis of NIS mRNA (and later, protein) expression in different tissues and organs. Since then, while using reverse transcription polymerase chain reaction (RT-PCR) technique, human NIS (hNIS) mRNA was detected in the thyroid, salivary gland, lacrimal ducts, parotid gland, submandibular gland, pituitary gland, pancreas, testis, mammary gland, gastric mucosa, prostate, ovaries, kidney, and placenta (8,9,10,11,12). As expected, the expression of the hNIS mRNA was also clearly observed in both the thyroid and parotid glands, by Northern blot analysis (10).

NIS is the fifth member of the sodium/solute carrier family 5A (SLC5A5; according to the Online Mendelian Inheritance in Man (OMIM) classification). This is a family of proteins that mediate the active transport of negatively-charged solutes (for instance, I-) into the cytoplasm using an electrochemical Na+ gradient (13). The SLC5A5 family of transporters belongs to a solute carrier super-family including 45 different solute carrier families and one organic anion transporter family (14,15). The rat NIS (rNIS) was the first member to be cloned by functional screening of a human cDNA library from FRTL-5, a rat thyroid cell line, in *Xenopus laevis* oocytes (7). In the same year another report described cloning of the human NIS (hNIS) using cDNA prepared from human papillary carcinoma tissue; they amplified the hNIS cDNA fragment using primers derived from the nucleotide sequence of the rat mRNA of rNIS (16). Subsequently, Tazebay et al. (17) showed that the transport of iodide in lactating mammary glands is also mediated by NIS. Later, the mouse sodium iodide symporter was cloned from the thyroid and lactating mammary gland tissues (18,19).

The gene encoding the human iodide transporter was mapped to chromosomal location 19p13.2-p12 using fluorescence in situ hybridization (8). The coding sequence of the hNIS gene is encoded by 15 exons, and the exon-intron junctions were also clearly described (8).

Structure of the Na^+/I^- Symporter

The hNIS cDNA encodes a 643-amino acid protein with 84% homology to both the rat and mouse genes (16). Generation of an anti-NIS antibody allowed researchers to analyze the structure and the post-translational modifications that lead to the mature functional protein. The cytoplasmic location of the carboxy terminus of NIS was confirmed using indirect immunofluorescence in FRTL-5 cells (20). Moreover, by using this antibody, membrane fractions from FRTL-5 cells or COS cells transfected with NIS cDNA revealed a prominent immunoreactive polypeptide with a molecular weight of about 87 kDa, different from the predicted mass of the protein (65 kDa). This difference was attributed to post-translational modifications at three putative Asparagine (Asn) residues at positions 225, 485 and 497 by N-linked glycosylation (20,21), two of which (residues 485 and 497) were located in the predicted sixth extra-cellular loop. Site directed mutagenesis of these putative glycosylation sites demonstrated that NIS is processed at three Asn sites instead of two. This placed the third glycosylation site (previously predicted in the third intracellular loop at position 225) in the cytoplasmic side of the membranesince N-linked glycosylation occurs at exposed extracellular facing sites in the endoplasmic reticulum during protein processing (22). Moreover, by using an amino-terminus FLAG-tagged NIS, engineered by site directed mutagenesis, Levy et al. (22) (1998) showed that non-permeabilized as well as permeabilized cells were stained by anti-FLAG antibodies, an observation suggesting that the amino terminus of NIS is located at the extra-cellular side of the plasma membrane. Based on that, the authors suggested a revised model for the secondary structure of NIS, in which the amino terminus faces extracellularly. According to the current model (Figure 1), NIS is an intrinsic membrane protein, with 13 transmembrane helices (22).

Function of the Na^+/I^- Symporter

The sodium/iodide symporter is the transporter responsible for the active transport of iodide from the blood stream into cells. This intracellular iodide is then used in different physiological processes. The uptake process is sodium dependent (23) and NIS co-transports Na+ and I- with a

stoichiometry of 2 Na^+:1 I^- (24). The sodium-driven transport of iodide is maintained by an ouabain sensitive sodium-potassium ATPase which provides the energy required for this process (25,26). NIS is also capable of transporting other ions with less affinity, including ClO_3^-, SCN^-, $SeCN^-$, NO_3^-, Br^-, BF_4^-, IO_4^-, BrO_3^-, but not perchlorate (ClO_4^-) (24). In fact, ClO_4^- is a well-known competitive inhibitor of iodide transport as well as NO_3^-, BF_4^-, SCN^-, 2,4-dinitrophenol, and cardiac glycosides (1,3,24).

Iodide Requirement in Hormone Biosynthesis and Development

Thyroid hormone biosynthesis requires the presence of inorganic iodide with the presence of an iodide trapping system as the first limiting step in this process (1,3,26). Iodide is an essential and covalently bound constituent of the thyroid hormones triiodothyronine (T_3) and thyroxin (T_4). NIS, located at the basolateral membrane of thyrocytes (27), transports iodide into the cells which is then transported across the apical membrane into the follicular lumen (or colloid) by different anion transporters, such as pendrin and apical iodide transporter (28,29). In the colloid, thyroid peroxidase covalently incorporates transported iodide into tyrosine residues of the thyroid hormone precursor, thyroglobulin, in a process known as organification (30,3). Iodinated thyroglobulin is then endocytosed, followed by phagolysosomal hydrolysis of the iodinated thyroglobulin releasing the thyroid hormones, which are then released into the blood stream. This process ismainly controlled by the thyroid stimulating hormone (3). Importantly, thyroid hormones are essential for proper growth and maturation of the skeletal muscles, the nervous system and the lungs of a fetus and a developing newborn (2).

As mentioned before, NIS is also expressed in the lactating mammary glands and functions to secrete iodide into mothers' milk (17), thus providing the first supply of iodide to the newborn to be used for thyroid hormone biosynthesis. To date, the biological relevance of NIS expression and iodide transport in organs other than the thyroid and the lactating mammary gland is not clear and further research is required to reveal the significance of iodide in the physiological processes in these other organ systems.

Regulation of Na^+/I^- Symporter Gene Expression in the Thyroid Gland

In the thyroid gland, thyroid stimulating hormone's (TSH) binding action elevates the intracellular level of cyclic AMP (cAMP), which, in turn, is an important modulator of gene expression in thyrocytes (31,32). The effect of TSH on iodide uptake in the thyroid was first reported in the 1960s when researchers described increased iodide uptake in the thyroid gland of rats treated with TSH in a cycloheximide dependent manner. This suggested that TSH is actually responsible for the synthesis of an enzyme that mediates iodide uptake (33). Subsequently, by using bovine thyroid cells, Knopp and co-workers confirmed this observation (1970) as well as an inhibitory effect of actinomycin D when added together with TSH. In contrast, cells treated with actinomycin D after two hours of TSH treatment displayed how iodide uptake was stimulated normally, suggesting that TSH treatment resulted in the synthesis of a specific RNA molecule. They also found that cyclohexamide blocked iodide uptake when added with TSH. However, if TSH and cyclohexamide were washed out after two hours, then iodide uptake developed normally (34). In the same study, they also observed a similar effect

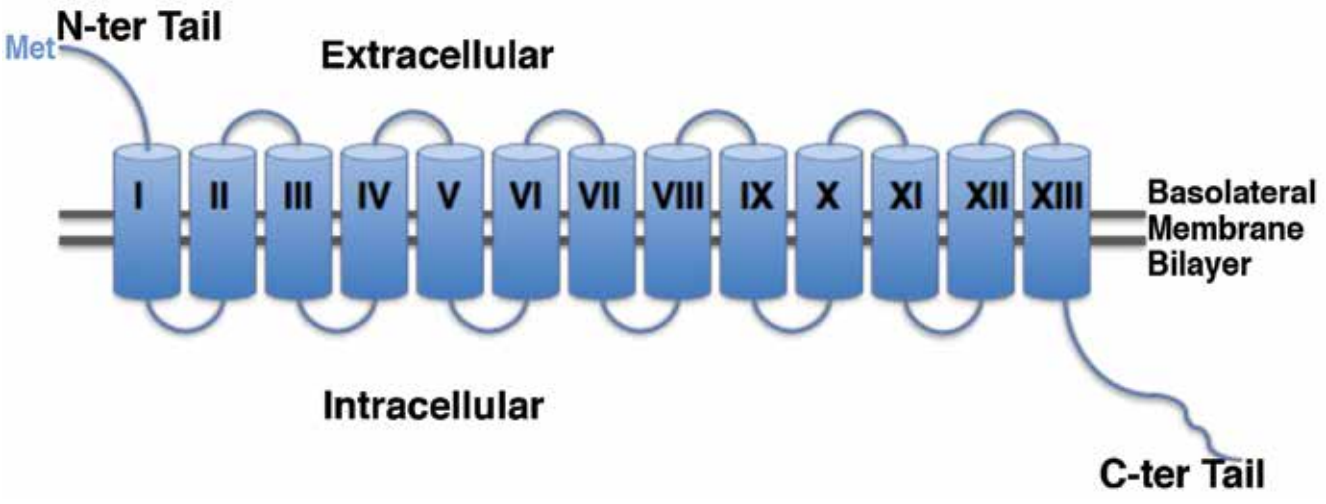


Figure 1. Secondary structure of Na^+/I^- symporter embedded in basolateral membrane bilayer. Transmembrane domains of Na^+/I^- symporter crossing the basolateral membrane bilayer are indicated by numbers (I-XIII). The start codon (Met) of Na^+/I^- symporter is indicated. N-terminal tail of the symporter has an extracellular localization, while the C-terminal part is cytoplasmic. Modified from Levy et al. (22) (1998)

of TSH when they incubated the cells with cAMP. Cellular levels of cAMP responded to increasing or decreasing concentrations of TSH concluding that TSH in thyroid cells activates adenyl cyclase so that cAMP production is augmented. This increase causes the production of a specific RNA molecule which in turn induces the formation of specific stimulatory protein (34).

These early findings actually suggested a regulatory action of TSH on the expression of NIS in the thyroid gland. Regulation of NIS expression at the transcriptional level was more evident in research studies carried out after the cloning of the NIS gene (7,16,18,19), thus supporting results of earlier reports concerning regulation of NIS expression in thyrocytes. It has been shown that TSH activates the transcription of NIS via cAMP in a cyclohexamide-dependent manner (35). Later on, several reports characterized this TSH stimulated NIS transcription indicating that in the thyroid gland, TSH regulates NIS dependent iodide transport both at post-translational and at transcriptional levels (20,35,36,37,38). Clues for TSH mediated post-translational regulation of NIS came from studies in which membrane vesicles (prepared from FRTL-5 cells that lost iodide uptake as a result of prolonged deprivation of TSH) retained iodide uptake after stimulation by TSH, suggesting that NIS protein is present in the vesicles and a mechanism other than transcription might be required for proper NIS activity (39,37).

The regulatory effect of TSH on NIS protein was illustrated later on; researchers determined that the half-life of NIS protein increases from 3 days to 5 days in the presence of TSH and that NIS is a phosphoprotein, whose phosphorylation is mediated by TSH (37). Moreover, TSH was found to modulate the intra-cellular distribution of NIS; in the presence of TSH, NIS is mainly located at the plasma membrane, whereas in TSH deprived cells, NIS was translocated to intra-cellular compartments (37).

TSH dependent expression of NIS was mediated at the transcriptional levelby the adenylate cyclase-cAMP pathway (3,36). Several groups isolated the 5' regulatory region of rat and human NIS genes in order to study cis- and trans-acting elements that regulate NIS transcription in thyrocytes (40,41,42,43,36). It was reported that a novel transcription factor, named "NIS TSH-responsive factor-1" or NTF-1, mediates the transcriptional regulatory effect of TSH, itself being mediated by cAMP, on NIS promoter through a TSH responsive element (TRE) located between positions -420 and -385 of the rat NIS promoter in a thyroid specific manner (44).

The thyroid transcription factor-1 [TTF-1; (45)], a member of the Nk2 family of homeobox-containing genes in Drosophila, was implicated in the regulation of several thyroid specific genes such as thyroid peroxidase (TPO), thyroglobulin (Tg) and thyroid stimulating hormone receptor (TSH-R) (46). It was also found to activate the transcription of NIS in thyroid cells, and functional TTF-1 binding sites were found between nucleotide positions -245 to –230 bp of the rNIS promoter (40). Mutations in the NTF-1 binding site (TRE) causing loss of the TSH response also resulted in a decrease in the TTF-1-induced promoter activity, suggesting that TTF-1-mediated thyroid specific expression of NIS is controlled by the TSH/cAMP-pathway (40).

Sodium-iodide Symporter Upstream Enhancer as a Cis-Regulatory Element

The isolation and cloning of the promoter and upstream regulatory region of the rat NIS gene facilitated the search for cis- and trans-acting genetic elements mediating thyroid-specific and TSH-regulated transcriptional activation (40,41,36). These studies resulted in the identification of a thyroid-specific transcriptional regulatory cis-acting element at the 5'-flanking region of the rat NIS gene (36). This enhancer region (NIS upstream enhancer, or NUE), is located between nucleotides -2495 to -2264 and contains binding sites for Pax8, a paired domain factor that is present both in the thyroid and kidney, and TTF-1, a homeodomain containing protein present in the developing thyroid, lung and diencephalon. In DNase I footprinting studies carried by Ohno et al. (36) (1999), it has been shown that Pax8 actually binds to two sites in this newly identified enhancer. Mutational analysis of these binding sites has shown that Pax8 binding as well as NIS transcription is reduced when these sequences are modified, suggesting a functional role for Pax8 in NUE transcriptional activity. TTF-1 also binds at two different sequences in NUE element, and one of these two binding sites overlaps with Pax8 binding site, while the other is closely located (~20 nucleotides) but distinct from the first site. However, Pax8 (and not TTF-1) is required for the transcriptional activation and cAMP stimulation of the NUE. Further work revealed that a cAMP responsive element (CRE) proximal to NUE can be recognized by various members of the AP-1 and CREB family of transcription factors that modulate the transcriptional activity of NUE. Furthermore, using tethered dimers of b-Zip molecules, it has been shown that specific homo- or hetero-dimers of AP-1 can synergistically stimulate NUE activity in concert with Pax-8 (47). The Human NIS upstream enhancer (hNUE) was also identified; it was found to be localized at –9847 to –8968 bp relative to the hNIS gene start codon. It contains functional Pax8 and TTF-1 binding sites and a CRE-like sequence (48). The enhancer was shown to be cell specific; it activates NIS transcription only in thyroid cell lines, and not in MCF-7 breast cancer or JEG-3 choriocarcinoma cells (48). Recent studies revealed novel transcription factors upregulating NIS transcription in a TSH dependent manner by specific interactions with hNUE; these were β-catenin (49), forkhead transcription factor, FoxE1 (50), and hairy and enhancer of split-1, Hes-1 (51). Also, sterol regulatory element binding proteins

(SREBPs) modulate NIS transcription in response to TSH not by interacting with NUE, but with the 5'-UTR of NIS (52).

Regulation of Na^+/I^- Symporter by Retinoids in the Thyroid

The ability of thyroid tumors to retain iodide uptake was used for decades for the treatment and diagnosis of thyroid cancer. However, especially in cases with advanced tumorigenesis, thyroid cells suspend this characteristic of radioiodide uptake due to progressive tumor-associated dedifferentiation of thyrocytes, thus leading to a rather ineffective radioiodide therapy [for a review on this, please see (53)]. In earlier reports, it has been shown that in patients with radioiodide resistant tumors, treatment with retinoic acid (RA; a well known agent with differentiation-inducing properties) reactivates the iodide uptake mechanism, and thus restores the possibility of radioiodide-based therapy (54,55). Further characterization of this stimulatory effect of RA in thyroid cell models revealed that RA treatment of normal non-transformed thyrocytes resulted in decreased iodide uptake and reduced NIS expression. On the other hand, both NIS mRNA and iodide uptake were elevated in human follicular thyroid carcinoma cell lines, suggesting that RA treatment could be used to up-regulate NIS expression and thus iodide uptake in tumor cells to be targeted differentially by radioiodide treatment (56).

Retinoic acids are derivatives of vitamin A, which play an important role in several physiological processes during embryonic development and in adult life (57). They are also known for their potent proliferation-inhibiting and differentiation-inducing properties. Retinoic acid signals are mediated by nuclear receptors (Retinoic acid receptors, RAR and Retinoic X receptors, and RXR), action of which can be seen as receptor-receptor interactions, receptor-DNA interactions, andinteractions with other regulatory proteins (57). Transcription activation function of RARs is mediated by binding to DNA sequences called retinoic acid response elements (RARE) in the promoter of target genes (58). The binding site of RARs may vary, depending on the target gene, and the consensus sequence is a hexamer (PuGG/TTCA). The classical RARE is composed of two direct repeats of this core motif, which are usually separated by 5 nucleotides, although functional direct repeats separated by 1, 2 or 10 nucleotides have been also reported (58,59). The molecular determinants controlling RA induced NIS expression in thyroid cells were investigated; RA exerts its up-regulatory effect on hNIS promoter through a RARE located at -1375 relative to the ATG codon (60). It has been shown that RAR binds to this element, specifically DR_{10} (AGGTCA-n10-GGGTCC), mediating activation of NIS transcription in response to RA stimulation, and that the RA stimulation and RAR binding were abolished due to mutations in either half site of this element (60). This evidence of a direct stimulatory action of RA on NIS expression in thyroid cell lines, as well as the success in RA-redifferentiation prior to radioiodide therapy, encouraged investigators to study also the feasibility of radioiodide therapy after RA treatment in cancer patients with tumors of also other origins (61,62).

Functional Retinoic Acid Response Elements in Multiple Introns of Na^+/I^- Symporter Gene

Using the genome Vista software tools (63), the flanking sequences andthe NIS gene were analyzed for conserved regions in the genomes of human, mouse and rat (64). This study was based on the previously shown correlation between conserved gene expression patterns and the conserved regulatory cis-acting elements (65). The results of the analysis revealed several conserved regions which are involved in the regulation of the transcription of this gene. As expected, Alotaibi et al. (64) (2010) first showed that the immediate upstream sequence in front of the NIS transcription start site including the previously described minimal promoter (66) and a RA responsive site (60) responded well to RA in terms of activation of NIS transcription (64). Furthermore, they have demonstrated the significance of intronic sequences in regulating NIS expression: the first intron of NIS contains both DR2-type elements and one overlapping DR10 element, the latter directly interacting with RAR and acting as an enhancer of the gene. Interestingly, multiple introns of NIS gene (7 out-of 14 introns) contain identical functional RARE sequences interacting with the NIS promoter (presumably by formation of a DNA-loop) and regulate the transcriptional activation of the symporter (64). Future molecular studies will determine possible roles of intronic cis-acting nuclear receptor binding sites in organ specific regulation of NIS gene expression.

Estradiol and ERα are Involved in the Transcription of Na^+/I^- Symporter

Previous studies have shown that in a rat thyroid cell line model, FRTL-5, activation of ER pathway by E2 down-regulates thyroid NIS gene expression (67,68). Interestingly, in mammary gland cell lines, the RA-dependent expression of NIS was strictly correlated with the presence of ERα, and the analysis of NIS expression in MCF-7 cells where ERα was suppressed by RNA interference revealed the importance of this factor in both basal and tRA induced expression of NIS (69). In the same study, the unliganded ERα (apo-ERα) was shown to play an essential role for holding the NIS gene at a transcriptionally competent state. This would indicate the absence of transcriptionally competent NIS gene loci in ERα- mammary cell lines, and thus explain the lack of tRA-responsive NIS expression in these cells (69). Concerning possibilities of a direct action via ERα, the authors detected a novel ERE sequence

conserved in human, rat and mouse genomes in proximity (9 base pairs) of NIS TATA element, with the capacity to activate gene expression in luciferase reporter assays in analyzed ER-positive mammary cell line models (69). In fact, such a close localization of TATA and ERE elements in NIS promoter is very unusual considering that all previously characterized ERE elements were shown to be localized at relatively distant positions to transcription start sites in corresponding genes [although varying remarkably between +23,088 and -2687 (70)].

Cytokines Modulate Na^+/I^- Symporter Expression Rather Negatively

Effects of cytokines, such as tumor necrosis factor alpha (TNF-α) and beta (TNF-β), interferon-gamma (IFN-γ), interleukins 1-alpha (IL-1α) and beta (IL-1β), and transforming growth factor-beta (TGF-β) on NIS gene regulation have been assayed by several groups in FRTL-5 cells. All of these factors were found to down-regulate NIS mRNA expression in a time and dose dependent manner (9,71,72,73,74,75,76). Thus it would be correct to state that the effect of cytokines on TSH-induced NIS expression is rather negative. Related with this, there are recent efforts to assess the effectiveness of specific inhibitors of cytokine pathways on an increased NIS expression and radioiodide transport function in advanced thyroid cancers (77).

Clinical Significance of Iodide Transport in Diagnosis and Therapy of Thyroid Carcinomas

The ability of the thyroid gland to transport iodide is an absolute requirement for the synthesis of T_3 and T_4. Significantly, iodide transport mediated by functional NIS expression is also observed in abnormalities of the thyroid such as thyroid nodules and thyroid cancer (78). Thus, the function of NIS -as the key transporter of iodide- has emerged as a valuable tool for the diagnosis and treatment of thyroid cancer, and for decades radioactive iodide played a major therapeutic role in the postoperative management of differentiated thyroid carcinoma (DTC) because of its effectiveness to ablate remnant thyroid tissue and metastases. Moreover, the degree and pattern of iodide accumulation in the thyroid, as revealed by scintigraphic imaging, is used as an aid in the differential diagnosis of thyroid nodules. Thus, radioiodide ($^{131}I^-$ or $^{123}I^-$) and also pertechnetate ($^{99m}TcO_4^-$) transport activity of NIS have successfully been used in the detection, treatment, and follow-up of thyroid cancers (79).

It has previously been reported in a number of studies that patients with refractory and advanced thyroid cancer may not fully benefit from radioiodide therapy due to insufficient TSH-induced functional NIS expression (16,80,77). By using quantitative RT-PCR techniques, Lazar et al. (81) (1999) have previously shown that NIS mRNA expression was significantly decreased in 40 out of 43 thyroid cancer cases (38 papillary and 5 follicular), and also in about the same percentage of cold adenomas. In the same study, a positive correlation was found between the expression levels of NIS and other thyroid specific proteins such as TPO, Tg, and TSH-R, indicating the link between low expression and dedifferentiation mechanisms. Along the same lines, higher tumor stages were also associated with low NIS expression. Importantly, NIS gene expression levels were also detected to be very low in oncogene-transformed rat thyroid cell models, indicating an inverse correlation between oncogene activation and NIS expression in thyroid cancer models (82). Lower levels of functional NIS expression were also detected in metastatic thyroid tumor tissues. Park et al. (83) (2000) studied correlations in NIS expression between primary thyroid tumors and their metastatic lesions (23 papillary carcinomas, and 7 pairs of primary and lymph node metastatic tissues), indicating variable levels of NIS mRNA expression that were significantly lower than those in healthy thyroid. Furthermore, in a number of cases where NIS expression was detected in the primary tumor site, it was completely absent in their lymph node metastasis, indicating that NIS expression in the primary thyroid tumor could not be used to predict the level of possible radioiodine accumulation in the metastatic lesions.

Retinoic acids (RA) and their derivatives are potent molecules that have been used for redifferentiation therapy of many cancers because of their differentiation-inducing and proliferation-inhibiting abilities (84). They are typically ligands of a class of nuclear receptors called the retinoic acid receptors (RAR and retinoid x receptors; RXR). It has been shown that patients with poorly DTC lacking iodide transport responded to treatments with RA and showed an increased radioiodine transport (54,85,86). It was reasonable to conclude that increased radioiodine transport was a reflection of an increased sodium iodide transporter activity and probably transcription (86). Schmutzler et al. (60) (2002) studied the involvement of RA and RAR in the transcription of NIS using human follicular thyroid carcinoma cell lines and found a dimeric retinoic acid responsive site (DR^{10}) at -1375 relative to the ATG start codon of the human NIS gene. Their data showed that this site was responsive to RA stimulation (2.5 fold increase), and that blocking mutations in either half site abolished RAR binding to this element and thus the loss of RA response. Thus, the up-regulatory effect of tRA on thyroid NIS expression was established at the molecular level with several clinical trials successfully demonstrating RA redifferentiation effects in previously dedifferentiated thyroid tumors and their metastases (87,88,89).

A Few Words on Non-Thyroidal Iodide Transport

Even though the iodide transport function is mostly associated with the thyroid gland, functional expression of NIS has also been reported in healthy mammary

gland specifically during late-pregnancy and in lactation (90,17). Interestingly, NIS expression was also observed in a high percentage of human breast cancer specimens (with various pathologies) in contrast to no expression in normal tissues obtained from reductive mammoplasties (17,91). These results suggest that radioiodide administration may be effective as an adjuvant to surgical treatment of primary breast cancer, and/or as a tool in the diagnosis and treatment of metastatic disease (17). A major characteristic of the healthy thyroid gland is that it exhibits NIS activity for life, within boundaries set by thyroid regulatory factors such as thyroid stimulating hormone and iodide itself (74,37). In contrast, the potential effectiveness of radioiodide therapy in breast cancer depends on whether NIS becomes functionally expressed in malignant mammary cells, given that it is not functionally expressed in healthy cells, except during pregnancy and lactation. It is notable that a single transport protein (i.e., NIS) catalyzes the same fundamental process (active Na+-dependent iodide transport) in both tissues, but is regulated differently in each of them. These differences affect not only how NIS functions under normal conditions, but also how it can play a role in cancer management in both tissues.

Unlike the thyroid gland (being the only known organ to incorporate iodide into thyroid proteins), in mammary gland cells iodide is secreted into the milk, and this difference creates a challenge for the application of an effective dose of radioiodide for the treatment of malignant cells of the breast (92). Clearly, alternative strategies for detection of micrometastatic disease and for more effective and targeted systemic therapies are needed to improve survival in breast cancer, which remains the leading cause of cancer deaths in women (ages of 20-59) in developed countries (93).

Several researchers have reported the possible use of radioiodide for the treatment of cancers by forced expression of NIS in tumors of several origins, such as in prostate cancer (94,95), hepatoma (96), glioma (97), neuroendocrine tumor cells (98), head and neck squamous cell carcinoma (99), colon cancer (100,101), pancreatic tumors (102), and in ovarian tumor xenografts (103). The challenge here is the method of delivering NIS expressing DNA selectively to the tumor. So far, studies mentioned above reported the successful use of viral vehicles such as retroviral or adenoviral particles, but more comprehensive studies are certainly required for more effective results, and for a wider range of cancer models to be targeted.

Conclusion

Thyroid diseases characterized by excess or deficient production of thyroid hormones, enlargement of the gland, presence of aberrant nodules, neoplastic proliferation, and auto-immune syndromes are frequently encountered in endocrinological practice, and thyroid ailments in endocrinology clinics are surpassed in numbers only by diabetes. Since the mid 1940's, differential expression of thyroid NIS in different pathological conditions leading to a differential biodistribution of iodide isotopes in tissues with different histological and pathological characteristics have made the radioiodide transport system, NIS, a crucial factor for the diagnosis, treatment, or evaluation of pathological thyroid conditions. When NIS is functionally expressed to a sufficient degree in cancerous cells of thyroid origin (and also of mammary glands), use of radioiodide emerges as a powerful potential diagnostic and therapeutic tool. Thus, radioiodide therapy has been used in clinics for decades for the treatment of thyroid cancer, where the goal is to deliver a lethal dose of gamma-radiation to the tumor without affecting the surrounding healthy tissue.

As stated in this paper, a considerable amount of molecular studies has already been carried-out concerning transcriptional regulation of NIS in the thyroid gland, and a substantial amount of studies are being performed to establish novel therapeutic/diagnostic procedures involving small molecules (inhibitors/inducers) and hormonal modalities (TSH, RA, estradiol, etc.) for a refined molecular control of NIS activity in targeted versus untargeted tissues (104), as NIS is expressed not only in thyroid follicular cells but also in lactating breast, gastric mucosa, lacrimal ducts, and salivary ducts. Accordingly, these NIS-expressing tissues are also subject to radioiodine-induced damage during therapeutic procedures. The side effects of radioiodine therapy include temporary or permanent salivary gland disfunction, temporary gastro-intestinal upset, lacrimal duct obstruction, gonadal disfunction, and possible secondary malignancies. Future studies for the molecular protection of non-targeted secondary iodide transporting tissues by, for instance, transiently shutting-down tissue-specific NIS expression will certainly provide an optimal use of radioiodide in the clinical management of thyroid cancer. One of the challenges on this issue is how to have a standard procedure in different cases. This could probably be solved by standardizing the doze of radioiodide, which could be calculated based on the size of the thyroid, the size of the functional part, and with an estimation of metastatic volume. Another challenge is how to protect non-targeted healthy tissue and/or other NIS expressing organs. As mentioned earlier, shutting down the tissue specific expression of NIS from non-targeted organs would be of great benefit, as this also directly affects also the dose received by the specific target tissue (neoplastic target or the metastasis), and thus the effectiveness of radiotherapy.

We also believe that an extensive study of cis- and trans-acting factors regulating the NIS gene in the mammary gland might prove extremely valuable and informative for the efforts of establishing a novel diagnostic and/or therapeutic protocol against lethal breast diseases. In addition, finding candidate small molecules, with molecular effects similar to those of TSH

and/or RA in thyroid cells, which could specifically stimulate the functional expression of NIS in other cellular models are also of utmost importance for paving the way for a radioiodide therapy in tissues that do not typically benefit from it.

Acknowledgements

Research in our laboratories have been generously supported throughout the years by multiple TÜBİTAK grants (101T075; 104T231; 109T049; 109T925; 114Z349 to Uygar Halis Tazebay and 114Z245 to Hani Alotaibi) for which we are grateful.

Authorship Contributions

Concept: Hani Alotaibi, Uygar Halis Tazebay, Analysis or Interpretation: Hani Alotaibi, Merve Tuzlakoğlu-Öztürk, Uygar Halis Tazebay, Literature Search: Hani Alotaibi, Merve Tuzlakoğlu-Öztürk, Uygar Halis Tazebay, Writing: Hani Alotaibi, Uygar Halis Tazebay.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Wolff J. Transport of iodide and other anions in the thyroid gland. Physiol Rev 1964;44:45-90.
2. Stubbe P, Schulte FJ, Heidemann P. Iodine deficiency and brain development. Bibl Nutr Dieta 1986;38:206-208.
3. Carrasco N. Iodide transport in the thyroid gland. Biochim Biophys Acta 1993;1154:65-82.
4. Brown-Grant K. Extrathyroidal iodide concentrating mechanisms. Physiol Rev 1961;41:189-213.
5. Hertz S, Roberts A, Salter WT. Radioactive iodine as an indicator in thyroid physiology. IV. The metabolism of iodine in Graves' disease. J Clin Invest 1942;21:25-29.
6. Vilijn F, Carrasco N. Expression of the thyroid sodium/iodide symporter in Xenopus laevis oocytes. J Biol Chem 1989;264:11901-11903.
7. Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. Nature 1996;379:458-460.
8. Smanik PA, Ryu KY, Theil KS, Mazzaferri EL, Jhiang SM. Expression, exon-intron organization, and chromosome mapping of the human sodium iodide symporter. Endocrinology 1997;138:3555-3558.
9. Ajjan RA, Kamaruddin NA, Crisp M, Watson PF, Ludgate M, Weetman AP. Regulation and tissue distribution of the human sodium iodide symporter gene. Clin Endocrinol (oxf) 1998;49:517-523.
10. Spitzweg C, Joba W, Eisenmenger W, Heufelder AE. Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complementary deoxyribonucleic acids from salivary gland, mammary gland, and gastric mucosa. J Clin Endocrinol Metab 1998;83:1746-1751.
11. Mitchell AM, Manley SW, Morris JC, Powell KA, Bergert ER, Mortimer RH. Sodium iodide symporter (NIS) gene expression in human placenta. Placenta 2001;22:256-258.
12. Spitzweg C, Dutton CM, Castro MR, Bergert ER, Goellner JR, Heufelder AE, Morris JC. Expression of the sodium iodide symporter in human kidney. Kidney Int 2001;59:1013-1023.
13. Dohan O, De la Vieja A, Paroder V, Riedel C, Artani M, Reed M, Ginter CS, Carrasco N. The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. Endocr Rev 2003;24:48-77.
14. Wain HM, Bruford EA, Lovering RC, Lush MJ, Wright MW, Povey S. Guidelines for human gene nomenclature. Genomics 2002;79:464-470.
15. Wain HM, Lush MJ, Ducluzeau F, Khodiyar VK, Povey S. Genew: the human gene nomenclature database, 2004 updates. Nucleic Acids Res 2004;32:255-257.
16. Smanik PA, Liu Q, Furminger TL, Ryu K, Xing S, Mazzaferri EL, Jhiang SM. Cloning of the human sodium lodide symporter. Biochem Biophys Res Commun 1996;226:339-345.
17. Tazebay UH, Wapnir IL, Levy O, Dohan O, Zuckier LS, Zhao QH, Deng HF, Amenta PS, Fineberg S, Pestell RG, Carrasco N. The mammary gland iodide transporter is expressed during lactation and in breast cancer. Nat Med 2000;6:871-878.
18. Perron B, Rodriguez AM, Leblanc G, Pourcher T. Cloning of the mouse sodium iodide symporter and its expression in the mammary gland and other tissues. J Endocrinol 2001;170:185-196.
19. Pinke LA, Dean DS, Bergert ER, Spitzweg C, Dutton CM, Morris JC. Cloning of the mouse sodium iodide symporter. Thyroid 2001;11:935-939.
20. Levy O, Dai G, Riedel C, Ginter CS, Paul EM, Lebowitz AN, Carrasco N. Characterization of the thyroid Na+/I- symporter with an anti-COOH terminus antibody. Proc Natl Acad Sci USA 1997;94:5568-5573.
21. Paire A, Bernier-Valentin F, Selmi-Ruby S, Rousset B. Characterization of the rat thyroid iodide transporter using anti-peptide antibodies. Relationship between its expression and activity. J Biol Chem 1997;272:18245-18249.
22. Levy O, De la Vieja A, Ginter CS, Riedel C, Dai G, Carrasco N. N-linked glycosylation of the thyroid Na+/I- symporter (NIS). Implications for its secondary structure model. J Biol Chem 1998;273:22657-22663.
23. Bagchi N, Fawcett DM. Role of sodium ion in active transport of iodide by cultured thyroid cells. Biochim Biophys Acta 1973;318:235-251.
24. Eskandari S, Loo DD, Dai G, Levy O, Wright EM, Carrasco N. Thyroid Na+/I- symporter. Mechanism, stoichiometry, and specificity. J Biol Chem 1997;272:27230-27238.
25. Wolff J, Halmi NS. Thyroidal iodide transport. V. The role of Na-K-activated, ouabain-sensitive adenosinetriphosphatase activity. J Biol Chem 1963;238:847-851.
26. Baker CH, Morris JC. The sodium-iodide symporter. Curr Drug Targets Immune Endocr Metabol Disord 2004;4:167-174.
27. Chambard M, Verrier B, Gabrion J, Mauchamp J. Polarization of thyroid cells in culture: evidence for the basolateral localization of the iodide "pump" and of the thyroid-stimulating hormone receptor-adenyl cyclase complex. J Cell Biol 1983;96:1172-1187.
28. Bidart JM, Mian C, Lazar V, Russo D, Filetti S, Caillou B, Schlumberger M. Expression of pendrin and the Pendred syndrome (PDS) gene in human thyroid tissues. J Clin Endocrinol Metab 2000;85:2028-2033.
29. Rodriguez AM, Perron B, Lacroix L, Caillou B, Leblanc G, Schlumberger M, Bidart JM, Pourcher T. Identification and characterization of a putative human iodide transporter located at the apical membrane of thyrocytes. J Clin Endocrinol Metab 2002;87:3500-3503.
30. Igo RP, Mahoney CP, Mackler B. Studies of the biosynthesis of thyroxine. I. Purification and properties of a particulate iodide peroxidase from thyroid tissue. J Biol Chem 1964;239:1893-1897.
31. Ikuyama S, Shimura H, Hoeffler JP, Kohn LD. Role of the cyclic adenosine 3',5'-monophosphate response element in efficient expression of the rat thyrotropin receptor promoter. Mol Endocrinol 1992;6:1701-1715.
32. Armstrong R, Wen W, Meinkoth J, Taylor S, Montminy M. A refractory phase in cyclic AMP-responsive transcription requires down regulation of protein kinase A. Mol Cell Biol 1995;15:1826-1832.
33. Halmi NS, Granner DK, Doughman DJ, Peters BH, Muller G. Biphasic effect of TSH on thyroidal iodide collection in rats. Endocrinology 1960;67:70-81.
34. Knopp J, Stolc V, Tong W. Evidence for the induction of iodide transport in bovine thyroid cells treated with thyroid-stimulating hormone or dibutyryl cyclic adenosine 3',5'-monophosphate. J Biol Chem 1970;245:4403-4408.

35. Kogai T, Endo T, Saito T, Miyazaki A, Kawaguchi A, Onaya T. Regulation by thyroid-stimulating hormone of sodium/iodide symporter gene expression and protein levels in FRTL-5 cells. Endocrinology 1997;138:2227-2232.
36. Ohno M, Zannini M, Levy O, Carrasco N, di Lauro R. The paired-domain transcription factor Pax8 binds to the upstream enhancer of the rat sodium/iodide symporter gene and participates in both thyroid-specific and cyclic-AMP-dependent transcription. Mol Cell Biol 1999;19:2051-2060.
37. Riedel C, Levy O, Carrasco N. Post-transcriptional regulation of the sodium/iodide symporter by thyrotropin. J Biol Chem 2001;276:21458-21463.
38. Pasca di Magliano M, Di Lauro R, Zannini M. Pax8 has a key role in thyroid cell differentiation. Proc Natl Acad Sci USA 2000;97:13144-13149.
39. Kaminsky SM, Levy O, Salvador C, Dai G, Carrasco N. Na(+)-I- symport activity is present in membrane vesicles from thyrotropin-deprived non-I(-)-transporting cultured thyroid cells. Proc Natl Acad Sci USA 1994;91:3789-3793.
40. Endo T, Kaneshige M, Nakazato M, Ohmori M, Harii N, Onaya T. Thyroid transcription factor-1 activates the promoter activity of rat thyroid Na+/I- symporter gene. Mol Endocrinol 1997;11:1747-1755.
41. Tong Q, Ryu KY, Jhiang SM. Promoter characterization of the rat Na+/I-symporter gene. Biochem Biophys Res Commun 1997;239:34-41.
42. Behr M, Schmitt TL, Espinoza CR, Loos U. Cloning of a functional promoter of the human sodium/iodide-symporter gene. Biochem J 1998;331:359-363.
43. Ryu KY, Tong Q, Jhiang SM. Promoter characterization of the human Na+/I- symporter. J Clin Endocrinol Metab 1998;83:3247-3251.
44. Ohmori M, Endo T, Harii N, Onaya T. A novel thyroid transcription factor is essential for thyrotropin-induced up-regulation of Na+/I-symporter gene expression. Mol Endocrinol 1998;12:727-736.
45. Guazzi S, Price M, De Felice M, Damante G, Mattei MG, Di Lauro R. Thyroid nuclear factor 1 (TTF-1) contains a homeodomain and displays a novel DNA binding specificity. EMBO J 1990;9:3631-3639.
46. Damante G, Di Lauro R. Thyroid-specific gene expression. Biochim Biophys Acta 1994;1218:255-266.
47. Chun JT, Di Dato V, D'andrea B, Zannini M, Di Lauro R. The CRE-like element inside the 5'-upstream region of the rat sodium/iodide symporter gene interacts with diverse classes of b-Zip molecules that regulate transcriptional activities through strong synergy with Pax-8. Mol Endocrinol 2004;18:2817-2829.
48. Taki K, Kogai T, Kanamoto Y, Hershman JM, Brent GA. A thyroid-specific far-upstream enhancer in the human sodium/iodide symporter gene requires Pax-8 binding and cyclic adenosine 3',5'-monophosphate response element-like sequence binding proteins for full activity and is differentially regulated in normal and thyroid cancer cells. Mol Endocrinol 2002;16:2266-2282.
49. Sastre-Perona A, Santisteban P. Wnt-independent role of β-catenin in thyroid cell proliferation and differentiation. Mol Endocrinol 2014;28:681-695.
50. Fernandez LP, Lopez-Marquez A, Martinez AM, Gomez-Lopez G, Santisteban P. New insights into FoxE1 functions: identification of direct FoxE1 targets in thyroid cells. PLoS One 2013;8:e62849.
51. Ferretti E, Tosi E, Po A, Scipioni A, Morisi R, Espinola MS, Russo D, Durante C, Schlumberger M, Screpanti I, Filetti S, Gulino A. Notch signaling is involved in expression of thyrocyte differentiation markers and is down-regulated in thyroid tumors. J Clin Endocrinol Metab 2008;93:4080-4087.
52. Ringseis R, Rauer C, Rothe S, Gessner DK, Schütz LM, Luci S, Wen G, Eder K. Sterol regulatory element-binding proteins are regulators of the NIS gene in thyroid cells. Mol Endocrinol 2013;27:781-800.
53. Brent GA, Kogai T. Cancer: Novel target to enhance radioiodine uptake in thyroid cancer. Nat Rev Endocrinol 2013;9:508-509.
54. Simon D, Köhrle J, Schmutzler C, Mainz K, Reiners C, Röher HD. Redifferentiation therapy of differentiated thyroid carcinoma with retinoic acid: basics and first clinical results. Exp Clin Endocrinol Diabetes 1996;104(Suppl 4):13-15.
55. Kogai T, Kanamoto Y, Che LH, Taki K, Moatamed F, Schultz JJ, Brent GA. Systemic retinoic acid treatment induces sodium/iodide symporter expression and radioiodide uptake in mouse breast cancer models. Cancer Res 2004;64:415-422.
56. Schmutzler C, Winzer R, Meissner-Weigl J, Köhrle J. Retinoic acid increases sodium/iodide symporter mRNA levels in human thyroid cancer cell lines and suppresses expression of functional symporter in nontransformed FRTL-5 rat thyroid cells. Biochem Biophys Res Commun 1997;240:832-838.
57. Pfahl M, Chytil F. Regulation of metabolism by retinoic acid and its nuclear receptors. Annu Rev Nutr 1996;16:257-283.
58. Giguere V. Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling. Endocr Rev 1994;15:61-79.
59. Kato S, Sasaki H, Suzawa M, Masushige S, Tora L, Chambon P, Gronemeyer H. Widely spaced, directly repeated PuGGTCA elements act as promiscuous enhancers for different classes of nuclear receptors. Mol Cell Biol 1995;15:5858-5867.
60. Schmutzler C, Schmitt TL, Glaser F, Loos U, Köhrle J. The promoter of the human sodium/iodide-symporter gene responds to retinoic acid. Mol Cell Endocrinol 2002;189:145-155.
61. Spitzweg C, Scholz IV, Bergert ER, Tindall DJ, Young CY, Göke B, Morris JC. Retinoic acid-induced stimulation of sodium iodide symporter expression and cytotoxicity of radioiodine in prostate cancer cells. Endocrinology 2003;144:3423-3432.
62. Abu J, Batuwangala M, Herbert K, Symonds P. Retinoic acid and retinoid receptors: potential chemopreventive and therapeutic role in cervical cancer. Lancet Oncol 2005;6:712-720.
63. Frazer KA, Pachter L, Poliakov A, Rubin EM, Dubchak I. VISTA: computational tools for comparative genomics. Nucleic Acids Res 2004;32:273-279.
64. Alotaibi H, Yaman E, Salvatore D, Di Dato V, Telkoparan P, Di Lauro R, Tazebay UH. Intronic elements in the Na+/I- symporter gene (NIS) interact with retinoic acid receptors and mediate initiation of transcription. Nucleic Acids Res 2010; 38:3172-3185.
65. Negre B, Casillas S, Suzanne M, Sanchez-Herrero E, Akam M, Nefedov M, Barbadilla A, de Jong P, Ruiz A. Conservation of regulatory sequences and gene expression patterns in the disintegrating Drosophila Hox gene complex. Genome Res 2005;15:692-700.
66. Venkataraman GM, Yatin M, Ain KB. Cloning of the human sodium-iodide symporter promoter and characterization in a differentiated human thyroid cell line, KAT-50. Thyroid 1998;8:63-69.
67. Furlanetto TW, Nguyen LQ, Jameson JL. Estradiol increases proliferation and down-regulates the sodium/iodide symporter gene in FTRL-5 cells. Endocrinology 1999;140:5705-5711.
68. Kogai T, Schultz JJ, Johnson LS, Huang M, Brent GA. Retinoic acid induces sodium/iodide symporter gene expression and radioiodide uptake in the MCF-7 breast cancer cell line. Proc Natl Acad Sci USA 2000;97:8519-8524.
69. Alotaibi H, Yaman EC, Demirpençe E, Tazebay UH. Unliganded estrogen receptor-alpha activates transcription of the mammary gland Na+/I- symporter gene. Biochem Biophys Res Commun 2006;345:1487-1496.
70. Bourdeau V, Deschenes J, Metivier R, Nagai Y, Nguyen D, Bretschneider N, Gannon F, White JH, Mader S. Genome-wide identification of high-affinity estrogen response elements in human and mouse. Mol Endocrinol 2004;18:1411-1427.
71. Ajjan RA, Watson PF, Findlay C, Metcalfe RA, Crisp M, Ludgate M, Weetman AP. The sodium iodide symporter gene and its regulation by cytokines found in autoimmunity. J Endocrinol 1998;158:351-358.
72. Pekary AE, Hershman JM. Tumor necrosis factor, ceramide, transforming growth factor-beta1, and aging reduce Na+/I-symporter messenger ribonucleic acid levels in FRTL-5 cells. Endocrinology 1998;139:703-712.
73. Spitzweg C, Joba W, Morris JC, Heufelder AE. Regulation of sodium iodide symporter gene expression in FRTL-5 rat thyroid cells. Thyroid 1999;9:821-830.

74. Eng PH, Cardona GR, Fang SL, Previti M, Alex S, Carrasco N, Chin WW, Braverman LE. Escape from the acute Wolff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleic acid and protein. Endocrinology 1999;140:3404-3410.
75. Eng PH, Cardona GR, Previti MC, Chin WW, Braverman LE. Regulation of the sodium iodide symporter by iodide in FRTL-5 cells. Eur J Endocrinol 2001;144:139-144.
76. Caturegli P, Hejazi M, Suzuki K, Dohan O, Carrasco N, Kohn LD, Rose NR. Hypothyroidism in transgenic mice expressing IFN-γ,gamma in the thyroid. Proc Natl Acad Sci USA 2000;97:1719-1724.
77. Lakshmanan A, Scarberry D, Green JA, Zhang X, Selmi-Ruby S, Jhiang SM. Modulation of thyroidal radioiodide uptake by oncological pipeline inhibitors and Apigenin. Oncotarget 2015;6:31792-31804.
78. Wollman SH, Reed FE. Radioiodide transport in an iodide-concentrating tumor of thyroid gland. Am J Physiol 1960;199:417-418.
79. Dadachova E, Carrasco N. The Na/I symporter (NIS): imaging and therapeutic applications. Semin Nucl Med 2004;34:23-31.
80. Haq M, Harmer C. Thyroid cancer: an overview. Nucl Med Commun 2004;25:861-867.
81. Lazar V, Bidart JM, Caillou B, Mahe C, Lacroix L, Filetti S, Schlumberger M. Expression of the Na+/I- symporter gene in human thyroid tumors: a comparison study with other thyroid-specific genes. J Clin Endocrinol Metab 1999;84:3228-3234.
82. Trapasso F, Iuliano R, Chiefari E, Arturi F, Stella A, Filetti S, Fusco A, Russo D. Iodide symporter gene expression in normal and transformed rat thyroid cells. Eur J Endocrinol 1999;140:447-451.
83. Park HJ, Kim JY, Park KY, Gong G, Hong SJ, Ahn IM. Expression of human sodium iodide symporter mRNA in primary and metastatic papillary thyroid carcinomas. Thyroid 2000;10:211-217.
84. Bastien J, Rochette-Egly C. Nuclear retinoid receptors and the transcription of retinoid-target genes. Gene 2004;328:1-16.
85. Simon D, Koehrle J, Reiners C, Boerner AR, Schmutzler C, Mainz K, Goretzki PE, Roeher HD. Redifferentiation therapy with retinoids: therapeutic option for advanced follicular and papillary thyroid carcinoma. World J Surg 1998;22:569-574.
86. Schmutzler C, Köhrle J. Retinoic acid redifferentiation therapy for thyroid cancer. Thyroid 2000;10:393-406.
87. Grünwald F, Menzel C, Bender H, Palmedo H, Otte R, Fimmers R, Risse J, Biersack HJ. Redifferentiation therapy-induced radioiodine uptake in thyroid cancer. J Nucl Med 1998;39:1903-1906.
88. Koerber C, Schmutzler C, Rendl J, Koehrle J, Griesser H, Simon D, Reiners C. Increased I-131 uptake in local recurrence and distant metastases after second treatment with retinoic acid. Clin Nucl Med 1999;24:849-851.
89. Simon D, Körber C, Krausch M, Segering J, Groth P, Görges R, Grünwald F, Müller-Gartner HW, Schmutzler C, Köhrle J, Röher HD, Reiners C. Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study. Eur J Nucl Med Mol Imaging 2002;29:775-782.
90. Cho JY, Leveille R, Kao R, Rousset B, Parlow AF, Burak WE Jr, Mazzaferri EL, Jhiang SM. Hormonal regulation of radioiodide uptake activity and Na+/I- symporter expression in mammary glands. J Clin Endocrinol Metab 2000;85:2936-2943.
91. Wapnir IL, van de Rijn M, Nowels K, Amenta PS, Walton K, Montgomery K, Greco RS, Dohan O, Carrasco N. Immunohistochemical profile of the sodium/iodide symporter in thyroid, breast, and other carcinomas using high density tissue microarrays and conventional sections. J Clin Endocrinol Metab 2003;88:1880-1888.
92. Zuckier LS, Dadachova E, Dohan O, Carrasco N. The endogenous mammary gland Na(+)/I(-) symporter may mediate effective radioiodide therapy in breast cancer. J Nucl Med 2001;42:987-988.
93. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. Ca Cancer J Clin 2000;50:7-33.
94. Spitzweg C, O'connor MK, Bergert ER, Tindall DJ, Young CY, Morris JC. Treatment of prostate cancer by radioiodine therapy after tissue-specific expression of the sodium iodide symporter. Cancer Res 2000;60:6526-6530.
95. La Perle KM, Shen D, Buckwalter TL, Williams B, Haynam A, Hinkle G, Pozderac R, Capen CC, Jhiang SM. In vivo expression and function of the sodium iodide symporter following gene transfer in the MATLyLu rat model of metastatic prostate cancer. Prostate 2002;50:170-178.
96. Haberkorn U, Henze M, Altmann A, Jiang S, Morr I, Mahmut M, Peschke P, Kübler W, Debus J, Eisenhut M. Transfer of the human NaI symporter gene enhances iodide uptake in hepatoma cells. J Nucl Med 2001;42:317-325.
97. Cho JY, Shen DH, Yang W, Williams B, Buckwalter TL, La perle KM, Hinkle G, Pozderac R, Kloos R, Nagaraja HN, Barth RF, Jhiang SM. In vivo imaging and radioiodine therapy following sodium iodide symporter gene transfer in animal model of intracerebral gliomas. Gene Ther 2002;9:1139-1145.
98. Schipper ML, Weber A, Behe M, Göke R, Joba W, Schmidt H, Bert T, Simon B, Arnold R, Heufelder AE, Behr TM. Radioiodide treatment after sodium iodide symporter gene transfer is a highly effective therapy in neuroendocrine tumor cells. Cancer Res 2003;63:1333-1338.
99. Gaut AW, Niu G, Krager KJ, Graham MM, Trask DK, Domann FE. Genetically targeted radiotherapy of head and neck squamous cell carcinoma using the sodium-iodide symporter (NIS). Head Neck 2004;26:265-271.
100. Mitrofanova E, Unfer R, Vahanian N, Kane S, Carvour M, Link C. Effective growth arrest of human colon cancer in mice, using rat sodium iodide symporter and radioiodine therapy. Hum Gene Ther 2005;16:1333-1337.
101. Scholz IV, Cengic N, Baker CH, Harrington KJ, Maletz K, Bergert ER, Vile R, Göke B, Morris JC, Spitzweg C. Radioiodine therapy of colon cancer following tissue-specific sodium iodide symporter gene transfer. Gene Ther 2005;12:272-280.
102. Dwyer RM, Bergert ER, O'connor MK, Gendler SJ, Morris JC. Adenovirus-mediated and targeted expression of the sodium-iodide symporter permits in vivo radioiodide imaging and therapy of pancreatic tumors. Hum Gene Ther 2006;17:661-668.
103. Dwyer RM, Bergert ER, O'connor MK, Gendler SJ, Morris JC. Sodium iodide symporter-mediated radioiodide imaging and therapy of ovarian tumor xenografts in mice. Gene Ther 2006;13:60-66.
104. Wapnir IL, Goris M, Yudd A, Dohan O, Adelman D, Nowels K, Carrasco N. The Na+/I- symporter mediates iodide uptake in breast cancer metastases and can be selectively down-regulated in the thyroid. Clin Cancer Res 2004;10:4294-4302.

Review
Mol Imaging Radionucl Ther 2017;26(Suppl 1):102-117 DOI:10.4274/2017.26.suppl.12

A Concise Atlas of Thyroid Cancer Next-Generation Sequencing Panel ThyroSeq v.2

Tiroid Kanser Genomiğinin Güncel Klinik Atlası: ThyroSeq v.2 Yeni Jenerasyon Dizinleme Paneli

Jorge Alsina[1], Raul Alsina[1], Seza Gulec, MD, FACS[2]
[1]*Florida International University Herbert Wertheim College of Medicine, Miami, USA*
[2]*Florida International University Herbert Wertheim College of Medicine, Departments of Surgery and Nuclear Medicine, Miami, USA*

Abstract

The next-generation sequencing technology allows high out-put genomic analysis. An innovative assay in thyroid cancer, ThyroSeq® was developed for targeted mutation detection by next generation sequencing technology in fine needle aspiration and tissue samples. ThyroSeq v.2 next generation sequencing panel offers simultaneous sequencing and detection in >1000 hotspots of 14 thyroid cancer-related genes and for 42 types of gene fusions known to occur in thyroid cancer. ThyroSeq is being increasingly used to further narrow the indeterminate category defined by cytology for thyroid nodules. From a surgical perspective, genomic profiling also provides prognostic and predictive information and closely relates to determination of surgical strategy. Both the genomic analysis technology and the informatics for the cancer genome data base are rapidly developing. In this paper, we have gathered existing information on the thyroid cancer-related genes involved in the initiation and progression of thyroid cancer. Our goal is to assemble a glossary for the current ThyroSeq genomic panel that can help elucidate the role genomics play in thyroid cancer oncogenesis.
Keywords: Thyroid cancer, next generation sequencing, ThyroSeq

Öz

Tiroid nodüllerinde kanser teşhisi ve tiroid kanserlerinin prognostik değerlendirilmesi için geliştirilmiş en güncel yöntem DNA dizinleme teknolojisine dayalı ThyroSeq® testidir. Bu test ince iğne aspirasyon materyalinde genetik profilleme yaparak tiroid kanserinin tetiklenmesinde ve gelişmesinde rol alan mutasyon ve genetik değişiklikleri tespit etmektedir. Bu makalede bu test ile tespit edilebilen genetik değişikliklere konu olan genlerin yapı ve fonksiyonlarını kısaca tanımlıyoruz. Tiroid kanseri onkogenezine bir ölçüde ışık tutan bu test için sözlük görevi tutan bir makale sunulmaktadır.
Anahtar kelimeler: Tiroid kanseri, yeni jenerasyon dizinleme, ThyroSeq

Introduction

Thyroid nodules are prevalent in the general population. Most thyroid nodules are benign and the clinical challenge is to accurately identify those nodules that are malignant and need to be surgically removed (1). Moreover, the extent of initial surgical treatment requires better understanding of particular tumor biology beyond conventional definitions. Molecular pathology is the new paradigm in cancer diagnosis and prognostication. Thyroid cancer develops and progresses through accumulation of genetic alterations, which does serve as important diagnostic, prognostic, and predictive biological markers (2). Next-generation sequencing technology allows high out-put

Address for Correspondence: Seza Gulec, MD, FACS, Florida International University Herbert Wertheim College of Medicine, Departments of Surgery and Nuclear Medicine, Miami, USA
E-mail: sgulec@fiu.edu

genomic analysis. An innovative assay in thyroid cancer - ThyroSeq® - was developed for targeted mutation detection by next generation sequencing technology in fine needle aspiration and tissue samples. ThyroSeq v.2 next generation sequencing panel offers simultaneous sequencing and detection in >1000 hotspots of 14 thyroid cancer-related genes and for 42 types of gene fusions known to occur in thyroid cancer (3). ThyroSeq is being increasingly used to further narrow the indeterminate category defined by cytology for thyroid nodules. From a surgical perspective, understandably this provides prognostic and predictive information as it relates to determination of surgical strategy. Both the genomic analysis technology and the data collection for the cancer genome atlas are rapidly developing.

This paper reviews basic genomic information on 23 thyroid cancer-related genes involved in thyroid cancer. We have detailed information in regards to the location, and function of these genes in normal thyroid cells. We also report gathered information as to the consequences mutations to these 23 genes can have on thyroid cancer initiation and progression. Our goal is to provide a detailed glossary for ThyroSeq mutation panel.

Molecular Markers of ThyroSeq Next-Generation Sequencing Panel

B-RAF

The *B-RAF* gene, located on chromosome 7q34, encodes *B-RAF* serine-threonine kinase, which functions as an intracellular effector of the *RAS*/MAPK signaling cascade (Figure 1). This is one of the three isoforms of the RAF serine-threonine kinase and the predominant isoform found

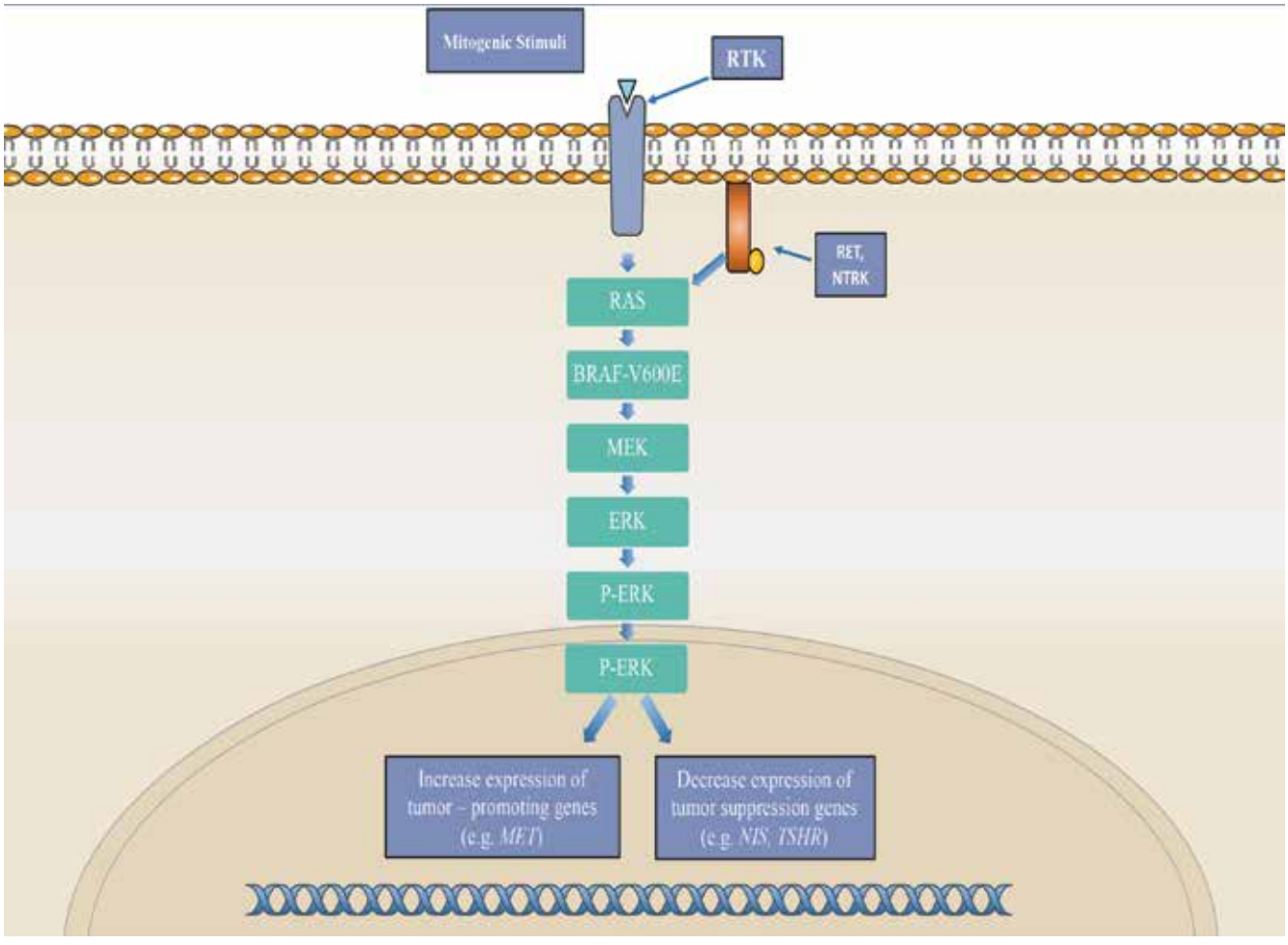


Figure 1. MAPK/ERK pathway

**This pathway begins with a mitogenic stimulus binding to a receptor tyrosine kinase, activating it. This triggers a cascade of protein activation, beginning with RAS and culminating with ERK. Once ERK is phosphorylated, it enters the nucleus and influences transcription, increasing expression of tumor-promoting genes and decreasing expression of tumor-suppressing genes. MAPK signaling can also be stimulated by genetic alterations in proteins RET and NTRK*

in thyroid follicular cells. In wild-type forms of this gene, the *B-RAF* protein is activated through binding of a *RAS*-GTP protein complex with the *B-RAF*'s *RAS* binding domain along with simultaneous conformational changes in the protein. Once activated, the *B-RAF* protein phosphorylates the next protein in the signal cascade-MEK and ERK. The protein's function contributes to the *RAS*/MAPK pathway's role in cell proliferation, migration, and differentiation (4,5).

The most common *B-RAF* mutation found in thyroid carcinomas is a point mutation at residue 600 involving a substitution from valine to glutamate (V600E). This mutation results in the constitutive activation of the *B-RAF* protein and subsequently the *RAS*/MAPK pathway. The activation of the *B-RAF* protein seems to be caused by a disruption of the hydrophobic interactions between its activation loop and the ATP binding site. In wild-type *B-RAF*, these hydrophobic interactions help maintain the protein in an inactive conformation. When disrupted, *B-RAF* remains in an active, catalytic conformation. This results in the constitutive phosphorylation of its downstream targets (4).

The *B-RAF* V600E point mutation is most prevalent in papillary thyroid carcinomas (PTC)-the most common form of well differentiated thyroid carcinoma-found in 45% of PTC cases. Though it is rare in follicular variants of thyroid carcinoma, *B-RAF* is an ideal genetic marker for use in a thyroid cancer sequencing panel. It is found in all forms of thyroid carcinoma and seems to play a very important role early in tumorigenesis as a driver mutation (4,5,6).

RAS

The *RAS* genes consist of a family of highly homologous isoforms: *K-RAS*, *N-RAS*, and *H-RAS*. Located on chromosomes 12p12.1, 11p5.5, and 1p13.1 respectively, all three genes encode G-proteins located on the inner surface of the cell membrane. These proteins help convey signals from receptor tyrosine kinases (RTKs) to the pathways *RAS* regulates: *RAS*/MAPK and PI3K/AKT signaling cascades (Figure 2). Once bound to GTP, the *RAS* proteins proceed to activate the *RAS*/MAPK pathway. The *RAS*-GTP complex quickly becomes inactive as a result of the protein's innate GTPase activity (7).

Point mutations in the *RAS* gene are the most common mutations. Mutations in codons 12 and 13 lead to an increased affinity for GTP. Mutations in codon 61 lead to inactivation of the *RAS* protein's innate GTPase function. Overall, these mutations result in the constitutive activation of the *RAS* protein and thus the activation of the downstream signaling pathways it regulates, a critical step in thyroid tumorigenesis. Thyroid carcinoma has been associated with mutations in all three isoforms of the *RAS* genes, though it seems the more prevalent mutated isoform is that of *N-RAS*. However, the actual pattern of isoform frequencies in thyroid carcinoma remains unclear (4,7).

RAS mutations occur with variable frequency in all types of thyroid follicular-derived tumors. *RAS* point mutations are most common in follicular thyroid carcinoma (FTC), occurring in 40-50% of tumors, as well as in poorly differentiated and anaplastic thyroid carcinoma (PDC and ATC). It is more infrequent in PTCs, occurring in about 10% of tumors. The prevalence of *RAS* mutations in thyroid carcinoma make it a viable genetic marker as well as a useful prognostic tool, given that, studies suggest it may increase the potential for malignant transformation and tumor progression (7).

CTNNB1

The *CTNNB1* gene, located on chromosome 3p21, encodes a cytoplasmic protein known as β-catenin. This protein plays several important roles in the cell. It is involved in E-cadherin mediated cell to cell adhesion, found primarily in adherens junctions. It is also an intermediate in the Wnt signaling pathway. Once activated by proteins upstream in the pathway, β-catenin is able to accumulate in the cytoplasm and eventually is translocated into the nucleus. In the nucleus, the protein works with T-cell factor/lymphoid enhancing factor (TCF/LEF) to modulate gene expression (8,9).

When β-catenin is not activated, free cytoplasmic levels are kept low through ubiquitin-proteasome degradation (Figure 3). However, point mutations in exon 3 of *CTNNB1* confer added stability to the protein and thus inhibit the degradation of β-catenin. This results in the accumulation and localization of β-catenin in the cytoplasm and nucleus of the cell and the constitutive activation of its targeted genes (ex: Cyclin D1, C-MYC, C-JUN) (8,10,11).

Mutations in *CTNNB1* are most commonly found in PDC and ATC but not in well differentiated forms. It is typically described as a late event in thyroid cancer progression, but dysregulation of β-catenin has been shown to contribute to tumor proliferation (10,11).

CTNNB1 can serve as a useful marker for PDC and ATC. Its involvement in the Wnt pathway contributes to its viability as a marker, given this pathway is constitutively active in 50% of PDC and ATC and it is involved in the dedifferentiation process of thyroid carcinoma (Figure 4) (10). This gene is thus a useful diagnostic marker as well as prognostic tool to determine the progression of the cancer and its propensity to spread.

NTRK1

The *NTRK1* gene, which is located on chromosome 1q21-22, encodes the neurotrophic RTK-1, also known as TrkA. It is a high affinity receptor for nerve growth factor-β (NFG-β) that provides instructions for the growth and development of nerve cells. It also induces the proliferation of a number of cell types, such as lymphocytes and keratinocytes. Once it is

bound to NFG-β, *NTRK1* dimerizes and autophosphorylates its five tyrosine residues, which then act as binding sites for several target proteins such as *RAS* and PI3K. By activating these proteins, *NTRK1* subsequently activates the *RAS*/MAPK and PI3K/AKT signaling cascades, thus mediating NGF effects on the proliferation and differentiation of cells (12,13).

Mutations in the *NTRK1* gene are frequently found in PTC. These mutations involve chromosomal rearrangements between *NTRK1* and at least three other identified genes: TPM3, TPR, and TFG. The chimeric oncogenes that are formed (TRK oncogenes) encode constitutively active RTKs. It remains active due to the fact that its kinase domain and the tyrosine binding sites are preserved in the chimeric oncogene. The only portion that is lost is a portion of its extracellular domain. It seems that the extracellular domain may contain important regulatory elements. This provides constant activation of the protein signals for cell proliferation, contributing to PTC (12,13).

About 60% of PTC carry chimeric oncogenes created through chromosomal rearrangement. Though *RET* rearrangement and not *NTRK1* is the most prevalent chimeric oncogene detected in PTC, thyroid epithelium seems to have a propensity for chromosomal rearrangements. That fact, along with *NTRK1* association with pathways implicated in thyroid carcinoma (*RAS*/MAPK and PI3K/AKT) make this gene a viable marker for diagnostic and prognostic purposes. Nevertheless, further investigation is necessary to better understand its role in thyroid carcinogenesis (13,14).

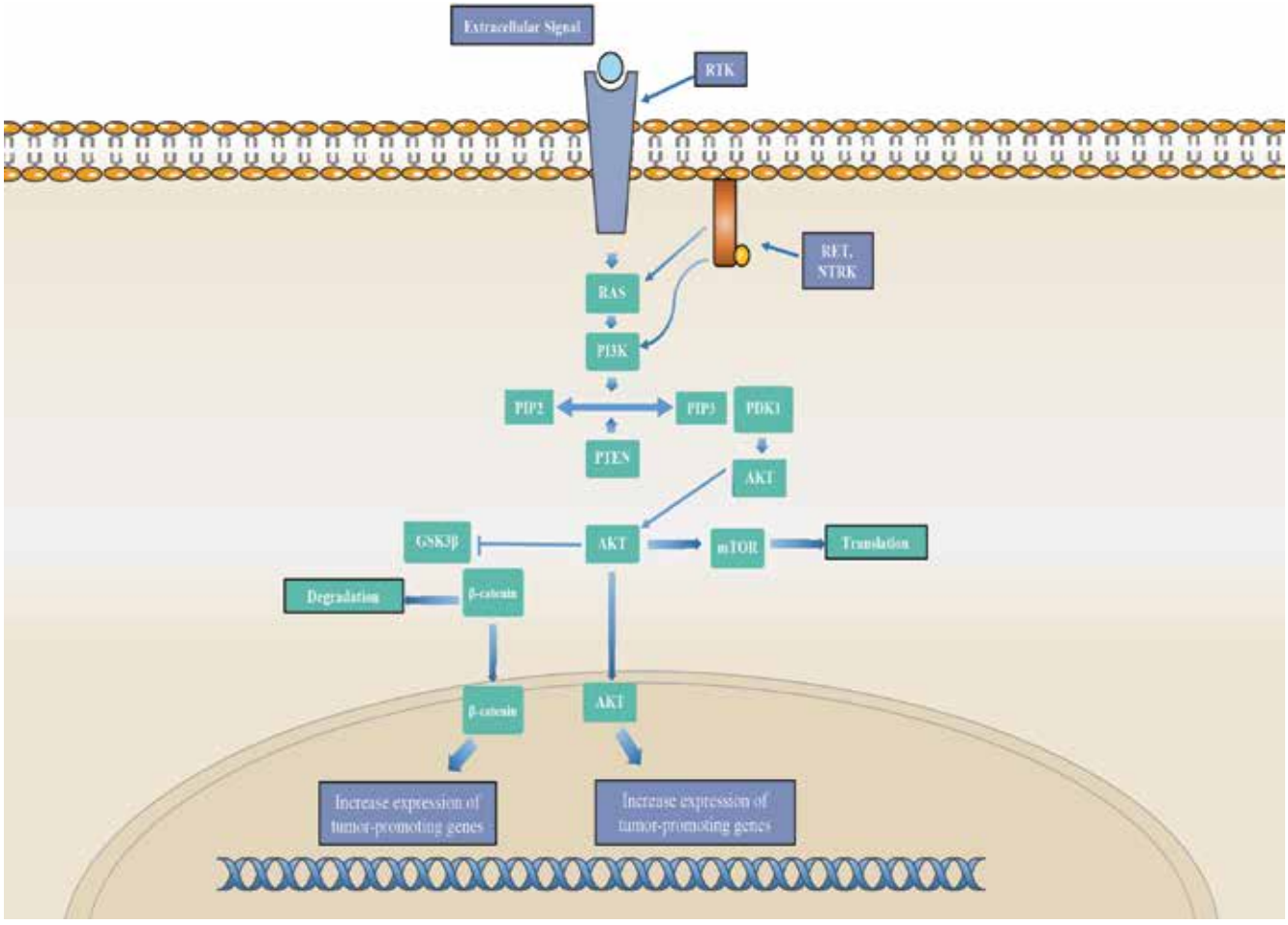


Figure 2. PI3K/*AKT* pathway

**The pathway begins with an extracellular stimuli activating a receptor tyrosine kinase, triggering the activation of RAS followed by PI3K. Activated PI3K catalyzes the conversion of phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3), a step that is regulated by PTEN. PIP3 activates the 3-phosphoinositide-dependent protein kinase 1, which targets and phosphorylates AKT. Phosphorylated AKT then enters the nucleus, where it increases the expression of tumor-promoting genes. Activated AKT also produces changes in the cytoplasm by activating mTOR and thus promoting translation. AKT can also phosphorylate glycogen synthase kinase 3-β (GSK3-β), inactivating the protein. GSK3-β inhibition results in a concomitant stimulation of β-catenin, allowing it to enter the nucleus and increase expression of tumor-promoting genes*

NTRK3

Another member of the neurotrophic tyrosine receptor kinase family, *NTRK3* encodes a RTK with a high affinity for its ligand neurotrophin-3. The gene is located on chromosome 15q25. Once bound, *NTRK3* autophosphorylates and proceeds to phosphorylate target proteins primarily involved in the *RAS*/MAPK and PI3K/AKT signaling pathways. Primarily expressed in the central nervous system, *NTRK3* is involved in cell proliferation and differentiation as well as neuronal cell processes (14,15).

The mutation of *NTRK3* observed in thyroid carcinomas is a chromosomal rearrangement involving ETV6, a gene encoding a transcription factor from the ETS transcription factor family. This particular oncogenic fusion involves the fusion of exons 1-4, the SAM domain, of ETV6 and exons 12-18, the tyrosine kinase domain, of *NTRK3*. This results in the constitutive activation of the RTK. It is this constant activation that contributes to thyroid carcinogenesis (15,16).

Chromosomal rearrangements of the NTRK genes are uncommon in sporadic PTC, only occurring in about 2% of cases. However, there is higher prevalence of these chimeric oncogenes in pediatric and radiation-related PTC, with the majority of ETV6-*NTRK3* fusions demonstrating the follicular variant of PTC. Mutations resulting in *RAS*/MAPK activation in radiation-related PTC tend to differ from those found in sporadic PTC as there tends to be more chromosomal rearrangements than point mutations (15,16).

RET

The *RET* gene is a proto-oncogene that is highly expressed in parafollicular C-cells. Located on chromosome 10q11.2, this gene encodes a RTK that is involved in the *RAS*/MAPK

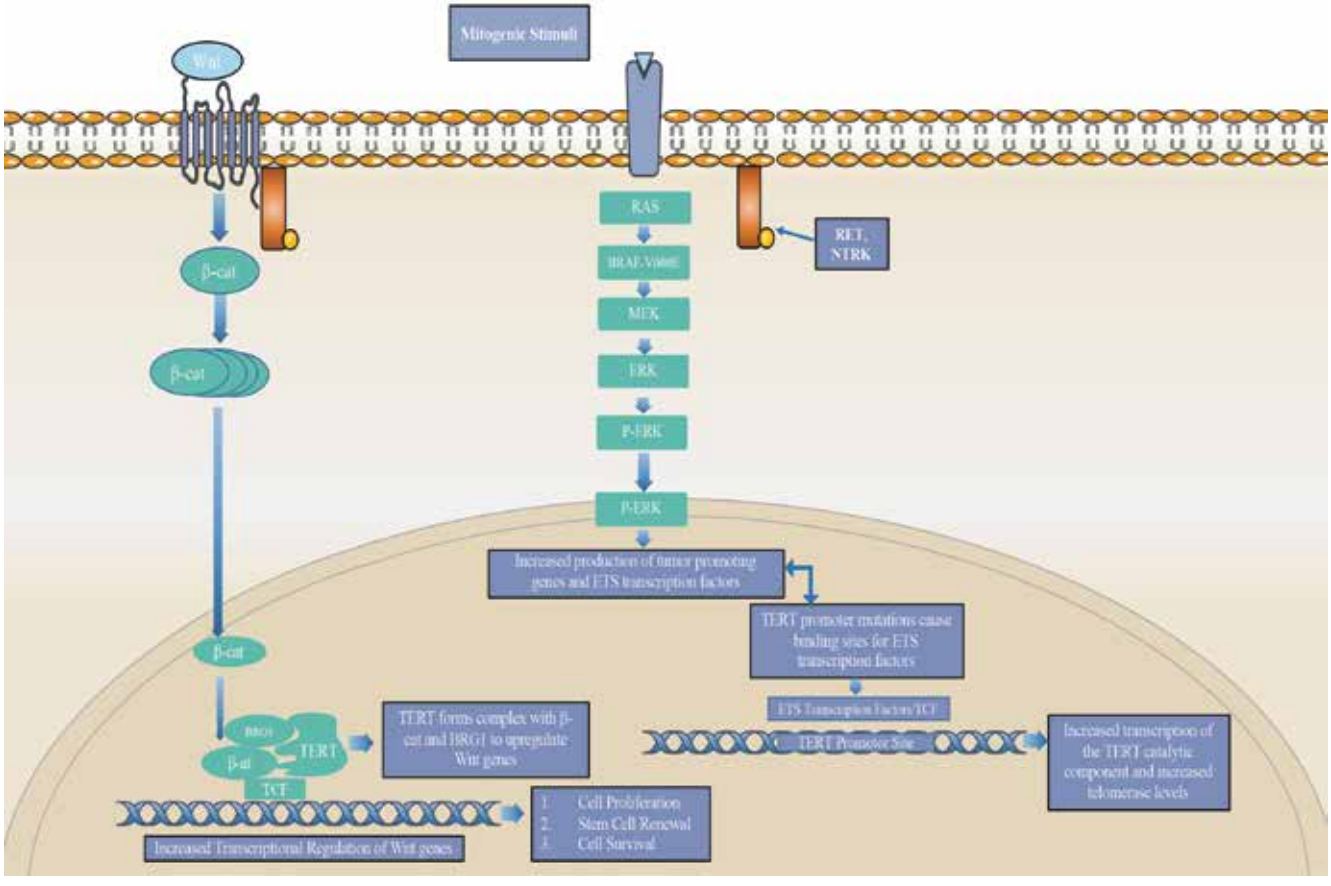


Figure 3. *CTNNB1*/*TERT* signaling

**Dysregulation of telomerase is an almost universal feature of cancer, either through genetic amplification of the gene's own locus or through crosstalk activity with other signaling pathways. In this pathway mutations to the promoter site of TERT (C228T and C250T) increase the transcription of TERT through the creation of consensus binding sites for ETS transcription factors. Constitutive action of the MAPK pathway due to B-RAF or RAS mutations leads to upregulation of TERT from ETS transcription binding activity. TERT also possess a role as a transcriptional modulator of the Wnt-β-catenin signaling pathway. Wnt stimulation at the plasma membrane leads to increased levels of β-catenin in the cytoplasm, and translocation to the nucleus. In the nucleus β-catenin forms a complex with TERT and the Wnt transcription factor BRG1. Once formed this complex binds to the promoters of Wnt target genes regulating the expression of these oncogenes*

signaling pathway. The *RET* protein responds to signals from the cell's environment and then proceeds to activate itself through autophosphorylation. Once activated, the *RET* protein elicits changes within the cell often involving cell proliferation and development (4,17).

Chromosomal rearrangements involving *RET* represents one of the most common causes of PTC. At least 11 rearrangement variants have been isolated but the most common translocations are *RET*/PTC1 and *RET*/PTC3. The aforementioned rearrangements are intrachromosomal paracentric inversions and account for the vast majority of variants found in PTC. These rearrangements result in a constitutively active *RET* protein, leading to activation of the *RAS*/MAPK pathway and driving thyroid carcinogenesis (17,18).

The *RET*/PTC1 rearrangement involves interaction between *RET* and the *H4* gene. This particular rearrangement is the dominant type in sporadic PTCs. The *RET*/PTC3 rearrangement involves interaction between *RET* and the *ELE1* gene. This particular rearrangement is the dominant type in radiation-related PTCs, which tend to be more aggressive and exhibit a higher frequency of chromosomal rearrangements (4,17).

AKT1

AKT1 codes for the *AKT1* kinase, one member of a family of homologous isoforms. Located on chromosome 14q32.322, *AKT1* kinase helps regulate cell growth and survival as well as the process of apoptosis, mainly through interaction with signaling pathways like PI3K/AKT. The protein contains a pleckstrin homology domain, making it a target for phospholipids generated by PI3K activity. Once activated through phosphorylation, *AKT1* binds to chaperone proteins and proceeds to phosphorylate its downstream effectors. Through this pathway, activated *AKT1* is able to inhibit apoptosis and induce cell growth in thyroid cells (19).

Activation of *AKT1* is known to promote thyroid tumorigenesis in Cowden's syndrome (CS). It is the overexpression and overactivation of *AKT1*, rather than mutation of the *AKT1* gene, that contributes to the pathogenesis of sporadic thyroid cancers, particularly in follicular thyroid cancers (FTC). In FTCs, activation of *AKT1* kinase and the localization of the protein in the nucleus promotes the PI3K/AKT pathway, contributing to tumor invasion and metastasis. It seems that overexpression of *AKT1* is capable of promoting cell

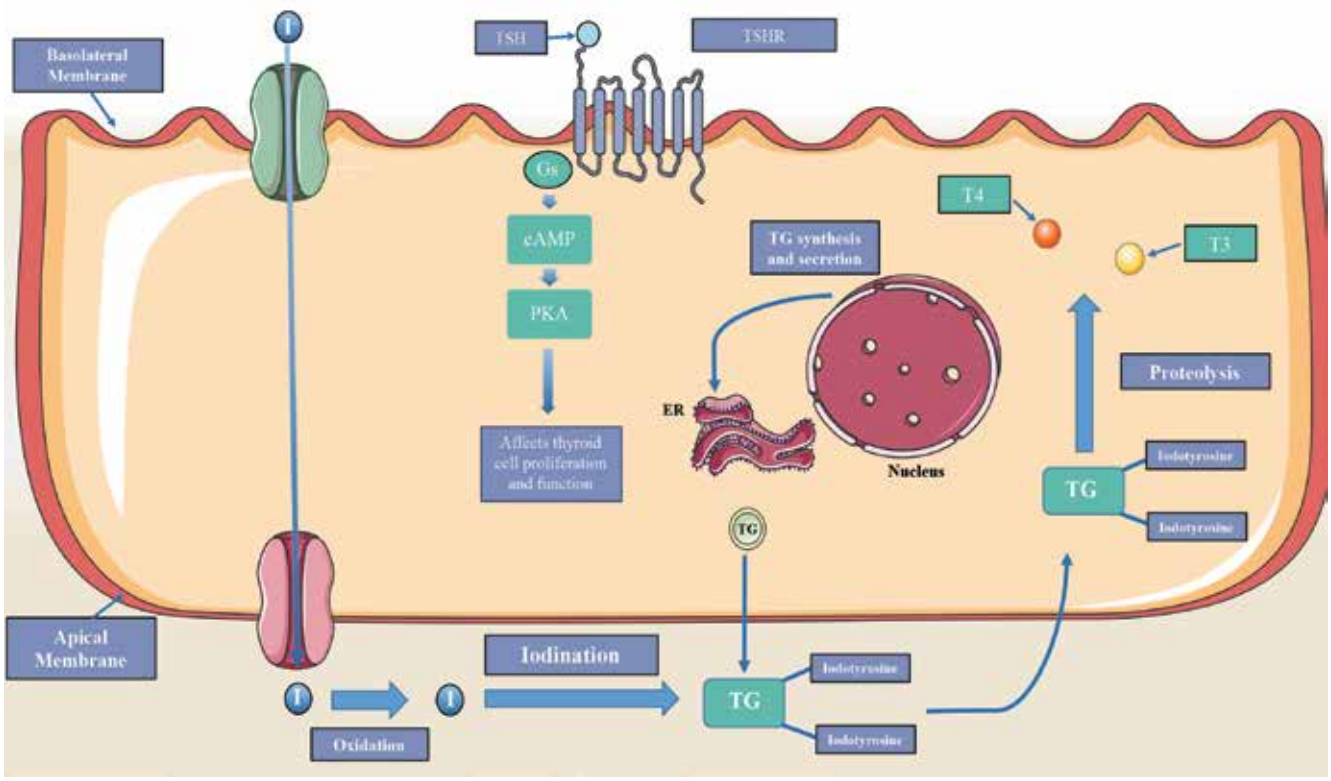


Figure 4. Thyroid hormone signaling

**Follicular cells contain thyroid stimulating hormone receptors (TSHR). Once bound to the hormone TSH, this G-protein coupled receptor generates the second messenger cyclic AMP. This second messenger activates protein kinases that trigger signal cascades responsible for thyroid cell proliferation and function. TSH stimulation of TSHR also leads to iodide transport into the cell via the sodium iodide symporter. This iodide is oxidized and then bound to tyrosine residues on thyroglobulin (TG), a process catalyzed by thyroperoxidase. This primed form of TG can then be cleaved through proteolysis in the cell to generate the thyroid hormones T3 and T4*

proliferation, sensitization to TSH, and inhibiting apoptosis, but it is not sufficient to transform thyroid cells by itself (19,20).

This alteration in *AKT1* occurs in about 15% of metastatic thyroid cancers, while also occurring in small fractions of ATCs and FTCs. Studies have shown that there is an association between *AKT1* activation and tumor aggressiveness. This association with metastasis in thyroid carcinoma was found in both PTC and FTC. However, the aggressiveness seen is likely attributed to a combination of genetic alterations rather than solely on dysregulation of *AKT1* activity (19,20,21).

TERT

The *TERT* gene, located on chromosome 5p15.33, encodes the protein telomerase reverse transcriptase. It is the catalytic subunit of the telomerase enzyme and determines telomerase activity. The *TERT* subunit associates with a RNA component TERC as well as additional components to form the telomerase enzyme. Telomerase serves an important role in synthesizing and maintaining telomeres, which helps prolong the life of cells in the body. When telomeres become too short, apoptosis is triggered in the cell. As a result, telomerase activity is highly regulated and expressed at very low levels in most tissues in the body (22).

Dysregulation of telomerase is an almost universal feature of cancer, with over 90% exhibiting overexpression of the enzyme. This imparts cancer cells with an infinite capability to divide. The up-regulation of *TERT* that is seen could be the result of epigenetic deregulation or genetic amplification of the gene's locus (Figure 3). Other factors contributing to the dysregulation of *TERT* expression involve changes in transcription factors that target the *TERT* gene. Such factors include *TP53* and the ETS transcription factors. Both are disrupted in thyroid carcinoma as *TP53* is often mutated and ETS transcription factors are stimulated by several oncogenes including *RAS*. These associations indicate a relationship between an up-regulation of *TERT* expression and the transformation of thyroid cells. One recently discovered mechanism has been mutations in the *TERT* promoter, the most important regulatory element of telomerase activity (22,23).

These promoter mutations, C228T and C250T, seem to increase the transcription of *TERT*, likely through the creation of consensus binding sites for ETS transcription factors. Promoter mutation C228T is the most common one found in thyroid carcinoma with no overlaps reported in the mutations. There seems to be a higher prevalence of *TERT* promoter mutations in more advanced forms of thyroid carcinoma like ATC. Studies have shown a strong association between *TERT* promoter mutations and *B-RAF* or *RAS* mutations as well. The evidence uncovered so far shows that mutations in the *TERT* promoter seems to represent a late stage genetic event in tumorigenesis as well as an indicator of aggressiveness given its prevalence in ATC (24,25).

GNAS

The *GNAS* gene provides instructions for the stimulatory α-subunit of heterotrimeric G protein complexes. The gene is located on chromosome 20q13.3. This subunit stimulates the activity of adenylate cyclase and controls the production of several hormones that help regulate the activity of the endocrine glands such as the thyroid gland. Mutations in *GNAS* are often detected at codons 201 and 227. These mutations result in the constitutive activation by impairing GTP hydrolysis. This in turn results in the constitutive activation of the cyclic AMP (cAMP) cascade. The increase in cAMP levels results in thyroid cell proliferation and increased thyroid hormone production. Despite this disruption in function, these mutations are almost never malignant (20,26).

Activating mutations of *GNAS* has been shown to overactivate thyroid stimulating hormone receptor (*TSHR*) signaling. This often leads to benign, hyperfunctional follicular thyroid adenomas (FTAs). Mutation of *GNAS* is also the main cause for autonomously functioning thyroid nodules. Studies show that mutations in *GNAS* resulting in overactivation of the G protein alpha subunit leads to a hyperfunctioning thyroid. Serving as a potential marker for benign thyroid nodules, *GNAS* does not seem to serve as a driver of thyroid carcinogenesis. However, it seems likely that this disruption in thyroid function in conjunction with other genetic alterations plays a role in thyroid carcinoma (26).

PIK3CA

PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, is a gene located on chromosome 3q26.3. It encodes the p110 alpha subunit of the PI3K kinase. This particular subunit is the catalytic subunit and is responsible for the kinase function of the protein. PI3K plays a very important role in the PI3K/AKT signaling pathway, one of the major pathways implicated in thyroid carcinoma. As part of this signaling pathway, PI3K helps regulate cell proliferation, motility, adhesion, and cell cycle progression. The pathway begins with signals transmitted from the environment to the inside of the cell through RTKs. These RTKs then engage PI3K, recruiting the protein to the cell membrane and generating phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 acts as a second messenger, recruiting protein kinases phosphoinositide-dependent protein kinase 1 (PDK1) and AKT to the membrane. Once activated via phosphorylation, these kinases target their downstream effectors, producing concomitant changes to cell growth and differentiation. This pathway is regulated by the *PTEN* lipid phosphatase, which dephosphorylates PIP3 and thus shuts off the PI3K/AKT pathway (27).

The PI3K pathway is frequently activated in thyroid carcinoma and studies have reported the overexpression of *PIK3CA* in FTC and ATC. The most common *PIK3CA* mutations observed with oncogenic effects tend to cluster around exons 9 and 20. These mainly somatic mutations result in the constitutive activation of PI3K. Consequently, this leads to overactivation of the PI3K/AKT pathway and thyroid tumorigenesis. Genetic alterations have been found in all differentiated stages of thyroid carcinoma. However, it seems to be the most frequent in ATC, occurring in about 24% of ATC cases. Given the occurrence of *PIK3CA* mutations in all stages of thyroid carcinoma, it supports the implication of the PI3K/AKT signaling pathway in both the initiation and progressive dedifferentiation of the disease (27,28,29).

PTEN

PTEN, phosphatase and tensin homolog, encodes a phosphatase enzyme found in almost all tissues in the body. Located on chromosome 10q23.3, *PTEN* plays an important role in the PI3K/AKT pathway. Once activated via phosphorylation, *PTEN* works to terminate the signaling pathway through its phosphatase activity. It converts PIP3 into PIP2and shuts off the PI3K/AKT pathway and reduces the recruitment of kinases PDK1 and AKT. As a result, this protein helps regulate cell proliferation and differentiation (28,29).

A tumor suppressor gene, *PTEN* is found mutated frequently in thyroid tumors. Point mutations, deletions, and promoter methylation in *PTEN* have been reported in thyroid carcinoma, particularly undifferentiated ones like ATC. Overall, studies have shown that *PTEN* inactivation is a critical step in thyroid tumor progression. This is evident in the fact that *PTEN* is downregulated or absent in highly malignant thyroid carcinoma like ATC, though mutations have been found in more differentiated forms. *PTEN* downregulation has been reported in about 37% of FTCs. Ultimately, this inactivation results in the overactivation of the PI3K/AKT signaling pathway, resulting in rampant cell proliferation (28,29,30).

PTEN mutations tend to be most frequent in ATC, with an occurrence in about 15% of cases. Germline mutations of *PTEN* is also a leading cause of CS, a disorder that can result in benign and malignant tumors. Individuals with CS have a 10% risk of developing thyroid cancer. This association between CS and *PTEN* further supports its role in thyroid tumorigenesis (27,29,30,31).

TSHR

The *TSHR* gene, located on chromosome 14q31, encodes the *TSHR*. This G protein-coupled receptor spans the membrane of follicular cells in the thyroid. Once activated by thyroid stimulating hormone (TSH), *TSHR* produces corresponding effects in the cell via second messengers like cAMP. This intracellular signaling plays an important role in thyroid cell proliferation and maintenance of thyroid function (Figure 4). Deregulation of *TSHR* seems to play an important role in thyroid carcinogenesis (20).

Studies have shown an association between high levels of TSH and the development of thyroid nodules. The overactivation of *TSHR*, whether through activating mutations in *TSHR* or *GNAS*, has also been shown to lead to hyperfunctional FTA. However, these tumors are rarely malignant, suggesting that *TSHR* could serve a dichotomous role - protecting against malignancy of thyroid tissue but also promoting carcinogenesis likely when activated by other oncogenic alterations. Mouse models have shown that *TSHR* is required for thyroid carcinogenesis. It is still uncertain whether this supports *TSHR*'s role in the initiation of thyroid carcinoma or it is simply due to the *TSHR*-dependent generation of thyroid cells from which carcinogenesis initiates (20,32,33).

Epigenetics has shown to play an integral part in the role of *TSHR* in thyroid carcinoma. Hypermethylation of the *TSHR* promoter has been frequently found in thyroid carcinoma, while it is unmethylated in normal and benign thyroid tumors. This epigenetic silencing is most prevalent in PTC, with a frequency of 34-59%. Epigenetic silencing via hypermethylation is also prevalent in more undifferentiated forms of thyroid carcinoma. Studies have also shown a strong association with *B-RAF* mutations in PTC. This relationship supports the theory that *TSHR* silencing is a secondary genetic event in thyroid carcinogenesis and not essential for the initiation of thyroid carcinoma (32,33).

The silencing of *TSHR* not only contributes to the development of thyroid carcinoma, but it desensitizes the thyroid tumors to radioiodine therapy through its interaction with sodium iodide symporter (NIS) in iodide uptake. The suppression of *TSHR* hinders the cancer cells' ability to concentrate iodine. Though no silencing mutations have been found thus far, it seems that methylation of *TSHR* serves as a marker for thyroid malignancy (33).

TG

The *TG* gene located on chromosome 8q24, encodes the prohormone *TG*. This protein, found in thyroid follicular cells, acts as a precursor for the thyroid hormones, serving as the matrix from which they are produced. It acts as a protein storehouse and when the thyroid hormones are needed, *TG* is altered and broken down to release the needed hormones. Tyrosine residues on the *TG* protein are iodinated, priming the protein for modification into the thyroid hormones T3 and T4 (Figure 4). The synthesis of *TG* is regulated by TSH, with TSH serving to stimulate production (34).

Mutations in *TG* have been reported in thyroid carcinoma. They tend to be more frequently found in differentiated

thyroid carcinomas, like PTC. The majority of PTCs with a mutation in *TG* tend to also carry a mutation in one of the genes in the MAPK signaling pathway. Though rarely mutated, *TG* does seem to contribute to the malignancy of the cancer. As the cancer dedifferentiates, the expression of *TG* seems to decrease, with it becoming absent in ATC. These genetic changes do not seem to be sufficient to initiate thyroid carcinogenesis, but the hormonal imbalance produced seems to aid in its progression (34,35).

Changes in *TG* are more commonly associated with dyshormonogenesis, occurring with a frequency of 25%. Individuals with the disorder tend to develop goiters, often due to chronic stimulation by TSH. Studies have shown that individuals with these dyshormonogenic goiters and *TG* mutations have an increased risk of developing aggressive forms of thyroid carcinoma.

TG also serves as a tool for monitoring thyroid cancer recurrence and metastasis after treatment and thyroidectomy. Serum *TG* levels are carefully monitored and upon stimulation by TSH, if levels rise too high it serves as a strong indicator of metastases. Such an association helps support the role of *TG* dysregulation in the development of thyroid carcinoma (35,36,37,38).

PAX8/PPARγ

A chromosomal rearrangement found in thyroid carcinoma is that between the genes *PAX8* and *PPARγ*, *PAX8* encodes a transcription factor that is essential for normal thyroid development. The *PPARγ* gene encodes a nuclear receptor part of the PPAR subfamily. This receptor forms heterodimers with retinoid X receptors and helps regulate the transcription of several genes, playing a major role in adipogenesis and lipid metabolism (3,39).

This rearrangement is the result of a translocation between chromosome regions 2q13 and 3p25. Highly expressed in thyroid follicular cells, it seems this fusion protein plays a role in thyroid tumorigenesis. The exact mechanism is still unknown. Some studies show that *PAX8/PPARγ* has a dominant negative effect on wild type *PPARγ*, which is believed to act as a tumor suppressor. The fusion protein contains the transcriptional regulatory domains of both proteins. As a result, it is capable of modulating their respective downstream pathways, usually targeting *PAX8*-responsive promoters (39,40).

PAX8/PPARγ is most frequently found in FTCs, with an occurrence rate of about 30%, and occurs rarely in the follicular variant of PTC. The presence of this rearrangement has no major impact on the prognosis of thyroid carcinoma. Though still much remains unknown about this chromosomal rearrangement, evidence points to its potential as an oncoprotein and genetic driver of thyroid carcinoma (4,39,40).

IGF2BP3/IMP3

The *IGF2BP3* gene is located on chromosome 7p11.2, and encodes for the insulin-like growth factor-2 (IGF-2) mRNA-binding protein 3. *IGF2BP3* is a RNA-binding protein consisting of two RNA-recognition motifs in its N¬-terminal part and four KH homology domains in the C-terminal region. Its primary function is as a translational activator of the IGF-2 gene, while also functioning as a post-transcriptional regulator of cell proliferation, and differentiation during embryogenesis. It is important to note that *IGF2BP3* is a member of the highly conserved IGF2BP family of mRNA-binding proteins along with IGF2BP1 and IGF2BP2. As a member of the IGF2BP family *IGF2BP3* shares a high amount of similarity to the other three members at the amino acid level but differs in terms of its expression. *IGF2BP3* is abundantly found in all tissues during embryonic stage, but it's expression in normal mature tissue is confined to the placenta and reproductive tissue (41,42,43).

IGF2BP3 has been the member of the IGF2BP family to be most associated with various cancer types such as lung, ovarian, pancreatic, and colorectal. Its role in tumor progression has been suggested to be by promoting cell growth, proliferation and resistance to ionic radiation via an IGF-2 dependent manner. Recent studies have found *IGF2BP3* to play a role in increasing the invasive potential of tumor cells *in vitro* (42,43).

Due to the high transcript levels of *IGF2BP3* in neoplastic cells and near undetectable presence in most adult tissue, it's been suggested as a potential biomarker. Through the use of immunoassays, its expression has been exclusively observed in malignant thyroid cancers of follicular origin, with a small portion of classical PTC, and was observed in 59% of cases for poorly differentiated thyroid carcinoma variants. However, through the use of reverse transcription polymerase chain reaction (RT-PCR) and qRT-PCR assay, it was demonstrated that *IGF2BP3* was overexpressed in thyroid carcinoma tissue when compared to benign thyroid lesions. *IGF2BP3*'s overexpression in thyroid carcinoma, its near undetectable levels in adult tissue, and its potential to distinguish between benign and malignant tissue make it an excellent candidate for the thyroid cancer sequencing panel (41,44,45,46).

KRT7/KRT20

The *KRT7* and *KRT20* genes both belong to the keratin gene family, which consists of 54 functional genes. The *KRT7* gene can be found in type 2 cluster on chromosome 12, while the *KRT20* gene is located in the type 1 cluster on chromosome 17. The protein products of these genes are subdivided into type 1 and type 2 based on their molecular weights. *KRT7* and KRT 20 both encode for cytokeratin proteins that are intermediate filament forming proteins exclusively found in epithelial tissue. The filaments

are formed from the heterodimer pairing of type 1 and type 2 cytokeratin proteins in equimolar amounts. The intermediate filaments created by cytokeratin proteins aid in forming the structural framework of epithelial cells, regulate cell size, growth, division, as well as protection against mechanical stressors (46,47).

Adenocarcinomas compromise the largest group of human epithelial malignancies and can occur in different organs. *KRT7* and *KRT20* along with the other members of the keratin gene family have been utilized as diagnostic markers to determine the origin tissue, due to the characteristic cell-type, differentiation and functional status-dependent expression pattern of cytokeratin in epithelial cells. In most adenocarcinomas, the expression levels of *KRT7* and *KRT20* are variable and thus are often screened for together (47).

Current literature on *KRT7/KRT20* combined screening of thyroid neoplasms is scarce. Almost all thyroidal tumor's expression levels for *KRT7* and *KRT20* have been found to be positive and negative, respectively. In fact, one study looked into the immunohistochemical profile of 43 different types of primary and metastatic thyroid neoplasms. They found that excluding anaplastic carcinomas, 79% of thyroid tumors and 94% of metastatic thyroid carcinoma cases reacted positively with the antibody. While the study found a positive result for *KRT7*, none of the thyroid tumors or their metastases reacted at all to the *KRT20* antibody. A second retrospective study examined 153 thyroid carcinoma samples for *KRT7/KRT20* immunohistochemically, and they found that all papillary carcinomas, follicular carcinomas, and medullary carcinomas were *KRT7* positive and *KRT20* negative. Due to the fact that *KRT7* has been positively stained in almost all thyroid neoplasms while *KRT20* has consistently been undetectable, *KRT7* and *KRT20* are excellent candidates for the thyroid cancer sequencing panel (48,49,50).

TP53

The gene *TP53* encodes tumor protein p53, located on chromosome 17p13.1. This protein is a nuclear transcription factor that functions as a tumor suppressor. P53 contains three functional domains: a transactivation domain, a DNA binding domain, and an oligomerization domain. In the nucleus, it binds directly to DNA in order to help prevent mutated or damaged DNA from dividing. The protein either activates other genes to fix any damages or triggers cell cycle arrest and apoptosis (Figure 5). As a result, this protein plays a crucial role in regulating cell division and preventing tumor formation (51).

Any impairment in the function of *TP53* can lead to destabilization of the genome and an accumulation of mutations. Most changes involve point mutations in exons 5-8, deletions, or inactivation through indirect mechanisms such as methylation. The now inactivated *TP53* protein becomes unable to bind to DNA and thus is unable to repair damages that arise in the DNA. The protein, as well as damages to the DNA, just accumulates in the nucleus. These type of changes are usually restricted to poorly differentiated thyroid cancers like ATC, reportedly occurring in 60% of cases (8,51).

TP53 exhibits tremendous oncogenic potential, as it is mutated in about 50% of all human cancers. However, inactivating mutations of *TP53* are found in only about 10% of thyroid carcinomas overall. In well differentiated thyroid carcinomas, *TP53* mutations have a reported relevance ranging from 0 to 25%. Though it seems other mechanisms of *TP53* inactivation play a role in thyroid carcinogenesis, as p53 protein is more commonly overexpressed with a frequency of 11-59% (51,52).

CALCA

CALCA (*CALCA-1*) gene is a member of the calcitonin gene family consisting of four known genes, *CALCA-1*, *CALCA-2*, *CALCA-3*, and *CALCA-4* all containing nucleotide sequence homologies. The *CALCA* gene located on chromosome 11 encodes for two separate peptide hormones. This gene consists of six exons in which exon 4 and 5 contain the sequences for the calcitonin and calcitonin gene related peptide (α-CGRP), respectively. Post-transcriptional processing of the *CALCA* gene in thyroid C cells and neural tissue results in tissue specific production of calcitonin and α-CGRP mRNAs. CT is a 32 amino-acid peptide hormone with tissue specific functional roles in the central nervous, skeletal, renal and gastrointestinal systems. However, CT's major role is in calcium and phosphate metabolism. CT modulates calcium levels by binding with CT receptors to inhibit osteoclast motility. All of CT's activities occur through its binding to the calcitonin receptor, which couples its binding to the activation of cAMP and PKC signaling pathways (53,54,55).

Due to the uncharacteristically high concentrations of calcitonin, serum CT is classically used as a diagnostic biomarker for MTC. Studies have demonstrated that preoperative serum CT levels can be strong predictors for tumor size and remission. Preoperative CT serum levels above 1000 pg/mL correspond to 25.0 mm tumor sizes, while levels below 100 pg/mL were associated with 3.0 mm tumor size. Distant metastases are the main cause of death in patients diagnosed with MTC. Metastasis is initially presented in 50% of MTC cases. Postoperative serum CT levels are an important procedure in screening for biochemical remission. CT doubling times have been found to be associated with progressive or stable disease in 80% of patients. In a study they found doubling times shorter than 25 months to have a 94% predictive value of progressive disease along with a predictive value of 86% for patients with a doubling time longer than 24 months (53,56,57,58,59).

CALCA detection studies and procedures involve detection of this peptide from blood serum. There are limitations to these assays due to needing stimulation tests, and possible intra-assay variation. Some research has found that detection of *CALCA* gene transcripts presented higher clinical sensitivity, specificity, and positive predictive values of 86.67, 97.06, and 92.86%, respectively. In addition to these promising results their readings mirrored measurements obtainable through a pentagastrin stimulation test. Due to its tissue specific production and role in preoperative and postoperative diagnosing of MTC, *CALCA* is a viable candidate to include in the thyroid cancer mutation panel (53,60).

MET

MET, also known as c-*MET*, encodes a proto-oncogene that is located on chromosome 7q31. A member of the RTK family, the *MET* receptor elicits its effects by binding to its ligand, hepatocyte growth factor (HGF). Once bound to HGF, the *MET* receptor dimerizes and thus becomes activated and helps promote cell proliferation, survival, and motility. These effects are largely the result of activation of MAPK and PI3K pathways through *MET* signaling. This process is tightly regulated and any disruption can result in tumorigenesis (61).

In thyroid carcinoma, what is most commonly seen is a deregulation of *MET*, most commonly in PTC. This deregulation often involves overactivation or overexpression of *MET*, which has been shown to promote tumor growth. This activation can be the result of activating point mutations often located in the tyrosine kinase domain, or paracrine signaling due to increased sensitivity to HGF. The altered *MET* gene acts as a mitogenic factor and helps promote cell motility and invasion (61,62).

Expression levels of *MET* tend to be low and tightly regulated in normal thyroids. However, it is amplified in 75% of PTCs, as well as in many FTCs. This overexpression seems to be secondary to other driver mutations like *RAS* and *B-RAF*. Despite its secondary role, evidence points to increased *MET* signaling having a significant role in the pathogenesis of thyroid carcinoma (62,63,64).

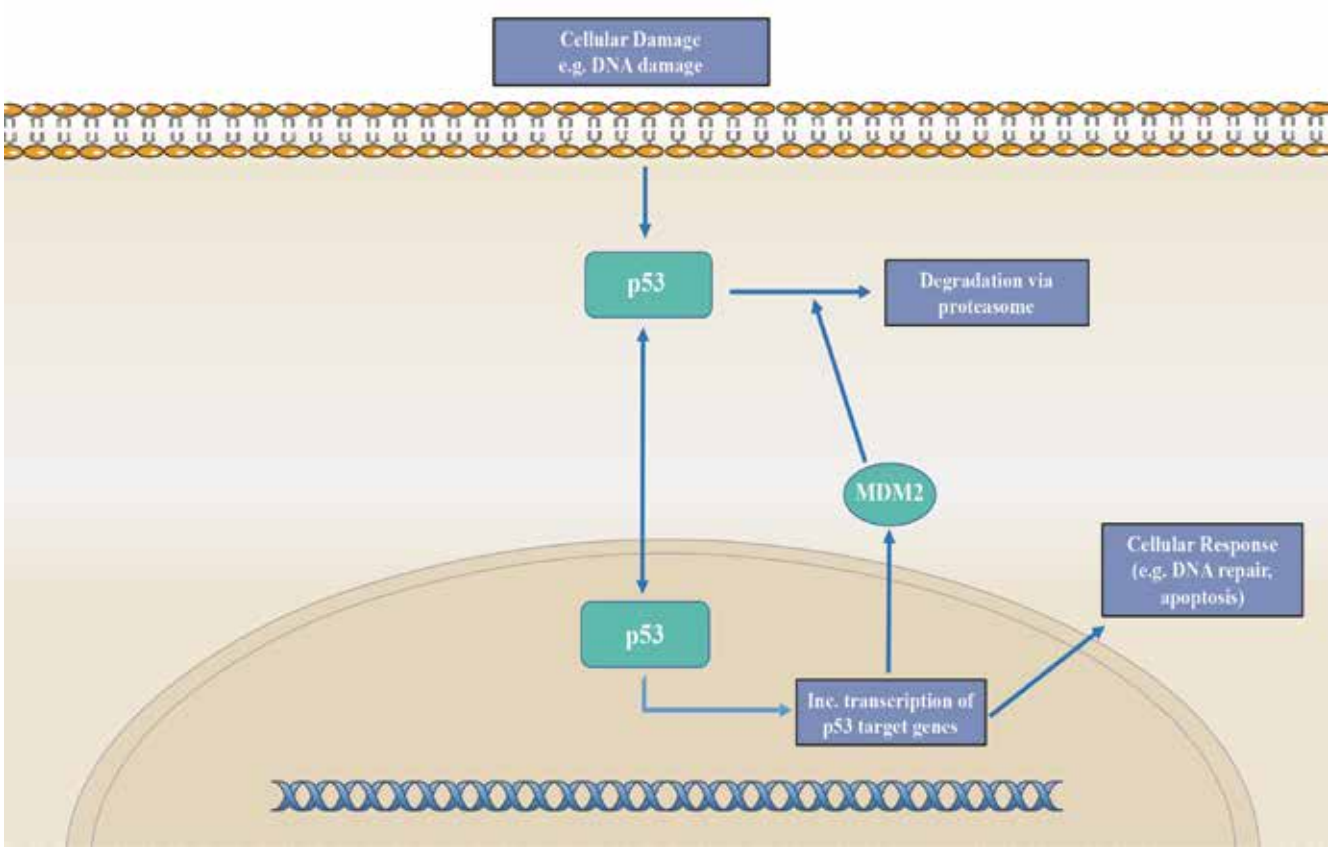


Figure 5. P53 pathway

**Signals of cellular stress, such as DNA damage, triggers activation of p53. Once activated, this nuclear transcription factor travels to the nucleus and binds to DNA, increasing expression of target genes involved in DNA repair and triggering apoptosis. p53 activity also induces expression of MDM2 as well. MDM2 serves to regulate p53 activity, inhibiting transcription of p53 and promoting its degradation. Overall, this pathway functions as an auto-regulatory feedback loop*

EIF1AX

This X-linked gene, located on Xp22.12, encodes the eukaryotic translation initiation factor 1A. This particular protein is required for the formation of the preinitiation complex (PIC). It does so by stabilizing the binding of the initiator tRNA (*Met*-tRNA) with the 40S ribosomal subunit and the translation initiation factor eIF2. This is an integral step in the translation of mRNA into proteins, as it is required for the binding of the PIC to the 5' end of capped mRNA and finding the proper start codon (65,66).

Mutations in *EIF1AX* have been found in both PTC and ATC. The most common sites of mutation are the N-terminal domain and a C-terminal splice acceptor site between exons 5 and 6. The C-terminal mutations seem to be specific to thyroid carcinoma. In PTC, it is rare and occurs in a mutually exclusive manner, found in only 1-2% of tumors. But, it is more frequent in ATC, found in 10% of tumors. It seems that these mutations result in an overactive *EIF1AX*, as it has been shown to increase cell proliferation (65,66,67,68).

EIF1AX mutations tend to coexist with *RAS* mutations and other driver mutations of thyroid carcinoma like *TP53*. This co-occurrence tends to be found predominantly in poorly differentiated thyroid carcinomas like ATC. This strong association suggests that *EIF1AX* is not sufficient enough to transform thyroid carcinoma into a malignant form. Mutations in *EIF1AX* have also been associated with poor prognosis in PDCs. The mechanisms of *EIF1AX* mutation in thyroid carcinoma are still unclear, especially in terms of its association with *RAS* mutations (65,66,67,68).

TTF1

Also known as NK2 homeobox 1, this gene encodes thyroid transcription factor 1. The *TTF1* protein is expressed in the epithelial cells of the thyroid with a heterogeneous distribution. As a homeoprotein, it plays an important role in thyroid development, cell growth, and differentiation. Essential for thyroid organogenesis, *TTF1* helps modulate thyroid function through its regulation of gene expression in the thyroid, activating the transcription of thyroid-specific genes like thyroglobulin (*TG*) and thyroperoxidase (TPO) (69,70).

The mechanisms by which *TTF1* contributes to thyroid carcinoma still remains unclear. Though the gene is expressed in thyroid carcinoma, its level of expression becomes reduced as cell dedifferentiation progresses. This change in expression could be the result of epigenetic silencing, likely achieved through hypermethylation and histone H3 modification. As a result, ATC exhibits the lowest levels of *TTF1*. In thyroid carcinoma as a whole it seems likely that *TTF1*'s molecular functions, DNA binding, and transcription activation are probably disrupted. That could explain why most undifferentiated thyroid carcinomas have little to no *TG* or TPO proteins, both targets of *TTF1* transcription factor activity (41,42,43).

Germline mutations in *TTF1* have also been shown to play a role in thyroid carcinoma, specifically in PTC. These mutations seem to promote cell proliferation as well as activate signaling pathways like PI3K/AKT. The germline mutations observed in *TTF1* have also been associated with multinodular goiter. This provides further evidence of a progression from a benign multinodular goiter to malignant thyroid carcinoma, such as PTC. Given the important role *TTF1* plays in maintaining thyroid architecture and function, it is not surprising that any abnormalities in the gene and its product contribute to the development of thyroid carcinoma (70,71,72).

PTH

The *PTH* gene encodes a member of the parathyroid family of proteins and is located on chromosome 11p15.3. This particular protein binds to the parathyroid hormone receptor and helps regulate blood calcium and phosphate levels in the body. Abnormal levels of *PTH* tend to be associated with parathyroid diseases, like hyperparathyroidism. However, there is evidence to suggest a possible link between *PTH* and the development of thyroid carcinoma (73).

Primary hyperparathyroidism (PHPT) is a disease that has been associated with thyroid carcinoma. PHPT results from an excessive secretion of *PTH*, which causes an elevation of calcium in the blood. The most common form of thyroid carcinoma associated with PHPT is a hereditary form of MTC known as multiple endocrine neoplasia type 2A (MEN2A). Carriers of the MEN2A gene with a mutation at codon 634 concomitantly suffer from PHTP with a frequency of 10-25%. Such an association provides evidence to support the role of *PTH* dysregulation in thyroid carcinoma (73,74).

Associations between non-medullary thyroid carcinoma and PHPT have also been reported. With rates ranging from 2-10%, thyroid malignancy seems to coexist with PHPT, albeit not frequently. A strong relationship has also been found between ionizing radiation and the simultaneous development of PHPT and thyroid carcinoma. *PTH* may also contribute to the development of thyroid carcinoma through chromosomal rearrangement with PAD1. This rearrangement results in an overexpression of cyclin D1 and thus an increase in cell proliferation. Though some changes to *PTH* may be the result of metastatic invasion of the parathyroid by thyroid carcinoma, the evidence available shows that the role of *PTH* in thyroid malignancy overall warrants further investigation (75,76,77).

SCL5A5

The *SCL5A5* gene encodes the NIS. NIS is a glycosylated protein with 13 trans-membrane domains and functions

to actively transport one I^- and two Na^+ ions from the sodium ion gradient established by the Na^+/K^+ ATPase. NIS expression at both the transcriptional level and posttranscriptional level is modulated by the effects of TSH. TSH causes elevated levels of endogenous cAMP in the thyroid cells. These cAMP levels play a role in stimulating several cis-regulatory elements found in the promoter and upstream enhancer sequences on the *SCL5A5* gene on chromosome 19 (78,79).

The *SCL5A5* gene contains an upstream enhancer sequence (NUE) reported to have both a Pax-8, and cAMP-response element (CRE) binding sites. Pax-8 and cAMP-response element modulator (CREM) transcription factors bind to these cis-elements on the upstream enhancer via cAMP-signaling pathways. These elements are stimulated through cAMP activation of protein kinase-A (PKA) dependent pathways in thyroid cells. PKA dependent activation phosphorylates basic-leucine zipper (B-ZIP) proteins, CREM, activating transcription factor-1 (ATF-1), and cAMP-response element binding protein (CREB). Studies have found that the binding of these transcription factors are necessary for the NUE's cAMP induced activity on thyroidal responsive genes such as TPO, *TG*, *TSHR*, and NIS (Figure 6). While CREB binding is important for SLC5A5 transcriptional regulation, full SLC5A regulation is not established without PAX-8 binding. TSH binding increases the expression and translocation of the anti-oxidative stress factor Ref-1. Ref-1 is a nuclear enzyme that reduces Pax-8 and mediates its binding to the NUE sequence. Impaired translocation of this nuclear enzyme has been reported in anaplastic, papillary cell lines as well as in thyroid cancer tissue. TSH also plays a role in the posttranscriptional regulatory role in the transport of NIS to the basolateral membrane (78,79).

While no somatic mutations to NIS have yet been identified, alterations in other genes have been found to be associated with NIS impairment in differentiated thyroid cancers.

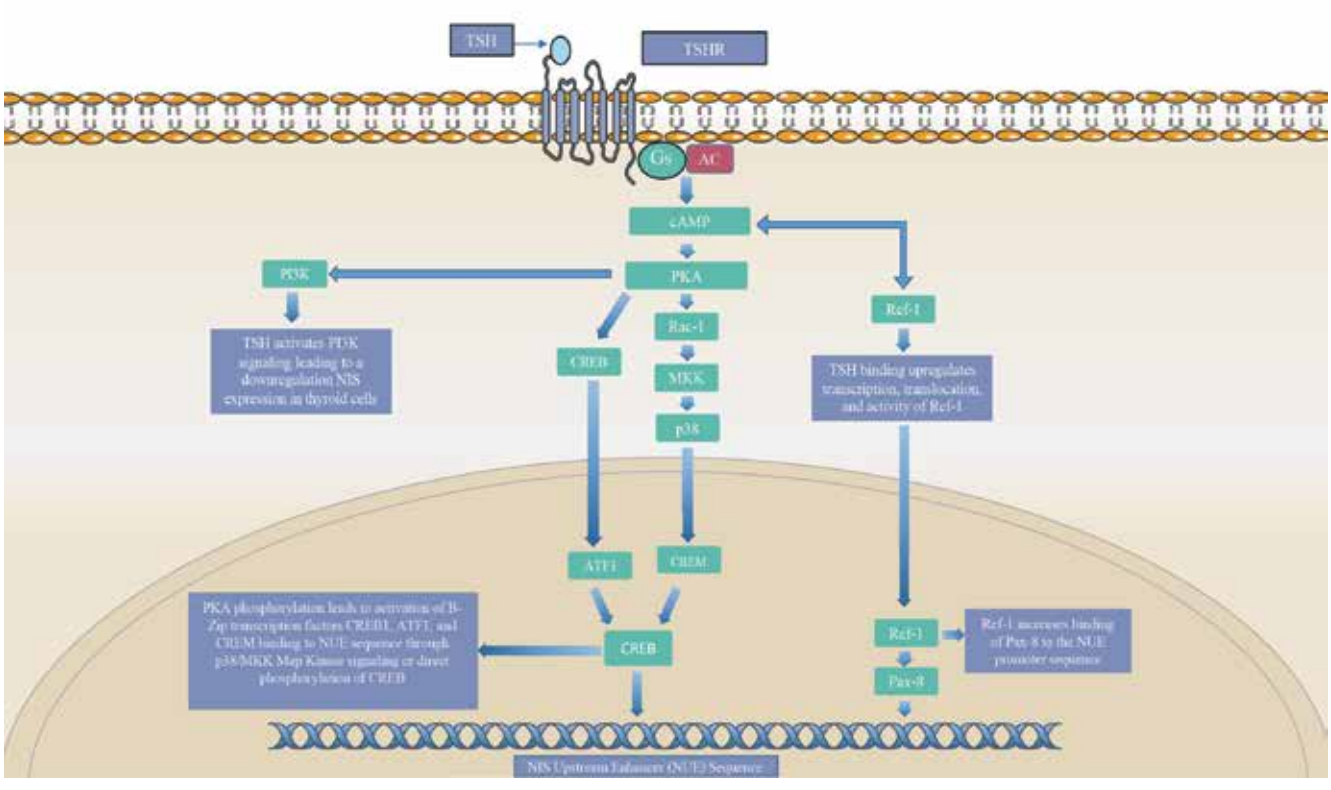


Figure 6. Sodium iodide symporter gene signaling

**The sodium Iodide symporter is a transmembrane protein that plays a crucial role in the uptake of iodine by thyroid cells. The SCL5A5 gene is regulated by the activity of TSH on the sodium iodide symporter upstream enhancer sequence (NUE). In this pathway TSH binding to TSHR stimulates an increase in cyclic AMP (cAMP) levels in the cell. Elevated levels of cAMP lead to the activation of protein kinase-A (PKA) dependent p38/MAPK and PKA-cAMP-response element binding protein (CREB) pathways. The p38/MAPK pathway leads to the recruitment of basic-leucine zipper transcription factors ATF1, and cAMP-response modulator to the nucleus. In the nucleus these transcription factors bind to the cAMP-response element binding sites in the NUE and activating the SCL5A5 gene. PKA can also directly phosphorylate the cAMP-CREB to directly regulate cAMP responsive genes such as TSHR, and SCL5A5. Full activation of the SCL5A5 gene is not reached until the PAX-8 transcription factor bindings to the NUE promoter site. TSH signaling upregulates the transcription and nuclear translocation of the oxidative stress factor Ref-1. Ref-1 activates and recruits PAX-8 to bind to the NUE promoter site, leading to full SCL5A5 gene activity. Inhibition of SCL5A5 activity can also occur due to the elevated levels of cAMP. These elevated levels can trigger activation of the PI3K signaling pathway leading to the downregulation of the SLC5A5 gene*

B-RAF^{V6000E} mutations reduce NIS mRNA expression by inducing the secretion of transforming growth factor-β (TGF-β). TGF-β acts via the SMAD signaling pathway in an MEK/ERK independent pathway. *RET*/PTC rearrangement impairs the activity of PAX-8 and PKA. Reducing the activity of these two proteins has been demonstrated to reduce recruitment of B-ZIP proteins and reduced binding to the PAX-8 and CRE elements in NUE (79,80,81).

NIS expression has been reported to be reduced in differentiated thyroid carcinomas. One study found reduced NIS mRNA expression in PTC tissue samples when compared to adjacent normal thyroid tissue. NIS expression was also found to be generally reduced in benign thyroid nodules compared with adjacent normal tissue, but to a lesser extent than in PTC tissue samples. It has also been reported that NIS gene expression is impaired in hypo-functioning thyroid tumors when compared to normal thyroid tissue. Quantification of *SCL5A5* mRNA levels should be added to the classical prognostic factors currently used to predict the outcomes of patients with differentiated thyroid cancer. *SCL5A5* is an excellent candidate for the thyroid cancer mutation panel (82,83,84).

Conclusion

Thyroid carcinoma is the result of an accumulation of genetic changes that impact its initiation and progression. These genetic alterations, thus, serve as both diagnostic and prognostic markers of thyroid carcinoma. ThyroSeq NGS allows for the use of small amounts of DNA to test for a very broad range of mutations and other genetic alterations, helping identify molecular profiles for the different types of thyroid carcinoma. The genotyping of thyroid samples provides invaluable insight in determining the proper course of treatment when faced with a cytologically indeterminate thyroid nodule.

The genetics of thyroid cancer provides us with a greater understanding of the pathophysiology of the disease than is possible from its cellular morphology. The presence of mutations in the molecular pathology profile can not only help more accurately determine malignancy in the thyroid nodules but help better predict the biology of thyroid carcinoma which will have paramount importance to tailor treatment strategies. The presence of certain genetic alterations may prove indicators of aggressiveness of the cancer and predictors for metastasis. As more studies are conducted, and the genomic profile of thyroid carcinoma is further elucidated, the utility of genomic profiling in the diagnosis and prognostication of thyroid cancer will be solidified.

Authorship Contributions

Surgical and Medical Practices: Seza Gulec, Concept: Seza Gulec, Jorge Alsina, Design: Seza Gulec, Jorge Alsina, Raul Alsina, Data Collection or Processing: Jorge Alsina, Raul Alsina, Seza Gulec, Analysis or Interpretation: Jorge Alsina, Raul Alsina, Seza Gulec, Literature Search: Jorge Alsina, Raul Alsina, Seza Gulec, Writing: Jorge Alsina, Raul Alsina, Seza Gulec.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Vigneri R, Malandrino P, Vigneri P. The changing epidemiology of thyroid cancer: why is incidence increasing? Curr Opin Oncol 2015;27:1-7.
2. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol 2011;7:569-580.
3. Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE. Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab 2013;98:E1852-1860.
4. Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Mod Pathol 2008;21(Suppl 2):S37-43.
5. LiVolsi VA. Papillary thyroid carcinoma: an update. Mod Pathol 2011;24(Suppl 2):S1-9.
6. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R, Cancer Genome P. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004;116:855-867.
7. Howell GM, Hodak SP, Yip L. RAS mutations in thyroid cancer. Oncologist 2013;18:926-932.
8. Patel KN, Singh B. Genetic considerations in thyroid cancer. Cancer Control 2006;13:111-118.
9. Isaeva AV, Zima AP, Saprina TV, Kasoyan KT, Popov OS, Brynova OV, Berezkina IS, Vasil'eva OA, Ryazantseva NV, Shabalova IP, Litvinova LS, Pak YD, Novitskii VV. Comparative Evaluation of beta-Catenin and E-Cadherin Expression in Liquid Aspiration Biopsy Specimens of Thyroid Nodules. Bull Exp Biol Med 2016;161:288-291.
10. Garcia-Rostan G, Camp RL, Herrero A, Carcangiu ML, Rimm DL, Tallini G. Beta-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. Am J Pathol 2001;158:987-996.
11. Sastre-Perona A, Riesco-Eizaguirre G, Zaballos MA, Santisteban P. ss-catenin signaling is required for RAS-driven thyroid cancer through PI3K activation. Oncotarget 2016 doi: 10.18632/oncotarget.10356.
12. Pierotti MA, Greco A. Oncogenic rearrangements of the NTRK1/NGF receptor. Cancer Lett 2006;232:90-98.
13. Vaishnavi A, Le AT, Doebele RC. TRKing down an old oncogene in a new era of targeted therapy. Cancer Discov 2015;5:25-34.
14. Greco A, Miranda C, Pierotti MA. Rearrangements of NTRK1 gene in papillary thyroid carcinoma. Mol Cell Endocrinol 2010;321:44-49.
15. Lannon CL, Sorensen PH. ETV6-NTRK3: a chimeric protein tyrosine kinase with transformation activity in multiple cell lineages. Semin Cancer Biol 2005;15:215-223.
16. Leeman-Neill RJ, Kelly LM, Liu P, Brenner AV, Little MP, Bogdanova TI, Evdokimova VN, Hatch M, Zurnadzy LY, Nikiforova MN, Yue NJ, Zhang M, Mabuchi K, Tronko MD, Nikiforov YE. ETV6-NTRK3 is a common chromosomal rearrangement in radiation-associated thyroid cancer. Cancer 2014;120:799-807.
17. Giordano TJ, Kuick R, Thomas DG, Misek DE, Vinco M, Sanders D, Zhu Z, Ciampi R, Roh M, Shedden K, Gauger P, Doherty G, Thompson NW, Hanash S, Koenig RJ, Nikiforov YE. Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. Oncogene 2005;24:6646-6656.

18. Marotta V, Guerra A, Sapio MR, Vitale M. RET/PTC rearrangement in benign and malignant thyroid diseases: a clinical standpoint. Eur J Endocrinol 2011;165:499-507.
19. Vasko V, Saji M, Hardy E, Kruhlak M, Larin A, Savchenko V, Miyakawa M, Isozaki O, Murakami H, Tsushima T, Burman KD, De Micco C, Ringel MD. Akt activation and localisation correlate with tumour invasion and oncogene expression in thyroid cancer. J Med Genet 2004;41:161-170.
20. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 2013;13:184-199.
21. Ringel MD, Hayre N, Saito J, Saunier B, Schuppert F, Burch H, Bernet V, Burman KD, Kohn LD, Saji M. Overexpression and overactivation of Akt in thyroid carcinoma. Cancer Res 2001;61:6105-6111.
22. Martinez P, Blasco MA. Telomeric and extra-telomeric roles for telomerase and the telomere-binding proteins. Nat Rev Cancer 2011;11:161-176.
23. Heidenreich B, Rachakonda PS, Hemminki K, Kumar R. TERT promoter mutations in cancer development. Curr Opin Genet Dev 2014;24:30-37.
24. Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, Sun H, El-Naggar AK, Xing M. Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer 2013;20:603-610.
25. Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, Ibrahimpasic T, Ghossein RA, Fagin JA. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab 2013;98:E1562-1566.
26. Hsiao SJ, Nikiforov YE. Molecular approaches to thyroid cancer diagnosis. Endocr Relat Cancer 2014;21:T301-313.
27. Garcia-Rostan G, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R, Hermsem MJ, Herrero A, Fusco A, Cameselle-Teijeiro J, Santoro M. Mutation of the PIK3CA gene in anaplastic thyroid cancer. Cancer Res 2005;65:10199-10207.
28. Guerra A, Di Crescenzo V, Garzi A, Cinelli M, Carlomagno C, Tonacchera M, Zeppa P, Vitale M. Genetic mutations in the treatment of anaplastic thyroid cancer: a systematic review. BMC Surg 2013;13 (Suppl 2):S44.
29. Nozhat Z, Hedayati M. PI3K/AKT Pathway and Its Mediators in Thyroid Carcinomas. Mol Diagn Ther 2016;20:13-26.
30. Smith JR, Marqusee E, Webb S, Nose V, Fishman SJ, Shamberger RC, Frates MC, Huang SA. Thyroid nodules and cancer in children with PTEN hamartoma tumor syndrome. J Clin Endocrinol Metab 2011;96:34-37.
31. Xu B, Ghossein R. Genomic Landscape of poorly Differentiated and Anaplastic Thyroid Carcinoma. Endocr Pathol 2016;27:205-212.
32. Kartal K, Onder S, Kosemehmetoglu K, Kilickap S, Tezel YG, Kaynaroglu V. Methylation status of TSHr in well-differentiated thyroid cancer by using cytologic material. BMC Cancer 2015;15:824.
33. Khan MS, Pandith AA, Masoodi SR, Wani KA, Ul Hussain M, Mudassar S. Epigenetic silencing of TSHR gene in thyroid cancer patients in relation to their BRAF V600E mutation status. Endocrine 2014;47:449-455.
34. Whitley RJ, Ain KB. Thyroglobulin: a specific serum marker for the management of thyroid carcinoma. Clin Lab Med 2004;24:29-47.
35. Alzahrani AS, Baitei EY, Zou M, Shi Y. Clinical case seminar: metastatic follicular thyroid carcinoma arising from congenital goiter as a result of a novel splice donor site mutation in the thyroglobulin gene. J Clin Endocrinol Metab 2006;91:740-746.
36. Francis Z, Schlumberger M. Serum thyroglobulin determination in thyroid cancer patients. Best Pract Res Clin Endocrinol Metab 2008;22:1039-1046.
37. Rubio IG, Medeiros-Neto G. Mutations of the thyroglobulin gene and its relevance to thyroid disorders. Curr Opin Endocrinol Diabetes Obes 2009;16:373-378.
38. Mazzaferri EL, Robbins RJ, Spencer CA, Braverman LE, Pacini F, Wartofsky L, Haugen BR, Sherman SI, Cooper DS, Braunstein GD, Lee S, Davies TF, Arafah BM, Ladenson PW, Pinchera A. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 2003;88:1433-1441.
39. Raman P, Koenig RJ. Pax-8-PPAR-gamma fusion protein in thyroid carcinoma. Nat Rev Endocrinol 2014;10:616-623.
40. Placzkowski KA, Reddi HV, Grebe SK, Eberhardt NL, McIver B. The Role of the PAX8/PPARgamma Fusion Oncogene in Thyroid Cancer. PPAR Res 2008;2008:672829.
41. Slosar M, Vohra P, Prasad M, Fischer A, Quinlan R, Khan A. Insulin-like growth factor mRNA binding protein 3 (IMP3) is differentially expressed in benign and malignant follicular patterned thyroid tumors. Endocr Pathol 2009;20:149-157.
42. Lederer M, Bley N, Schleifer C, Huttelmaier S. The role of the oncofetal IGF2 mRNA-binding protein 3 (IGF2BP3) in cancer. Semin Cancer Biol 2014;29:3-12.
43. Bell JL, Wachter K, Muhleck B, Pazaitis N, Kohn M, Lederer M, Huttelmaier S. Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? Cell Mol Life Sci 2013;70:2657-2675.
44. Findeis-Hosey JJ, Xu H. The use of insulin like-growth factor II messenger RNA binding protein-3 in diagnostic pathology. Hum Pathol 2011;42:303-314.
45. Jin L, Seys AR, Zhang S, Erickson-Johnson MR, Roth CW, Evers BR, Oliveira AM, Lloyd RV. Diagnostic utility of IMP3 expression in thyroid neoplasms: a quantitative RT-PCR study. Diagn Mol Pathol 2010;19:63-69.
46. Asioli S, Erickson LA, Righi A, Jin L, Volante M, Jenkins S, Papotti M, Bussolati G, Lloyd RV. Poorly differentiated carcinoma of the thyroid: validation of the Turin proposal and analysis of IMP3 expression. Mod Pathol 2010;23:1269-1278.
47. Karantza V. Keratins in health and cancer: more than mere epithelial cell markers. Oncogene 2011;30:127-138.
48. Lam KY, Lui MC, Lo CY. Cytokeratin expression profiles in thyroid carcinomas. Eur J Surg Oncol 2001;27:631-635.
49. Bejarano PA, Nikiforov YE, Swenson ES, Biddinger PW. Thyroid transcription factor-1, thyroglobulin, cytokeratin 7, and cytokeratin 20 in thyroid neoplasms. Appl Immunohistochem Mol Morphol 2000;8:189-194.
50. Weber T, Lacroix J, Weitz J, Amnan K, Magener A, Holting T, Klar E, Herfarth C, von Knebel Doeberitz M. Expression of cytokeratin 20 in thyroid carcinomas and peripheral blood detected by reverse transcription polymerase chain reaction. Br J Cancer 2000;82:157-160.
51. Malaguarnera R, Vella V, Vigneri R, Frasca F. p53 family proteins in thyroid cancer. Endocr Relat Cancer 2007;14:43-60.
52. Morita N, Ikeda Y, Takami H. Clinical significance of p53 protein expression in papillary thyroid carcinoma. World J Surg 2008;32:2617-2622.
53. Schifter S. Expression of the calcitonin gene family in medullary thyroid carcinoma. Peptides 1997;18:307-317.
54. Masi L, Brandi ML. Calcitonin and calcitonin receptors. Clin Cases Miner Bone Metab 2007;4:117-122.
55. Pacini F, Castagna MG, Cipri C, Schlumberger M. Medullary thyroid carcinoma. Clin Oncol (R Coll Radiol) 2010;22:475-485.
56. Ball DW. Medullary thyroid cancer: therapeutic targets and molecular markers. Curr Opin Oncol 2007;19:18-23.
57. Laure Giraudet A, Al Ghulzan A, Auperin A, Leboulleux S, Chehboun A, Troalen F, Dromain C, Lumbroso J, Baudin E, Schlumberger M. Progression of medullary thyroid carcinoma: assessment with calcitonin and carcinoembryonic antigen doubling times. Eur J Endocrinol 2008;158:239-246.
58. Cohen R, Campos JM, Salaun C, Heshmati HM, Kraimps JL, Proye C, Sarfati E, Henry JF, Niccoli-Sire P, Modigliani E. Preoperative calcitonin levels are predictive of tumor size and postoperative calcitonin normalization in medullary thyroid carcinoma. Groupe d'Etudes des Tumeurs a Calcitonine (GETC). J Clin Endocrinol Metab 2000;85:919-922.
59. Machens A, Schneyer U, Holzhausen HJ, Dralle H. Prospects of remission in medullary thyroid carcinoma according to basal calcitonin level. J Clin Endocrinol Metab 2005;90:2029-2034.

60. Camacho CP, Lindsey SC, Melo MC, Yang JH, Germano-Neto F, Valente Fde O, Lima TR, Biscolla RP, Vieira JG, Cerutti JM, Dias-da-Silva MR, Maciel RM. Measurement of calcitonin and calcitonin gene-related peptide mRNA refines the management of patients with medullary thyroid cancer and may replace calcitonin-stimulation tests. Thyroid 2013;23:308-316.
61. Organ SL, Tsao MS. An overview of the c-MET signaling pathway. Ther Adv Med Oncol 2011;3:S7-S19.
62. Wasenius VM, Hemmer S, Karjalainen-Lindsberg ML, Nupponen NN, Franssila K, Joensuu H. MET receptor tyrosine kinase sequence alterations in differentiated thyroid carcinoma. Am J Surg Pathol 2005;29:544-549.
63. Zenali M, deKay J, Liu Z, Hamilton S, Zuo Z, Lu X, Bakkar R, Mills G, Broaddus R. Retrospective Review of MET Gene Mutations. Oncoscience 2015;2:533-541.
64. Parameswaran R, Brooks S, Sadler GP. Molecular pathogenesis of follicular cell derived thyroid cancers. Int J Surg 2010;8:186-193.
65. Landa I, Ibrahimpasic T, Boucai L, Sinha R, Knauf JA, Shah RH, Dogan S, Ricarte-Filho JC, Krishnamoorthy GP, Xu B, Schultz N, Berger MF, Sander C, Taylor BS, Ghossein R, Ganly I, Fagin JA. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. J Clin Invest 2016;126:1052-1066.
66. Cancer Genome Atlas Research N. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676-690.
67. Kunstman JW, Juhlin CC, Goh G, Brown TC, Stenman A, Healy JM, Rubinstein JC, Choi M, Kiss N, Nelson-Williams C, Mane S, Rimm DL, Prasad ML, Hoog A, Zedenius J, Larsson C, Korah R, Lifton RP, Carling T. Characterization of the mutational landscape of anaplastic thyroid cancer via whole-exome sequencing. Hum Mol Genet 2015;24:2318-2329.
68. Karunamurthy A, Panebianco F, S JH, Vorhauer J, Nikiforova MN, Chiosea S, Nikiforov YE. Prevalence and phenotypic correlations of EIF1AX mutations in thyroid nodules. Endocr Relat Cancer 2016;23:295-301.
69. Katoh R, Kawaoi A, Miyagi E, Li X, Suzuki K, Nakamura Y, Kakudo K. Thyroid transcription factor-1 in normal, hyperplastic, and neoplastic follicular thyroid cells examined by immunohistochemistry and nonradioactive in situ hybridization. Mod Pathol 2000;13:570-576.
70. Kimura S. Thyroid-specific transcription factors and their roles in thyroid cancer. J Thyroid Res 2011;2011:710213.
71. Ngan ES, Lang BH, Liu T, Shum CK, So MT, Lau DK, Leon TY, Cherny SS, Tsai SY, Lo CY, Khoo US, Tam PK, Garcia-Barcelo MM. A germline mutation (A339V) in thyroid transcription factor-1 (TITF-1/NKX2.1) in patients with multinodular goiter and papillary thyroid carcinoma. J Natl Cancer Inst 2009;101:162-175.
72. Fabbro D, Di Loreto C, Beltrami CA, Belfiore A, Di Lauro R, Damante G. Expression of thyroid-specific transcription factors TTF-1 and PAX-8 in human thyroid neoplasms. Cancer Res 1994;54:4744-4749.
73. Sharretts JM, Simonds WF. Clinical and molecular genetics of parathyroid neoplasms. Best Pract Res Clin Endocrinol Metab 2010;24:491-502.
74. Pacini F, Castagna MG, Cipri C, Schlumberger M. Medullary thyroid carcinoma. Clin Oncol (R Coll Radiol) 2010;22:475-485.
75. Sidhu S, Campbell P. Thyroid pathology associated with primary hyperparathyroidism. Aust N Z J Surg 2000;70:285-287.
76. Morita SY, Somervell H, Umbricht CB, Dackiw AP, Zeiger MA. Evaluation for concomitant thyroid nodules and primary hyperparathyroidism in patients undergoing parathyroidectomy or thyroidectomy. Surgery 2008;144:862-866; discussion 866-868.
77. Carling T. Molecular pathology of parathyroid tumors. Trends Endocrinol Metab 2001;12:53-58.
78. Kogai T, Taki K, Brent GA. Enhancement of sodium/iodide symporter expression in thyroid and breast cancer. Endocr Relat Cancer 2006;13:797-826.
79. Kogai T, Brent GA. The sodium iodide symporter (NIS): regulation and approaches to targeting for cancer therapeutics. Pharmacol Ther 2012;135:355-370.
80. Bizhanova A, Kopp P. Minireview: The sodium-iodide symporter NIS and pendrin in iodide homeostasis of the thyroid. Endocrinology 2009;150:1084-1090.
81. Riesco-Eizaguirre G, Rodriguez I, De la Vieja A, Costamagna E, Carrasco N, Nistal M, Santisteban P. The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer. Cancer Res 2009;69:8317-8325.
82. Ward LS, Santarosa PL, Granja F, da Assumpcao LV, Savoldi M, Goldman GH. Low expression of sodium iodide symporter identifies aggressive thyroid tumors. Cancer Lett 2003;200:85-91.
83. Trouttet-Masson S, Selmi-Ruby S, Bernier-Valentin F, Porra V, Berger-Dutrieux N, Decaussin M, Peix JL, Perrin A, Bournaud C, Orgiazzi J, Borson-Chazot F, Franc B, Rousset B. Evidence for transcriptional and posttranscriptional alterations of the sodium/iodide symporter expression in hypofunctioning benign and malignant thyroid tumors. Am J Pathol 2004;165:25-34.
84. Ringel MD, Anderson J, Souza SL, Burch HB, Tambascia M, Shriver CD, Tuttle RM. Expression of the sodium iodide symporter and thyroglobulin genes are reduced in papillary thyroid cancer. Mod Pathol 2001;14:289-296.