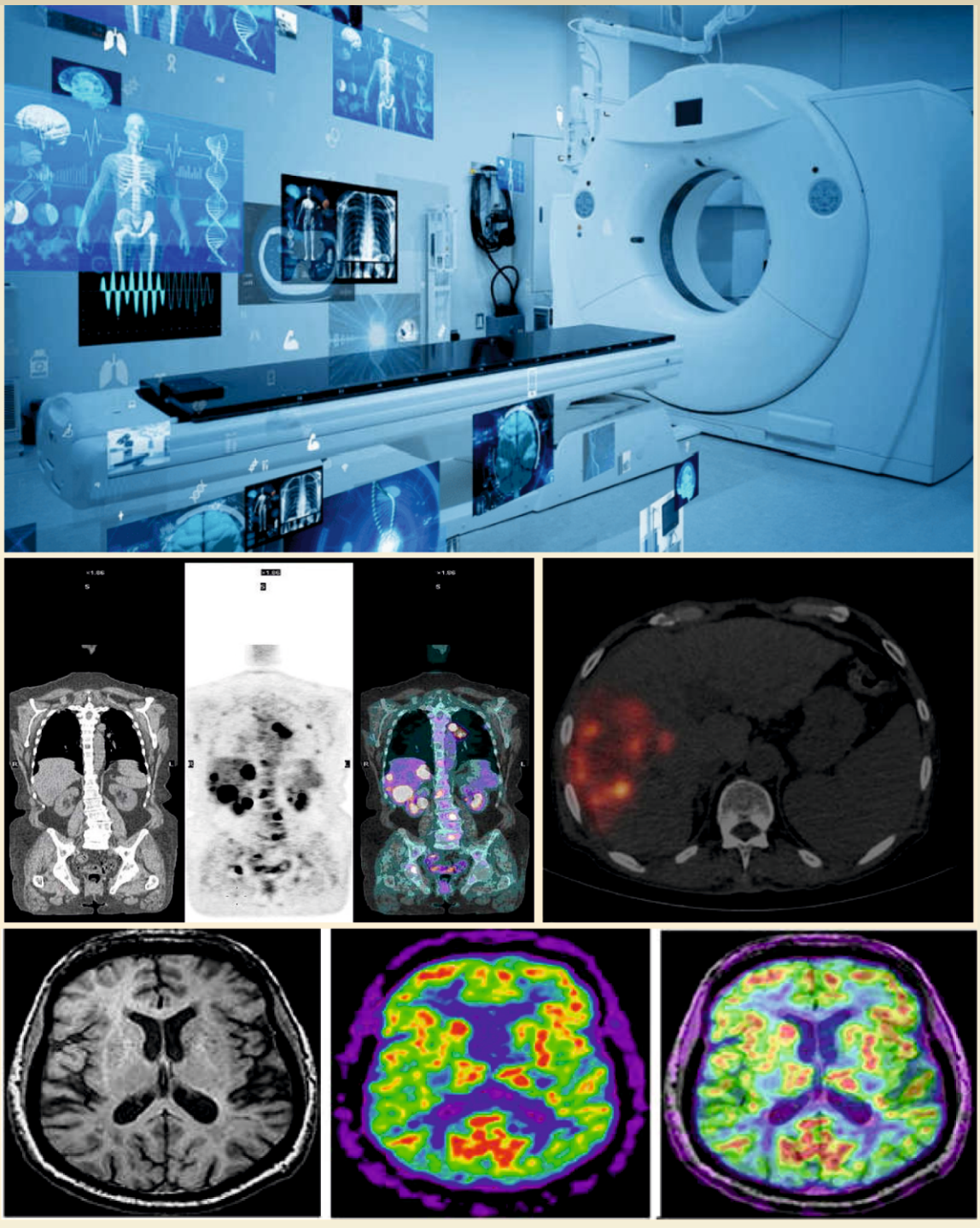
IANCAS Bulletin

DESIGN, DEVELOPMENT AND APPLICATIONS OF RADIOPHARMACEUTICALS - PART II



Vol. XX No. 2 www.iancas.org.in January 2025

IANCAS Bulletin

Design, Development and Applications of Radiopharmaceuticals-Part II

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A Publication of
INDIAN ASSOCIATION OF NUCLEAR CHEMISTS AND ALLIED SCIENTISTS (IANCAS)
(Reg. No. MAH/232/1984/GBBSD)
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President's Message

Radiopharmaceuticals, as the name suggests, are radioactive compounds used in nuclear medicine and play a crucial role in modern healthcare, particularly in the fields of diagnosis and therapy. The diagnostic radiopharmaceuticals are injected or ingested by the patient to give images with good contrasts to provide valuable information about the function and structure of organs and tissues using specialized imaging techniques like PET or SPECT. This allows doctors to detect diseases such as cancer, heart conditions, and neurological disorders at early stages. The therapeutic radiopharmaceuticals are used in targeted therapies, particularly for cancers. By delivering radiation directly to tumor cells, these treatments minimize damage to healthy tissue while effectively treating the disease. With advancements in radiopharmaceuticals, healthcare providers can offer more personalized, precise treatments, improving patient outcomes and reducing side effects of the drugs.

My colleagues from the Radiopharmaceuticals Division, BARC have earlier published an IANCAS Bulletin entitled 'Design, Development and Applications of Radiopharmaceuticals-Part I', which covered the basic aspects of radiopharmaceuticals, radiopharmaceutical design, preparation and quality control. I am happy to know that the second part of the Bulletin is being brought out by IANCAS now. This Bulletin is a sequel to the earlier Bulletin which will now cover other interesting aspects such as image reconstruction (Chapter 1) and introduce the reader to different diagnostic (Chapter 2) and therapeutic radiopharmaceuticals (Chapter 3) which are currently in clinical practice. Wherever applicable, the mechanism of targeting action by the radiopharmaceutical is briefly explained. It is evident that the radiopharmaceuticals require rigorous preclinical evaluation followed by different phases of clinical trials before it can finally be used for regular clinical use. Chapter 4 of the Bulletin thoroughly explains different aspects of preclinical evaluation of any new radiopharmaceutical. It is always interesting to know how a radiopharmaceutical is finally used in a clinical set-up and how nuclear medicine physicians gather patient information from scans which will then be used for clinical management of the disease of the patient. Chapter 5 of the Bulletin enlightens the reader on this subject.

I would like to extend my sincere appreciation to the guest editors for their dedicated efforts in compiling and editing this comprehensive Bulletin. The expertise and commitment of the contributors of this bulletin have been instrumental in bringing together such valuable content that enhances the understanding of radiopharmaceuticals and their applications in healthcare. Finally, I commend their hard work in making this Bulletin one of the important volumes for the scientific community.

Dr. P. K. Mohapatra
President, IANCAS

From Secretary's Desk

Indian Association of Nuclear Chemists and Allied Scientist (IANCAS) was founded in 1981 with an objective of popularizing Nuclear and Radiochemistry, Applications of Radioisotopes, and Nuclear Techniques among the scientific community in India. For this purpose, IANCAS is continuously organizing seminars, workshops and publishing periodic thematic Bulletins focused on fundamentals of Nuclear and Radiochemistry and applications of radioisotopes in education, research, agriculture, medicine and industry. With active participations of the life-members, IANCAS has become one of the popular associations for popularizing the subject of Nuclear and Radiochemistry across the country.

IANCAS through its various outreach programs motivate the young researchers and scientists to apply Nuclear and Radiochemistry based methods in their respective research field. In addition, IANCAS life-members through IANCAS activities motivate students to pursue a career in the field of Nuclear Science. For the promotion of Nuclear Science among the researchers, IANCAS has instituted three awards; (i) Dr. M. V. Ramaniah Memorial Award (ii) Dr. Tarun Dutta Memorial Award, and (iii) Prof. H. J. Arnikar best thesis Award. All these three Awards are conferred annually. Dr. M. V. Ramaniah Memorial Award is conferred to an outstanding scientist for the significant contributions in the field of Nuclear and Radiochemistry during his/her lifetime. Dr. Tarun Dutta Memorial Award is given to a scientist (below 45 years age) having significant contributions in the field of Nuclear and Radiochemistry including the applications of radioisotopes. Prof. H. J. Arnikar best thesis Award is given for the PhD thesis focused on Nuclear and Radiochemistry and application of radioisotopes etc.

IANCAS publishes thematic Bulletins on the topics directly related to the Nuclear Science and Technology with the financial support from BRNS, DAE. These Bulletins are distributed free to all IANCAS life-members, and are made freely available at IANCAS website (www.iancas.org.in) for download. The association's popular book on "Fundamentals of Nuclear and Radiochemistry" is widely sought amongst the academia, researchers and students from DAE and Universities. In the series of IANCAS Bulletins, the present Bulletin titled "**Design, Development and Applications of Radiopharmaceuticals-Part II"** aims at giving practical applications of radiopharmaceuticals in diagnosis and therapy.

Information about the workshops, Awards and various activities of IANCAS are available on the website (www.iancas.org.in). All the publications of IANCAS including Bulletins and books are also available at the website.

Dr. Sandeep Kumar Sharma
Secretary, IANCAS

From Editor's Desk

The most significant societal applications of radioisotopes and radiation are presently practiced in healthcare, particularly in the field of Nuclear Medicine. This involves both diagnostic and therapeutic uses, with some treatments combining both, a practice known as theranostics. Nuclear Medicine offers painless internal or external treatments for various diseases, either through radiation therapy alone or in combination with conventional treatments. The success of Nuclear Medicine depends entirely on the availability of Radiopharmaceuticals, which are created by combining radioisotopes, sourced from nuclear reactors or particle accelerators (cyclotrons), with appropriate carrier ligands. The Present Thematic Bulletin on "**Design, Development and Applications of Radiopharmaceuticals - Part II"** aims at giving practical applications of diagnosis and therapy in details in five chapters by experts as given below :**Chapter 1**: Basic Principles of SPECT and PET: How Images are Recreated; **Chapter 2**: Diagnosis Using Radiopharmaceuticals; **Chapter 3**: Therapeutic Radiopharmaceuticals: Translation from Laboratory to Nuclear Medicine Clinics over the Last Two Decades in India; **Chapter 4**: Pre-clinical Evaluation of Radiopharmaceuticals; and **Chapter 5**: Clinical Applications of Nuclear Medicine in Cancer and Non-oncological Disorders: A Case Based Approach.

On behalf of IANCAS, I thank all the authors and contributors of the chapters for sharing their research and applied works. Without their valuable contributions, this Bulletin would not have been possible. I sincerely thank both the Guest Editors, Dr. Tapas Das, Head, Radiopharmaceuticals Division (RPhD), BARC and Dr. Madhava B.Mallia, SO/H, RPhD, BARC for sparing their valuable time and helping to shape this Bulletin (second in the series) into its current form. I also thank EC of IANCAS for the continued support and interest in bringing out more thematic bulletins like the present one.

On behalf of IANCAS, sincere thanks and acknowledgement are extended to **BRNS, DAE** for financial support towards publications of such thematic bulletins, aligned with the mandate of DAE for societal applications of **Radioisotopes and Radiation.** This bulletin, like earlier ones, will also be available online in the IANCAS website: www.iancas.org.in

With Regards

Raghunath Acharya
Editor, IANCAS

Preface

This IANCAS bulletin is in continuation to the previous bulletin titled 'Design, Development and Applications of Radiopharmaceuticals - Part I' published earlier (Vol: XIX, No.: 2, Sep 2023). Part-I of the bulletin highlighted the peaceful applications of radioisotopes and radiation, introducing readers to the concept of nuclear medicine and its significance in modern healthcare. The following chapters provided a brief overview of freeze-dried radiopharmaceutical kits, which have simplified the preparation of diagnostic radiopharmaceuticals in busy hospital radiopharmacies. The section also covered PET radiopharmaceuticals and various quality control techniques used to ensure the safety and effectiveness of radiopharmaceuticals administered to patients for diagnosis or therapy.

Part-II of the bulletin begins with a clear explanation of the nuclear imaging process and how the images are reconstructed by the gamma camera. This process allows nuclear medicine physicians to assess the functional status of various organs and detect the presence or absence of diseases. The following chapters provide a brief overview of the diagnostic and therapeutic radiopharmaceuticals currently in use in clinical nuclear medicine. It is important to note that newly developed radiopharmaceuticals must undergo several phases of rigorous pre-clinical and clinical testing before they can be introduced into routine clinical practice. One chapter is dedicated to explaining the various types of preclinical studies a radiopharmaceutical must undergo before becoming suitable for clinical use. The final chapter of the bulletin offers a glimpse into how radiopharmaceuticals are applied in clinical practice and how the information obtained from nuclear medicine imaging aids in the clinical management of various disorders in the human body.

As with the previous bulletin, the editors and authors of this edition have made every effort to present the content in a clear and simple manner, guiding readers through the fascinating journey of radiopharmaceuticals from the research laboratories to the bedside without causing boredom. We extend our gratitude to all the authors for sharing their knowledge and expertise, as well as for dedicating their valuable time to compile the articles in this bulletin. While we have made every effort to ensure the articles are free of errors, there may be some inadvertent oversights and we do apologize for the same. We hope readers from diverse backgrounds find the articles in this bulletin informative, and that Parts 1 and 2 of the IANCAS bulletins on radiopharmaceuticals together offer a comprehensive overview of their applications. Enjoy your reading!

Dr. Madhava B Mallia
Dr. Tapas Das

Guest Editors

Dr. Madhava B Mallia is presently the head of Radiopharmaceuticals Chemistry Section in Radiopharmaceuticals Division of Bhabha Atomic Research Centre, Mumbai. He completed his MSc in Applied chemistry from Cochin University of Science and Technology. After a brief stint in National Chemical Laboratory, Pune, he joined the 45th Batch BARC Training School in 2001. He was awarded with Homi Bhabha gold medal for securing 1st rank in BARC Training School in Chemistry discipline. In 2002, after successful completion of the training program, he joined Radiopharmaceuticals Division, BARC. His main area of research is the development of Technetium-99m-tracers, with small biomolecules, antibodies and peptides as targeting vectors, for diagnosis, with a possible extension of the idea to corresponding rhenium-186/188 analogues for therapy. Development of freeze-dried kits for the preparation of technitium-99m-Hynic TOC for diagnosis of neuroendocrine tumors, rhenium-188-HEDP for bone pain palliation, rhenium-188-lipiodol for liver cancer therapy, etc. are some of his significant contributions to radiopharmaceuticals program in India. He is a 'Professor' of Chemical Sciences and recognized Ph.D. Guide of Homi Bhabha National Institute (HBNI), Mumbai. He has over 50 research articles in various peer reviewed journals and he is also the recipient of the DAE-Scientific and Technical Excellence Award (2015), DAE-Group achievement awards (2015, 2017) and Dr. Tarun Datta Memorial Award (2019). He served as subject expert for Regional Training Program of IAEA in Thailand (2018) and participated as consultant for IAEA Technical Meeting on New Generation of Technetium-99m kits (2021, 2022).

Dr. Tapas Das is presently working as Head, Radiopharmaceuticals Division of Bhabha Atomic Research Centre (BARC), Mumbai. He had joined DAE in 1998 after completing his post-graduation in Chemistry and one-year OCES Program from 41st batch of BARC Training School. He had obtained Ph.D. from University of Mumbai in 2004. His research field of interest includes production of radioisotopes and development of radiopharmaceuticals for diagnostic and therapeutic applications. Dr. Das was instrumental in developing, demonstrating and deploying several state-of-the-art radiopharmaceuticals and freeze-dried kits, which are now regularly used in various hospitals of our country. He has received several awards such as, Prof. H.J. Arnikar Best Thesis Award (2005), Tarun Datta Memorial Young Scientist Award (2011), DAE Young Scientist Award (2008), DAE Scientific & Technical Excellence Award (2015), and multiple DAE Group Achievement Awards (2009, 2017, 2018). He has served as Technical Co-operation Expert of International Atomic Energy Agency (IAEA, Vienna, Austria) to Republic of Korea, Kazakhstan and Indonesia. He has also served as Consultant of IAEA. He is a 'Professor' of Chemical Sciences and recognized Ph.D. Guide of Homi Bhabha National Institute (HBNI), Mumbai. Dr. Das is a fellow of Maharashtra Academy of Sciences (2023) and one of the Editors of the journal 'Applied Radiation and Isotopes' (published by Elsevier). Dr. Das has published 110 research articles in various peer-reviewed international journals.

Chapter 1
Basic principles of SPECT and PET: How images are recreated

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***Abstract**: Nuclear imaging techniques provide a non-invasive route for understanding physiological changes and monitoring the biological processes happening inside the body. The method involves administration of a gamma emitting radiopharmaceutical into the body through intravenous route. Subsequently, the time-dependent or time-independent distribution of the radiopharmaceutical is monitored using machines called 'Gamma Camera', which provide a pictorial representation of the distribution of radioactivity in the body. These pictorial representations called 'Scans' forms the basis for nuclear medicine physicians to extract clinical information for further management of the patient. This chapter attempts to explain in layman terms, how the gamma camera recreates the final clinical image.*

***Keywords**: Nuclear medicine, SPECT, PET, radiopharmaceuticals, imaging, resolution, gamma camera*

Introduction

'A picture is worth a thousand words'. This adage summarily describes the power of an image to convey information. Sight is one of the important sensory faculties of human beings, and animals, to gather information about their surroundings. Therefore a brief discussion on this topic would provide a suitable platform to understand nuclear imaging and subsequently, nuclear imaging techniques.

Let us assume a leaf floating in a slow moving river (Figure 1). What information can we gather using this leaf? Our knowledge on basic physics would immediately tell us that (a) we can understand the direction of movement of the river by seeing the direction of movement of the leaf and (b) calculate the approximate 'speed' of the flowing water by determining distance covered by the leaf in certain time. Though the above discussion looks obvious, it is important to understand the role of certain other factors which permit this observation possible. *Light* and our *eyes* are those two important factors without which acquisition of above information is not possible (Figure 2).

Technically, the light from the sun (it may be light from other sources also) reflected by the leaf reach our eyes which makes this observation possible. The leaf in this example 'traces' the movement of the river and hence, we can call it a '**Tracer**'. It is the reflected light from the tracer which permits our eyes to see the leaf. Thus the reflected light from the leaf may be called as the '**Signal**'. Eyes are our '**Detectors**' which record the light signals from the leaf (**tracer**) and our brain (**signal processor**) interprets it as a leaf. It is obvious from the above discussion that, a **Tracer** emitting **signals** (signals can be light or any other electromagnetic radiation) and a **detector** which can efficiently detect those signals are three important as well as essential factors to create an image. Though several other examples could be quoted, the above example of leaf in a slow moving river will suffice to understand how nuclear imaging works.

Figure 1. A leaf floating in river can be used a tracer to determine the direction and speed of flow

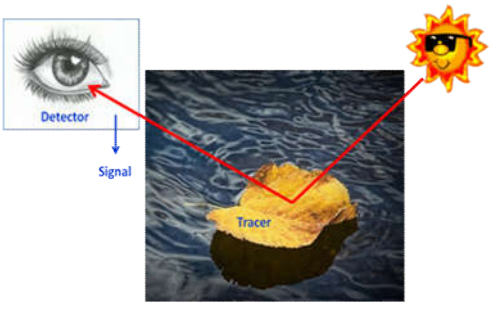


Figure 2. The sunlight, leaf and eyes together helps to determine the direction and speed of flow of the river

Nuclear imaging

The most significant attribute of nuclear imaging is its ability to provide functional information of various organs in human body, which no other technique can provide as on today. Though functional MRI (fMRI) can provide similar information, it cannot match the sensitivity of nuclear techniques. While MRI, CT, X-ray etc. provide only diagnostic information, nuclear medicine provides options both for diagnosis as well as therapy of various diseases including cancer [1-4]. In essence, nuclear medicine is probably the finest example of peaceful uses of radiation.

Nuclear imaging refers to the construction of images using nuclear radiations, generally gamma radiations, to understand the biological processes in body, functional information of various organs, presence or absence of certain disease such as cancer, so on and so forth to the detection and staging of Alzheimer's and Parkinson's disease. Nuclear imaging can even assist surgeons to pin-point the location of the diseased tissue to be removed. Though a thorough discussion on nuclear imaging is beyond the scope of this monograph, it is an irrefutable fact that nuclear imaging is playing a central role in modern day diagnostic medicine.

Radioisotopes are the chief source of nuclear radiation. Therefore, it is natural that nuclear imaging involves the use of the radioisotopes. However, a natural question would be 'why nuclear radiations? Why not light?' The answer lies in what we want to image first of all. As described in the above paragraph, nuclear imaging tries to uncover what is happening inside the body. To achieve this objective, it is necessary to use a 'signal' that can penetrate human tissue and reach the detector kept outside the body. Ordinary light, therefore, cannot be a choice for this purpose. For a similar reason, the inability to penetrate human tissue and reach the detector, other nuclear radiations such β^- or α are also not used for diagnostic imaging.

Basic procedure in nuclear imaging involves introducing a suitable radiotracer safe for administration into human body, which can monitor certain functions or detect the presence of diseases. Additionally, the radiotracer should have certain characteristics to be a good diagnostic agent. The radiotracer can be administered into the body either by oral route or intravenous injection.

Gamma camera

A blind man cannot savour the beauty of nature. It is our eyes that make all the difference. Our eye produces images with highest resolution among all the man made cameras available as on date. Unfortunately, our eyes are designed only for visible light. Therefore, to detect other types of radiation such as gamma radiation, specialized devices are required. Gamma camera or 'Anger camera' are medical devices which can 'scan' human or animal body, injected with a gamma-emitting radiotracer. It is designed to detect, record and process the gamma radiations emanating from the body into an image showing the distribution of radiotracer in the body at that point of time. Figure 3 shows Hal O. Anger with his 'Anger camera'. For comparison, a modern day gamma camera is also shown in Figure 3.

Though a detailed discussion on gamma camera technology is beyond the scope of this Chapter, essential components of a gamma camera are mentioned below.

- Radiation detector (gamma camera head, Figure 4)
    - Scintillation crystal
    - Photomultiplier tubes

Figure 3. Hal O. Anger with his invention: Gamma camera (left) and modern day gamma camera (right)

- Collimator
- Electronics

A scintillation crystal and a set of photomultiplier tubes together form the radiation detector or gamma camera head. This is probably the most important part of gamma camera. Inside view of gamma camera head is schematically shown in Figure 5. Scintillators are materials which emit visible light when gamma radiations interact with them. These scintillations, which are proportional to the energy of gamma radiation, are detected by photomultiplier tubes arranged behind the scintillation crystal (see Figure 5) and form the primary signal for image construction.

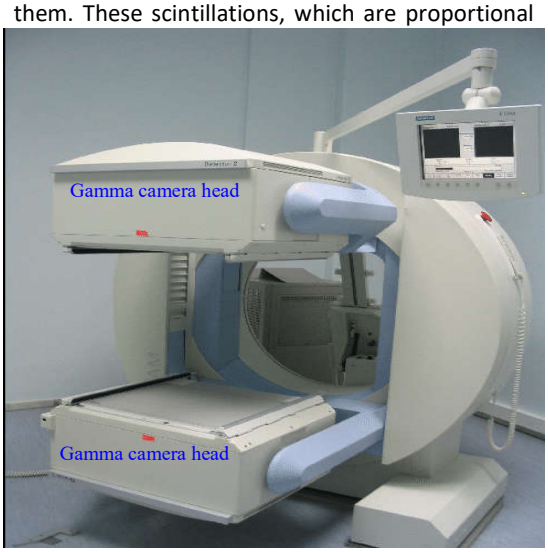


Figure 4. Gamma camera head

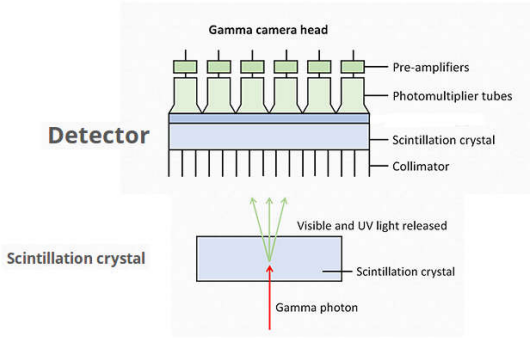


Figure 5. Gamma camera head

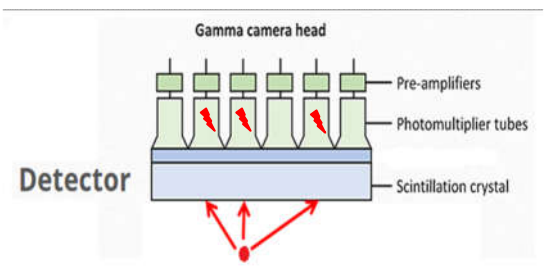


Figure 6. Consequence of not having a collimator

Nal(Tl) crystal (20 inch x 20 inch typically) is used as scintillator in gamma imaging. In modern instrument, however, the detector designs may vary. The working principles and details of photomultiplier tube and other associated electronics are beyond the scope of this monograph. Another important component of the detector is the collimator. The radiations emitted by the radioisotope are isotropic. Therefore, it is possible that the gamma radiation released from the patient's body can exit at any angle and hit the detector in a location that does not necessarily correlate with the point of its origin. This scenario is depicted in Figure 6. Figure 6 shows three gamma radiations (it can be more than three!) emitted from the radioactive sample (depicted as red dot) interacting at three different locations in the scintillation crystal. Consequently, signal will be produced in three photomultiplier tubes. Each PMT corresponds to a coordinate on the scintillation crystal. This is then mapped out onto a matrix for image construction. Each time a gamma photon is detected, it is mapped on to its corresponding coordinate within the image. It is obvious that in the absence of a collimator there will be difficulty in deciding the location of actual source of radiation. This will drastically affect the quality of final image and reliability of the information that can be obtained from the image.

To overcome this, a collimator is used in which only gamma photons that travel perpendicular to the collimator will be accepted. Those travelling at an angle will hit the collimator septum (usually lead), be absorbed and, therefore, will not contribute to the image. This scenario is depicted in figure 7. Gamma camera comes with different designs. While earlier gamma cameras had a single head, modern gamma cameras have two or three heads. Additional camera heads has the benefit

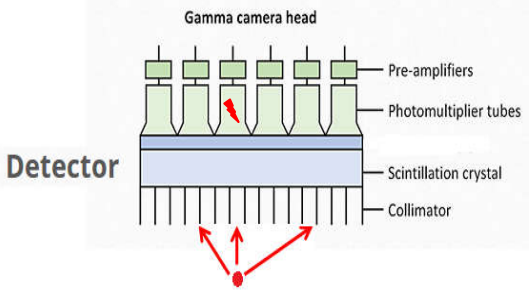


Figure 7. Role of collimator

that it significantly reduces the image acquisition time.

Scanning procedure and whole-body scan

Scanning or image acquisition is the process of recording the distribution of radiotracer in the body. The patient administered with the radiotracer is generally asked to lie on a couch and the gamma camera head is positioned over (or under) the area of the body to be scanned (Figure 8).

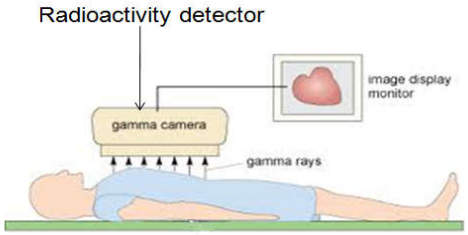


Figure 8. Gamma scanning

Sometimes, the gamma camera head, slowly and steadily, moves over the patient to acquire the 'whole-body image'. Once the acquisition is completed, the computer processes the acquired signal and presents it as a 2-D (two dimension) image (called 'scan') showing the distribution of radiotracer in the body. Typical whole-body scan of a patient injected with ^{188}Re-HEDP (HEDP: Hydroxyethyl diphosphonate) is shown in Figure 9. ^{188}Re-HEDP accumulates in bone cancer lesions.

Besides emission of beta particles, ^{188}Re also emits gamma radiations which make gamma imaging possible. The dark spots on the image are indicative of accumulation of radiotracer in bone

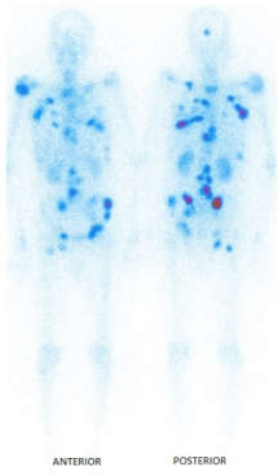


Figure 9. Typical whole-body scan of a patient injected with ^{188}Re-HEDP

cancer lesions of the patient. It is clear from this figure that nuclear imaging technique provides a non-invasive way of obtaining information on the location as well as the extent of the disease. To complete the discussion, anterior and posterior image mentioned in Figure 9 refers to the images obtained by scanning the patient from front or back, respectively.

Tomography

The word tomography is coined from two words 'tomos' meaning slice and 'graphe' meaning to write. Tomography is representation of a three dimensional object as a collection of slices. For example, a loaf of bread may be considered to be made up of a collection of individual slices (Figure 10). While the whole bread does not permit inspection of the inner portion, slicing permits close analysis of the interior that makes up the whole bread. Tomography has significant role in clinical imaging through SPECT (Single Photon Emission Computed Tomography), PET (Positron Emission Tomography), CT (Computed Tomography) etc. Some of these techniques will be discussed in details in the following sections [5].

Figure 10. Tomography is the representation of a three dimensional object as a collection of slices, like that of a loaf of bread

SPECT

As the name suggests, SPECT employs a gamma photon emitting radiotracer. It would be more prudent to start the discussion on SPECT by addressing the question why we need it in the first place. The whole-body bone scans in Figure 9 show the distribution of the bone cancer lesions in the patient's body. Though the image provides useful clinical information, it does not provide any information on how deep the lesions are located in the body (a 2D image lacks depth). While such information may only have limited clinical value as far as a bone scan is concerned, there are other clinical scenario where information on depth of the lesion is important for clinical management of the disease. Figure 11 shows the whole-body image of a liver cancer patient. The cancer lesions in the liver can be seen as dark spots. A surgeon attempting surgical removal of the cancer lesion

would be interested to know exact location of the lesion before the patient is brought to surgical table since that will make his task easy and efficient. The two-dimensional whole-body scan lacks this information. Tomographic technique provides a solution to this problem by recreating a three dimensional image of the lesion from a set of two dimensional images of the lesion acquired from different angles.

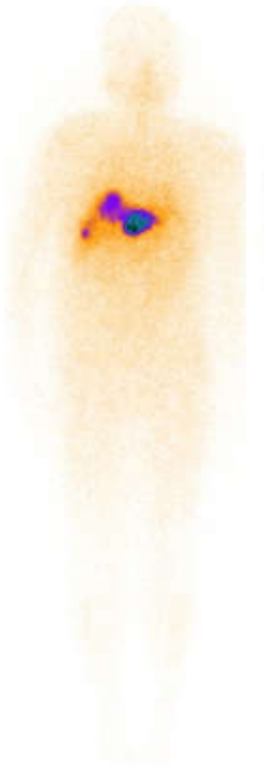


Figure 11. Whole-body image of a liver cancer patient

Figure 12 shows the camera positions for planar imaging and SPECT. During planar imaging the camera records the image only in one perspective; while in SPECT, the camera rotates around the patient, acquiring 2D planar images at different angles, which then forms the input image set for reconstructing the image in 3D (tomograph). Though a thorough discussion on the mathematical equation involved in the reconstruction of the 3D image from a set of 2D images is beyond the scope of this chapter; a brief and simplied description of the method may be useful to the readers to understand the process of image reconstruction.

The method is described using a set of four spheres as an object for imaging (Figure 13). As

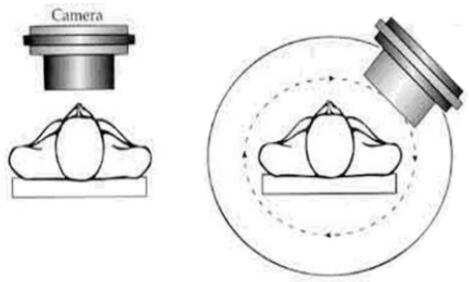


Figure 12. Schematic diagram indicating planar imaging and SPECT

shown in Figure 13, the camera records images of the objects from different angles. In this example, camera acquires 8 images from 8 angles. The image that the camera sees from a particular angle is also shown in Figure 13. It is important to note that though our object (set of 4 spheres) is 3D, the image acquired by the camera is 2D!! This is easy to understand because a sphere when viewed from any one side appears like a circle. Now to reconstruct the 3D image, a computer sums up the individual 2D images following certain mathematical procedures. 'Back projection' is one of the different methods used for image reconstruction. Figure 14 may be helpful to the reader to imagine the concept of back projection. Figure 14(a) shows the 2D images of the object acquired by the camera from different angles (refer figure 13) and Figure 14(b) shows the projection of individual 2D images towards the centre. It could be noticed that intensity of projections in certain area at the centre is enhanced upon back projection of the 2D images obtained earlier. These are exactly the locations of the original 3D object (see Figure 13)!! This is nothing but reconstruction of the 3D image from a set of 2D images.

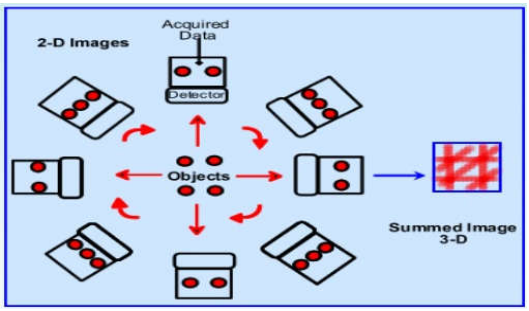
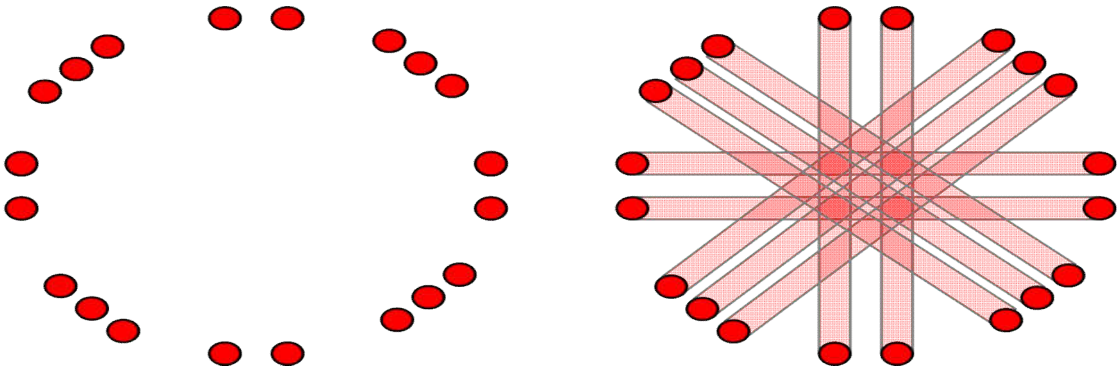


Figure 13. Schematic diagram showing the image of objects as seen by the detector from different angles. The 3D image is reconstructed from the 2D images from different angles



(a) (b)

Figure 14. Schematic diagram showing the reconstruction of the 3D image from the 2D images of the objects from different angles

It should be noted that the actual procedure of back projection involves steps to filter out unwanted signals which will result in sharper image of the original object. It also goes without saying that more the number of 2D images acquired from different angles, better will be the quality of the reconstructed image.

PET

An image may be viewed as a collection of closely spaced points in space. For example, a line is formed by a number of closely spaced points in one dimension (Figure 15).

Figure 15. A line can be imagined as a collection of closely spaced points

It essentially means that if we know the coordinates of each points of an image in space, the image can be reconstructed. The issue in SPECT is that the gamma photon getting registered in the detector does not carry information regarding its point of origin. For example, in Figure 16 the gamma photon registered at the detector (shown as red dot) could have originated from any point along the line (shown within body). Due to this uncertainty, SPECT employs mathematical techniques such as back projection for reconstructing the image.

However, in PET, the inherent nature of positron itself provides a means to calculate the point of origin of the signal being detected. In PET, as the name suggests, a positron emitting radiotracer is administered to the patient. Positron is a positive electron (β^+) emitted from the decaying nucleus. Being antimatter, it does not have existence in real world. Positron emitted from the nucleus with certain initial energy, continuously loses its energy to the surroundings through collisions, Columbic

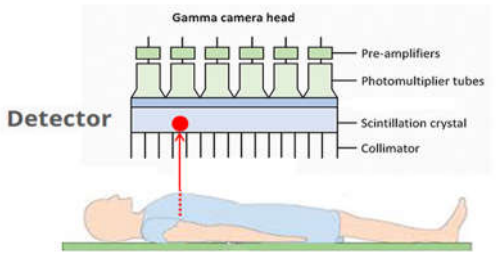


Figure 16. Schemetic diagram indicating the emission of a gamma photon from the body and its detection in the detector

interactions or other modes of energy loss associated with the charge particles. Once the positron attains near zero energy, it combines with an electron from its surroundings through a process called 'annihilation'. During this process, both the electron and positron cease to exist and an energy equivalent to their rest masses (1022 keV) appears as two gamma photos of 511 keV each. From the point of annihilation, these two photons fly away in opposite directions at close to 180° angle to each other. It is these two 511 keV photons that are central to PET imaging.

Unlike in a SPECT camera with one, two or three gamma detectors, a PET camera uses an array of detectors arranged in the form of a ring (Figure 17) with the detectors placed diametrically opposite to each other and connected in a 'coincidence mode'. Coincidence mode of detection of gamma radiation and its significance will be clear from

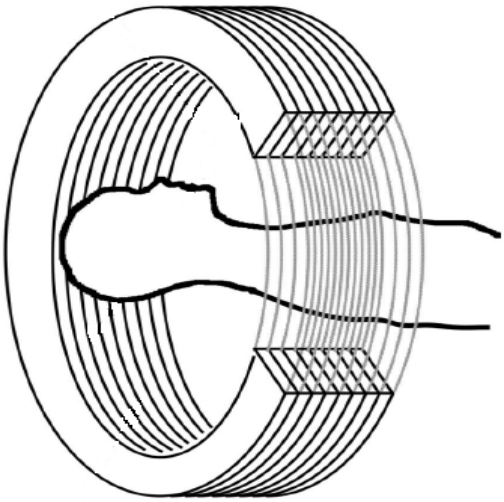


Figure 17. Schematic diagram showing positioning of a patient in PET machine

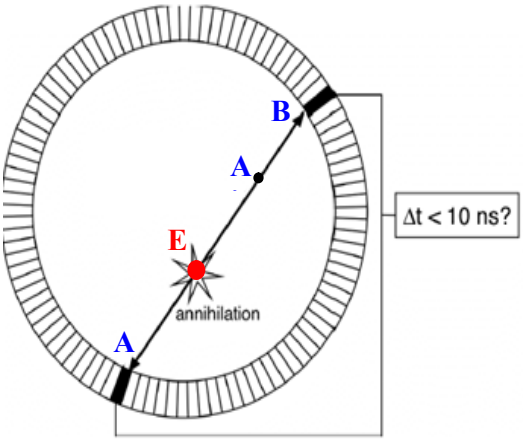


Figure 18. Schematic diagram showing annihilation event and the coincident detection of the two photons in detectors placed 180 degree configuration

discussion that follows. The patient administered with a positron emitting radiotracer is asked to lie on a couch (similar to the procedure followed for SPECT) which then slowly moves through the detector for acquiring the image. Here also, a scintillator is used for detecting the gamma radiations coming out of the patient's body. The only difference is that here a more dense scintillator, BGO (Bismuth germanium oxide), is employed instead of NaI(Tl) used in SPECT. A denser scintillator is needed for PET because of the higher energy (511 keV) of gamma photons to be detected.

As mentioned earlier, the annihilation event will lead to formation of two gamma photons moving away from each other at nearly 180°. This information can be used to calculate approximate location of annihilation event. However, in order to do so, one must make sure that the two gamma photons detected are from the same annihilation event and not from different events. This is achieved through coincidence measurements. One must remember that the two gamma photons start from the point of annihilation at the same time. Therefore, it is assumed that if two photons are detected in opposite detectors within a time window of say 10 ns (nano seconds), they are from the same annihilation event. Figure 18 shows an annihilation event (**E**) and coincidence detection of the resultant photons at nearly opposite detectors (Point **A** and **B**). The annihilation event has taken place along with line **AEB**. It is also evident that the detector **A** will register the arrival of gamma photon first since it is closer to the point of annihilation **E**. Now, if 'Δt' is the time difference between the registration of gamma photons in detector **A** and detector **B**, then $\Delta t \times c$ (c is the velocity of light) is the extra distance travelled by the second gamma photon to reach the detector **B** (distance **A'B**). Since the distance between the two detectors (**AB**) and extra distance travelled by one of the gamma photos from annihilation event (**A'B**) is known, point of annihilation can be calculated. This is how *time of flight* PET reconstructs images. Compared to SPECT, PET images are of high resolution because of the precise determination of the origin of gamma photons. A quick reference comparing SPECT and PET technology is provided in Table 1.

Fusion/Hybrid imaging: PET/SPECT-CT & PET/SPECT-MRI

Fusion or Hybrid imaging refers to a process of exact superimposition of physiological information from a nuclear medicine study with the anatomic information from CT or MRI (Magnetic resonance imaging). Though PET as well as SPECT provides 3-D images of radiotracer distribution in patients, uncertainty in determining the exact location of the lesions is a problem in taking the surgical decisions based on the these scans.

CT uses x-rays to acquire anatomical images. CT achieves excellent anatomical resolution, tissue differentiation and high imaging speed, but offers little functional information, largely depending on size and morphology to differentiate tumour from normal structures. Combining PET/SPECT and CT has the potential to improve lesion localization, increase specificity, minimize interpretative pitfalls and allow fast, low-noise attenuation correction. A typical CT image, PET image and resulting fusion/hybrid image is shown in Figure 19.

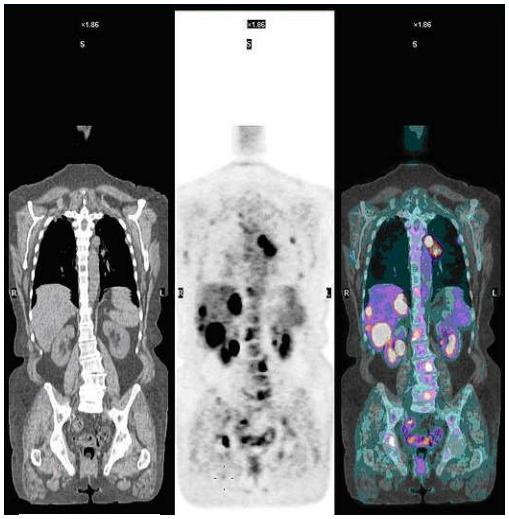


Figure 19.. A typical CT image, PET image and the corresponding fusion/hybrid image

PET/SPECT-MRI is similar in concept to the PET/SPECT-CT. The main difference is the source of electromagnetic radiation which is X-rays in CT and radio waves in case of MRI. Anatomical information provided by MRI is superior to CT due to greater inherent contrast resulting from differences in proton density and magnetic relaxation properties of tissue with respect to differences in tissue density. MRI offers range of relevant, quantitative information on tumor body related to blood flow, vascular and tissue spaces, hypoxia, cellularity and metabolic concentrations without exposing to radiation (such as x-rays in the case of CT). A typical MRI/PET scan before and after fusion is shown in Figure 20.

There are several advantages of fusion/hybrid imaging. Few advantages are quoted below.

- Accurate identification of the margins of a tumor/metastasis
- Better identification of small recurrent tumors obscured by scar tissue at site of incipient radiation or post-operative necrosis.
- Detecting large tumors that lay in clinically inaccessible areas, such as the hypopharynx or maxilla.
- Locating the primary lesion in unknown primary tumors.
- Better staging of tumors.
- Offers better guidance to clinical management including planning (radiation therapy planning), guidance of biopsy, surgery or radiation therapy.
- Provides structural and functional information in the same image.
- Improving confidence in diagnosis when one modality alone is not definitive.

Conclusions

In a direct comparison with respect to the image resolution, PET still scores over SPECT. However, significant technological advances in hardware such as signal amplifiers, detectors, camera head and collimator designs and image reconstruction algoriths have significantly reduced the resolution gap between the two techniques. Replacement of bulky photomultiplier tubes (PMTs) with position-sensitive PMTs (PSPMTs), avalanche photodiodes (APDs), and silicon PMTs, have helped to achieve higher detection efficiency along with improved energy and spatial resolutions. These advances have significantly reduced the image acquition time minimizing the patient discomfort. With the arriaval of new SPECT machines with image resolution approaching that of PET, the technetium-99m radiopharmaceuticals may play a more significant role in diagnostic nuclear medicine, competing with the PET radiopharmaceuticals.

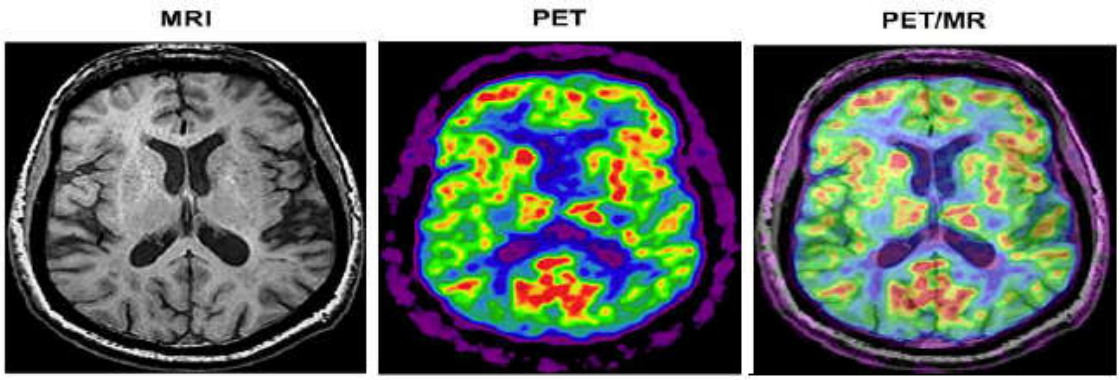


Figure 20. A typical MRI/PET scan before and after fusion

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Chapter-2

Diagnosis using radiopharmaceuticals

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Abstract: *Radiopharmaceuticals are radioactive agents used for either diagnosis or therapy of a diseased condition. The radiopharmaceuticals used for diagnosis usually emit either gamma rays or positrons and their in vivo distribution is mapped using SPECT or PET modalities, respectively. Nuclear medicines are capable of targeting the physiological function and metabolic activity of diseased organs which occur before any anatomical changes. This provides significant information leading to early detection of the diseased condition and its better staging which can determine treatment options and prognosis. Diagnosis using radiopharmaceuticals has gained significant importance in the recent past with the advancement of SPECT and PET techniques. This chapter summarizes the radiotracers for SPECT and PET imaging.*

Keywords: *Radiopharmaceuticals, myocardial imaging, brain imaging, renal imaging, lungs imaging, skeletal imaging, infection imaging, tumor imaging, hypoxia imaging.*

Introduction

Diagnostic Radiopharmaceuticals are radiochemical formulations or radiolabeled drugs with a constant composition which can be administered either orally or intravenously with adequate safety, for the purpose of carrying out diagnosis of a diseased condition of an organ or cancers. In diagnostic radiopharmaceuticals, radioisotope used is either a gamma emitter or a positron emitter. In the former case, the radiopharmaceutical is used for SPECT (Single Photon Emission Computed Tomography) imaging, while in the latter the diagnostic radiopharmaceutical is used for PET (Positron Emission Tomography) imaging. This chapter gives a brief description about several SPECT and PET radiotracers currently used or under research for diagnosis of diseased state of body.

Myocardial imaging

Myocardial perfusion imaging

Myocardial Perfusion Imaging (MPI) is a non-invasive imaging method that shows how well blood flows (perfuses) through the myocardium. It can show areas of the heart muscle that aren't getting enough blood flow. MPI is useful in patients for non-invasive and early detection of narrowed or blocked heart arteries (angina). Usually, MPI is done in both stress (by exercise or by injection of vasodilators such as dipyridamole, adenosine etc.) and resting conditions [1]. During the stress test, the accumulation of the tracer in normal cells is higher as compared to that in ischemic cells (due to low blood flow), thus resulting in a defect in stress image. Under rest condition, there is high uptake in the normal cells, which due to high perfusion washes out from the cells after some time. At the same time, the low uptake in ischemic cells washes out slowly due to less perfusion of blood. As a result, equilibrium is reached after some time post-injection, wherein the uptake in normal cells and ischemic cells become almost equivalent. Thus, an image showing a defect in stress conditions which reduces in rest conditions signifies ischemic but viable tissue. In case of an infarct cell (dead cell), the uptake of tracer would be negligible in both stress and rest conditions. Thus, a defect in both resting and stress conditions signify infarct tissues.

SPECT agents

(a) Thallium-201 (^{201}TlCl)

Although thallium belongs to group IIIA, the thallous ion behaves like K^+, because both are monovalent and have similar ionic radii. Tl^+ is transported through the cell membrane via active transport by the Na^+-K^+ adenosine triphosphate (ATPase) enzyme. It has a high first pass myocardial extraction (85%). Thallium-201 is cleared rapidly from the blood via the kidneys.

For imaging studies, the tracer is injected intravenously and 5-10 min post-injection the stress test is done. The stress test is completed within 30 min. Further, the patient is asked to rest for 3-4 hrs and then the rest images are taken. Since ^{201}Th mimics K^+, its gets redistributed in the myocardium and thus a single injection is sufficient for both the stress and rest test. This unique property of ^{201}Tl makes it one of the most used procedures in nuclear cardiology.

^{201}Tl imaging has several limitations, such as:

i. The physical characteristics of the isotope are suboptimal. Thallium emits mainly mercury X-

rays at 69 to 83 keV, which is not ideal for SPECT imaging.

ii. The low energy also causes problems because of attenuation within the body.

iii. The relatively long physical half-life (73 hours) and long biological half-life (10 days), provides unnecessary radiation dose to the kidneys. This limits the amount of ^{201}Tl [74-111 MBq (2-3 mCi)] being administered in patients.

iv. ^{201}Tl is obtained from cyclotron making it an expensive radioisotope for imaging.

(b) ^{99m}Tc-Sestamibi (Cardiolite®)

^{99m}Tc is obtained from ^{99}Mo/^{99m}Tc generator which makes it a cheaper radionuclide compared to cyclotron produced ^{201}Tl. ^{99m}Tc decays by emission of 140 keV gamma rays which is ideal for imaging with SPECT cameras. These 140 keV gamma emissions also help in overcoming the problems of soft tissue attenuation. Further in contrast with the kinetics of thallium, ^{99m}Tc has a shorter half-life (6 hours). Therefore, larger doses of ^{99m}Tc-labeled tracer can be administered in patients for imaging.

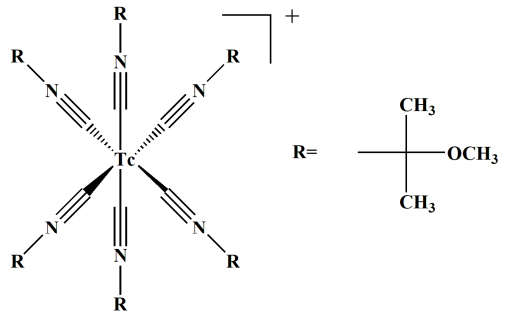
Hexakis-2-methoxy-2-isobutyl isonitrile technetium(I) (sestamibi) (Fig 1a) is a lipophilic cationic complex. The initial myocardial accumulation of sestamibi, like thallium, is proportional to regional myocardial blood flow, with a first-pass extraction fraction (65%) lower than that of thallium. However, a higher dose of sestamibi [740 MBq (20 mCi)] is administered as compared to ^{201}Tl which compensates for lower extraction [2].

Sestamibi is taken up by the myocytes due to its lipophilicity, which helps it in crossing the biological membrane. Once sestamibi accumulates within the myocardial cell, it is associated with negatively charged mitochondria (as ^{99m}Tc-sestamibi has unipositive charge). It clears via the hepatobiliary system which leads to low heart/liver ratios. Since ^{99m}Tc-sestamibi does not show the property of redistribution, separate injections are required for stress and rest tests. The stress test is usually done 15 min post-injection. In case of rest test, the imaging is usually performed 60 min post-injection to allow for clearance from the liver.

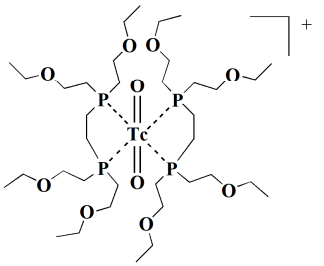
Since isonitriles are volatile and unstable compounds, sestamibi is available in stabilized form as copper tetrafluoroborate adduct, $[Cu(MIBI)_4]BF_4$. On heating the adduct at elevated temperature, it decomposes and the free ligand MIBI (methoxy isobutyl isonitrile) can then complex with ^{99m}Tc.

(c) ^{99m}Tc(V)-Tetrofosmin (Myoview®)

^{99m}Tc(V)-Tetrofosmin (Fig 1b) has a lower myocardial extraction coefficient (54%) than sestamibi. Similar to sestamibi, it rapidly accumulates in myocardium by passive diffusion. But unlike ^{99m}Tc-sestamibi, it has more rapid hepatobiliary clearance which allows for imaging soon after injection and also reduces the impact of



(a) Structure of ^{99m}Tc-sestamibi



(b) Structure of ^{99m}Tc-Tetrofosmin

Figure 1. Structures of ^{99m}Tc-based myocardial perfusion imaging agents

unwanted liver uptake (better heart/liver ratios). Tetrofosmin, unlike sestamibi, can be labelled at ambient temperature. Since ^{99m}Tc-Tetrofosmin also does not show the property of redistribution, it is injected separately for acquiring images under stressed and rest conditions.

PET agents

(a) ^{82}Rb-Rubidium Chloride (Cardiogen-82®)

^{82}Rb is a positron emitter ($t_{1/2}$=75 s; $E_{\beta+}$ = 3.15 MeV) and a monovalent cation analogue of potassium and therefore, it is used in PET imaging of the heart. Since ^{82}Rb has a short half-life, it is possible to carryout multiple examinations of the same patient within a small period. The first pass extraction of ^{82}Rb by the myocardium is 65-75% at normal blood flow. For rest test, 1.48-2.22 GBq (40-60 mCi) of activity is intravenously injected and the imaging is done 4-6 min after injection. Then stress is induced using vasodilator, and again similar quantity of radioactivity is injected into the patient for recording images under stressed

condition. Since ^{82}Rb is available from ^{82}Sr/^{82}Rb generator, it is an attractive radionuclide for PET imaging. ^{82}Sr has a half-life of 25.5 days and decays to ^{82}Rb by electron capture. ^{82}Sr is loaded on a SnO_2 column (loaded using NH_4OH/ NH_4Cl) and ^{82}RbCl is eluted with 0.9% NaCl solution.

(b) ^{13}N-Ammonia

Nitrogen-13 ($t_{1/2}$= 10 min; $E_{\beta+}$ = 1.2 MeV) ammonia ($^{13}NH_3$) is the most commonly used perfusion tracer for PET. When injected, ammonia is extracted by myocardial tissue with a very high extraction fraction (70%-80%), at which point it is converted to N-13 glutamine. Like rubidium, the Na/K ATPase pump takes up ammonia, because it exists in solution as cationic NH_4^+ form. For imaging studies, 555-740 MBq (15-20 mCi) of tracer activity is intravenously injected. ^{13}N is a cyclotron produced radionuclide. Due to the short half-life of ^{13}N, the cyclotron must be available in close proximity to the PET/CT scanner.

Myocardial metabolic imaging

The heart requires constant supply of energy to sustain its pumping function, which is derived majorly from metabolism of fatty acids and glucose. In fasting state, the concentration of free fatty acids in blood plasma and its uptake in myocytes is high leading to suppression of glucose oxidation. Glucose is not normally the major myocardial fuel, but in cases when glucose level in plasma is high (such as after a meal), oxidation of glucose increases and fatty acid oxidation is largely suppressed. In ischemic conditions when the oxygen supply to the heart muscles reduces, a shift in oxidation is observed from fatty acids to glucose. Glucose is then oxidized by anaerobic glycolysis which requires less oxygen consumption. This shift in metabolism in heart can be assessed using radiolabeled glucose or fatty acid analogues. Myocardial metabolic markers are important to assess the regions of stunned myocardium wherein the blood supply has been restored (such as after revascularization) but the myocardial metabolism has not been restored. This can help in prognosis after surgeries such as revascularization.

(a) ^{18}F-FDG (PET tracer)

^{18}F-FDG (fluorodeoxyglucose) is an analogue of glucose and once taken up by cardiomyocytes via GLUT transporters, it gets phosphorylated to ^{18}F-FDG-6-phosphate by hexokinase. ^{18}F-FDG can be used as a metabolic marker for assessment of myocardium viability. In ischemic myocardium (where oxygen supply is less), higher accumulation of ^{18}F-FDG is observed as compared to normal cells due to the dominant anaerobic glucose metabolism. On the other hand, in the infarcted scar tissue, ^{18}F-FDG accumulation is absent due to non-availability of glucose metabolism. A region with high ^{18}F-FDG accumulation but reduced myocardial perfusion indicates ischemic but viable myocardium. Region with high ^{18}F-FDG accumulation with high myocardial perfusion indicate stunned or hibernating myocardium. ^{18}F-FDG is also used for diagnostic imaging of cancer. More on ^{18}F-FDG, including the structure of the molecule, is discussed in Section 8(a).

(b) ^{123}I-BMIPP (SPECT tracer)

^{123}I ($t_{1/2}$ = 13.22 h; E_γ = 159 keV) labeled β-methyl-*p*-iodophenylpentadecanoic acid (BMIPP) (Fig 2) is a fatty acid marker used for imaging of myocardium. It is currently an approved radiopharmaceutical in Japan [3]. BMIPP is a long chain fatty acid which passively diffuses into the myocardium cells. It then undergoes β- oxidation inside the cells. Since BMIPP has a methyl group in its β- position, its oxidation is slow in the cells. Thus, it is retained for a long time in the myocytes and can be imaged using SPECT cameras. In ischemic regions, the uptake of ^{123}I-BMIPP is low as compared to the normal cells. The region of perfusion-metabolic mismatch (^{123}I-BMIPP defect larger than perfusion defect) indicates the presence of ischemic myocardium.

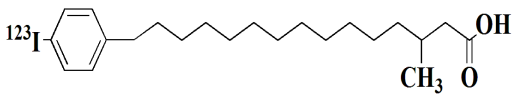


Figure 2. Structure of ^{123}I-BMIPP

Brain imaging

Brain perfusion imaging agents

A brain perfusion scan is a type of test that shows the amount of blood taken up by the brain. This provides information on the perfusion abnormalities in the brain such as infarction, stroke, tumor etc.

The principle of brain imaging is governed by a mechanism called the Blood-Brain Barrier (BBB), which is a highly selective semi-permeable border that separates the circulating blood from the brain and extracellular fluid in the Central Nervous System (CNS) (Fig 3). It allows the passage of hydrophobic molecules (O_2, CO_2, hormones) and small fat-soluble molecules by passive diffusion, as well as by the active transport of molecules using specific transport proteins such as glucose, water and amino acids that are crucial to neural function.

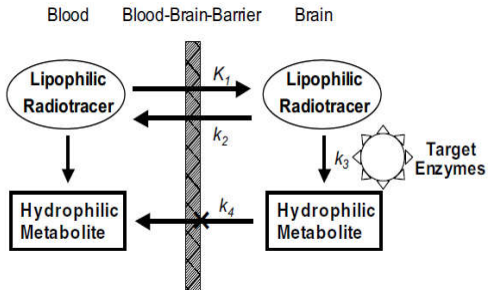


Figure 3. Schematic représentation of penetration and retention of a lipophilic radiotracer across blood brain barrier

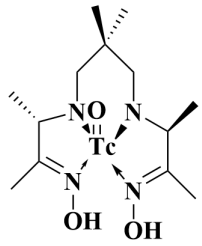
But it restricts the passage of pathogens, the diffusion of solutes in the blood, and large or hydrophilic molecules into the cerebrospinal fluid.

Brain perfusion imaging agents, used to detect areas of the brain deprived of normal blood supply, should be able to cross the BBB, retain there for sufficient period of time and its brain uptake should be greater than that in blood circulation. For permeability across BBB passively, biomolecules should be small, lipophilic and neutral, while active pathway involves the entry mediated by a carrier protein or enzyme (Fig 3).

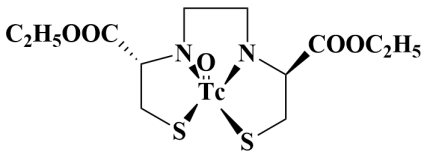
In early days, small and hydrophilic complexes of ^{99m}Tc namely, ^{99m}Tc-citrate, ^{99m}Tc-DTPA and ^{99m}Tc-glucoheptonate were used to evaluate the integrity of BBB. These agents cannot cross the intact BBB, but can be applied for brain imaging when the BBB is damaged, for example, by tumor or head trauma. On the other hand neutral and lipophilic complexes of ^{99m}Tc such as, ^{99m}Tc-d,l-HMPAO (Hexa-Methyl-Propylene Amine Oxime) and ^{99m}Tc-l,l-ECD (Ethylene Cysteine Diester) were synthesized and perused with an aim to cross the intact BBB.

(a) ^{99m}Tc-HMPAO (Ceretec®)

^{99m}Tc-Hexa-Methyl-Propylene Amine Oxime (^{99m}Tc-HMPAO) (Fig 4a) was the first neutral lipophilic technetium compound able to pass through the intact blood brain barrier. Due to the presence of asymmetric carbon atoms in the ligand, different stereoisomers of the active substance and the labelled compounds are known. The brain uptake of the d and l (trans) isomers is high while the meso (syn) isomer does not show acceptable accumulation in the brain tissues. The trans isomers are intracellularly transformed into hydrophilic compounds and retained in the brain cells. The brain uptake of ^{99m}Tc-HMPAO is proportional to the perfusion in the tissues. d,l-HMPAO can form a complex with glutathione present in brain and gets retained there while meso form is not recognised by glutathione. After penetration of the BBB, ^{99m}Tc complex of HMPAO dissociates and complexes with intracellular proteins, rendering it unable to re-diffuse through the BBB and thus gets trapped in the brain. However, ^{99m}Tc-HMPAO complex is not very stable and undergoes degradation. Around 370-740 MBq (10-20 mCi) of ^{99m}Tc-HMPAO is injected intravenously and the imaging is done 0.3-2 hrs after administration of the radiopharmaceutical [1].



(a) ^{99m}Tc-HMPAO



(b) ^{99m}Tc-ECD

Figure 4. Structures of ^{99m}Tc-complexes for brain perfusion imaging

(b) ^{99m}Tc-ECD, (Neurolite®)

^{99m}Tc-ECD (Ethylene Cysteine Diester) is a neutral lipophilic complex that localizes in the brain by crossing the BBB via passive diffusion (Fig 4b). In the brain, an enzyme catalysed hydrolysis of one of the ester groups to carboxylic acid results in the formation of an anionic and hydrophilic complex, which cannot diffuse across the BBB, thus preventing the washout from the brain. ^{99m}Tc-ECD exists in two stereoisomeric forms l,l and d,l; both of which exhibit brain uptake, but only the former exhibits brain retention. Slow hydrolysis in blood and rapid hydrolysis in brain tissue to the more hydrophilic metabolites results in high brain uptake and retention as they cannot diffuse back once metabolized.

The injected dose of ^{99m}Tc-ECD is 370-740 MBq (10-20 mCi) and imaging is done 30 min to 1 h after injection. The uptake of ^{99m}Tc-d,l-HMPAO and

^{99m}Tc-l,l-ECD in the brain is according to the regional Cerebral Blood Flow (rCBF), thus they can be used for monitoring the changes of rCBF in various conditions such as cerebrovascular diseases, stroke, chronic ischemia, early detection of dementia, evaluation of brain injury, assessment of brain death etc.

Receptor specific radiolabeled brain imaging agents

There are many radiolabeled targeting molecules for imaging of various Central Nervous System (CNS) receptors. Besides ability to exhibit binding to receptors with high affinity and selectivity, radiolabeled agents for neurology should show minimum permeability of their radio-metabolites across BBB post-metabolism in brain.

SPECT agents

^{99m}Tc-TRODAT

Parkinson's disease (PD) is a neurological disorder associated with a progressive degeneration of dopaminergic neurons. Current diagnostic procedures based on clinical criteria can be incorrect in the early stages of the disease. SPECT and PET imaging using radioligands with affinity for the dopaminergic system could be used in diagnosis of PD at an early stage.

One of the most important dopaminergic binding sites is the dopamine transporter (DAT), which is located on dopamine neurons and serves a critical role in dopamine neurotransmission. A significant reduction in the density of DAT has been reported in patients with PD and Alzheimer's disease, while an increased density was measured in patients with Attention Deficit Hyperactivity Disorder (ADHD). ^{99m}Tc-labeled tropane derivative, ^{99m}Tc-TRODAT-1 (Fig 5a), which binds to the dopamine transporter, could be used to image the dopaminergic system. ^{99m}Tc-TRODAT is a neutral lipophilic complex capable of penetrating the BBB.

PET agents

(a) ^{18}F-FDG

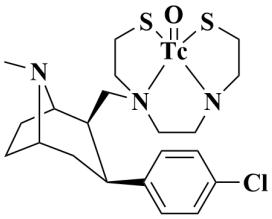
^{18}F-FDG crosses the BBB via the GLUT1 transporter, is metabolized in the brain cells and gets trapped there. For imaging studies, 370-555 MBq (10-15 mCi) of the tracer activity is intravenously injected. It is used for detection of epilepsy, recurrent tumors and dementias like Alzheimer's etc [1]. In cases such as epilepsy, the blood flow and brain metabolism increase at the focal region causing an increase in uptake of ^{18}F-FDG at the focal region. While in between seizures, a decrease in blood flow and brain metabolism happens leading to decrease in uptake of ^{18}F-FDG. ^{18}F-FDG is also useful in differentiating between recurrent brain tumor and necrotic brain tissue. An increased uptake of ^{18}F-FDG is observed in recurrent brain tumor while decreased uptake is observed in necrotic tumor [1].

(b) ^{18}F-DOPA

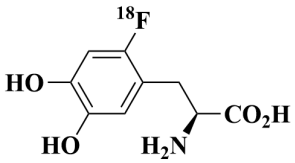
4-Dihydroxy-6-^{18}F-fluoro-l-phenylalanine (^{18}F-DOPA) is the metabolic tracer, which is the precursor of dopamine (Fig 5b). It is taken up by the dopaminergic nerve terminals. It is converted to ^{18}F-Fluorodopamine by DOPA-decarboxylase and gets stored there. It is used for early detection of PD or other neurodegenerative diseases.

(c) ^{11}C-MET

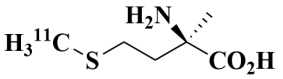
Radiolabeled amino acids are used for PET imaging of brain tumors because of their increased uptake in tumor tissues but low uptake in normal brain cells, resulting in an improved tumor-to-brain



(a) ^{99m}Tc-TRODAT



(b) ^{18}F-DOPA



(c) ^{11}C-Methionine

Figure 5. Structures of receptor specific brain imaging agents

contrast as compared to ^{18}F-FDG. Amino acids are taken up by the brain cells with the help of amino acid transporters. The amino acid PET tracer, ^{11}C-methyl-l-methionine (MET) (Fig. 5c) is used for diagnosis and management of adult patients suffering from brain tumors such as embryogenic tumors, glioneural tumors, lymphomas,

meningiomas and low-grade gliomas. MET differentiates between benign and malignant lesions in adults with high sensitivity and specificity with comparatively low background activity in normal brain tissue. It is also useful in monitoring chemotherapy in patients with brain tumors [4]. Dose of ^{11}C-MET in adults is usually 200-250 MBq (5.4-6.8 mCi).

Renal imaging

The basic unit of the renal system is a nephron consisting of glomerulus and a renal tubule (Fig 6). The nephron filters the blood plasma from glomeruli followed by selective absorption of required materials by the tubules and also secretion of materials by tubules (which are not filtered by glomeruli) in to the urine for balancing the electrolyte concentration and pH of the urine. Under normal conditions, the wastes and excess fluid become part of the urine to be excreted by the body so as to prevent their build up in blood.

Renal failure is impairment of excretory kidney function. Renal imaging is usually applied for the assessment of renal function expressed as Glomerular Filtration Rate (GFR) or Effective Renal Plasma Flow (ERPF). GFR is the volume of the fluid filtered from the glomerular capillaries into the Bowman's capsule per unit time. GFR is equal to renal clearance ratio when a solute is filtered freely and is neither absorbed nor secreted by the tubules (perfusion of the kidneys). ERPF shows the rate at which plasma flows through the kidneys (filtered or secreted by tubules) and is measured in terms of quantity of injected tracer excreted per minute in the urine. ERPF is a measure of the functional status of the kidneys.

Clinical methods to evaluate kidney function include measurements of urea or creatinine levels in urine. However, these serum indicators of GFR become abnormal only when renal function is significantly compromised. Over 50% of renal function may be lost before a rise in serum creatinine occurs. On the contrary, radionuclide imaging techniques can be used for early assessment of kidney functionality, which is required in cases such as, kidney transplant or during chemotherapeutic treatment etc. [5].

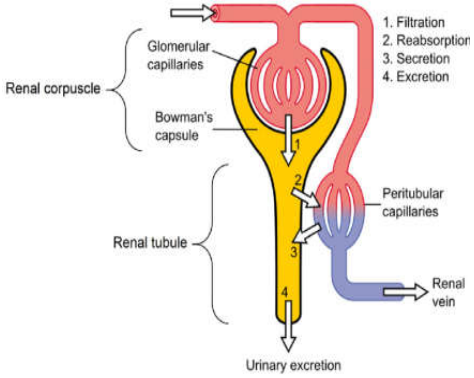


Figure 6. Nephron physiology showing urine formation (Source: Wikipedia)

Image acquisition in renal scintigraphy

Interpretation of the renal images provides information about the obstruction or functional abnormalities in the kidney. For the purpose of interpretation, graphs known as renogram are generated by plotting activity associated with a region of interest (ROI) against time (Fig 7). A renogram has the following three main segments.

Segment a: The vascular phase which signifies the arrival of the tracer and is around 30 secs long.
Segment b: It represents the renal accumulation of tracer before the excretion;
Segment c: Excretion of the tracer into the urine.

Segments b and c help in diagnosis of obstructive diseases of the kidneys. The period between injection and peak renal activity is called renal transit time which under normal conditions is around 3-5 min in adults. It can be prolonged in cases of renal arterial stenosis, dehydration or pooling in the renal pelvis. Obstructive diseases such as, acute tubular necrosis may result in delayed excretion, flattening segment c of the graph. In order to differentiate between functional and mechanical obstruction, furosemide (diuretic) is intravenously injected a few minutes after peak renal activity. Functional obstruction is alleviated by furosemide and the renogram becomes normal. If the obstruction is mechanical (like stones) little or no change occurs in the renogram after furosemide injection.

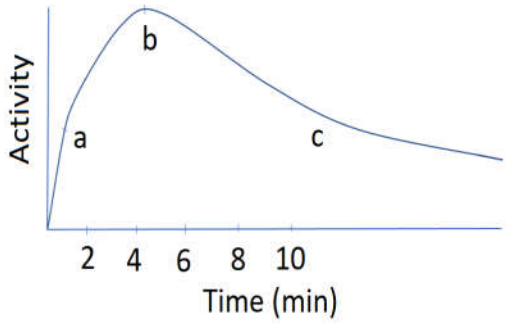
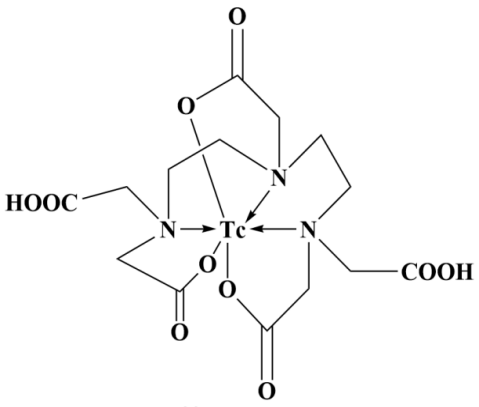


Figure 7. A schematic normal renogram showing different segments a, b and c

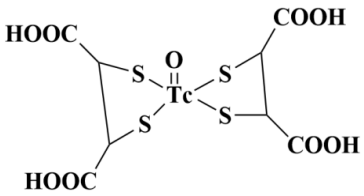
Renal imaging agents

(a) ^{99m}Tc-DTPA (Penetate®)

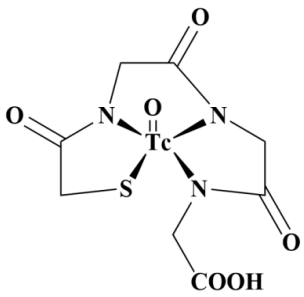
For the evaluation of GFR, ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) is used



(a) ^{99m}Tc-DTPA



(b) ^{99m}Tc-DMSA



(c) ^{99m}Tc-MAG3

Figure 8. Structures of ^{99m}Tc-labeled renal imaging agents

(Fig 8a). Following intravenous administration, it is entirely filtered by glomeruli in the kidneys and neither secreted nor reabsorbed by the tubules. Thus, it can be used for measurement of GFR and provides information about the blood perfusion in the kidneys. This method presents several practical advantages such as simple preparation, availability and good reproducibility. For measurement purposes, around 370-555 MBq (10-15 mCi) of activity is injected intravenously and then serial imaging is done at 1-2 sec intervals for up to 2 min. This provides information about blood flow through the kidneys. Further dynamic images are taken every 30-120 s for next 30 min. This help in generating the renogram which provides information about the renal function.

(b) ^{99m}Tc(V)-DMSA

^{99m}Tc(V)-dimercaptosuccinic acid (DMSA) is another agent (Fig 8b), which localizes in the renal cortex, binds to the proximal tubules and accumulates in the kidney with time. DMSA uptake is correlated to GFR as well as to ERPF and provides morphological information of the kidneys. The usual dosage of intravenous injection is 74-185 MBq (2-5 mCi) and images are usually taken 3-4 h after injection.

(c) ^{99m}Tc-MAG3 (Technescan®) and **^{99m}Tc-EC**

^{99m}Tc-mercaptoacetyltriglycine (MAG3) (Fig 8c), is established as the renal tubular function agent. *p*-Amino hippuric acid, a tracer with 80% renal clearance via tubular secretion, represented the classic molecule for the determination of ERPF. Its analogue namely o-iodohippuric acid labeled with ^{131}I (^{131}I-OIH) was used earlier as a renal imaging agent. However, the tracer showed considerable disadvantages including high radiation exposure caused by the high energy beta-irradiation, poor imaging characteristics due to the high energy gamma of 364 keV which is far from optimal for commonly used gamma-cameras, and the long physical half-life of 8.03 days. Therefore, ^{99m}Tc-labeled MAG3 with comparable properties has gained popularity. 90% of MAG3 excretion occurs via tubular secretion. ^{99m}Tc-MAG3 [185-370 MBq (5-10 mCi)] is intravenously injected and is used to determine both the flow and function of the kidneys. ^{99m}Tc-ethylene dicysteine (EC), a metabolite of ethylene cysteine dimer (ECD), is another technetium-labeled renal tubular function tracer with imaging qualities similar to ^{99m}Tc-MAG3. The elimination of ^{99m}Tc-EC is principally via active tubular transport**.**

Imaging of lungs

Perfusion imaging of lungs is accomplished by using radiolabeled particles (^{99m}Tc-macroaggregated albumin, ^{99m}Tc-MAA) injected into the body. This helps to assess the presence of emboli (obstruction) or other abnormalities to the pulmonary blood flow. The macroaggregated (MAA) particles are of diameter range 10–100 µm. On account of their size, the intravenously administered ^{99m}Tc-MAA particles are trapped in

the lung capillary bed during the first pass of circulation and are thus employed to study perfusion in the lung tissue.

In order to assess the complete functionality of the lungs, a pulmonary ventilation/perfusion (V/Q) scan is done which involves two nuclear scan tests to measure breathing (ventilation) and circulation (perfusion) in all areas of the lungs. During the ventilation scan, gaseous radionuclides such as ^{133}Xe, ^{81m}Kr or ^{99m}Tc-DTPA in aerosol form is inhaled by the patient through a mouthpiece or mask that covers the nose and mouth. Technegas machine, which produces an aerosol of radioactive nanoparticles, specifically ^{99m}Tc-labeled carbon nanoparticles, can also be used. The ventilation part of the test looks at the ability of air to reach all parts of the lungs, while the perfusion part evaluates how well blood circulates within the lungs. Decreased uptake of the inhaled radioisotope may indicate an impaired ability to breathe, airway obstruction, or possible pneumonia.

Decreased circulation of the injected ^{99m}Tc-MAA indicates a problem with blood flow into or within the lungs. A localized area of decreased uptake with normal ventilation images (mismatched defect) suggests a pulmonary embolus or blood clot in the lungs, which leads to reduced perfusion beyond the obstruction.

It is also possible to perform PET scan using ^{68}Ga-labeled carbon nanoparticles (Galligas) for ventilation images, and with ^{68}Ga-MAA for perfusion images. ^{68}Ga ($t_{1/2}$= 68.1 min) is a PET radioisotope obtained from a ^{68}Ge/^{68}Ga generator system.

Imaging of skeleton/whole body

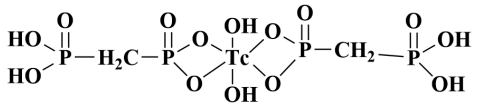
Metastasis of malignant tumors to bone is common, surpassed only by metastasis to the lungs and liver. Bone metastasis is a major complication of several solid cancers like the prostate, breast, lung, kidney, and thyroid cancers and it is associated with bone pain. Early detection of bone metastasis is important for effective patient management and improvement in quality of life.

(a) ^{18}F-NaF

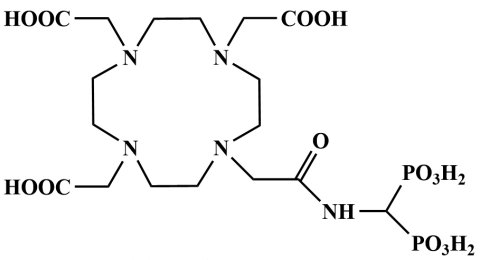
^{18}F-NaF is a PET radiopharmaceutical used for screening of patients with suspected bone metastases. Its mechanism of action involves chemisorptions of F^- ions onto hydroxyapatite. Hydroxyapatite is the major component and an essential ingredient of normal bone and teeth. $^{18}F^-$ on NaF exchanges rapidly for OH^- on the surface of the hydroxyapatite matrix [$Ca_{10}(PO_4)_6OH_2$] to form fluoroapatite [$Ca_{10}(PO_4)_6F_2$]. In case of a cancer lesion in bone tissue, the chemisorptions of fluoride gets enhanced and such regions exhibit intense signals in PET scans than those obtained corresponding to normal bone tissues. For imaging studies, 148 MBq (4 mCi) of activity is intravenously injected and imaging is done 15-30 min later as ^{18}F-NaF exhibits rapid bone uptake.

(b) ^{99m}Tc-MDP

^{99m}Tc-Methylene Diphosphonate is the simplest bisphosphonate (Fig 9a). Bisphosphonates have P-C-P unit which are biologically stable and are excreted unaltered from the human body. After intravenous injection, ^{99m}Tc- MDP is rapidly taken up into bone by chemisorption in the hydroxyapatite mineral component. It is suggested that the hydroxyapatite crystal remove the phosphonate component from ^{99m}Tc-MDP, which leaves reduced technetium free to bind to hydroxyapatite at another binding site. For imaging studies 370-740 MBq (10-20 mCi) of tracer is injected intravenously and the scanning is done 2-3 h after injection.



(a) ^{99m}Tc-MDP



(b) BPAMD

Figure 9. Structures of bone imaging agents

(c) ^{68}Ga-BPAMD

^{68}Ga-BPAMD (4-{[bis- (phosphono methyl)) carbamoyl] methyl}-7,10-bis(carboxymethyl)-1,4,7,10- tetraaza cyclododec-1-yl)acetic acid) (Fig 9b) is another radiolabeled bisphosphonate used for PET imaging of skeletal metastasis. ^{68}Ga-BPAMD gives better imaging with high resolution compared to the images obtained with bone-

seeking SPECT radiopharmaceuticals, such as ^{99m}Tc-MDP.

Imaging of infection and inflammation

Infection is the invasion of body tissues by microorganisms such as bacteria, virus, fungi, parasites etc., their multiplication and, the reaction of the body to the infectious agents and the toxins produced thereafter. Inflammation is the body's protective response to disease and may occur after infection, tumors, physical trauma or other conditions both local and widespread. The five classical signs of inflammation are fever, pain, redness, swelling, and loss of respective tissue function. While infection almost always causes inflammation, inflammation is not necessarily driven by microbial invasion – for example, atherosclerosis, trauma, ischemia, and autoimmune diseases. Nuclear imaging is a useful tool to differentiate between inflammation and infection, which could help in overcoming antibiotic resistance by curtailing indiscriminate use of antibiotics. Further, nuclear imaging can also help in early detection of infectious conditions such as sepsis, abdominal abscesses etc. which is required for better patient management.

(a) Radiolabeled leukocytes

Leukocytes or white blood cells (WBC) circulate in the blood and body fluids and are involved in counteracting foreign substances and disease. Leukocytes radio-labeled with ^{111}In or ^{99m}Tc are used for imaging of infections and inflammations. Leukocytes from the patient are labeled with ^{111}In-oxine *ex-vivo* and re-injected. For ^{99m}Tc-labeling, the leukocytes are incubated with ^{99m}Tc-sulfur colloids or ^{99m}Tc-exametazine (HMPAO) *ex-vivo* and then re-injected into the patient.

Imaging is done at three different time points mainly 30 min to 1 h (early image), 3 to 4 h (delayed image) and 20 to 24 h post-injection (late image). Any local increase of activity with time is a sign of infection, whereas an accumulation at 3 to 4 h with a decrease at 20 to 24 h is a sign of inflammation.[6,7]

Recently, *ex-vivo* radiolabeling of WBCs for PET imaging was attempted using ^{18}F-fluorodeoxyglucose (^{18}F-FDG) and ^{64}Cu. However, short half-life of ^{18}F (110 min) limits its usage for delayed imaging. Therefore, ^{64}Cu-WBCs would be more useful because of the long half-life of ^{64}Cu (12.7 h), which allows imaging up to 18-24 h after re-injection in patients [6, 7].

Radiolabeled leukocytes are considered as the gold standard for nuclear imaging of many infections. These scans are used in work-up of inflammatory bowel diseases, acute osteomyelitis, soft-tissue infections, and suspected infections of vascular and orthopaedic prostheses.

Even after being the gold standard for infection imaging, radiolabeled leukocytes have certain disadvantages. Firstly, the preparation of this radiopharmaceutical requires sterile conditions. The labeling procedure is complicated and time-consuming, taking up to 3 h even for a trained professional. Also, it requires the handling of contaminated blood, which pose health hazard to the technicians.

(b) ^{67}Ga-Citrate

^{67}Ga ($t_{1/2}$ = 3.26 days) is a cyclotron produced SPECT radioisotope which decays by electron capture and emits four major gamma energies suitable for imaging: 93, 184, 296, and 388 keV. ^{67}Ga-citrate, on injection into the blood stream binds to circulating transferrin, lactoferrin, and leukocytes *in vivo*. The ^{67}Ga-labeled transferrin and lactoferrin particles concentrate in areas of infection/ inflammation due to increased vascular permeability at the site. ^{67}Ga-citrate also binds to siderophores at the infection site. Siderophores are small, high-affinity iron-chelating compounds that are secreted by microorganisms such as, bacteria and fungi and serve primarily to transport iron across cell membranes.

Due to its low specificity, a high target-to-background ratio with ^{67}Ga-citrate imaging is achieved only after 48-72 h post-injection. Also, the long half-life of ^{67}Ga with high gamma energy leads to high radiation absorbed dose to the patients and staff. Due to these reasons recently, ^{68}Ga-citrate was developed for PET scanning, which resulted in optimal scanning time and better anatomic details.

Other experimental methods for imaging infections

(a) Cytokines

Cytokines and chemokines are group of proteins, peptides or glycoproteins secreted by the immune cells and are found in high concentrations at sites of infection. Thus, labeled cytokines have been used for imaging of infection. Strategies investigated have included ^{123}I- or ^{99m}Tc-labeled interleukin (type of cytokines) but with limited clinical success.

(b) Ubiquicidin

UBI or Ubiquicidin is an antimicrobial peptide (AMPs) designed to rapidly kill a broad spectrum of Gram-positive and Gram-negative bacteria. ^{99m}Tc labeled with ubiquicidin peptide fragment 29-41 (UBI_{29-41}: TGRAKRRMQYNRR) bind to the bacterial membrane and thus, selectively accumulate at the bacterial infection site. Recently developed PET analogues ^{68}Ga-NOTA-UBI_{29-41} and ^{68}Ga-NOTA-UBI_{31-38} showed high uptake in bacterial infected tissues in patients.

(c) Antibiotics and antimicrobials

Antibiotics are designed to specifically target invasive microbes. Thus, radiolabeled antibiotics can be used to visualize the site of infections. Many such antibiotics and antifungal agents are in different stages of clinical developments such as: ^{99m}Tc-Fluconazole, ^{99m}Tc/^{18}F-ciprofloxacin, ^{99m}Tc-ceftriazone, ^{99m}Tc-kanamycin, ^{99m}Tc-rifampicin.

Tumor imaging

Tumor is an abnormal mass of tissues in the body. Tumors may be benign, or malignant. Tumors, have some characteristics that are taken advantage of in radionuclide imaging. For example, tumors have increased metabolic activity, high vascular permeability, low oxygen concentration etc. With proper choice of radiopharmaceuticals suitable for evaluating any of these characteristics, tumor imaging can be accomplished.

(a) ^{18}F-FDG

2-Deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG) is a glucose analogue with ^{18}F substituted for the hydroxyl group at the C-2 position in the glucose molecule (Fig 10). It competes with glucose for active transport from blood to cells by various types of specific facilitative glucose transport proteins (GLUT). Once inside the cell, it is phosphorylated by hexokinase (whose mitochondrial form is greatly elevated in rapidly growing malignant tumors). The 2-hydroxyl group (-OH) in normal glucose is needed for further glycolysis, but this 2-hydroxyl group is missing in ^{18}F-FDG . Thus, FDG cannot be further metabolized in cells and gets trapped in the cell. As a result, the distribution of ^{18}F-FDG shows the distribution of glucose uptake and phosphorylation by cells in the body. Its enhanced uptake in tumor is based on the higher glucose metabolism in malignant tumors as compared to normal tissues. The imaging of ^{18}F-FDG is done under fasting conditions (at least 6 hours before imaging) in order to reduce the blood glucose level. This accentuates the difference in uptake of the tracer in normal cells and malignant cells leading to high contrast images.

^{18}F-FDG can be used for diagnosis, staging, and monitoring treatment of cancers, particularly in Hodgkin's disease, non-Hodgkin lymphoma, colorectal cancer, breast cancer, melanoma, and lung cancer. ^{18}F-FDG can also be used for the assessment of glucose metabolism in the heart, lungs and the brain.

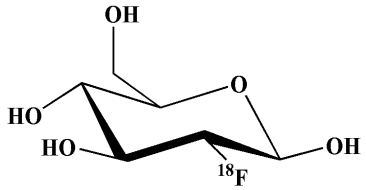


Figure 10. Structure of ^{18}F-FDG

(b) ^{123}I-MIBG (AdreView^{TM})

Metaiodobenzyl guanidine **(**MIBG) is a guanethidine analogue similar to neurotransmitter norepinephrine/ noradrenaline which are taken up by healthy adrenal cells and tumor cells.

MIBG binds to the cell membrane and gets actively transported into the cell. It is metabolized in the kidneys and slowly excreted via urine. ^{123}I-MIBG (Fig 11) is used for SPECT imaging mainly of adrenal medullary tumors such as, pheochromocytoma and adrenal-medullary hyperplasia. It is also used for imaging of some other neuroendocrine tumors including carcinoid tumor, medullary-thyroid carcinoma, paraganglioma, and neuroblastoma [9].

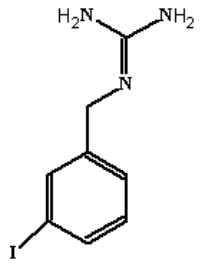


Figure 11: Structure of ^{123}I-MIBG

Tumor receptor imaging

Receptors are proteins usually present in the cell surface, which bind to ligands and release a messenger for further signal transduction. Ligands are molecules that bind to a receptor and can be a protein or peptide or another small molecule such

as a neurotransmitter, hormone, pharmaceutical drug or toxin.

While numerous receptors are found in most cells, each receptor will only bind with ligands of a particular structure. Many receptors are often over-expressed (100 to 1000 times) on primary human cancer cells compared to their low expression in normal tissues. Thus, ligands with extremely high affinities for these receptors form the molecular basis for imaging of such tumors [10].

Fibroblast activation protein imaging

Tumors consist of malignant cells as well as stromal cells which include vascular cells, inflammatory cells and fibroblasts. The stroma contributes to > 90% of the tumor mass in tumors such as breast, colon and pancreatic carcinoma. The fibroblasts associated with tumor (Cancer-Associated Fibroblasts-CAFs) facilitate the growth and migration of tumors to other organs. These fibroblasts over-express Fibroblast Activation Protein (FAP) which is a cell-surface serine protease. The expression of FAP in normal fibroblasts is very low making these proteins an attractive target for imaging of wide variety of cancers. FAP targeting small molecule for PET imaging, ^{68}Ga-FAPI (Fibroblast Activation Protein Inhibitors) has shown high affinity for FAP enzyme. In the clinical trials, ^{68}Ga-FAPI-04, (Fig 12) is shown to be effective for imaging of up to 28 types of cancer such as sarcomas, esophageal, breast, cholangiocarcinoma and lung cancer. FAPI imaging has various advantages over FDG such as, no dependency on blood sugar levels (so no excess uptake observed in brain and heart cells), no need for resting and the possibility of imaging even at 10 min post-injection which can help in reducing the waiting time of the patients and also reduction in the amount of activity injected. Further, FAPI ligands have DOTA chelator which can be complexed with therapeutic radionuclides (^{177}Lu, ^{90}Y etc) for treatment of cancer [8]. This can help in personalized treatment of cancer patients.

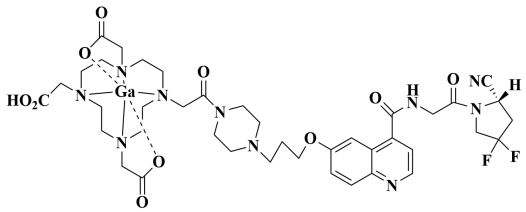


Figure 12. ^{68}Ga-FAPI-04

FAPI accumulation is seen not only in the tumors, but also at sites with tissue remodeling, where activated fibroblasts are present such as, chronic inflammation, activated arthrosis or in case of injured myocardium after myocardial infarction. Imaging using radiolabeled FAPI can help in diagnosis and prognosis in case of myocardial infarction as its uptake in normal heart cells is negligible.

Somatostatin receptor imaging

Somatostatin (SST) is a hormone produced by many tissues in the body, principally in the nervous and digestive systems. Human endocrine originated tumor cells often over-express the somatostatin receptors (SSTR1-5) with varying receptor density. While SSTR2 and SSTR5 is the most abundant, SSTR4 is rarely expressed in tumor cells. A high density of the SSTRs is found in neuroendocrine tumor (NETs) such as pituitary tumors, endocrine pancreatic tumors, carcinoid, paraganglioma, pheochromocytoma, medullary thyroid carcinoma, small-cell lung cancer, neuroblastoma, meningioma, and lymphoma. NETs are characterized by slow growth along with highly invasive malignancies. Their characterization by ^{18}F-FDG is often difficult because of their low metabolic rate.

Radiolabeled somatostatin cannot be used for imaging of NETs due to its short biological half-life (about 3 min) as it is rapidly degraded by enzymes. Currently, its biological analogue octreotide is most commonly used for imaging.^{111}In-DTPA-octreotide was the first registered commercial imaging agent for NETs. But it was found to have high uptake in liver. Further the low spatial resolution of SPECT makes it difficult to detect small cancer lesions. Another SPECT agent developed was ^{99m}Tc-Hynic-TOC (TOC = [Tyr^3] octreotide), which showed lower liver uptake as compared to ^{111}In-DTPA octreotide. Later PET based imaging agents were developed mainly: ^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-NOC (NOC = [1Nal^3]octreotide) and ^{68}Ga-DOTA-TATE (TATE = [Tyr^3]octreotate) (Fig 13). The main difference amongst these three tracers is their variable affinity to SSTR subtypes. All of them can bind to SSTR2 and SSTR5, while only DOTA-NOC shows good affinity for SSTR3 [10]. Gallium chelated octreotide derivatives exhibited higher SSTR2 specificity and lower kidney uptake as compared to ^{111}In-octreotide tracers. ^{68}Ga-DOTA-TOC was approved for PET imaging of neuroendocrine tumors in Europe (Austria, Germany, France) in 2016,while US-FDA approved ^{68}Ga-DOTA-TOC in 2019.

$\alpha_v\beta_3$ integrins imaging

Angiogenesis is the physiological process through which new blood vessels are formed from pre-existing vessels. Tumor angiogenesis is an essential requirement for tumor growth and metastasis,

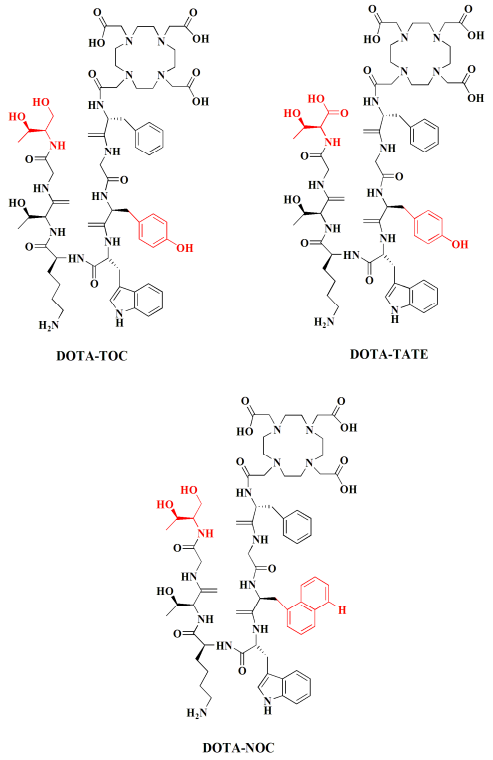


Figure 13. Structures of SSTR specific imaging agents (Red highlight shows the difference in the three agents)

where integrin $\alpha_v\beta_3$ plays a major role. The $\alpha_v\beta_3$ integrins are over-expressed on large number of activated endothelial cells during angiogenesis in contrast to the resting endothelial cells. Also, $\alpha_v\beta_3$ integrins are over-expressed in some tumors like osteosarcomas, neuroblastoma, glioblastomas, melanomas, lung carcinomas and breast cancer and help in their growth and metastasis. Thus, imaging of $\alpha_v\beta_3$ integrins is considered as a useful tool in management of cancer. The peptide motif, arginine-glycine-aspartic acid (RGD), is the most commonly used ligand for imaging due to its high affinity and specificity for $\alpha_v\beta_3$ integrins. A variety of RGD-based radiotracers are in clinical trials for imaging $\alpha_v\beta_3$ integrins [8,10]: ^{18}F-Galacto-RGD, ^{18}F-FPP(RGD)$_2$, ^{18}F-RGD-K$_5$, ^{18}F-fluciclatide, Al^{18}F-alfatide-I, Al^{18}F-alfatide-II, ^{68}Ga-NOTA-PRGD$_2$, ^{68}Ga-NOTA-RGD (Fig 14).

PSMA imaging

Prostate-specific membrane antigen (PSMA) is a transmembrane protein over-expressed in

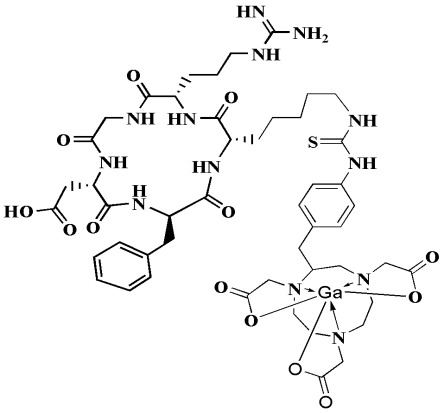


Figure 14. Structure of ^{68}Ga-NOTA-RGD for imaging of $\alpha_v\beta_3$

prostate cancer, bladder carcinoma, schwannoma and tumor neovasculature of many solid tumors. Amongst the various PSMA-targeted peptide vectors investigated for accurate identification and staging of prostate cancer, 'glutamate-urea-lysin' Glu-NHCO-NH-Lys-(Ahx) containing pharmacophore has proven to be most effective. ^{68}Ga- PSMA-11 (Fig. 15) was the first FDA approved drug (in December, 2020) for PET imaging of PSMA-positive lesions in men with prostate cancer. In this radiotracer, the HBED-CC (HBED: N,N-bis(2-hydroxybenzyl)ethylene diamine-N,N-diacetic acid) chelator was conjugated with the γ-amine of lysine via an aminocaproic acid linker, which ensures reduced interaction between the chelator and the binding pocket of PSMA [10]. There are few ^{99m}Tc-based PSMA agents such as, ^{99m}Tc-MIP-1404, ^{99m}Tc-iPSMA, ^{99m}Tc-PSMA I&S and ^{99m}Tc-HYNIC-PSMA which are currently in different stages of clinical evaluation.

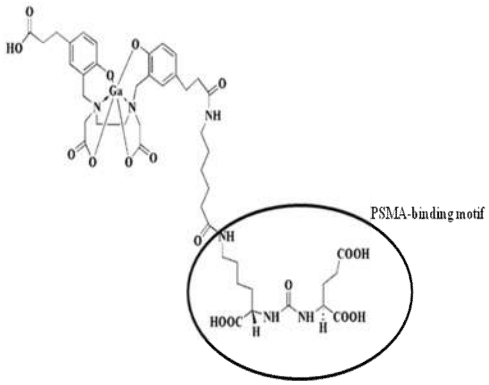


Figure 15. Prostate cancer imaging agent ^{68}Ga-PSMA-11

Tumor hypoxia

Tumor hypoxia is the situation where tumor cells have been deprived of oxygen. As a tumor grows,

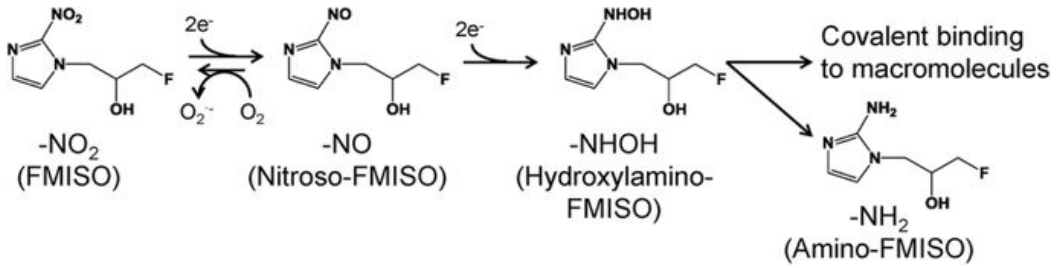


Figure 16. Uptake of ^{18}F-FMISO in the hypoxic cells

it rapidly outgrows its blood supply, leaving portions of the tumor with regions where the oxygen concentration is significantly lower than in healthy tissues. Thus, tumors contain mixtures of normoxic, hypoxic, and anoxic cells (dead cells). Radiation or chemotherapy can effectively eliminate well-oxygenated cells. However, the hypoxic regions in tumor are resistant towards chemotherapy as well as radiation therapy thereby compromising the prognosis of the cancer patient and ultimately contributing to relapse. Detection of hypoxic regions in tumor is important and once detected; treatment of such cancer patients can be tailored accordingly.

Tumor hypoxia imaging agents

(a) $^{15}O_2$

Inhalation of $^{15}O_2$ for PET imaging of tissue oxygen level is the 'gold standard'. Yet it is not a popular approach because of the short half-life of $^{15}O_2$ making imaging logistically and technically complex, challenging and expensive.

(b) ^{18}F-FMISO

Nitroimidazole ligands with suitable single electron reduction potential (SERP) (electron affinity close to but not more than that of oxygen else they would be toxic for mitochondria) are specific for hypoxia. These ligands after entering the cells, get reduced to corresponding amine derivatives through intermediate reduced products (nitroso and hydroxylamino) in an anaerobic environment followed by the intracellular retention post-binding with the thiol group of cellular macromolecules. In normoxic conditions, the first reduction step is reversible in nature forcing the washing out of the non-reduced nitroimidazole from the cellular compartment [11]. This process marks the selectivity of nitroimidazole-based ligands towards tumor hypoxia.

3-Fluoro-1-(20-nitro-10-imidazolyl)-2-propanol or ^{18}F-fluoromisonidazole (^{18}F-MISO) is the most studied tracer for evaluation of hypoxia and radioresistant tumors. Its uptake is inversely

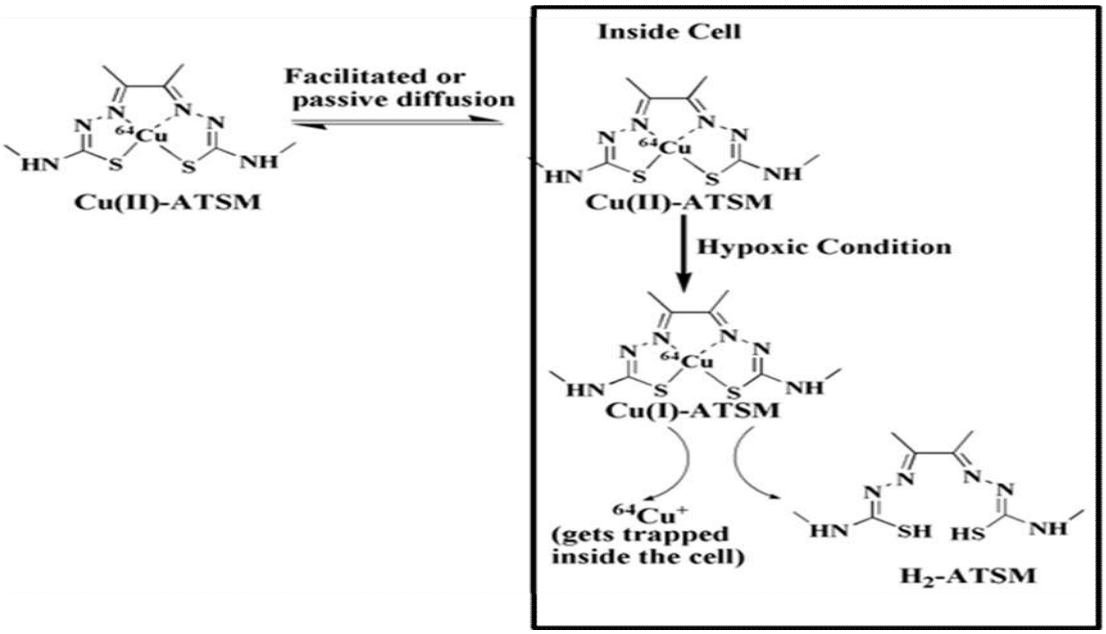


Figure 17. Mechanism of Uptake of ^{64}Cu-ATSM in hypoxic cells

proportional to O_2 level, that is, the degree of hypoxia is identified by increased uptake of ^{18}F-MISO, and its delivery to tumor is not restricted by perfusion [11-13]. (Fig. 16).

(c) ^{64}Cu-ATSM

Cu^{2+} complex of diacetyl-2,3-bis(N(4)-methyl-3-thiosemicarbazone (^{64}Cu-ATSM) (Fig. 17) has a low molecular weight and a high cell membrane permeability allowing it to diffuse easily from the bloodstream to surrounding cells. It has low reduction potential and gets readily reduced from Cu(II) to Cu(I) in hypoxic cells along with protonation of the ATSM ligand. Cu(I) then dissociates slowly from the ATSM ligand in cells with low oxygen concentration, becoming irreversibly trapped inside. It is thus found to be a potential marker for hypoxic cells.

Hypoxic markers are not used in routine clinical applications because of a number of limitations such as (i) slow uptake in hypoxic tumors, (ii) High uptake in non-target organs due to high lipophilicity, and (iii) radioactive metabolite products formed due to non-oxygen dependent metabolism, which circulate in the body giving wrong signals [13].

Conclusions

This chapter gives a glimpse of different radiopharmaceuticals currently used for diagnosis of various organs as well as cancer. Nuclear medicine non-invasively provides information about the functioning of different organs due to which it has become a popular diagnostic tool in the present day medical practice. Owing to its popularity, advanced research is is now being conducted for the development of new diagnostic radiopharmaceuticals based on nanoparticles, peptides, antibodies etc. Also, the same carrier ligands are being explored for therapeutic applications for treatment of cancer. This field is known as theranostics, which is a combination of diagnostic and therapeutic radiopharmaceuticals which helps in providing more personalized treatmentto the patient.

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Chapter 3
Therapeutic radiopharmaceuticals: Translation from laboratory to nuclear medicine clinics over the last two decades in India

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Abstract: Radiopharmaceutical therapy has emerged as a safe and effective strategy to treating various oncological and non-oncological diseases. In this treatment modality, radiation is systemically or locally delivered using radiopharmaceuticals that either bind preferentially to cancer cells or accumulate by physiological mechanisms. Radionuclides used in therapeutic radiopharmaceuticals decay by emission of particulates such as, α, β^- and Auger e, which are responsible for delivery of cytoxic payload to the dossed tissue. Apart from this, most of these emit photons that can be imaged, enabling non-invasive visualization of the biodistribution of the therapeutic agent. Compared with almost all other systemic treatment options, radiopharmaceutical therapy, which is regularly practiced for last several decades, has shown efficacy with minimal invasiveness and toxicity. In recent times, with the introduction of several newer radiopharmaceutical agents, the remarkable potential of this treatment has now emerged and recognized worldwide. The present article covers the fundamental properties and mechanisms of action of different categories of therapeutic radiopharmaceuticals and gives an account of their development and clinical translation in India over the last two decades.

Keywords: *Nuclear medicine, radiopharmaceuticals, therapy, cancer, radioisotope*

Introduction

The past two decades have witnessed tremendous growth in the development of engineered radiopharmaceuticals which can deliver cytotoxic dose of ionizing radiation to the disease cells with high degree of specificity [1]. Extensive studies have suggested that radiation therapy at the cellular level using target-specific radiolabeled molecular vectors or so called 'magic bullets' has the potential to reduce the risk of both short- and long-term side effects of treatment. Especially in oncology, this treatment modality enables even tiny deposits of cancer cells (metastases) to be killed throughout the body without causing any major side effects. Although targeted radionuclide therapy for cancer management gained momentum over last two decades, it is not really a new approach. One such therapy in nuclear medicine, called ^{131}I therapy, has been used to treat several types of thyroid cancer since the 1940s. In this treatment modality, when the reactor-produced radionuclide ^{131}I as simple [^{131}I]I$^-$ ion is ingested (as a pill or a liquid), it specifically accumulates in thyroid cancer cells left over after surgery and ablates them. Although the next-generation therapeutic radiopharmaceuticals are far advanced than the simple ionic [^{131}I]NaI, it is still one of the most widely used formulation for targeted cancer treatment. Engineered radiopharmaceuticals often consist of three main building blocks: a suitable therapeutic radionuclide, a targeting molecule (that recognizes and latches specifically onto disease cells), and a linker that joins the two. Such compounds could generally be injected, infused, inhaled, or ingested, and then make their way to the disease site.

The development of therapeutic radiopharmaceuticals is a multidisciplinary endeavor, requiring expertise in radiochemistry, radiobiology, oncology, pharmacology, medical physics and radionuclide imaging and dosimetry. After its introduction in human clinical use in the form of radioiodine therapy during 1940s, radiopharmaceutical therapy has been used as a treatment modality of last resort and available only in small clinical trials or as part of compassionate care from different hospitals having nuclear medicine departments. In fact, very few therapeutic radiopharmaceuticals such as [^{177}Lu]Lu-DOTATATE and [^{177}Lu]Lu-PSMA-617 have been recently accorded the status of United States Food and Drug Administration (US FDA) approved drugs for clinical use in cancer care [2, 3]. Though radiopharmaceutical therapy has been an 'orphan treatment' modality for many years, it is now developing a well-defined community of stakeholders all over the world. The remarkable potential of therapeutic radiopharmaceuticals directed against primary cancers as well as distant metastases, is now being increasingly recognized as an effective, safe and economically and logistically viable treatment modality, receiving renewed attention from both small and large pharmaceutical companies globally.

This article provides an overview of the radiochemistry and radiobiology aspects needed to understand the fundamentals of radiopharmaceutical therapy. The different categories of radiopharmaceutical therapy agents developed in India over the last two decades and in use in the clinics for the treatment of cancer are discussed. Notably, many other therapeutic radiopharmaceuticals are in preclinical development, but their discussion is beyond the scope of this articles. Similarly, a few therapeutic radiopharmaceutical formulations, namely, [^{131}I]NaI, [^{153}Sm]Sm-EDTMP etc. are in routine clinical use in India for last several decade. These will not be discussed here as there are extensive reviews on these topics [4, 5].

Basic mechanism and biological effects of radiopharmaceutical therapy

Primarily, the goal of targeted radiopharmaceutical therapy is to deliver therapeutic doses of ionizing radiation to specific disease sites for cure, disease control, or pain palliation. The selective localization of the radiopharmaceuticals in the disease site has been elaborately discussed in some other articles of the present series and is hence not discussed here. Briefly, the biological effects of radiopharmaceutical therapy are obtained by three different mechanisms:

i) Interaction of ionizing radiation with water in which chemically active free radicals (hydroxyl radical) are formed that can react with biomolecules (phospholipids, proteins, RNA, DNA, etc.), thereby irreversibly damaging the cells.
ii) Direct interaction of ionizing radiation with DNA in which single-strand breakage or double-strand breaks can occur. Therefore, therapeutic radionuclides must emit particulate radiation with relatively short path lengths thereby depositing the radiation energy at a short distance to spare surrounding non-target tissues. Single-strand breaks can be repaired while double strand DNA breaks are far more difficult to repair. When repairing DNA strand breaks, repair errors can also occur, causing mutations.
iii) During treatment of a tumor, the immune system can be activated against certain antigens of the tumor so that other tumor cells (possibly at a distance from the treated tumor) can be attacked by the immune system, this is known as the abscopal effect.

Dosimetry aspects in radiopharmaceutical therapy

Dosimetry gives answer to the fundamental question for radiopharmaceutical therapy that is how much dose of ionizing radiation is absorbed in the tumor in comparison with normal tissues and thus provides a measure of potential treatment success [5, 6]. The treatment outcome will also depend on the biological repair and radiosensitivity of the tumor cells. In dosimetry, the absorbed dose (D) is defined as the energy absorbed per unit mass of tissue. As implemented in radiopharmaceutical therapy, dosimetry may be thought of as the ability to perform the equivalent of a pharmacodynamic study in treated patients in real time. The ability to rapidly assay genetic and epigenetic characteristics of tumor samples comes closest to providing the kind of information that dosimetry provides regarding the potential efficacy and toxicity of a therapeutic radiopharmaceutical agent in an individual patient.

The mathematical formalism and tools available to perform dosimetry of therapeutic radiopharmaceuticals have evolved over time. Dosimetry calculations intended to assess risk are now performed for an anatomical geometry designed to represent the average patient population rather than any one particular patient. Nuclear imaging (SPECT/PET)-based, patient-specific dosimetry allows the distribution of the radiopharmaceutical agent in tumors and normal organs to be quantified. The amount of therapeutic radiopharmaceutical that concentrates in the tumor can be increased by increasing the administered activity, which also impacts the tumor- absorbed dose. Nevertheless, dosimetry analysis following a low-activity administration has the potential to inform the amount of activity required to administer for actual therapeutic application. In this context, it is essential to pin-point that organ toxicity is usually reflected not by a whole-organ absorbed dose but rather by absorbed dose ‘hot-spots’. This is particularly important if such regions of high absorbed dose correspond to organ sub-regions that are critical to organ function. A typical example is some therapeutic radiopharmaceuticals used for peptide receptor radionuclide therapy (PRRT) concentrate and are retained in the renal cortex, so the absorbed dose in the renal cortex better predicts toxicity than the absorbed dose in the whole kidney volume.

Table 1. Physical characteristics of a-emitting radioisotopes used in targeted therapy

Radionuclide	Half-life	Mode of decay	Energy (keV)	Principal γ-component E in keV (% abundance)
^{211}At	7.2 h	a, γ	5982.4	687.0 (0.3)
^{223}Ra	11.4 d	a, γ	5979.3	269.4 (13.6)
^{224}Ra	3.6 d	a, γ	1900.0	241.0 (3.9)
^{225}Ac	10.0 d	a, γ	5935.1	99.7 (3.5)
^{212}Bi	60.6 min	a, γ	6207.1	727.2 (11.8)
^{213}Bi	45.6 min	a, γ	5982.0	439.7 (27.3)
^{227}Th	18.7 d	a, γ	5900.0	236.0 (11.2)

Radionuclides for preparation of therapeutic radiopharmaceuticals

Different radionuclides are chosen for preparation of therapeutic radiopharmaceuticals based on decay characteristics and their potential coordination chemistry [4]. These radionuclides emit alpha particles, beta particles, and Auger electrons which emit radiation causing damage to the tissues. The following parameters must be evaluated while choosing the radionuclides:

i) Physical and biological half-life
ii) Energy of the different emitted particles and their penetration depth in tissues
iii) Daughter products
iv) Purity of the radionuclide
v) Size of the tumor
vi) Uptake and retention of radiopharmaceuticals within the tumor volume
vii) Stability of the radiopharmaceuticals under in vivo conditions and their toxicity.

Below, we briefly summarize the radiation effects caused by emission of alpha particles, beta particles, and Auger electrons.

Alpha (α) particle emitter

An alpha particle is made of a helium (^{4}He) nucleus with a +2-charge emitted by radionuclides while undergoing radioactive decay. Alpha particles have the highest LET which is nearly 80 keV/µm. Hence, they can deposit their whole energy within a cell diameter of 50-100 µm. The biological effectiveness and cytotoxic effect of alpha particles are 500 times greater than of beta particles. Primarily, alpha particles break the double strand of DNA within the cell nucleus, leading to delaying G2 phase and chromosomal damage. Thus, alpha therapy is suitable for treating small or microscopic sized tumors. Although more than 100 alpha emitting radionuclides are available, majority of them have inappropriate half-lives for therapeutic use and non-economical production route that restrict their applicability in nuclear medicine. Table 1 summarizes the nuclear properties of these α-emitters that are presently being used for targeted cancer therapy. Astatin-211 is one of the most important radionuclides that has been extensively studied in in vivo cancer models [7]. Not only does a sufficiently long half-life allow multiple synthetic procedures but it also produces only one alpha particle per decay which simplifies dosimetry calculations and minimizes off-targeting of daughter products. ^{211}At can be produced in cyclotrons by the nuclear reaction ^{209}Bi (α, 2n)^{211}At without involving nuclear fuel material as a target [7]. Another important candidate is ^{225}Ac which emits four alpha particles per decay [7]. However, the daughter products (^{221}Fr, ^{213}Bi, and ^{217}At) cause cytotoxicity to the healthy cells. The main problem associated with alpha therapy is the migration of multiple daughter products which give serious toxic effects to the healthy tissues.

Auger electron emitter

Auger electron arises when a radionuclide decays by electron capture (EC) or internal conversion (IC). During radioactive decay, when a vacancy is created in the inner electron orbital, it is filled by an outer shell electron. The energy difference created from this transition is transferred to another electron resulting in its final ejection from the atom. The ejected, low-energy electron is an Auger electron. On average, 5 to 35 Auger electrons are emitted per one decaying atom with energy ranges from a few eV to 1 keV. LET of these Auger electrons is 4-26 keV/µm with a penetration range < 0.5 µm in biological tissues. This maximizes the cytotoxic effect by breaking DNA double strands whilst generating reactive oxygen species (ROS). As maximum energy is deposited by Auger electrons adjacent to the decay site, the therapy requires precise dose delivery to the components inside the cells. The precise DNA double helix diameter, measuring 2 nm, exactly matches with the range of the maximum energy deposition by Auger electron. Due to this, Auger electrons are taken into consideration for the

treatment of small tumors or clusters of cancerous cells. A list of Auger electron-emitting radionuclides is summarized in Table 2. However, it is pertinent to mention that very few practically usable therapeutic radiopharmaceuticals have been developed to date with Auger electron emitters [8].

Additionally, another limitation of beta radiation is that, similar to alpha particles, they cannot provide a lethal dose to a single cancerous cell. However, the long-range crossfire effect gives beta radiation supremacy over targeted alpha therapy. The majority of therapeutic radiopharmaceuticals that have been clinically translated are prepared with β^- emitting radionuclides such as ^{131}I, ^{177}Lu and ^{90}Y [4].

Table 2. Physical characteristics of Auger electron emitting radioisotopes

Radionuclide	Half-life	Mode of decay[@]	Emission	Principal γ-component E in keV (% abundance)
^{111}In	2.8 d	EC (100%)	γ, Auger e^-	245.4 (94.2)
^{125}I	60 d	EC (100%)	γ, Auger e^-	35.49 (6.6)
^{103}Pd	16.9 d	EC (100%)	γ, Auger e^-	20 (64)

[@]*Only principal decay mode is mentioned; EC indicates decay by electron capture; E indicates energy*

Beta (β^-) particle emitter

A beta particle is a negatively charged high energy electron emitted during nuclear decay process. The β^- emitting radioisotopes are widely used in cancer treatment by means of internal radiotherapy. Nuclear decay characteristics of some beta emitters are listed in Table 3.

Compared to alpha and Auger electron emitters, LET of beta particle is much lower (0.1–1.0 keV μm^{-1}) and has a higher spatial penetration range which varies from 0.05 to 12 mm. These long-range beta particles act as a double-edged sword. On one hand, they can travel through several cell diameters which increases average dose to the tumor by breaking DNA and reactive oxygen species (ROS) generation, which permanently or partially arrests the cell cycle. This phenomenon makes them suitable candidates for treating bulky or large tumors. On the other hand, they also deliver doses to the surrounding healthy tissues.

Overall, choosing the appropriate radionuclide depends on the size and the position of the malignant tumor. As already discussed above, small clusters of tumors or the bulky tumors require different radiation doses for effective ablation of cancer cells. Furthermore, the radiolabeling and the purification process should be in accordance with the half-life of the corresponding radionuclide. The comparative evaluation of radiation damage caused by α, β^- and Auger electron emitting radionuclides is schematically illustrated in Figure 1.

Production of therapeutic radionuclides in India

The production of therapeutic radionuclides in India is solely dependent on the Dhruva research reactor located at BARC. Target chemicals are neutron irradiated from few days to several weeks at neutron flux in the range of 1.8×10^{13} to 1.8×10^{14} $n/cm^2/s$. Measured amounts of respective

Table 3. Physical characteristics of β^- emitting radioisotopes

Radionuclide	Half-life [15]	Mode of decay[@]	Energy (keV)[#]	Principal γ-component E in keV (% abundance) [16]
^{32}P	14.3 d	β^-	1710.6	No γ-ray
^{47}Sc	3.3 d	β^-, γ	600.1	159.4 (68.0)
^{67}Cu	61.8 h	β^-, γ	577.0	184.6 (48.7)
^{89}Sr	50.5 d	β^-	1496.6	No γ-ray
^{90}Y	64.1 h	β^-	2282.0	No γ-ray
^{131}I	8.0 d	β^-, γ	970.8	364.5 (81.2)
^{153}Sm	46.3 h	β^-, γ	808.4	103.2 (28.3)
^{166}Ho	26.8 h	β^-, γ	1854.5	80.6 (6.2)
^{169}Er	9.4 d	β^-, γ	351.2	No γ-ray
^{177}Lu	6.7 d	β^-, γ	498.2	208.4 (11.0)
^{186}Re	90.6 h	β^-, γ	1069.5	137.2 (8.6)
^{188}Re	16.9 h	β^-, γ	2120.4	155.0 (14.9)
^{198}Au	2.7 d	β^-, γ	1372.5	411.8 (95.5)
^{199}Au	3.1 d	β^-, γ	452.6	158.4 (36.9)

[@]*Only principal decay mode is mentioned;* [#]*For β^- particles maximum β^- energy is mentioned*

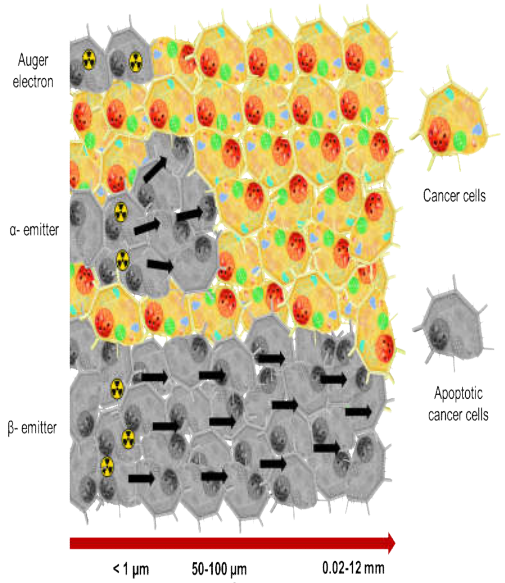


Figure 1. Comparison of radiation damage caused by α, β^- and Auger electron emitting radionuclides in cancer cells

target to be irradiated in the reactor for production of radioisotopes are weighed and sealed in standard aluminum capsules (22 mm diameter, 46 mm height) by cold welding. Certain precious target materials, particularly isotopically enriched targets are first encapsulated in quartz ampoules which are placed inside the irradiation containers. The amount of target is calculated and optimized for obtaining the desired radioactivity content on irradiation at a known neutron flux for adequate length of time duration. Activity of the radionuclide produced by thermal neutron activation depends on various factors such as isotopic abundance of the target nuclide, activation cross-section for neutron absorption and the neutron flux, the duration of irradiation and half-life of the product radionuclide. Presently, two tray rods namely, K-09 and H-07 in Dhruva are dedicated for radioisotope production.

The therapeutic radionuclides produced using the Dhruva reactor can be categorized into two types: (a) established radionuclides (those which are regularly produced and supplied for clinical uses) and (b) emerging radionuclides (those which are soon to be introduced for routine production and supply). In the following sections, we discuss the production routes and radiochemical processing/separation methodologies of these radionuclides.

Established radionuclides

^{131}I: Iodine-131 decays by emission of both β^--particles with a maximum energy of 0.61 MeV and γ photons [principal γ photon energy 364 keV (81%)]. The 8.04 d half-life is logistically favorable for shipment of ^{131}I radiopharmaceuticals to places far away from the reactors. With expanding areas of applications and growing interest in the use of ^{131}I-labeled radiopharmaceuticals, the domestic demands of ^{131}I have increased several folds over the last decade. In the quest for an effective method for large-scale routine production of ^{131}I to cater the increasing domestic requirements, we turned our focus towards the use of dry distillation technology owing to its appealing attributes. The dry distillation technology developed at BARC is facile, robust, efficient, easily up-scalable, generates minimum amount of radioactive waste and cost effective. Briefly, the procedure involves heating of neutron irradiated high purity TeO_2 target, purging the ^{131}I released using an inactive carrier gas and trapping it in NaOH solution containing Na_2SO_3 to obtain ^{131}I as radiochemically pure [^{131}I]NaI solution. The reported method has been successfully used for the routine production of ~ 1.48 TBq (40 Ci) of ^{131}I every week [9].

^{153}Sm: Samarium-153 [$T_{½}$ = 46.3 h] decays by emission of both β^--particles with a maximum energy of 0.81 MeV and γ photons [principal γ photon energy 103 keV (28%)]. Favorable nuclear characteristics coupled with feasibility of its large-scale production made this radioisotope a good choice for palliative care of painful skeletal metastases. Presently, ~185 GBq (~5 Ci) ^{153}Sm is produced fortnightly via ^{152}Sm(n,γ)^{153}Sm route for formulation of ^{153}Sm-EDTMP (EDTMP = ethylenediaminetetramethylene phosphonic acid) radiopharmaceutical for its use in palliative care of cancer patients suffering for painful skeletal metastases. For this, isotopically enriched Sm_2O_3 target (typically 98.6% in ^{152}Sm) is irradiated in Dhruva for 3-4 days at a thermal neutron flux of 1.4×10^{14} n/cm^2/s. The irradiated target is radiochemicaly converted to [^{153}Sm]$SmCl_3$ for further use. Typical specific activity of ^{153}Sm obtained is ~ 44 GBq/mg (~1.2 Ci/mg) at the end of irradiation.

Carrier-added ^{177}Lu: Lutetium-177 decays to stable ^{177}Hf with a half-life of 6.65 d by emission of β^- particles having E_{max} of 497 keV (78.6%), 384 keV (9.1%) and 176 keV (12.2%). The emission of low-energy gamma photons [E_γ=113 keV (6.6%), 208 keV (11%)] enable imaging and therapy with the same radiolabeled preparation and allow dosimetry to be performed before and during treatment as well. Two different strategies, namely, (i) direct thermal neutron activation of enriched (in ^{176}Lu) lutetium target and (ii) thermal

neutron activation enriched (in ^{176}Yb) ytterbium target leading to the formation of ^{177}Lu from the β^- decay of the short-lived activation product ^{177}Yb ($T_{½}$ = 1.9 h) could be utilized to produce ^{177}Lu [10]. The direct (n,γ) route offers large-scale ^{177}Lu production with specific activity adequate for targeted tumor therapy in nuclear reactors having thermal neutron flux of ~1.0×10^{14} n/cm^2/s or higher using enriched target (70% or more in ^{176}Lu). This is the least intricate route to access ^{177}Lu in the desired chemical form with minimum generation of radioactive waste, apart from being inexpensive [10]. In order to tap the potential of (n,γ)^{177}Lu production method for application of ^{177}Lu in the preparation of receptor-specific therapeutic radiopharmaceuticals, we have worked on feasibility of producing ^{177}Lu in adequate specific activity and in requisite purity by careful optimization of the irradiation parameters [10]. Radiopharmaceuticals Division is currently supplying 1110-1480 GBq (30-40 Ci) of radiopharmaceutical grade [^{177}Lu]$LuCl_3$ radiochemical every week to the leading nuclear medicine centers across India. The specific activity of indigenously produced ^{177}Lu available every week at nuclear medicine clinics in India is around ~740 GBq/mg (20 Ci/mg), considering the decay loss of 48 h during transit [10].

^{90}Y: Yttrium-90 [$T_{½}$ = 64.1 h, $E_{\beta(max)}$ = 2.28 MeV] is a pure β^- particle emitting radionuclide with well-established applications in targeted therapy. Yttrium has a relatively simple chemistry and its suitability for forming complexes with a variety of chelating agents is well established. The ^{90}Sr/^{90}Y generator is an ideal source for the long-term continuous availability of NCA ^{90}Y suitable for the preparation of radiopharmaceuticals for radionuclide therapy [11]. Fuel Reprocessing Division, BARC has developed the technology for the bulk separation of ^{90}Sr in pure from Cs-lean high-level waste (HLW) by solvent extraction using Tetra Ethyl Hexyl Di-GlycoAmide (TEHDGA) in IDA-Dodecane [12]. The recovered ^{90}Sr is used for milking out ^{90}Y [13]. An indigenously developed two-stage supported liquid membrane (SLM) based ^{90}Sr/^{90}Y generator system is utilized for milking NCA ^{90}Y, the quality of which is permissible as per the European Pharmacopeia [12, 13].

Apart from sourcing ^{90}Y from the SLM based ^{90}Sr/^{90}Y generator, the suitability of low specific activity ^{90}Y produced by ^{89}Y (n, γ) ^{90}Y route in in Dhruva reactor has been successfully demonstrated by our group in the treatment of arthritis of knee joints in the form of ^{90}Y-labeled hydroxyapatite (HA) microparticles [14]. Yttrium-90 is routinely produced by this route once in a month and supplied as [^{90}Y]YCl_3.

Emerging radionuclides

No-carrier-added ^{177}Lu: No-carrier-added (NCA) ^{177}Lu produced via ^{176}Yb(n,γ)^{177}Tb → ^{177}Lu route is becoming increasingly popular in the practice of targeted radionuclide tumor therapy. An electrochemical separation procedure based on selective electroamalgamation of Yb and purification procedure based on deposition of ^{177}Lu on a platinum electrode was developed by our group to obtain NCA ^{177}Lu in a clinically usable form [15]. Overall yield of the radiochemical separation and purification process developed was > 70% and it was reproducible in multiple batches. The effective specific activity of NCA ^{177}Lu was ~3.0 TBq/mg (80 Ci/mg). The feasibility of recovery of enriched (in ^{176}Yb) target in chemical form suitable for reuse in the production of a fresh batch of NCA ^{177}Lu was demonstrated, which makes the process economically viable.

^{166}Ho: Holmium-166 [$T_{1/2}$ = 26.9 h, $E_{\beta(max)}$ = 1.85 MeV, E_γ = 81 keV (6.4%)] also holds good promise as a therapeutic radioisotope in India, thanks to the feasibility of its large-scale production via ^{165}Ho (n, γ) ^{166}Ho reaction with adequate specific activity. Holmium-166 is produced with a specific activity of ~14.8 GBq/mg (~400 mCi/mg) at the end of irradiation in Dhruva reactor and its possible utility in the treatment of liver cancer has been evaluated [16].

^{47}Sc: Scandium-47 [$T_{½}$ = 3.35 d, $E_{\beta(max)}$ = 600 keV, Eγ = 159 keV] is another relatively new radioisotope that holds tremendous potential for use in cancer theranostics. The radioisotope can be produced in a research reactor by thermal neutron irradiation of enriched ^{46}Ca producing ^{47}Ca, which decays by β^- emission to ^{47}Sc. Our group has developed a viable method based on the selective electroamalgamation of Ca^{2+} ions for the clinical-scale separation of NCA ^{47}Sc from ^{47}Ca [17]. The overall yield of ^{47}Sc after the separation process was >80% and it was obtained with >99.9% radionuclidic purity in a form suitable for radiopharmaceutical preparation.

Clinical advances in therapeutic radiopharmaceuticals developed in India over the last two decades

Over the last two decades, a number of therapeutic radiopharmaceuticals have been developed in India, some of which have been translated to the clinic. Targeting ligands such as peptides, monoclonal antibodies, phosphonates,

small molecules, etc. have been evaluated as potential agents for targeted radiotherapy. An outline of the major research efforts from India that have been translated to the nuclear medicine clinic for potential applications in targeted therapy is given below.

Somatostatin receptor targeting therapeutic radiopharmaceuticals

Somatostatin receptors (sstr) are typically overexpressed in a variety of tumors of neuroendocrine origin in the pancreas, lung, intestine and thyroid [18]. Over the last two decades, there has been concerted research in the field of sstr targeting in nuclear medicine for both diagnostic and therapeutic indications [19, 20]. Five subtypes of somatostatin receptors (sstr1-sstr5) have been identified and cloned, out of which sstr2 receptors are predominantly expressed in neuroendocrine tumors (NETs) [21]. In this regard, ^{177}Lu-labeled 1,4,7,10-tetraazacyclododecane-N^{I},N^{II},N^{III},N^{IIII}-tetraacetic acid (DOTA)-coupled sstr analog (D)Tyr_3-octreotate (DOTATATE) has been established as the most extensively used therapeutic agent in the management of patients with inoperable or metastatic NETs [22]. The chemical structure of [^{177}Lu]Lu-DOTATATE is shown in Figure 2. This radiopharmaceutical is able to treat tumors and their metastases via internalization through sstr2, generally overexpressed on the cell membrane. This radiopharmaceutical has evolved during early 2000 and has been introduced in human clinical use in India 2006.

Till date, [^{177}Lu]Lu-DOTATATE is one the most extensively used therapeutic radiopharmaceutical in India. Significant volume of clinical studies with [^{177}Lu]Lu-DOTATATE have been performed during the last decade using medium specific activity ^{177}Lu produced by direct neutron activation route [10, 23]. Recently, our group prepared therapeutically relevant doses of [^{177}Lu]Lu-DOTATATE using

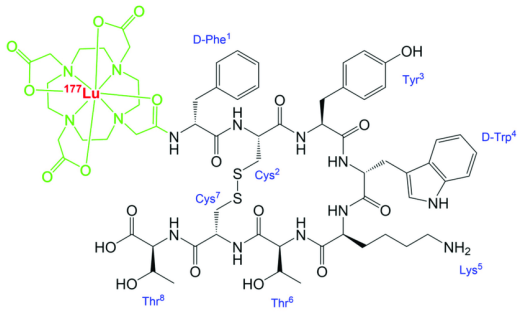


Figure 2. Chemical structure of [^{177}Lu]Lu-DOTATATE

electrochemically separated NCA ^{177}Lu [15]. The radiopharmaceutical was administered in patients with proven NET. A typical whole body SPECT image of a 67-y old male patient acquired at 4 h post-injection of 7.4 GBq dose of [^{177}Lu]Lu-DOTATATE is shown in Figure 3. The SPECT image shows selective accumulation of the radiopharmaceutical at targeted cancerous sites marked with arrows. No uptake of radioactivity was observed in skeleton which proved the *in vivo* stability of the radiolabeled agent. A systematic clinical and dosimetry study carried out in India showed that absorbed dose in tumor lesion and normal organs from the multiple cycle treatment in patients with metastatic neuroendocrine tumors using [^{177}Lu]Lu-DOTA-TATE from carrier added and no carrier added (NCA) ^{177}Lu are comparable [24].

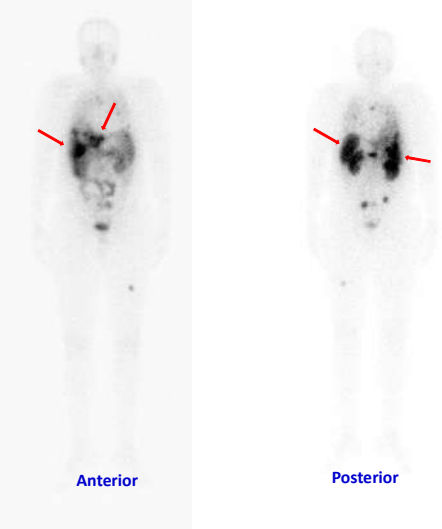


Figure 3. Typical SPECT image of a patient intravenously administered with 7.4 GBq of [^{177}Lu]Lu-DOTA-TATE, prepared using NCA ^{177}Lu separated by electrochemical route, at 4 h post-injection. Reproduced from [15]. Copyright Elsevier.

Off late, it has been observed that [^{177}Lu]Lu-DOTATATE therapy alone has lesser potential in the clinical setting of NETs with large bulky disease and nonhomogeneous sstr distribution, owing to lower energy ($E_{\beta max}$ = 497 KeV) and a shorter particle penetration range (maximum 2–4 mm) of ^{177}Lu. In large bulky NETs, ^{90}Y has the theoretical advantages because of a longer beta particle penetration range (a maximum soft tissue penetration of 11 mm) [25]. Therefore, a combination of ^{177}Lu and ^{90}Y is a theoretically sound concept that can result in better response in metastatic NET with large-bulky lesion and non-homogeneous SSTR distribution [25].

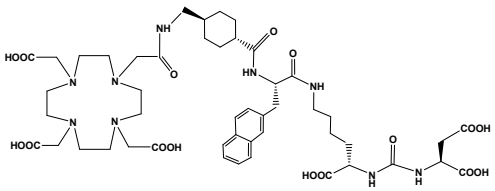


Figure 4. Structure of PSMA-617

Prostate specific membrane antigen targeting therapeutic radiopharmaceutical

Prostate cancer is among the most common malignancies in men globally, with 10-20% of the cases progressing to metastatic castration resistant prostate cancer (mCRPC). Approximately, 90% of mCRPC patients present with bone metastases causing severe pain, stress fractures, poor quality of life and morbidity. A variety of therapeutic procedures such as chemotherapy, androgen deprivation therapy and immunotherapy have conventionally been proposed in the treatment of mCRPC. However, very few therapeutic options are available for the patients who have the disease that progresses with steadily increasing serum prostate specific antigen (PSA) level. Especially for such patients, prostate specific membrane antigen (PSMA), a type II transmembrane glycoprotein overexpressed in prostate cancer, is an excellent target in the imaging and therapy of mCRPC [7, 26]. Several urea based peptidomimmetic agents have been reported for targeting PSMA, out of which the commercially available PSMA-617 (Figure 4) has received widespread attention for radiolabeling with ^{177}Lu [7, 26].

In fact, ^{177}Lu-PSMA-617 therapy which evolved within a very short span of time in the last decade has revolutionized the radionuclide therapy of mCRPC [27]. The growth of this radiopharmaceutical has been so rapid and huge that it has overtaken [^{177}Lu]Lu-DOTATATE as the most widely used ^{177}Lu-based radiopharmaceutical in clinical context. Utilizing medium specific activity ^{177}Lu produced by direct neutron activation route in medium flux research reactor, several clinical studies have successfully been performed by various groups in India, a typical example of which is provided in Figure 5 [28].

Radiopharmaceuticals of radioimmunotherapy (RIT)

Over the last several years, radiolabeled monoclonal antibodies are being increasingly used for personalized management of various types of cancer. Lutetium-177 is an important radioisotope in this regard as its relatively long half-life matches

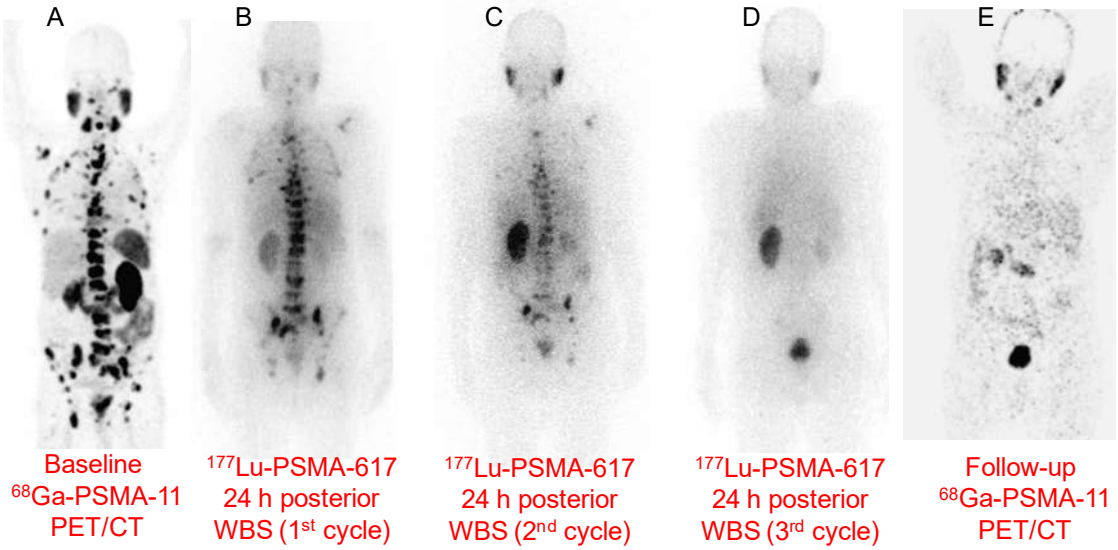


Figure 5. [^{177}Lu]Lu-PSMA-617 therapy in a 65-year-old man with mCRPC. (A) The baseline pre-therapy diagnostic [^{68}Ga]Ga-PSMA-11 PET/CT showed PSMA-avid extensive skeletal metastases, (B) Posterior whole body scintigraphy (WBS) 24 h after administration of first cycle of [^{177}Lu]Lu-PSMA-617, (C) Posterior WBS 24 h after administration of second cycle of [^{177}Lu]Lu-PSMA-617 showed remarkable reduction in uptake, (D) Posterior WBS 24 h after administration of third cycle of [^{177}Lu]Lu-PSMA-617 did not show any abnormal uptake, (E) After three cycles of therapy, the follow-up diagnostic [^{68}Ga]Ga-PSMA-11 PET/CT scan showed near complete metabolic response with resolution of the PSMA-avid metastases. Reproduced from [28]. Copyright Springer-Nature.

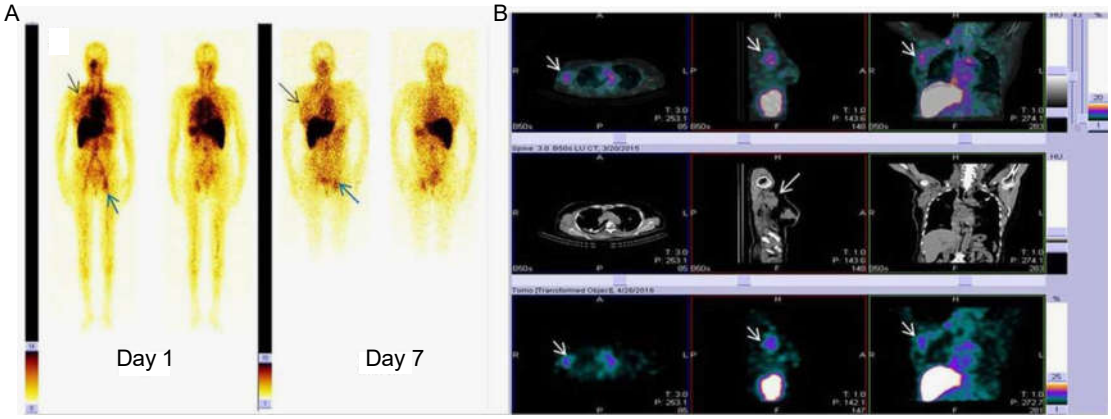


Figure 6. A 60-year-old breast cancer patient (HER2 +ve) was administered with [^{177}Lu]Lu-DOTA-trastuzumab therapy. (A) Whole body scan (WBS) at day 1 and day 7 post administration of [^{177}Lu]Lu-DOTA-trastuzumab. Tracer uptake can be observed in primary breast tumor (black arrow head). The bone metastasis in the acetabulum region was visualized at day 1 and day 7 (blue arrow heads). (B) SPECT/CT, CT, and SPECT images showing lymph node metastases which could not be localized on WBS (white arrow heads). Reproduced with permission from Wiley [30]

the pharmacokinetics of the antibodies. The first systematic clinical study with ^{177}Lu-labeled monoclonal antibody using low specific activity ^{177}Lu in India was reported by Yadav et al, wherein the authors studied the dosimetry of [^{177}Lu]Lu-DOTA-rituximab in 10 patients with relapsed/refractory non-Hodgkin's lymphoma [29]. In this study, rituximab (375 mg/m^2), followed by 1.85 GBq of [^{177}Lu]Lu-DOTA-rituximab was administered as a slow intravenous infusion and SPECT images were acquired and internal dose estimation was performed using OLINDA software. In another study, Bhusari et al formulated clinically relevant doses of [^{177}Lu]Lu-DOTA-trastuzumab and performed feasibility of radioimmunotherapy assessment in breast cancer patients [30]. The patient studies showed the localization of [^{177}Lu]Lu-DOTA-trastuzumab at primary as well as metastatic sites (HER2 positive) in the planar and SPECT/CT images (Figure 6). No tracer uptake was observed in HER2 negative patients that indicated the specificity of the radioimmunoconjugate (**Figure 6**).

Integrin $\alpha_v\beta_3$ targeting therapeutic radiopharmaceuticals

Integrin $\alpha_v\beta_3$, which is a receptor for the extracellular matrix proteins with the exposed arginine(R)-glycine(G)-aspartic acid(D) tripeptide sequence, plays an important role in angiogenesis during tumor growth and metastasis. For both early detection as well as treatment of rapidly growing solid tumors, the over-expression of integrin $\alpha_v\beta_3$ presents an interesting molecular target. Luna-Gutierrez et al [31] first reported the preparation and biological evaluation of ^{177}Lu-labeled cyclic RGD peptide derivatives in animal model. Realizing the scope of these agents targeted tumor therapy, our group optimized the protocol for formulation of clinically relevant doses of ^{177}Lu-labeled DOTA-coupled dimeric cyclic RGD peptide derivative E[c(RGDfK)]$_2$ (E = glutamic acid) (Figure 7) using medium specific activity ^{177}Lu produced via direct neutron activation route and demonstrated its potential in pre-clinical setting [32].

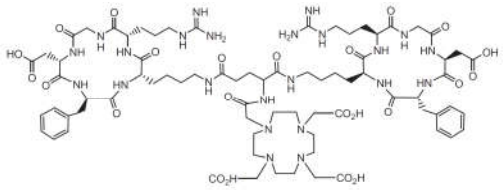


Figure 7. Structure of DOTA-E[c(RGDfK)]$_2$

Later, Parihar et al demonstrated the utility of [^{177}Lu]Lu-DOTA-E[c(RGDfK)]$_2$ in treatment of patients with differentiated thyroid carcinoma and presenting with thyroglobulin elevation with negative ^{131}I scintigraphy (TENIS) [33]. In fact, TENIS in thyroid cancer patients causes a serious management challenge due to limited treatment modalities. Recently, RGD peptides have been identified as effective agents for this purpose due to overexpression of integrin $\alpha_v\beta_3$ in differentiated thyroid cancer [33]. The authors reported the case of a 54-year-old female patient with papillary thyroid cancer who developed TENIS syndrome

after receiving 500 GBq of ^{131}I in cumulative doses. The patient experienced considerable adverse effects with hardly any clinical improvement on sorafenib therapy for 1 year. After [^{68}Ga]Ga-DOTA-E[c(RGDfK)]$_2$ PET/CT scan for evaluating the extent of the disease and pre-therapy assessment, she was administered with 5.5 GBq of [^{177}Lu]Lu-DOTA-E[c(RGDfK)]$_2$. Post-therapy follow-up (Figure 8) showed significant pain relief and reduced pre-sternal swelling, suggesting clinical benefit. At 4 months post-therapy, [^{68}Ga]Ga-DOTA-E[c(RGDfK)]$_2$ PET/CT scan showed reduced uptake in the cancerous lesions indicating clinical benefit (Figure 8).

Therapeutic radiopharmaceuticals targeting fibroblast activation protein (FAP)

The conventional approaches in radiopharmaceutical therapy have predominantly focused on tumor cells. Nowadays, it is increasingly recognized that the tumor stroma, a critical component of the tumor microenvironment (TME), plays a vital role in cancer development and progression. The tumor stroma is composed of cancer associated fibroblasts (CAFs), the extracellular matrix (ECM), various types of immune cells and tangled blood vessels. Among them, CAFs are the most important drivers of stromal interactions which can lead to tissue remodeling, tumorigenesis, tumor stiffness, disease progression, metastasis, modulating the immune response and treatment resistance formation. A key tool in the pro-tumorigenic role of CAFs is the fibroblast activation protein (FAP), which is a type II transmembrane serine protease that cleaves peptide hormones. Generally, FAP is overexpressed on the CAFs of over 90% of epithelial tumors such as breast, colorectal, head and neck, lung, ovarian, and pancreatic adenocarcinomas. Owing to limited FAP expression in normal tissues, it has been identified as a 'pan-tumoral' target for the molecular imaging and targeted therapy of cancer. Several FAP targeting radiolabeled small molecules have been designed and developed for use in nuclear medicine [34]. Among these, a series of quinoline-based FAP inhibitors (FAPIs) showed unprecedented tumor-to-organ selectivity in SPECT/PET imaging [34].

While the potential of these radiolabeled FAPIs as SPECT/PET imaging agents is incontestable, their suitability for therapy is impaired by their short tumor retention leading to suboptimal radiation doses to the tumor. For FAP-targeted radionuclide therapy, an evolving synthetic approach is to directly modify the molecular structure of FAPI ligands to enhance tumor uptake and prolong retention while preferably minimizing the accumulation in non-target tissues [35]. In the past, several molecular modification strategies have been developed to attain sustained accumulation of radiolabeled FAPI ligands in tumor leading to success of the treatment. The first approach is multimerization of high-affinity FAPI ligands to enhance residence time in FAP-positive tumors. Such as, dimeric FAPI ligands offer better chances of rebinding to their target, with slower off-rates than are seen with their monovalent counterparts [35]. Another promising strategy is to prolong the blood circulation of the radioligand by introduction of albumin-binder moieties for improving the tumor uptake and retention of

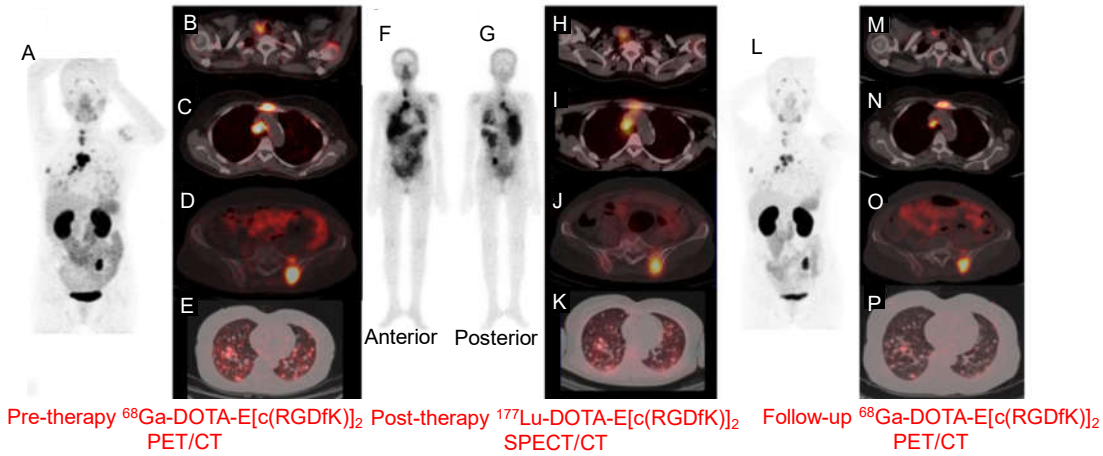


Figure 8. A 54-year-old woman with papillary thyroid carcinoma who developed TENIS syndrome after receiving 500 GBq of ^{131}I in cumulative doses, was administered with [^{177}Lu]Lu-DOTA-E[(cRGDfK)$_2$] therapy. [^{68}Ga]Ga-DOTA- E[(cRGDfK)$_2$] PET/CT was performed to evaluate disease extent and for pre-therapy assessment. Reproduced with permission from Springer-Nature [33]

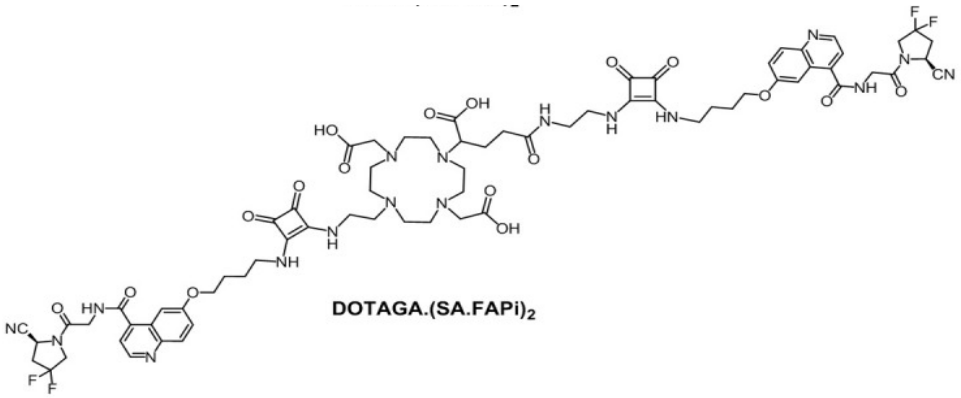


Figure 9. Structure of DOTAGA.(SA.FAPI)$_2$

radiopharmaceuticals [35]. Some of these engineered FAPI ligands are currently being investigated in and clinical settings as radiopharmaceutical therapy agents. In this endeavor, All India Institute of Medical Sciences, New Delhi in collaboration with Johannes-Gutenberg University, Mainz, Germany has evaluated ^{177}Lu-labeled modified FAP targeting ligand DOTAGA.SA.FAPI and its homodimeric counterpart DOTAGA.(SA.FAPI)$_2$ (Figure 9) in human patients [36]. The study revealed that [^{177}Lu]Lu-DOTAGA.(SA.FAPI)$_2$ had significantly longer median whole-body effective half-life (Figure 10) compared to that of [^{177}Lu]Lu-DOTA.SA.FAPI. The clinical dosimetry study demonstrated significantly higher tumor absorbed doses with [^{177}Lu]Lu-DOTAGA.(SA.FAPI)$_2$ compared to [^{177}Lu]Lu-DOTA.SA.FAPI [36]. Overall, it was concluded that [^{177}Lu]Lu-DOTAGA.(SA.FAPI)$_2$ is safe to treat various end-stage cancer patients.

Bone metastases targeting therapeutic radiopharmaceuticals

Bone is one of the common sites of metastases from various types of cancers, which include that of prostate, breast, lung, thyroid, lymphomas and sarcomas. Of these, primary cancers of prostate, breast and lung account for >80 % cases of bone metastases. These metastatic lesions on bone surface have huge impact in the quality of life of those patients, causing complications such as severe bone pain, pathologic fractures, and hypercalcemia. The management of bone pain involves a multidisciplinary approach involving systemic and nonsystemic treatments. However, many treatment options have limitations in their efficacy and duration. Some of them manifest significant adverse effects that seriously compromise the quality of life of the cancer patients. Over the last few decades, radiopharmaceuticals are being used widely as an alternate method for palliative care of metastatic bone pain [37]. In this regard, ^{153}Sm/^{177}Lu-EDTMP and ^{177}Lu-DOTMP have been clinically established as effective radiopharmaceuticals for bone pain palliation. A strategy which has recently evolved is to form well-defined and highly stable complex by keeping the bisphosphonate moiety out of the radiometal chelating framework and using the conventional bifunctional chelating approach for

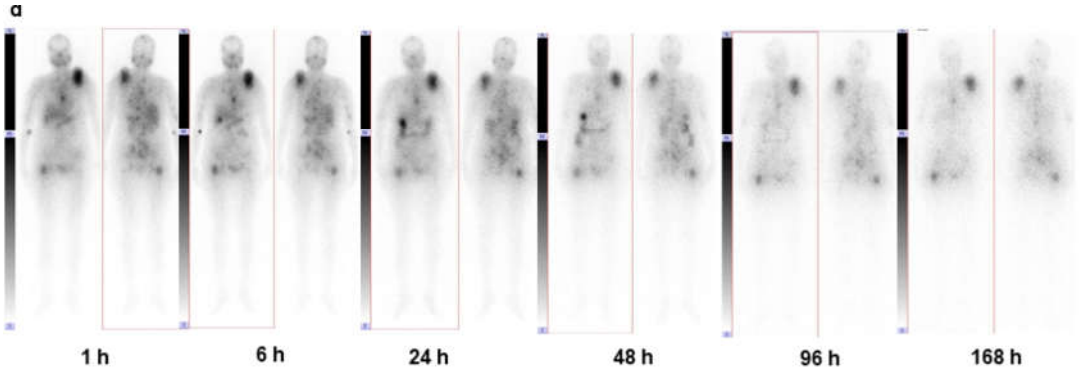


Figure 10. Serial [^{177}Lu]Lu-DOTAGA.(SA.FAPI)$_2$ whole-body scintigraphy images for dosimetry, after intravenous injection of 1.48 GBq of radiotracer, showing radiotracer retention in the metastatic sites until 168 h delayed images. Reproduced from [36]. Copyright MDPI.

complexation with radiometals such as, ^{68}Ga, ^{153}Sm and ^{177}Lu. In this regard, a new macrocyclic bisphosphonate amide of DOTA or BPAMD (Figure 11) has been developed and studied extensively in the form of its ^{177}Lu-compex. which guarantees

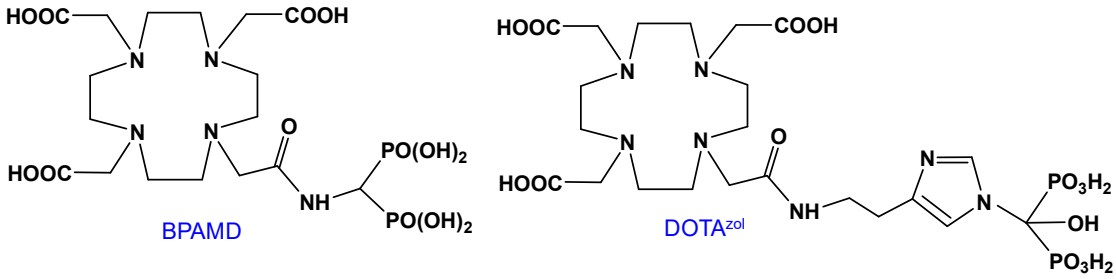


Figure 11. Structures of (A) BPAMD, (B) DOTA^{zol}

^{177}Lu-complexation with high thermodynamic stability and appreciable kinetic rigidity *in vivo* [38]. Consequently, BPAMD concentration in the radiopharmaceutical could be significantly lowered which aided towards targeting micrometastatic sites, resulting in enhanced therapeutic efficacy. Our group has developed a protocol for formulation of therapeutically relevant dose of [^{177}Lu]Lu-BPAMD using medium specific activity ^{177}Lu produced by direct ^{176}Lu (n,γ) ^{177}Lu reaction [39]. After establishing the efficacy of the product in preclinical settings, preliminary clinical investigations were carried out in limited number of patients with metastatic bone pain. Intense uptake of the radiopharmaceutical was observed in the metastatic skeletal lesions with insignificant uptake in any other major non-targeted organs. In all the patients, considerable reduction in pain was observed after one week without any adverse effects.

Despite promising clinical results obtained in palliative radiotherapy using [^{177}Lu]Lu-BPAMD, there is still much potential for improvement with regard to further enhancement of accumulation of the radiolabeled agent in bone metastases and minimizing the uptake in non-targeted organs. In this regard, the side chains on the central carbon atom of the bisphosphonate moiety play a significant role in the bisphosphonate's activity, i.e., in terms of affinity to hydroxyapatite (the constituent of bone). It has been found that an aromatic nitrogen atom in the side chain could cause building of another hydrogen bond and thereby also raise bone accumulation [97, 100]. One such bisphosphonate is zoledronic acid, which has also been found to influence biochemical processes. This molecule has been conjugated with chelator DOTA (DOTA^{zol}) (Figure 11). Yadav et al evaluated the safety and efficacy of [^{177}Lu]Lu-DOTA^{zol} as bone pain palliation agent in 40 patients suffering from bone metastasis due to variety of cancers [101]. The patients were treated with either 1 or 2 cycles of [^{177}Lu]Lu-DOTA^{zol}. The biodistribution and uptake of [^{177}Lu]Lu-DOTA^{zol} in a patient with skeletal metastases from prostate cancer is shown in Figure 12. Satisfactory treatment responses were observed in all the patients.

Radiopharmaceuticals for liver cancer therapy

Primary as well as metastatic liver malignancies are among the most prevalent causes of cancer related deaths worldwide. Among the different types liver malignancies, hepatocellular carcinoma (HCC) is the most frequent and constitutes 90% of cancers of liver globally. Selective internal radiation therapy (SIRT) using a suitable β^- emitting radionuclide is one of the most promising treatment modality of unresectable liver carcinoma. Intrinsically [^{90}Y]Y-labeled glass microsphere formulation is the most widely used radiotherapeutic agent for SIRT and is sourced from a commercial manufacturers in the name of TheraSphere® [42]. It is a radiochemical formulation consisting of millions of non-biodegradable glass microspheres, having diameter in the range of 20-35 µm, in sterile physiological saline and in which ^{90}Y is an integral constituent of the glass. For SIRT, [^{90}Y]Yttrium-glass microspheres are administered via intra-arterial route through hepatic artery. Malignant liver tumors are generally highly vascular and receive the majority of their blood supply from the hepatic artery, compared with liver parenchyma, which receives its blood supply primarily from the portal vein. Therefore, the intra-arterial injection of [^{90}Y]Yttrium glass microspheres can deliver the radioactive substance in local or regional manner, resulting in high radiation doses to tumor while sparing liver parenchyma [42]. Once administered in the hepatic artery, the microspheres preferentially lodge in the vasculature of the malignant hepatic cells delivering cytotoxic doses of ionizing radiation from ^{90}Y. One of the major

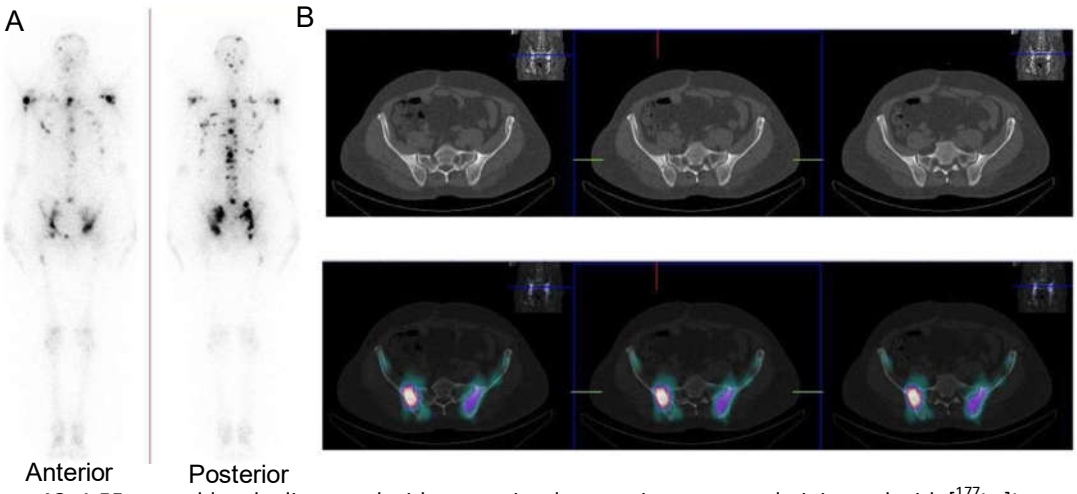
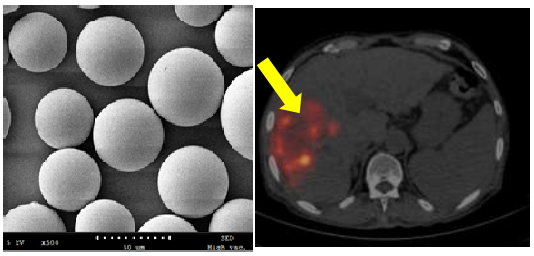


Figure 12. A 55-year-old male diagnosed with prostatic adenocarcinoma was administered with [^{177}Lu]Lu-DOTA^{zol}. (A) Post-therapy WBS 24 h post-administration showed uptake in multiple skeletal sites, (B) Post-therapy SPECT/CT showed uptake in the pelvic bone metastases. Reproduced with permission from Springer-Nature [41].

constraints in the broader utility of [^{90}Y]Yttrium glass microsphere in the treatment of liver cancer is the prohibitively high cost of the dose of commercially available formulation individual patient care. Taking it into consideration, BARC has developed a new [^{90}Y]Yttrium glass microspheres formulation which will be biosimilar to TheraSphere® and can be made available at an affordable cost. Yttria alumino silicate (YAS) glass microspheres having $20Al_2O_3$-$40SiO_2$-$40Y_2O_3$ (w/w) chemical composition and 20-36 µm particle size range have been synthesized following procedure developed indigenously and characterized (Figure 13a). Intrinsically ^{90}Y-labeled glass microspheres ([^{90}Y]YAS) were produced by thermal neutron irradiation of cold microspheres at a suitable thermal neutron flux in Dhruva reactor. [^{90}Y]YAS glass microspheres were produced with a specific activity of 137.7 ± 8.6 MBq/mg of microspheres irradiated, which correspond to ~ 6800 Bq of ^{90}Y radioactivity per microsphere. Radionuclidic purity of the formulations were >99.9%, desirable for human clinical applications [42].

Human clinical investigation of [^{90}Y]YAS glass microsphere formulation was carried out in patients with hepatocellular carcinoma at Tata Memorial Hospital, Mumbai. Customized doses of [^{90}Y]YAS glass microsphere formulation were prepared and administered through right hepatic artery with super selective approach. PET/CT images were recorded at 24 h post administration to ascertain the localization of the glass particles. In vivo PET imaging studies revealed target specific localization and near-complete retention of [^{90}Y]YAS glass microsphere formulation in the liver cancer site as desirable (Figure 13b). Further, there were no adverse side effects reported in the therapeutic procedure using [^{90}Y]YAS glass microsphere dose synthesized in-house [42].

Rhenium-188-labeled lipiodol is another clinically effective, economically viable radiopharmaceutical for SIRT of liver cancer. Freeze-dried kits of acetylated 4-hexadecyl-4,7-diaza-1,10-decanedithiol (AHDD), super six sulfur (SSS), and diethyl dithiocarbamate (DEDC), were used for the



(a) (b)

Figure 13. SEM image of YAS glass microspheres (a) and post-therapy transaxial PET/CT image of a 56 y male patient with right lobe hepatocellular carcinoma 24 h after administration of 3.4 GBq of [^{90}Y]YAS glass microspheres showing near-complete retention of the formulation in the diseased site

preparation of [^{188}Re]Re-lipiodol using freshly eluted ^{188}Re-sodium perrhenate from commercial ^{188}W/^{188}Re generator [43]. Limited clinical trials of [^{188}Re]ReN-DEDC/lipiodol, prepared using these two-vial kits was carried out in Tata Memorial Hospital (TMH), Mumbai, Kovai Medical Centre and Hospital (KMCH), Coimbatore, and recently in

All India Institute of Medical Sciences (AIIMS), New Delhi.

Radiopharmaceuticals for radiation synovectomy

In radiation synovectomy, beta-emitting radionuclides in colloidal or particulate form (1–10 µm size range) are intraarticularly injected into the affected synovial joints. This is an effective treatment modality in patients suffering from inflammatory and degenerative joint diseases [44]. In this treatment modality, the uptake of radionuclides occurs in the synovial lining cells, phagocytized by the outermost cellular layer of the synovial membrane and deliver radiation dose to the synovium sparing the surrounding tissue.

The design and fabrication of porous and biocompatible inorganic materials having high adsorption capacity for therapeutic radioisotopes can trigger development of 'next-generation' radiopharmaceuticals for use in radiation synovectomy. Nanoporous hydroxyapaptite (HA) microspheres of 1-10 µm diameter were synthesized by sol-gel technique followed by spray drying [45]. The synthesized microparticles exhibited remarkably high sorption capacity for ^{90}Y (141 ± 5 mg Y/mg) and ^{166}Ho (262 ± 3 mg Ho/mg), which enabled the use of low specific activity radioisotopes produced in medium flux research reactors. ^{90}Y labeled nanoporous HA formulation was administered in a patient with painful arthritis of knee joint. SPECT/CT image of the knee recorded after 1 h p.i. (Figure 14), showed diffused distribution of the radiopharmaceutical in the joint and its selective retention within the synovial cavity of the knee joint. The leakage of the radiolabeled formulation from the patient's knee was investigated by serial whole body SPECT scans recorded up to 7 d post-injection of [^{90}Y]Y-HA. The SPECT images did not show accumulation of radioactivity into any non-targeted organs, including liver, spleen and bones. Preliminary assessment of therapeutic efficacy of [^{90}Y]Y-HA carried out over a period of 1 month based on the information from the patient showed considerable improvement in the disease conditions such as, reduction in joint effusion, local pain and enhancement in the range of motion.

Apart from [^{90}Y]Y-HA, our group has also developed ready-to-use formulation ^{177}Lu-labeled hydroxyapatite microparticles ([^{177}Lu]Lu-HA) for treatment of inflammatory joint diseases of medium sized joints such as elbow and wrist. The developed formulation is currently in routine clinical use in India and significant improvement in disease condition was observed in patients treated with the formulation.

Conclusions and future directions

Radiopharmaceutical therapy can be considered as a safe and effective targeted approach to treating many types of cancer. This treatment modality has shown high efficacy with minimal toxicity compared to other systemic cancer treatment options. Different radioligands can be chosen to uniquely target molecular receptors or intracellular components, making them suitable for personalized therapy of cancer patients. Further research is still needed regarding specific targets, radioligand stability in vivo, toxic effects, crossfire, dosimetry, and bond stability with daughter nuclides. Targeted alpha therapy (TAT) has brought a new revolution in nuclear medicine. Hence, there is a great demand for α-emitters for design of TAT radiopharmaceuticals for affordable clinical use. There is also great demand for generator systems such as ^{90}Sr/^{90}Y generator that are presently not available. Therapy of relatively large sized malignant tumors would be greatly facilitated with the availability of ^{90}Y-based radiopharmaceuticals. Radiolabeling methods for preparation of TAT radiopharmaceuticals need improvements as to the in vitro and in vivo stability of the label, rapidity of the procedures and effective targeting. More radiochemistry optimization will be involved in attaching bifunctional groups to molecules, α-emitting radionuclides and their daughter products in order to secure the radiolabel and ensure localization in target tissues. In this endeavor, emerging new particle drug delivery systems, including the use of nanoparticles would continue to enhance efficacy and safety of targeted therapy. With a growing

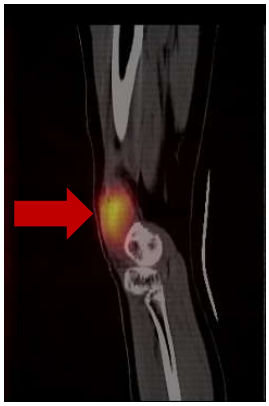


Figure 14. SPECT/CT image of arthritis affected knee joint recorded 1 h after administration of ^{90}Y-HA.

positive track record, public understanding and perception of the safety and success of radiopharmaceutical therapy is definitely going to improve in our country. If this occurs, then radiopharmaceutical therapy will be adopted as an increasingly mainstream cancer therapy approach and the investment needed to resolve issues of radionuclides supply. The planned new research reactor by the Department of Atomic Energy (DAE) exclusively for radioisotope production in India shall bring a paradigm shift in the super specialty of nuclear medicine. In the coming year, therapeutic radiopharmaceuticals will provide an increasing variety of rapid, personalized, practical, effective, and affordable treatments offering new hope to thousands of cancer patients in our country.

Acknowledgements

The authors are grateful to Dr. P.K. Mohapatra, former Director, Radiochemistry and Isotope Group, BARC and Dr. Tapas Das, Head, Radiopharmaceuticals Division, BARC for their encouragement and support. The authors would also like to place on record their sincere gratitude towards the contribution of all the colleagues from Radiopharmaceuticals Division, BARC, radiopharmacists and nuclear medicine physicians from different institutions in India whose works have been summarized in this chapter.

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Chapter 4
Pre-clinical evaluation of radiopharmaceuticals

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Abstract: *As with all other drugs, a robust pre-clinical program for biological evaluation of radiopharmaceuticals is essential before they enter clinical trials. Such a program involves in vitro studies (performed in cell culture / tissue samples / fluids) and animal testing (ex-vivo / in vivo). These modes are complementary and jointly generate useful information about the target affinity, target specificity, physiological distribution, pharmacokinetics, metabolism, efficacy and toxicity of the tested formulation. A well-designed pre-clinical development workflow generates the necessary data to obtain regulatory approval for clinical studies and eventual translation to use in patients, taking into account all ethical and financial considerations. This article offers an easy-to-understand overview of the goals, principles, key factors, methodologies, and expected results from a pre-clinical evaluation program.*

Keywords: Radiopharmaceuticals, Pre-clinical evaluation, animal testing, nuclear medicine

Introduction

Radiopharmaceuticals are used worldwide for diagnostic, therapeutic and disease monitoring purposes in nuclear medicine practice. With improved understanding of the molecular basis of disease initiation and progression, it is possible to develop more precise and personalized formulations. Also, there is greater focus now on therapeutic radiopharmaceuticals, which impact the physiological status of the patient.

For all drug formulations, including radiopharmaceuticals, the laws governing the conduct of clinical studies in human patients/ volunteers are justifiably strict. Moreover, the technical, financial and personnel resources required to conduct clinical trials in the prescribed manner are significant: Patient screening and enrolment into study groups, rigorous testing protocols, follow-up measures, data collection and analysis of results – sometimes from multiple centers – must be performed in compliance with the prevailing scientific, ethical and legal norms. A failure at the stage of clinical assessment represents a massive waste of the efforts and resources poured into this exercise.

It therefore makes sense that prior to clinical testing; all potential new ligands / formulations must undergo rigorous pre-clinical screening to identify the best performing candidates, worthy of clinical translation. Compared to patient studies, pre-clinical exercises performed in the laboratory are more economical, can be performed with a statistically more favorable number of subjects, are less subject to medico-legal restrictions and can provide deeper information about the operating principles and quantifiable efficacy of the formulation.

The design process for any radiopharmaceutical must ensure adherence to the following properties:

- High affinity and selectivity for the target, which translates to rapid uptake and adequate retention in the region of interest.
- Negligible binding to other non-specific components, which leads to minimal uptake and/or quick washout from non-target regions.
- Adequate in vitro and in vivo stability, with minimal presence of redistributing radio-metabolites.
- Minimal unintended toxic or pharmacologic effects.

To obtain regulatory approval to perform clinical studies for a newly developed formulation, a sufficiently compelling case must be made in terms of the findings of the pre-clinical assessment, of the safety and effectiveness of said formulation. Thus, analogous to the necessity of applying a battery of suitable analytical techniques to report on the physico-chemical characteristics of any substance, it is essential to have a comprehensive program of biological testing and characterization in a pre-clinical setting prior to clinical trials in humans.

Pre-clinical biological evaluation studies using cell lines, tissue sections and experimental animals represent a key stage of the process of developing novel radiopharmaceuticals. In pre-clinical bio-evaluation, the parameters tested may include target specificity and pharmacologic impact at the cellular level, physiological distribution pathway and pharmacologic impact within the test animal, metabolism, identification of organs vulnerable to ionizing radiation effects, and estimation of radiation doses that may be incurred during the

clinical studies. Characteristics like radiation dose and pharmacologic effect are especially important in assessment of therapeutic candidates. Additionally, even if most radiopharmaceuticals use dosages far below any possible pharmacological effect of the active biomolecules, toxicity profiles should also be studied in the case of development using new ligands. Conversely, pre-clinical evaluation may also be done for well-established radiopharmaceuticals, for the following reasons:

a. As a routine quality control assessment of such formulations.
b. As a benchmark to assess the performance of a new ligand.
c. As a marker to study the physiological basis of a disease or the effect of a drug.

As depicted in Figure 1, pre-clinical biological evaluation of radiopharmaceuticals falls into two major categories:

a. In vitro Studies – This refers to assays performed outside of a living animal. It includes studies with cultured cells, serum stability and tissue-based assays.

b. Animal Testing – In this case, the radiopharmaceutical formulation is administered inside a living animal for assessment of its behavior. Animal studies may be further classified as:

i) Ex-vivo – After administration of the formulation, the animals are sacrificed in the course of study and dissected to remove relevant organs/tissues. Blood and other body fluids may also be extracted for analyses. Measurements are carried out in the excised / extracted samples.

ii) In vivo – Both administration and real-time monitoring of the pathway of the radiopharmaceutical formulation is performed within a living animal. This requires the availability of scintigraphic imaging instruments.

Several properties can be tested across both in vitro and animal model studies. For example, affinity and specificity of the radiopharmaceutical for the target region can be studied in vitro using specific tumour cell lines and in animals bearing those tumours. Metabolism of a radio-formulation may be studied in vitro with extracted plasma/serum and exposure to human liver cells (liver being the major organ of metabolic breakdown of drugs), or by assessment of radio-metabolites in body fluids and tissue extracts post-administration in the living system. Pharmacological impact and/or toxicity may be studied both in vitro (using suitable cell cultures) and in animal models. In these cases, the different modes are not necessarily competitive; they can help to cross-verify inferences and complement each other in providing comprehensive

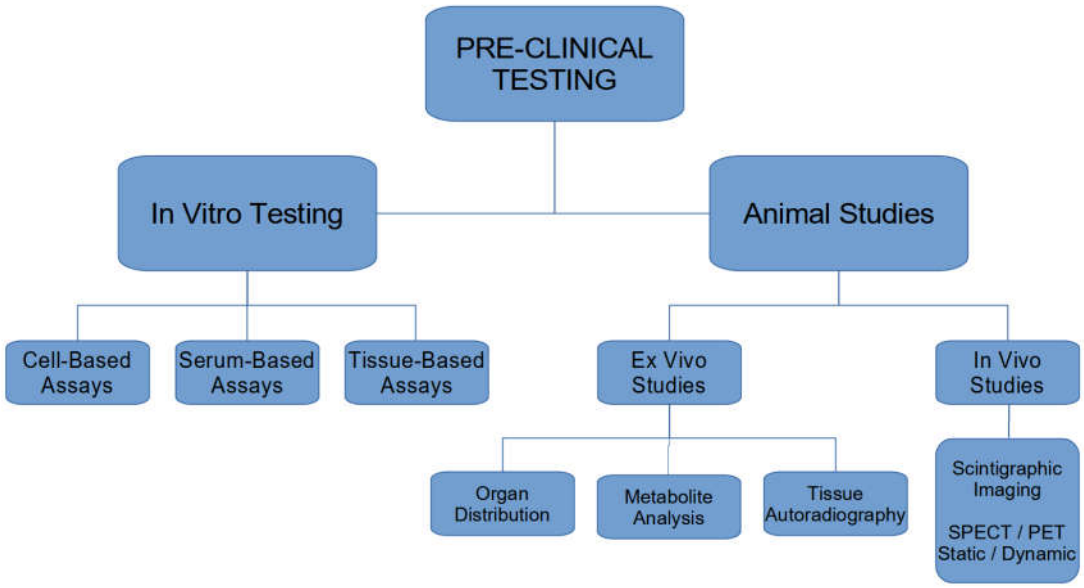

Figure 1. Pre-clinical biological evaluation of radiopharmaceuticals

information not available from individual approaches. Some parameters can be assessed only by specific testing approaches. For instance, mechanistic studies of the action of radiopharmaceuticals require access to an advanced in vitro molecular biology setup, while the distribution and pharmacokinetics of radiolabeled formulations, and dosimetric information need to be assessed using living animals. An all-round radiopharmaceuticals development program will therefore require access to both kinds of facilities.

To achieve optimal results in pre-clinical assessment of radiopharmaceuticals, a sequential workflow of carefully chosen study protocols must be designed, taking into account the nature of the formulation and the intended clinical application. Especially in animal testing, the formulation and testing protocols must mimic to the maximum extent possible, the application in human patients. Complete adherence to compliance with the Good Manufacturing Practices (GMP) vis-a-vis purity and sterility of the formulation is not normally mandatory in pre-clinical testing, but it must at least be ensured that any impurities or deviations at this stage will not interfere with the results of the biological evaluation studies. While assessing the pharmacology/toxicity of therapeutic formulations, this aspect becomes more crucial, and must be strictly adhered to.

In vitro testing

In vitro studies refer to test procedures that are conducted in a controlled environment outside of a living organism. In the current instance, in vitro testing means pre-clinical experiments involving cultured cells / fractionated sub-cellular components, plasma/serum or tissue samples (whole / homogenized). These may be derived from humans or animals. In contrast to ex-vivo studies, no radioactivity is present in the samples at the time of collection. Post-harvesting, the samples will be exposed to radiopharmaceuticals to study specific uptake / response mechanisms.

The main purpose of in vitro testing is to reduce as far as possible the magnitude of animal testing required by way of meticulous screening for lead compounds. Eliminating non-functional ligands helps to focus in identifying potentially hazardous / toxic materials before testing in living animals, where they may cause undue distress.

The following is a list of tests that may be considered a baseline for in vitro assessment of any novel targeted radiopharmaceutical; other tests may be added, based on their relevance to the intended application. Unless otherwise specified, the majority of the tests are common for both diagnostic and therapeutic formulations – in these cases, the term 'radiotracer' simply indicates the use of trace amount of radiolabeled molecule to study its behaviour. Additionally, Therapeutic radiopharmaceuticals must be tested for their pharmacologic impact as well as potential toxicity. In vitro assays may be broadly sorted as:

1. Cell Based Assays
2. Serum Based Assays
3. Assays with Tissue Samples

Cell based assays

Cell based assays account for the majority of in vitro testing protocols. Most in vitro cell studies for radiopharmaceuticals are performed with mammalian cell lines (human/animal origin). For assessment of microbial infection tracers, suitable bacterial cultures are employed. Cell studies may be sub-divided into several types based on the assay design and intended result. The specific sequence of carrying out these assays must be chalked out for optimization of reaction parameters and inferring of the results. Controls in the form of target-negative cell lines are advisable to verify specificity of tracer uptake / binding in the cell line.

a) Cell Uptake Studies

Cell uptake studies evaluate the ability of known concentration of the radiopharmaceutical to associate with a cell, which expresses the target. The total measured uptake is the sum of surface binding and/or internalization of the tracer. Uptake studies help to know if a proposed tracer is taken up in adequate proportion at a viable point in time (or superior to other tracersused for similar purpose) to function as a useful in-organism tracer. Care must be taken to track cell uptake over time, so as to estimate the optimal time interval from administration to accumulation in the region of interest. This will be useful in locking reaction parameters for further assays. While uptake reactions are typically performed at physiological temperature (37 °C), uptake specific to membrane receptor binding may be assessed by carrying out the reaction in refrigerated conditions (4-8 °C) to minimize internalization.

b) Cell Binding Studies

Binding studies evaluate the binding characteristics between a tracer and its target receptor. Estimation of affinity and binding kinetics can provide a quantitative value to assist

in the selection of which tracer to take forward, and what dose needs to be administered. For example, for a tracer to be useful in imaging, the target binding affinity should reach approximately 1 nM or better (since most targets have densities of 10 nM or less). Target affinity of the tracer may be measured directly using saturation assay (in which the concentration of tracer required to saturate the receptor sites is measured) or indirectly by inhibition assay (in which inhibition of the tracer binding by the native ligand is measured); accordingly it may be expressed as the dissociation constant (K_D) or the inhibition constant (K_i), respectively. Selectivity of binding to the target can be assessed by performing competition binding studies with receptors similar to the intended target. Cell uptake studies are usually carried out in whole cells, but binding studies can also be carried out with cell/tissue homogenates (e.g. membrane fractions) or sub-cellular extracts. Special care should be taken to verify that the affinity measurements are conducted at a time point when binding equilibrium has been established. Binding assays conducted using transfected cell lines that over-express specific receptors commonly result in affinity values differing from that of native cell lines and animal / human subjects.

c) Internalization Studies and Intracellular / Sub-cellular Distribution Studies

Internalization assays provide information about the ability of a radiotracer to enter intracellular space – they may quantify the degree of tracer internalization, or describe the mechanism of the process or both. Intracellular distribution studies assess the specific localization of the tracer within the cellular system. In some cases, the evaluation of tracer internalization may help to optimize radionuclide choice (for example, radionuclides retained within the cell are preferred to those that are excreted out) or to assess similarity of the tracer to the parent / native ligand. Internalization and intracellular distribution assays can help understand the mechanism and potential efficacy of radiotracers; this is especially crucial with alpha / beta emitting therapeutic radiopharmaceuticals. The most common internalization assay for radiotracers is cell uptake followed by acid wash. The acid wash solution presumably strips off all surface bound tracer molecules; measurement of remaining cell-associated radioactivity after an acid wash indicates the amount of internalized tracer. The acid wash procedure may however disrupt the cell membrane or may not necessarily release tracer from the membrane surface. Hence, there is a need to validate the suitability of the assay protocol before use.

d) Dissociation Study

Dissociation studies help to evaluate retention time of the tracer within the target cell. This is essentially an extended cell uptake study where, after an initial period of cell uptake, the cells are washed with pure cell culture medium and remaining bound material is quantified over time, often many hours. Cell uptake and cell dissociation can often be combined in one assay. Cell dissociation assays reveal the ability of the tracer to stay associated with the target, after allowing the host organism to clear tracer from the blood stream, for example.

e) Metabolite Analysis

Metabolite analysis helps to assess post-uptake metabolic transformation of the tracer. It may indicate functional similarity of the tracer to the native biomolecule. It also provides insight into the fate of the tracer, especially its post-metabolic release from the cell system, which is important from the point of view of usefulness and safety. The primary concern is with the generation of radio-metabolites, which can carry the radionuclide signal but whose distribution and clearance behaviour differ from the parent tracer. Thus, metabolite analysis also helps to validate the molecular position and specific strategy used to radiolabel a clinically useful ligand. Metabolite analysis of the tracer may be performed by exposing it to the target cells or to liver cells (liver being a major organ of drug metabolism). Either isolated cell suspensions or tissue homogenates may be used for these studies. The time allowed for incubation must be sufficient for metabolic degradation of the tracer. The selected assay (e.g. HPLC, TLC) must be able to differentiate between the tracer and its various metabolites. It must be ensured that metabolites are not generated due to microbial contamination of the suspension or chemical degradation of the tracer. While HPLC may be regarded as the gold standard, TLC protocols may be developed by correlation and used for day-to-day screening. Half-life of the radioisotope will have an impact on the sample preparation and analytical techniques used.

f) In vitro Efficacy Studies

Efficacy studies are applicable for therapeutic radio-formulations, which is performed to test the ability of the radiopharmaceutical to exert therapeutic action on target expressing cells at a relevant concentration. The impact of exposure of the formulations on cells may be measured using a

number of parameters including cell viability, toxicity, markers of apoptosis / programmed cell death. Techniques like flow cytometry can provide in-depth information about the pharmacologic impact of a radiopharmaceutical on the target cell line. Efficacy studies can be used to compare competing therapeutic approaches and even to assess the synergistic benefit of combining two or more approaches. The major challenge for correlation with animal experiments is in estimating the relevant dose that will be deposited on the area of interest.

g) Additional Assays

In addition to the above set of cellular assays, radiopharmaceuticals designed to target regions inside the brain may need to be tested for their ability to penetrate the Blood-Brain Barrier (BBB) and not be removed by efflux pumps like P-glycoprotein. In vitro models are available to test this property and are seen to correlate with real-world observations for BBB penetration of several ligands.

Infrastructure/Considerations for conducting cell-based studies

To perform the above-mentioned assays, a laboratory equipped for tissue culture protocols is a minimum requirement. This is an isolated, aseptic (germ-free) zone housing a laminar airflow cabinet, an incubation chamber for mammalian cells (typically, at 37 °C with 85% relative humidity and an atmosphere containing 5% CO_2), a centrifuge capable of separating whole cells/sub-cellular components and an optical microscope for examination of cells/tissue. Nutrient media, physiological buffers, supplements and other reagents used should be as per the available literature for the specific cell line. These may also need to be tailored as per the study – for example, use of folate-deficient media when studying tracers targeting folate receptors. Facilities propagating and storing cell lines will have additional infrastructure requirements. Detailed insights into the practical aspects of cell culture are widely available and beyond the scope of this monograph.

Serum based assays

These are typically stability/metabolism assays performed in serum or other body fluids, which may be obtained from a human/animal host. Serum stability assay evaluates stability of the tracer in the intended body fluid, most often blood. The objective of the assay is to predict the likelihood that the tracer retains its integrity when administered into the body. It also provides an idea of plasma protein binding of the tracer, which impacts circulation time and availability to the target. Serum stability assay is an important precursor to assess suitability of the developed tracer for animal studies. The same conditions and considerations that govern the conduct of cellular metabolism studies apply here: The time allowed for incubation must be sufficient for metabolic degradation of the tracer (here, it will be also dependent on the possible blood retention time). HPLC is a gold standard for metabolite analysis, but simpler alternatives like thin layer chromatography, solid phase extraction and liquid-liquid extraction may also be developed for day-to-day analysis/QC. The selected assay must be able to differentiate between the tracer and its various metabolites. It must be ensured that metabolites are not generated due to microbial contamination or chemical degradation of the tracer.

Assays with tissue samples (In vitro)

These assays involve assays with histological slices taken from relevant organs/tissues, which are exposed to the radiotracer under study. The distribution of the bound tracer in the tissue can be recorded using autoradiography; it may be correlated with other parameters (histology or immunohistochemistry) to make inferences about the affinity and specificity of tracer binding. This technique is applicable when the targets are membrane bound proteins or insoluble supramolecular aggregates (like amyloid plaques). It is usually performed with slices obtained from snap-frozen tissue, where the native structure of the protein is preserved. It may be noted that, as compared to assays with cell cultures or sub-cellular fractions, a better correlation with living systems is observed in tissue autoradiography of parameters like binding affinity, specificity and binding site density. However, proper calibration standards must be developed to obtain quantitative / semi-quantitative information about radioactivity distribution in tissue sections. The nature of radionuclide emissions, amount of radioactivity retained in the sample and the sensitivity of autoradiography equipment are important factors in the planning of these experiments. Tissue based assays are typically performed using tissue from the same animal species that will be used for subsequent pre-clinical evaluation – this maximizes the likelihood of translation of the in vitro results to the pre-clinical *in vivo* phase. The ultimate goal of radiopharmaceuticals is to be applicable to humans, hence it can also be considered to do histochemical studies with human origin tissue sections.

Autoradiography can either be performed with standard clinical imaging radiotracers or with a β^- /Auger electron emitter labeled analogs (for example ^{3}H instead of ^{11}C, or ^{125}I instead of ^{124}I). ^{3}H is an ideal radioisotope for autoradiography because the low energy of emitted electrons favors high resolution.

Animal testing – an overview

The development protocols described in this section require administration of the radiopharmaceuticals into live animals. With radiopharmaceuticals, the major objective of animal studies is to establish the pharmacokinetic properties of the formulation (distribution and clearance), and where applicable, the therapeutic efficacy of the preparation. For diagnostic radiotracers, the ability of the tracer to bind in vivo with the target and clear out from non-specific regions is paramount to its usefulness. Biodistribution studies may be used to assess novel/in-development radiopharmaceuticals or established tracers may be used as markers to assess in vivo properties of other drugs / substances. Factors to be considered in the experiment design include:

a) Selection of suitable animal model (species, gender, , age, weight, normal / disease model) and preparation of the animal for the study.
b) Match between the half-life of the radioisotope used and the kinetics of uptake / clearance.
c) Dosage and physical form of the preparation, route of administration.
d) Samples to be collected and measurement techniques used.
e) Data analyses and generation of results.

Some of these factors are discussed in the following sections.

Selection of animal species, housing and preparation of animals for experiments

Selection of animal species is based on the specific research question, the physiology of the model in comparison to human physiology, structure and size of the organ/region of interest and its amenability towards generating useful data, the need to generate a specific disease or knockout model, the type and number of measurements to be taken, facility and staff requirements. Like with other kinds of drugs, pre-clinical animal testing of radiopharmaceuticals is primarily done in rodent models (mice/rats) on account of their ease of handling and housing, rapid breeding cycle and availability of suitable disease / transgenic models. In contemporary radiopharmaceuticals research, cancer diagnosis/therapy and neurological studies are the major areas of exploration, and various rodent models are available for these applications. Other animals in which laboratory experiments may be carried out include rabbit, hamster, pig, monkey etc. Larger species offer the advantage that their physiology is often closer to human physiology and behavior, the dosimetry is more closely matched to humans, surgical interventions are simpler, it is possible to obtain larger blood samples for analysis and finally the larger structure sizes can be resolved with commercially available imaging systems. However, the disadvantages include higher costs, more involved handling, size logistics for radioactivity measurements, and finally the ethical concerns. For novel formulations it is advised to test in more than one model/ species to check on universality of the results obtained – this is relevant when attempting to translate the findings of pre-clinical studies to expectations in the clinical scenario.

Experimental animals must be housed in cages in secure and sanitary conditions. The cages should have a comfortable layer of bedding material, capable of absorbing moisture from animal urine and faeces. They should be periodically replaced (every 2-3 days) and sent for cleaning and sterilization between uses. Animals with compromised immune systems, used to generate tumour models of human cancer cell lines, require filter-topped individually ventilated cages to prevent cross-contamination. After induction of disease models and/or administration of radiopharmaceutical formulations, the cages should be tagged accordingly for proper identification during the period of study.

Preparation of the animal for the experiment depends on the type of study being done. For instance, if the study requires a tumor model (by induction or transplantation) or other disease model, then the specific model needs to be generated, following the prescribed protocols for the same. Tumour-bearing animals are the most common disease models in radiopharmaceuticals research. Excellent reviews on generation of animal models for pre-clinical cancer research are available in the literature. Tumor models may be generated by any of the following methods, depending on suitability and the specific research question:

a) Chemical induction using suitable carcinogens
b) By transplantation of tumor cells derived from the same species (syngeneic model). This can

Table 1. Assorted Parameters for Animal Testing of Radiolabeled Formulations

Sr. No.	Parameters	Mice	Rats
1	Typical body weight (at 6-8 weeks)	20-25 g	200-250 g
2	Injection volume (intravenous)	100-150 µL	Up to 1000 µL
3	Anaesthesia dosage:		
	i) Ketamine:Xylazine (intraperitoneal)	[100:10] mg/kg	
	ii) Isoflurane (inhalation)	4-5% for induction 2.5-3% for maintenance	
4	Blood collection volume:		
	i) Cardiac puncture (bleed-out)	0.6-1.2 mL	8.0-12.0 mL
	ii) Safe volume for repeat bleeds	0.1-0.2 mL	1.5-3.0 mL

be done in animals with a functioning immune system.

c) By transplantation of tumor cells derived from human / other animal species (xenograft model). This is usually only possible in immunocompromised hosts, where the foreign origin tumor can grow.

Tumor transplantation is normally done subcutaneously (under the skin). In some cases, it may be required to generate an orthotopic model by transplanting the tumor cells in the same organ for a more real-world representation of the disease (for instance, orthotopic liver cancer model by transplantation of hepatocellular carcinoma cells in the liver of the host animal). All ethical norms must be followed while developing animal models for cancer, microbial infection and other disease conditions, to ensure minimum possible discomfort or distress to the animal.

Certain procedures may require the animal to be anaesthetized, which should be as per prescribed protocols (See Table 1). Protocols like viable surgery or study of specific physiological processes may call for fasting of the animal – it is advised to keep the fasting period as short as possible (<7 hours), to avoid any serious impact on the animal's welfare parameters. The requirement of anesthesia (by inhalation/injection) and its possible effects on organ function and the pharmacokinetic behavior of the tested formulation must be ascertained before doing the study. Post-administration, the animals will need to be housed individually for the specified time-points to avoid commingling of excreted radioactivity.

Radioactive dose and time points of study

Taking into account specific activity of the prepared radiopharmaceutical and ensuring that the amount of ligand used has no pharmacologic impact, the radioactive dose delivered to the animal should be sufficient to take accurate ex-vivo /in vivo measurements. For efficacy studies of therapeutic formulations, the administered dose must be carefully chosen to induce required therapeutic effect in the animal model. It is necessary to consider a preliminary set of studies to assess pharmacokinetic behavior of the radiolabeled preparation prior to designing efficacy studies. The time-points selected for study must also be in sync with the design of the radiopharmaceutical; for example, animal studies for formulations with short-lived isotopes like Ga-68 ($t_{½}$ = 68 Min) or Tc-99m ($t_{½}$ = 6 h) would not benefit from longer time-points (>1 day).

Dose administration and blood sampling

The predominant route of administration for radiopharmaceuticals is intravenous – in rodent models the tail vein is a common injection site. The needle gauge for injection should be selected depending on the vein size and the fluidity of the formulation. The use of a previously installed catheter to administer the injection can help to limit dose exposure time. It is recommended to keep injection volume as low as possible (for typical examples, see Table 1). Some disease models call for more specialized protocols, like injection into the synovial joint of palliative therapy radiopharmaceuticals to manage arthritic pain symptoms, or administration using viable surgery into the hepatic vasculature for liver cancer therapy with trans-arterial radio-embolization formulations.

Blood sampling may be done to study the pattern of radioactivity concentration in the blood or to take samples for metabolite analysis. The highest amount of blood can be obtained via a direct cardiac puncture while sacrificing the animal in the course of study. But if repeated blood sampling is needed, then other sources, like the saphenous vein in the leg of the animal or the lateral tail vein, should be considered. In such cases blood collection must be done under anesthesia and it is advised to not draw more than 10% of total blood volume in a single bleed (See Table 1 for general guidelines). Blood sampling in a living animal should be performed by trained and experienced staff to minimize discomfort to the animals.

Animal sacrifice and management of carcasses

Animals may be sacrificed in the course of the experiment or at the completion of studies. Carbon dioxide saturation or anesthesia overdose are the typical methods of euthanasia. Cervical dislocation is a rapid technique that can be used in mice, but not for bigger animals. Post-experiment, the animal carcasses and viscera must be stored in deep-freeze conditions (≤-20 °C) for a minimum of 10 half-lives of the radioisotope to ensure maximal decay of the radioactivity (~99.9%), and checked for measurable residual activity prior to disposal as per norms.

Ethical considerations for animal testing

All the procedures related to experiments with laboratory animals discussed above are subject to the ethical norms governing the conduct of experiments with laboratory animals. The national body in India for enforcement of laboratory animal ethics is the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Additionally, all institutions conducting animal experiments must have an Institutional Animal Ethics Committee (IAEC), which approves research projects based on merit and monitors the conduct and progress of animal procedures carried out in these projects. Any animal experiment must adhere to the 3R principles of laboratory animal science:

a) Replacement – where possible, animal experiments must be replaced with other methods, like computer simulation, mathematical models, in vitro studies and artificial tissues/organs.
b) Reduction – Only the minimum number of animals needed to answer the stated research question may be used. This number should be based on statistical considerations. It is important to note that using too few animals is as wasteful as using too many animals, if the experimental conclusions are not statistically validated.
c) Refinement – Experiments must be performed in a manner causing minimum discomfort / distress in both magnitude and duration to the animals used.

References are available for guidelines on the implementation of the 3R principles for laboratory animal research.

The considerations for animal experiments discussed in this section are common to all animal testing protocols conducted for radiopharmaceuticals. As previously stated, they can be further sub-divided as *ex-vivo* and *in vivo* studies.

Ex-vivo animal testing

In these studies, the radiopharmaceutical is administered into the living animal. After the respective incubation time-points to allow for distribution and/or metabolism of the radiopharmaceutical in vivo, the animals are euthanised by suitable methods and relevant samples are excised/extracted for measurement and analyses. The following sections describe the different kinds of ex-vivo studies that may be performed for a radioactive formulation.

Ex-vivo organ distribution

This is the most common form of ex-vivo study with radiopharmaceuticals. It aims to quantitatively map the distribution and retention pattern of the radiolabeled formulation inside an animal host at specific time intervals post-administration. After allowing for distribution, the animal is euthanised and the relevant organs and tissues are excised for measurement of accumulated radioactivity. Measurement may be done on a dose calibrator (based on the principle of ionisation chamber) or a flat bed geometry solid scintillation detector. Alternatively, a representative sample of the tissue may be measured in a well-type scintillation counter, but the possibility of heterogeneous distribution of the pharmaceutical within the organ must be considered beforehand. For comparison, the radioactivity measurements in each organ/tissue may be represented as a percentage (%) of the total administered activity. Necessary extrapolations must be made to quantify total retention in the blood, muscle and bone tissues. Depending on the application of the radiopharmaceutical, specific ratios may need to be calculated: for tumor targeting tracers, the

ratios of retention in tumor to normal tissue and tumor to blood are critical. For myocardial perfusion agents, the ratio of accumulation in heart to lung and heart to liver are important.

The time-period covered in the study must ideally cover the pattern of uptake and retention of the tracer in the region of interest, as well as non-target uptake and washout, thereby providing a clear picture of the in vivo distribution and pharmacokinetics. Metabolic breakdown of the tracer may also influence the distribution of radioactivity in the body, which may need to be separately assessed. The specificity of a targeting radiopharmaceutical in vivo may also be tested by concomitant or prior administration of a blocking agent at a much higher concentration (≥100-fold) that will saturate the target sites.

Tissue autoradiography

Similar to in vitro autoradiography studies to assess tissue binding of radiolabeled formulations, autoradiography studies can also be done for tissue slices obtained from relevant organs/ regions in animals injected with radiopharmaceuticals. In this case, a more realistic picture of tissue accumulation is obtained based on the post-injection distribution of the formulation. Autoradiography may provide higher resolution than scintigraphic imaging instruments.

Metabolite analysis

For ex-vivo metabolite analysis, the animal host is first injected with the radiopharmaceutical to allow for in vivo distribution and clearance. At selected end-points post-administration, aliquots of body fluids like blood plasma/serum, cerebrospinal fluid, urine (using metabolic cages where urine is separately collected) etc. are drawn out for measurement of radioactive content and chemical identification of the radiolabeled formulation/its metabolites. The specific analytical protocols followed for metabolite analysis are the same as those described for in vitro serum based assays, typically chromatographic assessment and protein binding.

For a long time, ex-vivo studies have formed the bulwark of animal testing of radiopharmaceuticals, and certain data, like quantitative in vivo distribution of radiopharmaceuticals outside of the major organs, will continue to require some form of ex-vivo assessment. But it is now also possible to obtain in vivo assessment of many critical pre-clinical parameters either during development of new radiolabeled formulations or when using them as tracers for evaluation of other drugs. This is detailed in the following section.

In vivo animal testing

Basics of In vivo testing

In the case of in vivo animal testing, both administration of the radiolabeled formulation and subsequent measurement of the radioactivity distributed within the host are performed in the living animal. This is achieved by the use of scintigraphic imaging instruments, which can offer 2D or 3D images. The basic principle of scintigraphy is that the radiations emitted by the labeled formulation are captured with a suitable detection system that gathers information about the position and intensity of these emissions within the body, generating a map of the in vivo distribution of the radiopharmaceutical. While scintigraphy in the clinics has been available since more than half a century, dedicated scanners for small animals like rodents are a relatively newer phenomenon: early prototypes were demonstrated in the 2000's. Even now, only a handful of companies worldwide design and manufacture such machines. The main requirements for small animal imaging technology are:

a) High detection efficiency – for greater sensitivity
b) High spatial resolution – to accurately demarcate region of uptake
c) Low dead time – for reliable quantification of uptake data
d) Good temporal resolution – for better imaging of moving organs like heart.
e) Good energy resolution – for improved signal-to-noise ratio and management of multi-isotope imaging (in case of SPECT).

Since a single living animal may be observed over multiple time-points, a well-developed process of in vivo evaluation by imaging can help reduce animal usage for the biological assessments of formulations, and provide a more accurate picture of the pattern of uptake and washout over time of a radiolabeled formulation administered in vivo.

Types of In vivo scintigraphy

Depending on the nature of radionuclide used for labeling, scintigraphic images may be obtained by two main modalities:

a) SPECT / Single Photon Emission Computed Tomography
   This is for radiolabeled formulations that emit gamma radiations (like Tc-99m, Lu-177, I-131).

These gamma radiations are focused through a collimator and impinge on the scintillator crystal. After signal amplification through a series of dynodes, the scintillations are recorded as counts. By permitting only radiations entering at a specific angle to pass through and interact with the crystal, SPECT machines provide information about both position and intensity of the radioactive signal. Clinical SPECT has a resolution of 7-10 mm. However, dedicated small animal SPECT units using multiple pinhole collimators may offer up to sub-millimeter spatial resolution (with corresponding trade-off in sensitivity).

b) PET/Positron Emission Tomography:
Radiopharmaceuticals using positron emitting radionuclides (like F-18, Ga-68, Cu-64) are visualized by PET. This is based on coincidence detection. After emission from the parent radionuclide, the positron interacts with surrounding electrons and gets annihilated. In the process, it emits two high energy gamma photons (511keV each), which are released in opposite directions. Typical PET scanners have a ring detector that records a coincidence event when the two annihilation photons are detected within a time coincidence window (typically in nanoseconds). Depending on the initial positron energy, coincidence detection can provide accurate positional information about the radionuclide inside the body, and a higher signal to noise ratio than SPECT. Clinical PET scanners have better spatial resolution (4-5 mm) than SPECT scanner, while the best reported resolution thus far for small animal PET scanners is ~1 mm.

The scintigraphic information can be paired with anatomical information obtained from X-ray based Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) to give a 'fusion' image providing a clear picture of distribution of the radiopharmaceutical within the animal body. Nowadays, both clinical and pre-clinical imaging instruments can be fitted with multiple imaging modalities – a single instrument may be capable of a combination of SPECT, PET and CT/MRI.

Protocols in pre-clinical imaging

Whether using SPECT/PET modalities, a sequence of steps is involved while performing in vivo imaging and a number of factors must be taken into consideration for the same. Some of these are described below:

Preparation of the animal for imaging

For the experiment, the suitable animal (normal control or disease model) must be made ready for the imaging procedure. The normal practice is to house individual animals in separate cages. Anesthesia is administered to keep the animal motionless throughout the period of the scan, either by injection or inhalation. Injectable anesthetics (e.g. xylazine:ketamine) are long-acting and usually only one dose may be done per animal in one day, to avoid complications from anesthesia overdose. Thus the number of scans is limited by the 'period of effect' of the anesthetic. Inhalation anesthesia e.g. using isoflurane aerosol mixed with air/oxygen allows for much quicker recovery of the animal when removed from the atmosphere containing anaesthetic. It is safer, and allows for repeated sedation and multiple scan sessions in a single day.

The animal in sedated state is placed on the 'bed' provided with the imaging instrument. When isoflurane is used, the animal is exposed to anaesthetic atmosphere throughout the period of image acquisition. Small animal imaging beds typically have a warming pad to maintain their body temperature in anaesthetized state. In some cases, it may be necessary to monitor respiration and heart rate of the animal, for which provision must be made (e.g. when cardiac gating studies are performed, in which image capture must take into account the beating motion).

Specifically in the context of in vivo imaging, when animal tumor models are raised by transplantation, due consideration must be given to the location of tumor induction. For instance, generating a tumor lesion adjacent to the liver or bladder may be avoided to circumvent possible issues of competing emissions from these regions.

Image acquisition

This refers to the process of capturing radiation emissions from the formulation injected inside the animal host. In terms of protocol, there are two main types of imaging: a) Static Imaging b) Dynamic Imaging.

Static imaging at defined time intervals post-administration provides time averaged information about radiotracer distribution. It is a simpler protocol and can provide sufficient information for most comparisons. Hence, it is the dominant form of pre-clinical in vivo evaluation Dynamic imaging as the name suggests gives a 'moving image' view of the dynamic process of tracer uptake and interaction with the target. For

evaluation of radiopharmaceuticals that evaluate blood perfusion to organs like heart and lung, it can be useful. It is however a more involved acquisition process and data interpretation is also more complex.

Scanning time depends on the amount of injected radioactivity and pattern of retention in the region of interest – single scans may be completed even within a few minutes. Based on the radionuclide and formulation being studied, image acquisition procedures may extend from few hours (for small molecules tagged with short-lived radioisotopes) to some days (for antibodies/nanomaterials tagged with longer half-life radionuclides). After each individual scan, the animal can be returned to its cage till the next scan instance.

Image reconstruction and data analysis

Unlike 2D gamma scanners that can provide a real-time window of radiotracer distribution during the process of acquisition, multiplanar 3D imaging requires a process of 'reconstruction' of the acquired scintigraphic data to generate the image. In imagers with bundled CT modes, both CT and scintigraphic image reconstructions must be carried out. Image reconstruction software is usually provided with the instrument and the parameters of reconstruction can be customized by the user. Before obtaining quantitative images, a series of correction factors must be applied to the detector readings. Some of these are mandatory (e.g. normalization, decay correction, dead time correction) and are applied during data acquisition or image reconstruction. Commercial pre-clinical scanners may offer pre-defined correction factors which can be applied to the image data.

Most image acquisition software packages allow for storing of acquisition data in non-proprietary open formats like DICOM (Digital Imaging and Communications in Medicine). Discrete software packages are available for image processing and data analysis. Image processing helps to minimize visual artifacts and obtain a focused picture of the in vivo radiotracer behavior. Data analysis provides quantitative information about the retention pattern.

Pre-clinical toxicity studies

The safety properties of new radiopharmaceuticals intended for clinical application should be evaluated in pre-clinical studies prior to human studies. In the case of diagnostic radiopharmaceuticals, the unlabeled compound is typically administered at 'tracer dose' in which the amount of the ligand should not cause any toxic or pharmacologic effect. As per USFDA (food and Drug Administration of the United State) guidelines for diagnostic radiopharmaceuticals, pre-clinical toxicity in animal model should be evaluated via extended single dose toxicity protocol involving 14 days observation with evaluation of body weights, outward clinical signs, serum biochemistry, haematology and post-autopsy histopathology. For therapeutic radiopharmaceuticals, toxicity from both the radioactive and non-radioactive components of the formulation has to be considered. Potential toxicity from the non-radioactive component may be derived using a similar protocol as described above. To ascertain the potential toxicity from therapeutic radioactivity, dosimetry data may be drawn from pharmacokinetic studies performed in animal models. This can be extrapolated based on body weight and/or body surface area to determine the dose to be given in humans for clinical study, which will not generate any adverse effects.

Conclusion

With the continuous development of radiopharmaceuticals to address an increasingly sophisticated range of clinical applications, it becomes necessary to have a carefully composed pre-clinical testing program that will answer the relevant questions about effectiveness and safety of a formulation before it is taken up for use in humans. A combination of resources and training will need to be provided, and rigorous testing protocols must be developed and adapted to the specific needs of the formulation under study. An effective pre-clinical strategy will pay large dividends in terms of reducing the chances of failure upon clinical translation.

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Chapter 5
Clinical applications of nuclear medicine in cancer and non-oncological disorders: A case based approach

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Abstract*:* The present article portrays common day-to-day clinical applications of radiopharmaceuticals for both diagnosis and treatment of various disorders. For easy understanding, the points of learning have been made from real-world clinical examples and illustrations. As can be observed, the clinical applications have found use virtually in every organ system including cardiology and pulmonary systems, neuropsychiatry, oncology, gastroenteropancreatic-hepatology and nephrology disciplines. The hybrid PET-CT and SPECT-CT have been the major developments in the diagnostic domains, while radioiodine- and lutetium-177-based radiopharmaceuticals have been at the forefront of the therapeutic domains.

Keywords*:* PET-CT; SPECT-CT; Theranostics, Targeted Radionuclde Therapy; I-131 therapy; Thyroid Cancer; [^{68}Ga]Ga-DOTATATE/^{177}Lu-DOTATATE; [^{68}Ga]Ga-/[^{177}Lu]Lu-PSMA; Neuroendocrine tumor; prostate carcinoma.

Introduction

The last two decades have witnessed rapid expansion in the clinical applications of Nuclear Medicine, enhancing the medical practice in both diagnostic and therapeutic domains for a number of diseases including cancer and non-cancerous disorders. Two new developments in the specialty could be considered as the hallmark of such development: (a) *Molecular PET and hybrid PET-CT and SPECT-CT imaging* during the period of 2000-2010, and (b) *Targeted Radionuclide Therapy & Theranostics* primarily over the last decade (2010-present) were in the forefront of these developments. Both of these techniques are briefly mentioned below.

Hybrid nuclear medicine imaging (PET-CT and SPECT-CT)

The terms 'Hybrid Nuclear Medicine Imaging Systems' encompasses (i) Single Photon Emission Computed Tomography and Computed Tomography (SPECT/CT) and (ii) Positron Emission Tomography – Computed Tomography (*PET*/*CT*). Very recently there has been evolution of simultaneous Positron Emission Tomography and Magnetic Resonance Imaging (*PET*/*MRI*) as another promising hybrid imaging modality.

In both PET-CT and SPECT-CT, there is fusion of anatomical structure (provided by CT) and functional (PET/SPECT) information which allows a more precise diagnosis and higher levels of accuracy. A comparison of SPECT/CT to SPECT alone shows that the diagnosis is enhanced in nearly 30% of cases. Over the years, the molecular PET-CT imaging has grown into a powerful imaging modality that has brought about a revolution in diagnosis in (A) cancer (influencing staging including biopsies, treatment response assessment, detecting recurrence and guiding therapies) and also greatly aids diagnosis in (B) Cardiovascular disorders, (C) Neuropsychiatry and the (D) Infection and Inflammatory disorders. The role of PET-MRI is evolving at present: this modality harnesses the strengths of both PET and MRI and has great potential for the future diagnosis. Improved soft tissue contrast, added value of diffusion weighted imaging (DWI), decreased radiation dose (No ionizing radiation from MRI component) are some of the specific advantages of PET-MRI compared PET-CT.

Theranostics, targeted radionuclide therapies and precision oncology

The term 'Theranostics' implies the combining of diagnostic and therapeutic capabilities into a single agent. The aim is to develop more specific, individualized therapies, for various diseases in clinical oncology practice. Targeting the receptor over-expression in tumors has been a major development in the field the field of nuclear medicine. The two developments where this has found immense success are (a) [^{68}Ga]Ga-DOTATATE/^{177}Lu-DOTATATE based theranostics and somatostatin receptor based targeted radionuclide therapy known as 'Peptide Receptor Radionuclide Therapy' (PRRT) in metastatic and advanced neuroendocrine tumors and (b) [^{68}Ga]Ga-PSMA-11/^{177}Lu-PSMA-617 based theranostics and Peptide Receptor Radioligand Therapy (PRLT) in metastatic Castration Resistant Prostate Carcinoma (mCRPC).

The I-131 therapy for thyroid cancer, that has developed to be the cornerstone of management of thyroid cancer and some benign conditions (hyperthyroidism) for more than 70 years (the first

therapy undertaken by Saul Hertz in The Massachusetts General Hospital in 1941: the year 2016, marked the 75th anniversary of Dr. Saul Hertz first using radioiodine to treat a patient with thyroid disease), is based upon the sodium iodide symporter (NIS), an integral membrane protein residing in the basolateral membrane of thyroid epithelial cells. The NIS symports two sodium ions for every iodide ion, which leads to 20-40 fold concentration of iodine in the thyroid gland compared to its plasma concentration.

In addition to the above development in theranostics, there is a large number of newer developments in the therapeutic nuclear medicine expanding its horizon such as evolution of the α-emitting radionuclides for treatment demonstrating high cell-killing efficiencies, transarterial radioembolization (TARE) for liver tumors (such as unresectable hepatocellular carcinoma), radioimmunotherapy employing (i) ^{131}I/^{177}Lu-DOTA-Rituximab (Treatment of relapsed follicular, mantle cell, or other lymphomas) and (ii) ^{177}Lu-Trastuzumab (HER2 or Erb2/neu positive metastatic/advanced breast malignancies) and so on. In the present article, we have discussed the various clinical applications (traditional and newer applications) through illustrative case examples in organ-specific/system-specific manner. The ancillary discussions and learning points have been mentioned in respective case scenario.

Case 1. ^{131}I scan and therapy in hyperthyroidism uncontrolled with anti-thyroid medications (Thionamides)

Case history: This 35 year old female, presented with clinical symptoms of hyperthyroidism, was on regular doses of carbimazole for 2 years with suppressed thyroid stimulating hormone (TSH) and adequate thyroxine (T4) on present dose of anti-thyroid medication. The patient was referred for consideration of radioiodine therapy for the treatment of hyperthyroidism. Radioiodine (RAI) scan undertaken using ~25 µCi (925 kBq) ^{131}I showed diffused increased tracer uptake in mildly bulky thyroid gland. The 2 hrs' ^{131}I uptake was 14.79%, while the 24 hrs ^{131}I uptake 49.85% (Normal 24 hour uptake values have a range of 10-30%) [**Fig**.1]

Clinical disease course: She received 12.8 mCi (474 MBq) ^{131}I RAI therapy for thyrotoxicosis. On regular follow up, the patient is now asymptomatic, not on any thyroid medication and normal thyroid function test (TFT) parameters (Serum.T4 7.88ug/dL, Serum.TSH-3.82uIU/mL), indicating excellent result and disease control with one dose of I-131 therapy.

Learning points & implications for patient care. In addition to thyroid cancer, ^{131}I therapy is routinely employed for treating Graves' disease and toxic nodular goiter (both solitary toxic nodule and toxic multinodular goiter) and also used to reduce the size of nontoxic nodular goiters (primarily when surgery is contraindicated or refused).

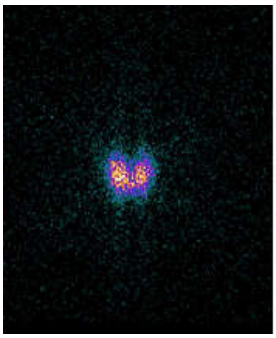

Figure 1. Radioiodine (RAI) scan undertaken with ~25 µCi (925 kBq) ^{131}I showing diffused increased tracer uptake in the thyroid gland (24 hrs. uptake: 49.85%; normal 24-hour uptake values have a range of 10-30%)

Case 2. ^{99m}Tc-pertechnetate thyroid scan for evaluation of patients with hyperthyroid /thyrotoxic symptoms

Case History: A 54-year-old female, presenting with chief complaints of pain in neck, palpitations, headache, weight loss, ultrasonography of thyroid showed mildly enlarged lobes of thyroid with altered echo pattern. The patient had suppressed TSH (<0.0005) and was referred for further evaluation of disease.

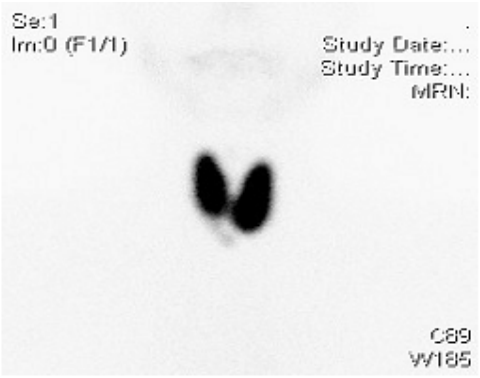

Figure 2. ^{99m}Tc-pertechnetate scan of the thyroid with 2-3 mCi (74-111 MBq), demonstrates both

lobes of the thyroid gland, mildly enlarged with normal shape at normal location, & increased tracer trapping (right>left). Calculated Thyroid uptake at 20 minutes: 16.4% (normal range of 0.50-4.50%). The present scan findings and uptake values are suggestive of hyper-functioning Graves' gland. ^{99m}Tc-pertechnetate uptake & scan has developed as a useful alternative in the centres that do not have uptake probe.

Learning Points & Implications for Patient care.
The two usual clinical applications of thyroid scans are: (a) differentiate the causes of hyperthyroidism and (b) assess thyroid nodules when the patient has hyperthyroidism (important value in diagnosing autonomous thyroid nodule).

Case 3. Radioiodine Ablation of Neck Residue in Differentiated Thyroid Cancer

Case History: 30 years old female, presented with neck swelling and diagnosed with differentiated carcinoma of thyroid (DTC), underwent total thyroidectomy with bilateral central compartment clearance and selective nodal dissection The histopathology report demonstrated Papillary Ca Thyroid with lymph node metastases, extra-thyroidal extension and lymphovascular invasion.

Clinical disease course: She received RAI ablation therapy with 143 mCi (5.20 GBq) of RAI and post-treatment scan before discharge from the isolation ward demonstrated avid concentration in the neck. 6 months following therapy, she reported excellent response to therapy given with negative large dose RAI scan (right hand image-Fig 3) and suppressed Serum thyroglobulin value of 0.4 ng/ml

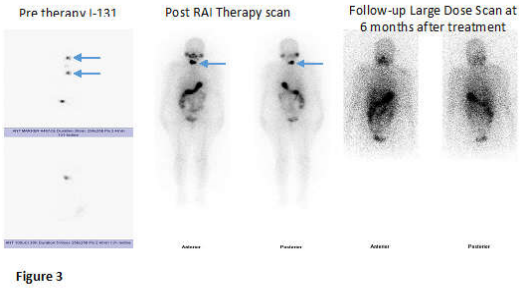

Figure 3. Post-surgery RAI scan which showed remnant thyroid tissue in thyroid bed (arrow marked in the images). She received 1 cycle of high dose RAI therapy with dose around 143 mCi (5.20 GBq). Follow up RAI scan 6 months later (right panel) shows near complete ablation of the remnant thyroid tissue.

Case 4. Radioiodine Therapy in Differentiated Thyroid Cancer with Pulmonary Metastases

Case history: 25 years old male, presented with neck swelling and diagnosed with differentiated thyroid carcinoma with lung metastasis underwent total thyroidectomy with bilateral central compartment clearance, the HPR suggesting Follicular Variant of Papillary carcinoma Thyroid with lymph node metastasis.

Clinical disease course: He reported reduction in RAI uptake in both lungs (right hand image- Fig 4), reduction in number and size of lung nodules on HRCT scan and serum thyroglobulin value reducing from >300 ng/mL post-surgery to 36 ng/mL suggestive of structural and biochemical response to therapy administered.

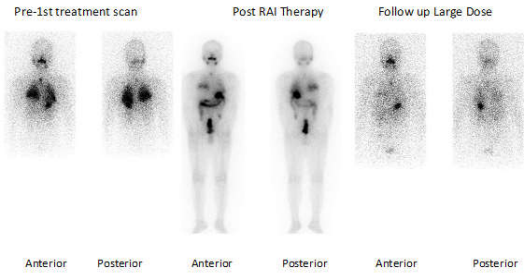

Figure 4. Post-surgery RAI scan (left panel) showed remnant thyroid tissue with extensive bilateral lung uptake and mediastinal lymph node. He received 3 cycles of high dose RAI therapy with dose around 200 mCi (7.4 GBq) per cycle. Follow up RAI scan (right panel) shows near complete resolution of metastatic lesions.

Learning points & implications for patient care.
Radioiodine therapy with I-131 is a widely accepted treatment for patients with lung metastasis from DTC. A number of studies have reported that ^{131}I-avid lung metastasis is curable and has an excellent prognosis (1). On the other hand, ^{131}I-non-concentrating pulmonary metastasis with high thyroglobulin levels has a relatively poor prognosis.

Case 5. Radioiodine non-concentrating FDG avid metastatic disease in thyroid cancer (TENIS syndrome)

Case history: A patient diagnosed of poorly differentiated thyroid carcinoma (PDTC), with rising serum thyroglobulin trend (stimulated Serum Tg - >400ng/mL) and negative post-surgery 1 mCi (37 MBq) I-131 scan (Fig 5 - left hand image). The FDG-PET/CT scan (Fig 5 - right hand image)

showed: FDG concentrating residual soft tissue lesions in neck (SUVmax 20.9) and anterior mediastinum (SUVmax 28.3) and non concentrating multiple bilateral lung nodules. FDG concentrating pre-vascular lymph node (SUVmax 52.9), FDG concentrating lytic sclerotic C7 and C5 vertebral lesions and soft tissue density luminal thickening is lower 3rd of esophagus - increased in size and uptake (SUVmax 22 vs. previous SUVmax 8.3), overall suggestive extensive metastatic disease that are I-131 negative.

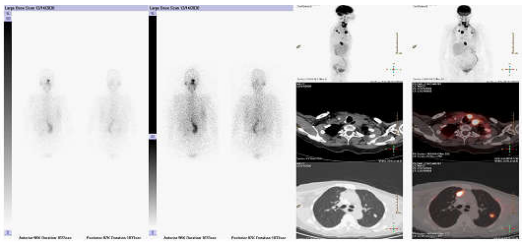

Figure 5. In a patient diagnosed of poorly differentiated thyroid carcinoma (PDTC), the ^{131}I-scan (left panel) and FDG-PET/CT scan (right panel) showing FDG-avid but non-RAI concentrating lesions.

Learning points & implications for patient care. Patient with thyroglobulin elevation but negative iodine scintigraphy, the TENIS syndrome, with FDG avid distant metastases usually a relatively aggressive disease course and poorer prognosis (2); on fast disease progression these patients are considered for tyrosine kinase inhibitors (sorafenib and lenvatinib).

Case 6. ^{177}Lu-DOTATATE Peptide Receptor Radionuclide Therapy (PRRT) in Metastatic Neuroendocrine Tumors (NETs)

Case history: 46 years old lady, presented with abdominal pain, got evaluated with CT scan showing pancreatic and bilobar liver lesions. The USG abdomen guided biopsy of the liver lesions demonstrated Grade 2 well differentiated NET (Mib-1 labeling index - 10%)

Clinical disease course: The patient received 'sandwich CHEMO-PRRT' with PRRT (^{177}Lu-DOTATATE) and capecitabine-temozolomide (CAPTEM) chemotherapy and showed near complete resolution after combined 3# of PRRT & 4# chemotherapy. Currently she is asymptomatic with no significant liver lesion noted in recent ^{68}Ga-DOTATATE PET/CT scan.

Learning points & implications for Patient care. ^{177}Lu-DOTATATE Peptide Receptor Radionuclide Therapy (PRRT) for Metastatic/Advanced Neuroendocrine Tumors (NETs) is a major development in the targeted treatment of Neuroendocrine Neoplasm and related malignancies (3), where the RMC, BARC has played a major role (initiated in 2010 at the centre), & completed a large number of patients making it one of the largest centers for this treatment.

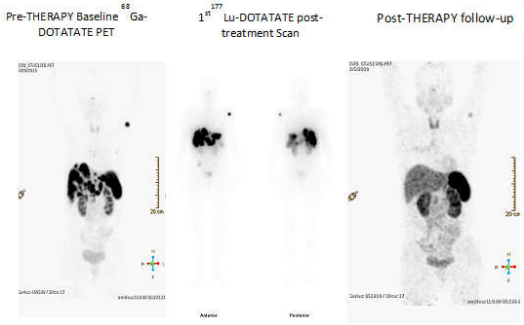

Figure 6. Left panel image shows pre-therapy ^{68}Ga-DOTATATE PET image depicting tracer avid metastatic liver lesions. After ^{177}Lu-DOTATATE PRRT (middle image showing post-therapy scan) follow-up scan showed near complete resolution of the liver lesions (right panel image).

Case 7. PSMA-based radio-ligand therapy (PRLT) with ^{177}Lu-PSMA-617 in metastatic castration resistant prostate carcinoma (mCRPC)

Case history: 63 years old gentleman was diagnosed in 2013 as locally advanced prostate carcinoma, post androgen deprivation therapy and local RT to prostate only (36Gy/6#/weekly) Dec 2014. He developed CRPC in June 2016 and received Docetaxel 4 cycles 3 weekly till Dec 2016. Was started on Abiraterone in Dec 2016 (received for 3 months) and subsequently received 6# Cabazitaxel till Oct 2017. Presented with further rising serum PSA levels and generalised weakness and extensive disease on ^{68}Ga-PSMA PET/CT scan (**Fig 7- left panel**).

Clinical disease course: He was then started with ^{177}Lu-PSMA-617 PRLT and received 4# with cumulative dose ~500mCi. His serum PSA level showed declining trend from 38.94ng/ml (Baseline) to 0.23ng/ml (post 4# of PSMA therapy), he gained weight and improved with generalised weakness and substantial improvement in the scan (**Fig 7-right panel**).

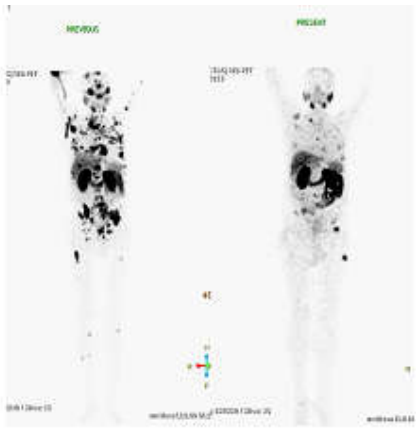

Figure 7. Left panel shows pre-therapy ^{68}Ga-PSMA-11 PET with tracer avid multiple metastatic skeletal lesions. Post ^{177}Lu-PSMA-617 therapy [4# with cumulative dose ~500 mCi (18.5 TBq)] follow up PET (right panel) shows significant reduction in number and size of the metastatic lesions.

Learning points & implications for patient care. ^{177}Lu-PSMA-617 PRLT is evolving as an important targeted therapeutic modality for the treatment of progressive metastatic prostate cancer. Targeted alpha-therapy (high linear energy transfer and shorter range) with ^{225}Ac-PSMA-617 is one step ahead for these groups of patients who show resistance to this form of therapy (4).

Case 8. FDG-PET/CT in oncology – Important role in treatment response evaluation

Case history: 14 years old male child, known case of progressive Hodgkin's lymphoma; received 2 cycles of second line chemotherapy. The present scan was for response assessment to administered chemotherapy.

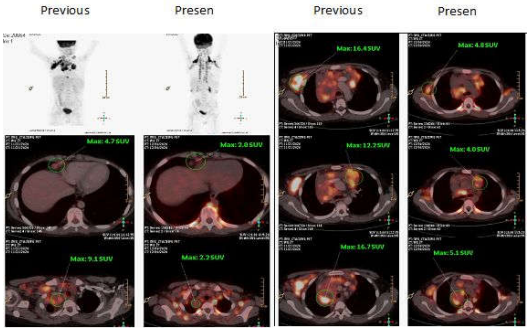

Figure 8. In this patient of lymphoma, as compared to older PET-CT (left panel), there is evidence of partial metabolic and morphologic regression (right panel) of previously seen in supra-diaphragmatic lymphadenopathy, paraaortic and retrosternal soft tissues. However, new ill-defined metabolic activity seen in ribs raises the suspicion of disease involvement.

Learning points & implications for patient care. Treatment response assessment is a major advantage of FDG-PET/CT imaging in cancer management. The **Deauville** five-point scale is usually adopted for routine clinical reporting and clinical trials using FDG PET-CT in the initial staging and assessment of treatment response in Hodgkin lymphoma (HL) and certain types of non-Hodgkin lymphomas (NHL). The other internationally-recommended treatment response scales include Positron Emission Tomography Response **Criteria** in Solid Tumours (**PERCIST**).

Case 9. FDG-PET/CT in neuropsychiatric disorders – Dementia workup

Case history: 52 years old female, presented with chief complaints of progressive forgetfulness and excessive sedation since last 8 months, though she is able to do daily activities, Mini-mental state examination (MMSE) Score 15/30. Neuronal and Cranial nerves and psychiatric evaluation unremarkable. The patient was referred for brain PET evaluation to rule out early Alzheimer's disease.

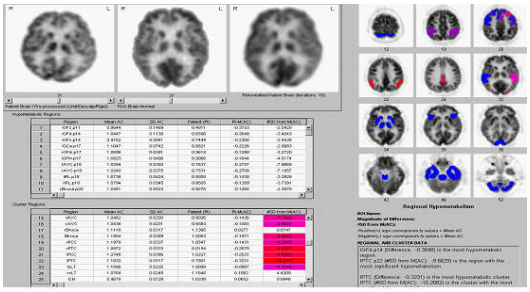

Figure 9. ^{18}F-FDG Brain PET processed images (NeuroQ software, Philips ^{TM}) showing areas of significant hypometabolism (reddish-pink highlighted areas) in bilateral parieto-temporal cortices, left medial frontal gyrus, posterior cingulate gyrus suggestive of Alzheimer`s disease.

Learning points & implications for patient care. The potentially areas of PET applications in the domain of neuropsychiatry include: differential diagnosis and evaluation of (i) dementia and its subtypes, (ii) detection of epileptic focus (iii) (5) Parkinson's disease and (iv) other hyperkinetic movement disorders (5).

Case 10. FDG-PET/CT in treatment monitoring of infection/inflammation

Case history: Known case of multi drug resistant Koch's spine on 2nd line anti-tubercular therapy (AKT) since 8 months, presented with pain radiating along left lower limb. USG showed well-defined abscess in left paravertebral region (17.5*4.5 cm) suggestive of psoas abscess. The MRI demonstrated ankylosis of C7-D1 with regression of the epidural abscess, osteolysis, and cortical destruction with PR paravertebral soft tissue seen from D11 TO S2 levels. The patient was referred for follow up PET-CT scan for disease status evaluation of current AKT regimen.

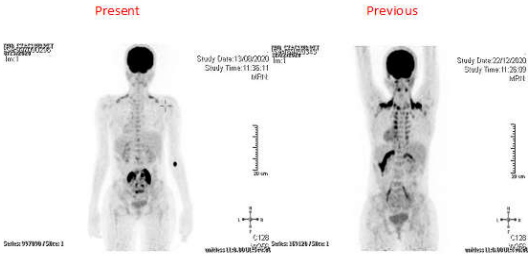

Figure 10. Post-treatment (AKT) ^{18}F-FDG-PET/CT scan (right panel) compared to previous PET CT scan (left panel) showing significant reduction in extent of spinal and para-spinal disease involvement in a patient of tuberculosis. Overall scan findings reveal partial metabolic response to anti-tubercular therapy given.

Learning points & implications for patient care. FDG-PET/CT has evolved over the last decade as a promising diagnostic modality for assessing several inflammatory and infectious diseases (6). This has direct and important relevance to the day to day clinical practice and is particularly relevant in developing countries including India.

Case 11. ^{99m}Tc-MIBI and FDG-PET/CT for cardiac viability study

Known patient of diabetes and hypertension from since 15 years (age 65 years), MI and 2D Echocardiography LVEF of 20% Referred for further cardiac evaluation
Findings: The LV cavity is dilated. The RV cavity also appeared dilated with heterogenous tracer uptake. Gated REST images showed global hypokinesia, predominantly of apical and antero-septal wall. LVEF~ 23%.

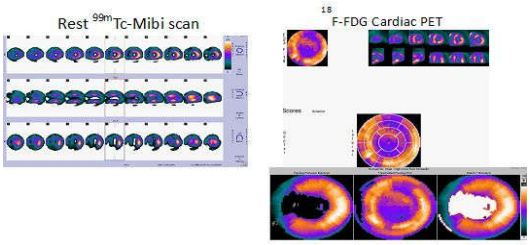

Figure 11. ^{99m}Tc-MIBI (left panel) myocardial perfusion images showing dilated LV cavity with apical and antero-septal wall hypoperfusion. ^{18}F-FDG PET myocardial viability study (right panel-processed on automated Emory Cardiac Toolbox) showing Perfusion-metabolism mismatch pattern in above-mentioned territory suggestive of hibernating myocardium.

Learning points & implications for patient care. The combination of myocardial perfusion study and FDG-PET cardiac imaging is indicated for patients with LV dysfunction due to coronary artery disease who are eligible for coronary revascularization and have resting myocardial perfusion defects in order to differentiate viable (i.e., hibernation) from non-viable myocardium (i.e., scar) (7).

Case 12. ^{99m}Tc-Macro Albumin Aggregates (MAA) Lung perfusion study for post-pneumonectomy lung function estimation

Case history: 38 year old male, known case of right hydropneumothorax with parenchymal atelectasis. Spirometry: Severe mixed restrictive and obstructive defect without significant reversibility. Chest X ray: Right lung consolidation. HRCT: Hydropneumothorax on right side with ICD in situ causing underlying parenchymal atelectasis. Fibrobronchiectatic and fibrocalcific changes noted in rest of right lung parenchyma. Fibronodular changes noted involving left upper lobe with few peribronchial confluent nodular opacities likely due to active disease. Planned for right decortication + upper lobectomy SOS pneumectomy SOS thoracoplasty

Scan undertaken: Lung perfusion scintigraphy performed with ^{99m}Tc-Macro Albumin Aggregates (MAA) administered intravenously. Scintigraphic images were acquired in multiple projections.

Quantitative perfusion analysis (Geometric mean):
Left lung (%): Upper zone- 17.6; Middle zone- 54.3; Lower zone- 22.9; Total Left lung (%)- 94.8%
Right lung(%): Upper zone- 1.0; Middle zone- 3.5;

Lower zone- 0.6; Total Right lung(%)-5.2% Overall perfusion of left lung is preserved and heterogenous, overall function is 94.8% of the total lung perfusion. Overall, the perfusion of right lung is significantly reduced, overall function is only 5.2% of the total lung perfusion. Calculated FEV1 by PFT was: 1.05L Plan of surgery - right lobectomy/ pneumonectomy. Thus, post right pneumonectomy: the expected residual FEV1 would be 0.99 liters.

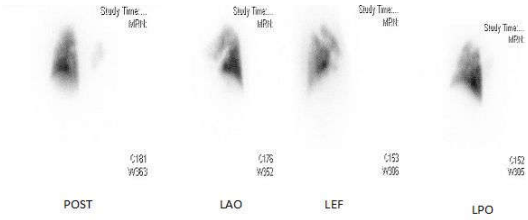

Figure 12. ^{99m}Tc-MAA lung perfusion scan images (in different oblique views) showing right lung perfusion severely reduced - in all 3 (upper, middle and lower) zones.

Case 13. Ruling out biliary atresia by ^{99m}Tc-mebrofenin hepatobiliary study

Case history: A case of neonatal jaundice, referred for ^{99m}Tc-Mebrofenin scan to rule out biliary atresia. Tracer concentration is noted in urinary bladder suggesting alternative route of excretion.

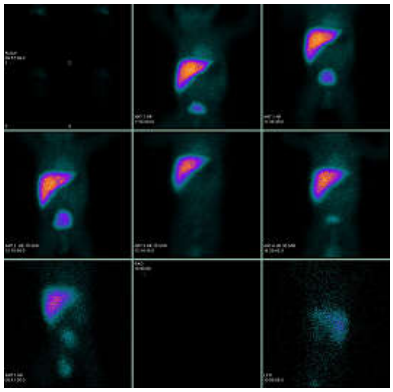

Figure 13. ^{99m}Tc-mebrofenin hepato-biliary scintigraphy scan shows fairly adequate hepatic uptake of the tracer but no obvious excretion even on delayed acquisition, a finding suggestive of biliary atresia

Impression: Hepatomegaly with reduced hepatocellular function. In view of non visualization of radiotracer in the gut even by 24 hours biliary atresia cannot be ruled out.

Learning points & implications for patient care. ^{99m}Tc-mebrofenin hepato-biliary scintigraphy also known as Cholescintigraphy (HIDA scan) plays an important role, to diagnose biliary atresia with relatively high accuracy (8).

Case 14. ^{99m}Tc-MIBI dual time-point imaging for detection of parathyroid adenoma

Case history: Patient presented with abnormal gait from last 1-year, diffuse osteopenia in visualized bones, serum PTH- 2081, Serum. Creatinine -0.6, Serum. Calcium-10 mg/dl, Serum Phosphorus -2.9 mg/dl, for scintigraphic evaluation of parathyroid disease.

Scan undertaken. ^{99m}Tc-MIBI Parathyroid Dual point imaging was performed dynamic images and static images of patient`s extended neck are acquired in anterior projection after 20 minutes and 2 hours.

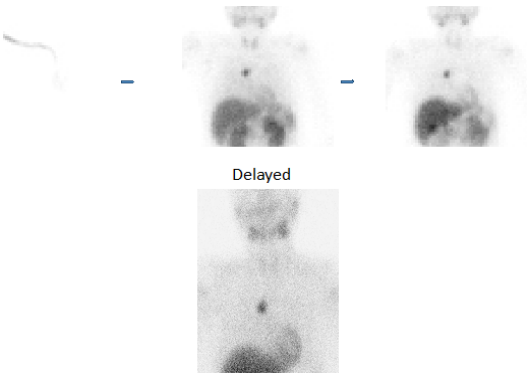

Figure 14. ^{99m}Tc-MIBI parathyroid scintigraphy scan shows homogenous tracer uptake in thyroid lobes. However, early and delayed images at 2 hours post-injection shows persistence of focal tracer concentration in the mid-chest region representing ectopically located parathyroid tissue/adenoma.

There is scintigraphic evidence of ^{99m}Tc-MIBI avid lesion in the mid-chest region that is likely to be isolated ectopic parathyroid adenoma in the given clinical context.

Learning points & implications for patient care. Dual-phase ^{99m}Tc-MIBI scintigraphy is based on the principle of time-related differential washout between the thyroid gland and a parathyroid tumor, where in the latter, the retention has been attributed to the high metabolic activity and mitochondria-rich oxyphil cell content of the tumor (9).

Case 15. Evaluating pyelonephritis and renal scars with ^{99m}Tc-DMSA renal scan in pediatric population

Case history: 9 years old male, case of left pelvi-ureteric junction obstruction and post left pyeloplasty, on follow up after that recently presented with fever and UTI.

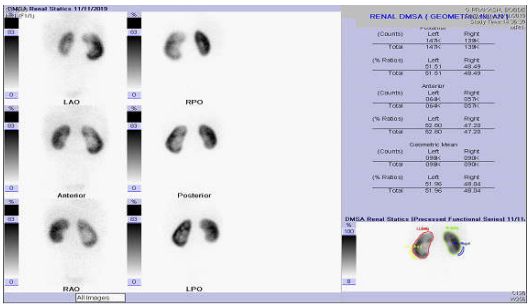

Figure 15. ^{99m}Tc (III) DMSA scintigraphy scan showing hydronephrotic left kidney, normal functioning cortical mass with evidence of cortical defect at its upper pole. Right kidney was normal in shape and having cortical function and have no evidence of any cortical defect.

Learning points & implications for patient care. The ^{99m}Tc-DMSA scan uses dimercaptosuccinic acid (DMSA) in assessing renal morphology, structure and function. The DMSA scan is considered as the most sensitive imaging technique for the detection of acute pyelonephritis (10).

Conclusion

Thus, in this treatise, we explored some day-to-day clinical applications of Nuclear Medicine both in diagnostic and therapeutic domains taking examples from our practice. As could be evidenced, the application of radioactive substances in both diagnosis and treatment of disease (both oncological and non-oncological) have seen rapid increase particularly over the last two decades. The principle and practice of Theranostics and Precision oncology have been greatly augmented by the recent developments in the specialty delivering targeted therapy in a number of cancers.

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