Production of high specific activity $^{177}\text{Lu}$ and formulation of $^{177}\text{Lu}$-DOTATATE for the treatment of neuroendocrine cancers

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Abstract

$^{177}\text{Lu}$ has emerged as one of the highly useful radionuclide for the development targeted radiotherapy agents owing to its favorable nuclear decay characteristics, adequately long half-life and ease of its large-scale production with sufficiently high specific activity and excellent radionuclidic purity using medium flux research reactors. Extensive studies carried out in the Radiopharmaceuticals Division in the past ten years to optimize the production of $^{177}\text{Lu}$ with adequately high specific activity has resulted the commercial deployment of $^{177}\text{LuCl}_3$ suitable for various radiotherapeutic applications. On the other hand, research on development of $^{177}\text{Lu}$-based radiotherapeutic agents has resulted in indigenous development of $^{177}\text{Lu}$-DOTATATE, an agent presently being used in six nuclear medicine centers of our country for the treatment of patients suffering from malignancies of neuroendocrine origin.

Introduction

$^{177}\text{Lu}$ is an attractive radionuclide for the development of targeted radiotherapeutic agents owing to its suitable nuclear decay characteristics [$E_{\beta\text{max}} = 0.49\text{ MeV}, E_\gamma = 113\text{ keV (6.4%) and 208 keV (11%)},$] comparatively longer half-life [$T_{1/2} = 6.65\text{ d}$] and ease of production with adequately high specific activity and excellent radionuclidic purity using the medium flux research reactors. [1]. The potential of $^{177}\text{Lu}$ in designing agents for therapeutic applications, more specifically targeted radiotherapy, was recognized as early as in 2000, in the Radiopharmaceuticals Division, BARC. Therefore, indigenous sourcing of this logistically ideal isotope in adequate quantities and specific activities became a necessity. Extensive studies have been carried out in the past ten years involving careful alterations of various irradiation parameters such as, flux and duration of irradiation, enrichment of the target etc., for the production of $^{177}\text{Lu}$ with adequately high specific activity and radionuclidic purity. Using the present reactor facilities of our Institute, $^{177}\text{Lu}$ suitable for the preparation of various kinds of radiotherapeutic agents, is being routinely produced and regularly supplied to several nuclear medicine centers of our country since 2007. A direct outcome of the extensive research carried out in our Division on the production of $^{177}\text{Lu}$ has resulted the commercial deployment of the radionuclide through BRIT in 2011. On the other hand, efforts toward the development of $^{177}\text{Lu}$-based radiotherapeutic agents has culminated in the indigenous formulation of $^{177}\text{Lu}$-DOTATATE, an agent presently being used at different nuclear medicine centres across the country for the
treatment of inoperable malignancies of neuroendocrine origin.

**Production of $^{177}$Lu**

$^{177}$Lu can be produced by two different routes, namely, by direct neutron activation of natural ($^{176}$Lu, 2.6%) or enriched (in $^{176}$Lu) Lu$_2$O$_3$ target and also by indirect route involving the irradiation of enriched (in $^{176}$Yb) Yb$_2$O$_3$ target followed by radiochemical separation of $^{177}$Lu from Yb isotopes [2]. The above two production routes lead to the product having different specific activities. Although the specific activity obtained in conventional (n,$\gamma$) activation is usually low; owing to the high thermal neutron capture cross-section of $^{176}$Lu ($\sigma = 2100$ b), direct neutron activation results in high specific activity of $^{177}$Lu. In fact the cross-section of $^{176}$Lu(n,$\gamma$)$^{177}$Lu is the highest encountered among all (n,$\gamma$) produced radionuclides presently used for therapy. Therefore, it is feasible to produce high specific activity $^{177}$Lu suitable for developing agents for targeted radiotherapy applications by simple (n,$\gamma$) reaction using enriched $^{176}$Lu as target in medium flux research reactors. However, a careful optimization of the time of irradiation is required in order to obtain the $^{177}$Lu with maximum specific activity as there will be considerable target burn up owing to the high thermal neutron capture cross-section of $^{176}$Lu ($\sigma = 2100$ b), direct neutron activation results in high specific activity of $^{177}$Lu. The $^{177}$Lu activity produced at the end-of-bombardment (EOB) as a function of irradiation time at three different thermal neutron fluxes has shown in Fig. 1. It is evident from the figure, that depending on neutron flux, the activity of $^{177}$Lu produced will be maximum after certain duration of irradiation, beyond which the activity will decrease owing to the high target burn up. Higher the thermal neutron flux of the reactor, shorter will be the time of irradiation for attaining maximum activity. Therefore, in order to obtain maximum specific activity using enriched $^{176}$Lu target, the time of irradiation must be judiciously decided depending on the neutron flux available for the irradiation.

$^{177}$Lu is produced by irradiation of enriched (74% and 82% in $^{176}$Lu) Lu$_2$O$_3$ target at a thermal neutron flux of $7 \times 10^{13}$-1 $\times 10^{14}$ n/cm$^2$.s for a period of 21 d at the DHRUVA reactor. Following irradiation, the target is dissolved in suprapure 1 M HCl by gentle warming inside a lead-shielded plant. The resultant solution was evaporated to near-dryness and reconstituted in ultrapure water. The last step of the process was repeated twice. A maximum specific activity of $\sim 1480$ GBq/mg (40 Ci/mg) was achieved when irradiation was carried out with 82% enriched target at a thermal neutron flux of $1 \times 10^{14}$ n/cm$^2$.s for 21 d. This corresponds to $\sim 36\%$ of the maximum achievable specific activity. The specific activity of $^{177}$Lu obtained was significantly higher compared to the theoretically calculated value under the irradiation conditions employed (12.9 atom %), accounting for only thermal neutron capture. The observed difference could be partially accounted for by considering the contribution from epithermal neutrons (resonance integral= 1087 b), which is not taken into account in theoretical calculations.

Fig. 1: Variation of $^{177}$Lu specific activity with respect to duration of irradiation at different thermal neutron fluxes

The radionuclidic purity of $^{177}$Lu could be determined by analyzing the gamma ray spectrum of radiochemically processed $^{177}$Lu sample. The major gamma peaks observed are at 72, 113, 208, 250 and 321 keV, all of which correspond to the photopeaks of $^{177}$Lu. The radionuclidic impurities that could be present in $^{177}$Lu are $^{176m}$Lu, formed by $^{175}$Lu (n,$\gamma$) $^{176m}$Lu ($\sigma = 16.4$ b) and $^{177m}$Lu produced via
The presence of $^{176m}$Lu ($T_{1/2} = 3.7$ h) can be eliminated by allowing 1 d cooling post-EOB. Therefore, $^{177m}$Lu ($T_{1/2} = 160.5$ d) could be the only possible radionuclidic impurity present in $^{177}$Lu. However, no peak corresponding to the photopeaks of $^{177m}$Lu (128, 153, 228, 378, 414, 418 keV) could be visible in gamma ray spectra. This is expected as the radioactivity due to $^{177m}$Lu produced will be insignificant and below the detectable limit on 21 d irradiation owing to its long half-life ($T_{1/2} = 160.5$ d) and comparatively low cross-section for its formation. The radionuclidic impurity burden due to the presence of $^{177m}$Lu in $^{177}$Lu could be determined by recording gamma ray spectrum of a sample aliquot after the complete decay of $^{177}$Lu (8-10 half-lives of $^{177}$Lu i.e. 55-70 days). The average level of radionuclidic impurity burden in $^{177}$Lu due to $^{177m}$Lu is reported to be $\sim 5.5$ kBq of $^{177m}$Lu / 37 MBq of $^{177}$Lu ($\sim 150$ nCi / 1 mCi) at EOB, which indicates $^{177}$Lu could be produced with 99.985% radiochemical purity using medium flux research reactors.

No carrier added (NCA) $^{177}$Lu [theoretical specific activity $40.33 \times 10^5$ GBq/g (1.09 $\times 10^5$ Ci/g)] can be produced following the indirect production route involving the neutron irradiation of enriched $^{176}$Yb target followed by radiochemical separation of $^{177}$Lu from the Yb isotopes. However, radiochemical separation of $^{177}$Lu activity from irradiated Yb$_2$O$_3$ target is difficult owing to the similarity in the chemistry of the two adjacent members of the lanthanide series. Presence of Yb isotopes in $^{177}$Lu will not only reduce the effective specific activity of the product but also interfere during the preparation of radiopharmaceuticals. Moreover, it can be shown by theoretical calculation that irradiation of 1 mg of 99% enriched (in $^{176}$Yb) Yb$_2$O$_3$ target at a reasonably high thermal neutron flux of $5 \times 10^{14}$ n/cm$^2$.s will produce only $\sim 5.55$ GBq ($\sim 150$ mCi) of $^{177}$Lu [1]. The use of enriched targets with low activation cross-section ($s = 2.4$ b for $^{176}$Yb($n,\gamma$)$^{177}$Yb) is not economical for isotope production, as a significant part of the target will be wasted.

$^{177}$Lu is being produced on a regular basis since the end of 2006 for carrying out clinical investigations of different

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**Development of $^{177}$Lu-DOTATATE for peptide Receptor Radionuclide Therapy (PRRT)**

PRRT using radiolabeled somatostatin analogues is a novel therapeutic modality for treatment of somatostatin receptor-positive tumors. $^{177}$Lu the promising radionuclide to develop new agents for PRRT which will be particularly useful for targeted therapy of smaller lesions owing to the small tissue penetration range of $\beta^-$ particles of $^{177}$Lu [3-4]. $^{177}$Lu-labeled DOTATATE, where TATE is a somatostatin analog octapeptide, was envisaged as a promising agent for the treatment of patients suffering from inoperable tumors of neuroendocrine origin, over-expressing somatostatin receptors.

$^{177}$Lu-DOTATATE was prepared in the laboratory with high radiochemical purity (>99%) following the optimized
protocol [3]. One of the challenges involved in carrying out targeted tumor therapy using $^{177}$Lu-DOTATATE is the preparation of patient doses as the radiolabeled agent has to be prepared with adequately high specific activity so that sufficient activity can be deposited in the cancerous lesions without saturating the limited number of available receptors. As the specific activity of $^{177}$Lu available at the time of preparation of the agent may vary considerably, it is crucial to optimize the labeling protocol, more importantly with respect to the amount of peptide, in order that it can be prepared with high radiochemical purity using minimum amount of DOTATATE. Therefore, an optimized protocol for the preparation of therapeutic dose ($5.55-7.4$ GBq, $150-200$ mCi) with maximum achievable specific activity and stability was developed. As per the protocol developed, $^{177}$Lu-DOTATATE, suitable for administration to the patients was prepared at the hospital radiopharmacies by adding the required volume of aqueous DOTATATE solution (1 $\mu$g/$\mu$L in de-ionized water) and $^{177}$LuCl$_3$ solution in 0.1 M ammonium acetate buffer (pH ~5) containing gentisic acid (40 mg/mL) such that DOTATATE:Lu molar ratio would be 4:1 [4]. Quick quality control of $^{177}$Lu-DOTATATE could be performed by paper chromatography using 50% acetonitrile in water as the eluting solvent. The extent of complexation can be subsequently determined with accuracy using HPLC [4]. More than 125 batches of $^{177}$Lu-DOTATATE have been prepared following this protocol till date at six different nuclear medicine centers of India with a radiochemical purity of >98% and specific activity of 32.74-65.49 GBq/$\mu$mol (885-1770 mCi/$\mu$mol).

$^{177}$Lu-DOTATATE is presently being used to treat patients with neuroendocrine tumors in six major hospitals across India wherein therapeutic doses of $^{177}$Lu-DOTATATE are prepared at the hospital radiopharmacies following the protocol developed. More than 150 patients suffering from various types of neuroendocrine originated tumors have been treated and more than 250 patient doses have been successfully dispensed to date. Fig. 3 shows the post-therapy scans (anterior and posterior) of patients with neuroendocrine originated primary cancer with extensive whole-body metastases and liver metastases, respectively, recorded at 1 d post-administration of 7.4 GBq (200 mCi) of $^{177}$Lu-DOTATATE.
of $^{177}$Lu-DOTATATE. Ongoing clinical investigations carried out in our country, revealed symptomatic relief for majority of the patients who underwent $^{177}$Lu-DOTATATE therapy.

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References