Approaches to Manufacturing Alpha Emitters For Radioimmunotherapeutic Drugs

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Abstract. Several alpha emitting isotopes have been proposed for radioimmunotherapy. To produce these nuclides reliably and in quantities needed, unique manufacturing approaches will be required. This paper describes the approaches that are being developed for the manufacture of 225Actinium (225 Ac) that decays to 213Bismuth (213 Bi) and the commercial manufacturing approaches. Oak Ridge National Laboratory (ORNL) currently supplies the actinium used for research and medical use. Today the ORNL 233U stockpiles only provide sufficient material for research quantities of 213 Bi. At the Institute for Transuranium Elements (ITU), in Karlsruhe, researchers have also developed a method of irradiating radium-226 with protons in a cyclotron to produce actinium-225 through the reaction $^{226}$Ra (p, 2n) $^{225}$Ac. Researchers from the Missouri University (MU), the Missouri University Research Reactor (MURR), MedActinium, Inc. and Los Alamos National Laboratory (LANL) are working on a collaborative effort to benchmark and optimize the production of $^{213}$Bi via neutron bombardment of $^{226}$Ra. MedActinium, Inc., in collaboration with commercial and institutional investigators at PG Research Foundation (PGRF) and Memorial Sloan Kettering Cancer Center (MSKCC), is developing commercial approaches to manufacturing these unique radioimmunotherapeutic drugs.

The alpha-emitters $^{212}$Bi, $^{213}$Bi, $^{225}$Ac, $^{211}$At, and $^{222}$Ra have very high cytotoxicity, high linear energy transfer, short path lengths (50-80 µm) and comparatively short radioactive half-lives. These properties along with their ability to conjugate with antibodies provide the potential for a breakthrough in radioimmunotherapy. The radiolabeled conjugates of these alpha emitters can selectively kill cancer cells and the absorbed dose ratios for cancer sites to whole body are far higher than for beta particles.

The most promising alpha particles appear to be $^{213}$Bi ($t_{1/2} = 46$ min) and $^{225}$Ac ($t_{1/2} = 10$ days). These alpha emitters have been extensively studied in pre-clinical evaluations [3, 5] and physician sponsored clinical trials [2,6] as promising radioimmunotherapeutic drugs.

In 1997 Memorial Sloan Kettering Cancer Center (MSKCC) developed generators to supply $^{213}$Bi and initiated the first human clinical trial with $^{213}$Bi labeled antibodies. This trial treated patients diagnosed with Acute Myeloid Leukemia (AML). The results of the trial indicated that the unique nature of the alpha particles’ high mass and energy in direct proximity to the cancer cell selectively killed individual cancer cells with minimal side effects to the patient.

Continuing studies at MSKCC have demonstrated the feasibility of using alpha radioisotopes to treat cancers such as those of the lung, prostate and breast. The treatment — Alpha Particle Immunotherapy (APIT) — combines the potent killing power of alpha particle-emitting radioactive atoms ($^{213}$Bi or $^{225}$Ac) with specific monoclonal antibodies to target cancer cells. In a clinical study, MSKCC treated 18 patients with AML in a dose-escalated study using $^{212}$Bi. In this study, the therapy successfully eliminated large numbers of tumor cells without significant clinical side effects to the patient [6]. This study also demonstrated the safety, feasibility, and antileukemic effects of $^{213}$Bi-HUM195 and resulted in the first proof-of-concept for system-targeted alpha particle immunotherapy to treat humans.

Concomitantly with the clinical study being performed at MSKCC, the processes and
controls necessary to commercially manufacture these radionuclides for radioimmunotherapeutic drugs are being developed. Commercialization of the radioimmunotherapeutic drug will require a reliable, sufficient, and economic supply of the radiochemical $^{213}$Bi. The supply of $^{225}$Ac and its daughter $^{213}$Bi is being sourced using the following four approaches:

- The extraction of $^{229}$Th from $^{233}$U that decays to $^{225}$Ac/$^{213}$Bi.
- Accelerator production of $^{225}$Ac through proton bombardment of $^{226}$Ra.
- Photon excitation of $^{226}$Ra producing $^{225}$Ra and subsequently $^{225}$Ac.
- Triple neutron capture in $^{226}$Ra leading to the production of $^{229}$Th.

The extraction process uses a generator to produce $^{225}$Ac by the radioactive decay of the parent $^{229}$Th. The shorter-lived daughter of interest is obtained through subsequent ion exchange separation and purification. These generators are used to produce actinium and bismuth isotopes at Oak Ridge National Laboratory (ORNL) and the Institute for Transuranic Elements (ITU). At the ITU, in Karlsruhe, Germany, 200 mg of $^{233}$Th is available and approximately 35 mCi of $^{225}$Ac can be extracted from it every two months [7]. ORNL and ITU supply about 60 mCi bimonthly of $^{225}$Ac for research and medical use through extractions from $^{229}$Th that has been previously separated from an inventory of $^{233}$U.

The $^{233}$U stockpile at ORNL (from $^{233}$U produced from molten salt breeder reactors in the 1960’s) also contains $^{232}$U that produces a mixture of $^{229}$Th and $^{229}$Th. The presence of $^{232}$U, as a contaminant in the $^{233}$U and the resultant decay products from $^{229}$Th, creates intense high-energy gamma radiation fields, which require special equipment and processing. This processing includes an extensive purification process that uses anion exchange to separate the thorium from uranium. Since the $^{229}$Th is typically in the ppm range, large quantities of $^{235}$U must be processed to produce useable quantities of $^{229}$Th.

The U.S. Department of Energy (DOE) has recently announced [8] its intent to seek commercial partners to process $^{231}$U to produce approximately 0.25 grams, or 50 mCi of $^{229}$Th. In addition, a recently announced Request for Proposal from DOE [9] is soliciting services to process all of the approximately 1 metric ton of uranium containing $^{231}$U currently in inventory for ultimate disposal. The $^{231}$U material will be processed to recover the $^{229}$Th, which will be used as a future source of $^{225}$Ac/$^{213}$Bi. The $^{233}$U will then be down-blended to produce a uranium oxide product suitable for long-term storage. By processing the $^{233}$U material prior to down-blending, this material will theoretically yield about 40 grams, or 8.5 Ci of $^{229}$Th. This would significantly expand the current inventory of available $^{229}$Th, from which $^{225}$Ac/$^{213}$Bi could be extracted and support commercial deployment of APIT.

Research is currently underway to study other methods for producing $^{225}$Ac/$^{213}$Bi. To fulfill the demand for ongoing projects and to prepare for future demand, ITU, DOE, and MedActinium are working on increasing production of $^{225}$Ac. ITU Researchers have patented the production of $^{225}$Ac through irradiation of $^{226}$Ra with protons from a cyclotron via the $^{226}$Ra (p, 2n) $^{225}$Ac reaction. ITU expects that under proper irradiation conditions, 1 Ci of $^{226}$Ra will produce over 1 Ci of $^{225}$Ac in two days [10]. MedActinium, Inc. is currently evaluating the feasibility of this process for potential commercial production. To date three $^{226}$Ra samples have been irradiated: a 1.4 µg sample irradiated for 5 days, a 1.4 µg sample irradiated for 18 days and an 11 µg sample irradiated for 35 days. Gamma-ray spectral measurements are carried out post-irradiation at times ranging from 3 days to 150 days.

The Missouri University (MU), the Missouri University Research Reactor (MURR), MedActinium, Inc. and Los Alamos National Laboratory (LANL) are working on a collaborative effort [12] to benchmark and optimize the production of $^{213}$Bi via neutron bombardment of $^{229}$Ra. This process involves the triple capture of neutrons, leading to the formation of $^{229}$Th, from which $^{225}$Ac/$^{213}$Bi can be extracted. This research involves both computational models to predict $^{225}$Ac/$^{213}$Bi production and experimental irradiations at the MURR followed by gamma spectroscopy analysis, to measure actual production rates. Irradiations are carried out in the graphite reflector of the reactor at a thermal flux of approximately $7 \times 10^{13}$ n/cm² sec and an epithermal flux of $2 \times 10^{13}$ n/cm² sec.

Table 1 gives a comparison between calculated and experimental results for the 11
μCi sample irradiated for 35 days at the MURR and measured at various time post-irradiation. The three isotopes listed (227Th, 228Th and 229Th) are the sequential result of the three-step neutron capture process. In general there is good agreement, although the experimental results are consistently 30% to 40% higher than predicted. To date, production of 229Th has been insufficient to accurately measure and therefore, irradiations at the High Flux Irradiation Facility (HFIR) at ORNL are being considered.

As a further benchmark, 229Th production has been compared to previous estimates assuming 25 day irradiations at the HFIR at ORNL at a thermal flux of 1 x 10^15 n/cm^2 sec and an epithermal flux of 4 x 10^14 n/cm^2 sec. Mirzadeh[13] has suggested that the yield of 229Th from 226Ra is approximately 7 mg per gram of 226Ra irradiated at a thermal flux of 1 x 10^15 neutrons/cm^2 sec for 24 days. Current calculations at Missouri University suggest a production of 1.8 mg of 229Th, while experiments at the MURR suggest 2.5 mg of 229Th. The disagreement between ORNL and MU results are primarily the result of differences in handling the cross sections in the calculations [14]. Additional verification of methods will likely reduce these differences.

If it is assumed that even the most conservative prediction of 1.8 mg of 229Th production is valid, the calculational model predicts an equilibrium 229Th production rate of approximately 1 mCi per day for a 10 gram 226Ra sample irradiated at HFIR. Current production costs from ORNL (i.e. the extraction of 229Th from 233U) are $14,000 per mCi [8]. Thus, it appears that neutron irradiation will be cost effective with current practice.

Approaches to commercially manufacturing these radionuclides must include the availability to produce sufficient quantity for research, development, pre-clinical studies, physician trials, clinical trials with patients and commercial supply. This supply is estimated to increase by a factor of 100 over the next five years if other APIT therapies are developed for cancers such as Non Hodgkin’s Lymphoma, prostrate, breast and others.

Production of 213Bi or 225Ac must be conducted using the applicable Food and Drug Administration (FDA) requirements (current Good Manufacturing Practices (cGMP). Commercialization of the radiopharmaceutical will require a cGMP source of the selected monoclonal antibody and bifunctional chelator as well. Conjugation of the radiometal to the antibody using the bi-functional chelator must also be performed under cGMP and must be stable in-vivo.

Finally, as required by the cGMP, the radiolabeled antibody must meet pre-determined specifications for yields, identity, chemical and radionuclidic purity, activity concentration, sterility and apyrogenicity.

The rapid preparation of a short-lived alpha particle emitting radiopharmaceutical for commercial use must be manufactured under a comprehensive quality management system based on the cGMP as described in Title 21 of the Code of Federal Regulations part 210 and 211(21 CFR 210,211). To be effective the systems of the commercial organization must include a QA organization independent of production, defined roles and responsibilities for all personnel, documented and controlled processes and procedures, and adequate facilities and equipment.

MedActinium, Inc. is designing a commercial facility and equipment with approaches that will meet these stringent requirements. The Alpha Radiopharmaceutical Facility will be designed, built, and validated to produce radiopharmaceuticals for patient use and process the radioimmunotherapeutic drug under cGMP requirements.

Operations in the building will include the manufacture, testing, packaging, distribution, and marketing of radiochemicals and radiopharmaceuticals. The facility is designed to meet FDA’s cGMP for design and construction and will also meet the radiological and environmental regulations of the State of Tennessee. The Alpha Radiopharmaceutical Facility, and associated equipment and personnel, will give MedActinium, Inc. the capacity to reliably supply its customers with radiochemicals and radiopharmaceuticals that meet regulatory and quality requirements.
grades of $^{229}\text{Th}$ and eliminate cross-contamination. This approach also allows for smaller shielded manipulator cells, reducing the area and materials contaminated, thereby reducing radioactive waste generation. The shielded manipulator cell ventilation system uses a novel negative pressure concept with internal HEPA/Carbon filter recirculation. This system continually removes airborne radioactive contamination and $^{226}\text{Ra}$, but contains the contaminants within the shielded confines of the cell. In addition, the system allows for removal of the filters for maintenance thus protecting workers from radiological exposure.

Rapid separation of the $^{225}\text{Ac}$ from $^{229}\text{Th}$ must be done “just-in-time”. The approach to maximizing $^{225}\text{Ac}$ is driven by the short half-life of both $^{225}\text{Ac}$ and its precursor $^{225}\text{Ra}$ ($t_{\frac{1}{2}} = 14.8$ days), which make it impractical to “stockpile” these materials for longer than a few weeks. MedActinium, Inc. has licensed proprietary separation processes from the PG Research Foundation, Inc. that maximizes the quantity of $^{225}\text{Ac}$ recovered to meet growing medical needs. These processes involve specialty chromatographic-type ion exchange resins that effectively separate Th, Ra, and Ac from various impurities. Chromatographic separations are distinct with nearly quantitative recovery of the desired radiochemical constituents. Furthermore, the separations technique can be configured to meet specific order requirements. Importantly, system performance can be validated using surrogates (e.g., $^{232}\text{Th}$ and $^{226}\text{Ra}$, spiked with $^{225}\text{Ac}$) as needed.

An example of an actinium recovery process is shown Figure 1 below. This process has been validated using $^{229}\text{Th}$ acquired from ORNL and also through surrogate testing. In this flowsheet, the Th (IV) is stored in an $(\text{NH}_4)_2\text{SO}_4$ solution until in growth of $^{225}\text{Ra}$ has approached radioactive steady state. At this point, the Th (IV)/$\text{SO}_4^{2-}$ solution is eluted on a cation-exchange resin as primary separation column that retains the Ac (III) and Ra (II) nuclides while greater than 98% of the Th (IV) elutes. After rinsing with $(\text{NH}_4)_2\text{SO}_4$, the Ra (II) and Ac (III) are stripped using concentrated HNO$_3$. Because trace Th (IV) may be present and because preservation of the $^{229}\text{Th(IV)}$ supply is critical, a guard column further removes Th (IV) impurities. The Th (IV) retained by the guard column may be recovered using the $(\text{NH}_4)_2\text{SO}_4$ solution at and recombined with the original $^{229}\text{Th(IV)}$ stock solution.

The Ra (II) and Ac (III) mixture is subsequently separated and the Ac (III) is recovered in 0.10 M HCl, which is seamlessly integrated into the multicolon column selectivity inversion generator for $^{212}\text{Bi}$. The Ra (II) in 6.0 M HNO$_3$ effluent from the Ac (III) purification may be recycled and used to strip future batches of Ra (II) and Ac (III) from the cation-exchange resin, as shown in Figure 2. This $^{225}\text{Ra}$ recycle permits the most efficient use of $^{225}\text{Ra}$ to produce $^{225}\text{Ac(III)}$, while simultaneously minimizing the losses of the $^{229}\text{Th}$ source material.

For many therapies currently under evaluation, the desired alpha emitter is $^{213}\text{Bi}$. However, the short half-life of $^{213}\text{Bi}$ presents unique challenges in administering consistent, high-quality radiopharmaceutical doses to the patient. By working with the medical customer and within the framework of FDA regulations, MedActinium has developed an automated prototype $^{213}\text{Bi}$ generator conjugation kit for use in a hospital setting. The kit aseptically extracts and purifies $^{213}\text{Bi}$ and remotely conjugates the mAb, which is then radiolabeled with the $^{213}\text{Bi}$. The alpha particle immunotherapeutic construct is then purified and transferred to a syringe ready for patient use.

Manufacturing the alpha emitters for radioimmunotherapeutic drugs require unique and specialized processes that must provide an adequate supply of the radiochemical. The processes being evaluated include the use of existing $^{233}\text{U}$ stockpiles, cyclotrons or linear accelerators and reactors. cGMP supply of the antibody and chelator as well as appropriate facilities and customized equipment are also being developed.

REFERENCES


9) DOE RFP DE-RP05-000R22860, 233U Disposition, Medical Isotope Production, and Building 3019 Complex Shutdown at the Oak Ridge National Laboratory.


12) U.S. Department of Energy Grant Number DE-FG05-01OR22891 to the Missouri University, Columbia, Missouri (Sept. 2001 – August 2003).


14) Personal Communication with Marc Garland, Bob Schenter and Saed Mirzadeh (June 19, 2002).

Table 1. MURR Experimental vs. Calculated Results (11 µg 226Ra sample, 35 day irradiation)

<table>
<thead>
<tr>
<th>Decay Time (days)</th>
<th>227Th Calc. (Bq)</th>
<th>227Th Exp. (Bq)</th>
<th>228Th Calc. (Bq)</th>
<th>228Th Exp. (Bq)</th>
<th>229Th Calc. (Bq)</th>
<th>229Th Exp. (Bq)</th>
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<tbody>
<tr>
<td>2.6</td>
<td>43,500</td>
<td>62,100 ± 200</td>
<td>92,984</td>
<td>98,000 ± 5,000</td>
<td>0.2</td>
<td>*</td>
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<tr>
<td>16</td>
<td>62,069</td>
<td>83,000 ± 200</td>
<td>92,884</td>
<td>117,000 ± 2,000</td>
<td>0.2</td>
<td>*</td>
</tr>
<tr>
<td>23</td>
<td>68,892</td>
<td>93,300 ± 200</td>
<td>90,886</td>
<td>121,000 ± 2,000</td>
<td>0.2</td>
<td>*</td>
</tr>
</tbody>
</table>

Figure 1. Example Scheme of Recovery of 225Ac