Poster #54

Radiolabeling of DOTA-conjugated Lintuzumab with ^{225}Ac: Comparison of ^{229}Th-produced and High-Energy Proton Accelerator-produced ^{225}Ac

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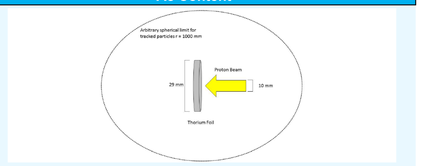
INTRODUCTION

Lintuzumab is a humanized monoclonal antibody (mAb) against CD33, an antigen widely expressed on myeloid stem cells and leukemic blast cells in patients with Acute Myeloid Leukemia (AML). Actinium Pharmaceuticals is advancing several targeted radio-immunotherapy programs utilizing Lintuzumab conjugated with the potent alpha emitting radionuclide Actinium-225 (^{225}Ac) to treat cancer patients. Currently, the supply of ^{225}Ac for clinical manufacturing is produced by a generator system from the decay of Thorium-229 (^{229}Th); however, the capacity of ^{229}Th generators to supply Ac-225 is limited (< 2 Ci/year). A highly promising source of Ac-225 supply is via high-energy linear proton accelerator (Linac) where ^{225}Ac is produced via irradiation of Thorium-232. Linac-produced ^{225}Ac, however, contains minor quantities (0.1-0.7% activity) of low energy ^{227}Ac which has a half-life of 21.8 years. ^{225}Ac has a half-life of 10 days. Because of the large differences in decay rates of these two isotopes, even at very low activity levels, the ^{227}Ac molecule is present in high quantities in the mixture. For example, at 0.3% activity, the molar ratio of ^{227}Ac to ^{225}Ac is approximately 2.3. At this level, the ^{227}Ac in linac preparations of ^{225}Ac may have a negative impact on labeling efficiency, stability and potency.

In order to assess the potential impact of the ^{227}Ac impurity on antibody labeling efficiency and other parameters, we conducted experimental studies where lintuzumab-DOTA conjugates were comparatively labeled with ^{225}Ac produced by both ^{229}Th generator and linac. In these studies, we compared radiolabeling efficiency and critical quality attributes of the radiolabeled finished drug product. Previously, *in vivo* mouse studies of linac-produced ^{225}Ac, free or DOTA-chelated, demonstrated similar biodistribution/dosimetry/toxicity profiles to ^{229}Th generated ^{225}Ac [1]. In our experimental scheme, a preparation of lintuzumab-DOTA conjugate was first prepared using a qualified manufacturing process. The lintuzumab-DOTA conjugate was then divided into two parts, and one part was radiolabeled with ^{229}Th generated ^{225}Ac and the second part with Linac-generated ^{225}Ac. Both ^{225}Ac radioisotope lots were supplied by the Department of Energy (DOE). Post-labeling, the radiolabeled lintuzumab-DOTA-Ac-225 preparations were passed through separate size exclusion chromatography columns to remove unlabeled ^{225}Ac from the preparation. The eluents were analyzed for radiochemical purity and immunoreactivity. Further, radiolabeling efficiency was determined for both ^{225}Ac radionuclide preparations. For verification of results, the study was repeated a second time with new lots of ^{225}Ac from each source. Our results demonstrated that, radiolabeling of lintuzumab-DOTA with ^{225}Ac generated by high energy proton accelerator exhibited similar characteristics in terms of radiolabeling efficiency, immunoreactivity and radiochemical purity to ^{229}Th generated ^{225}Ac, suggesting that the elevated molar concentration of low energy ^{227}Ac in linac preparations does not have a significant negative impact on the labeling of monoclonal antibodies for the generation of radioimmunoconjugates.

Challenges Associated with Accelerator Derived ^{225}Ac: Unavoidable ^{227}Ac Content

A)



B)

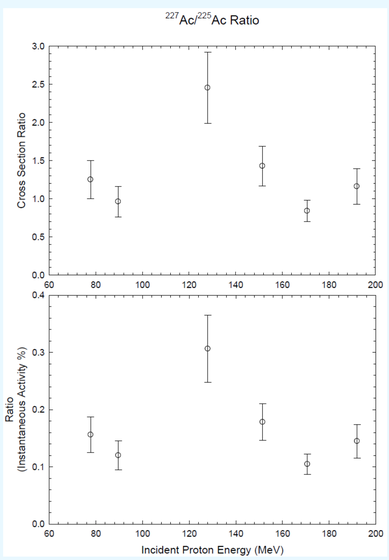


Figure 1: A) Simplified Geometry in PHITS (Particle and Heavy Ion Transport Code System) Calculations. B) ^{227}Ac/^{225}Ac ratio based on Cross Section and Instantaneous Activity. The lowest ^{227}Ac/^{225}Ac ratio measured for the lower energy IPF irradiations is at 90 MeV while the lowest ^{227}Ac/^{225}Ac ratio measured at BNL occurred at 174 MeV [2].

Figure 2: Impurities Present in ^{225}AC Produced from ^{229}Th vs ^{232}Th

A)

Thorium Cow Generated ^{225}Ac			
Radiosiotopes Batch 183026 Batch Analysis Date: April 02, 2018	Activity Level mCi	Activity Level %	Comments
^{225}Ac	42.9	99.99	
^{225}Ra	< 5x10^{-3}	< 1x10^{-2}	Not detected
^{224}Ra	< 5x10^{-4}	< 1x10^{-3}	Not detected
^{229}Th	< 2x10^{-4}	< 5x10^{-4}	Not detected
All fissionable material	< 3x10^{-5}	< 7x10^{-5}	Extrapolated from earlier runs

Accelerated Generated ^{225}Ac			
Radiosiotopes	Activity Level mCi	Activity Level %	Comments
^{225}Ac	2.2	99.99	
^{227}Ac	~0.02	~1	Extrapolated from earlier runs
^{223}Ra	< 2x10^{-4}	< 1x10^{-3}	Not detected
^{224}Ra	< 2x10^{-4}	< 1x10^{-3}	Not detected
^{227}Th	< 2x10^{-4}	< 1x10^{-3}	Not detected
^{229}Th	< 2x10^{-4}	< 1x10^{-3}	Not detected
^{140}Ba	< 2x10^{-4}	< 1x10^{-3}	Not detected
^{140}La	< 2x10^{-4}	< 1x10^{-3}	Not detected
^{241}Ce	< 2x10^{-4}	< 1x10^{-3}	Not detected

B)

	Ac225	Ac227
Specific Activity of Ac225 (Ci/g)	58,000	72.375
Activity per gram of Ac225 (Ci)	58,000	580
Amount based on Activity (g)	**1**	**8.01**

At 1% Activity, mass of ^{227}Ac is 8.01 times higher than mass of ^{225}Ac

Figure 2: A) Impurity analysis of ^{225}Ac generated from Thorium Cow (^{229}Th) vs Accelerator Process (^{232}Th). B) Calculation of mass ratio of ^{227}Ac/^{225}Ac based on presence of 1% Activity of ^{227}Ac.

Figure 3: Schematic of Conjugation and Labeling of Lintuzumab

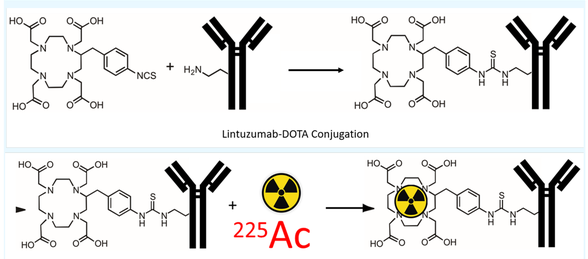


Figure 4: Retention time changes after DOTA Conjugation

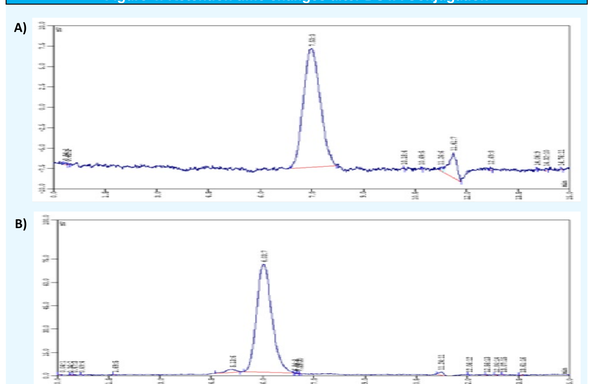
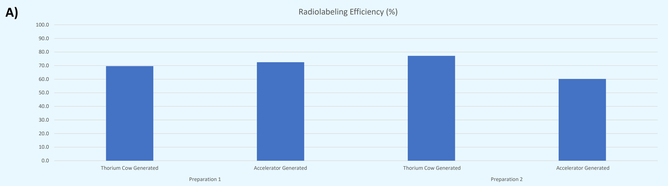


Figure 4: SEC-HPLC Analysis of A) Lintuzumab B) Lintuzumab-DOTA Conjugate

Figure 5: Radiolabeling Efficiency and Purity is Comparable for ^{229}Th vs ^{232}Th Generated ^{225}Ac

A)



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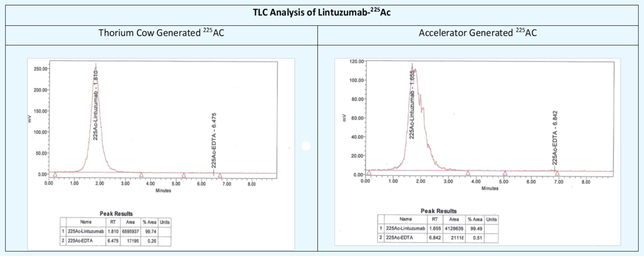


C)

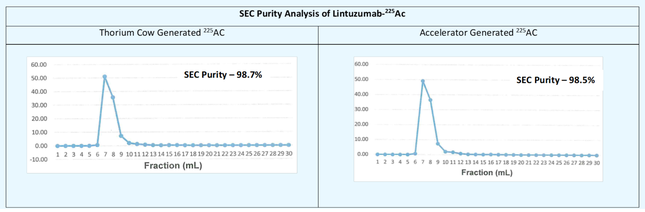


Figure 5: A) Radiolabeling Efficiency determination of Lintuzumab-^{225}Ac chelation process. B) Radiochemical Purity analysis of Lintuzumab-^{225}Ac using Instant Thin-Layer Chromatography (iTLC) to determine free Ac225. C) Radiochemical Purity Analysis of Lintuzumab-^{225}Ac using SEC-HPLC to determine HMW and LMW

Figure 6: Presumed Critical Quality Attributes are Comparable for ^{229}Th vs ^{232}Th Generated ^{225}Ac

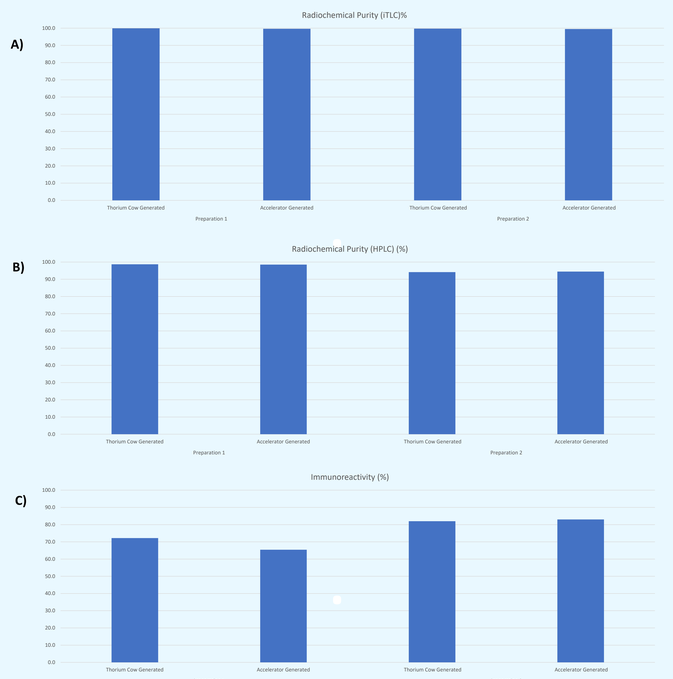
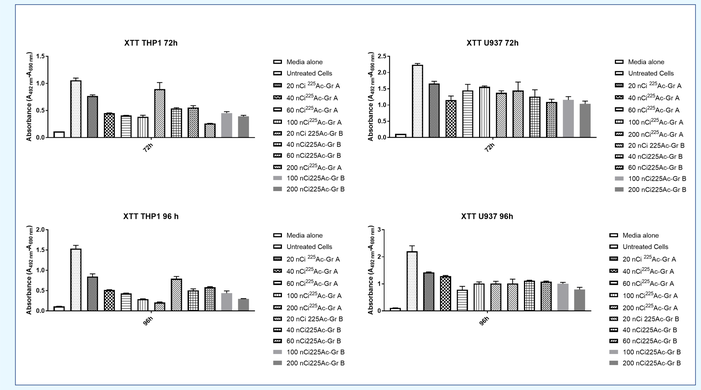


Figure 6: A) Radiochemical Purity by instant thin-layer chromatography . B) Radiochemical Purity analysis of Lintuzumab-^{225}Ac using SEC-HPLC. C) Immunoreactivity Analysis of Lintuzumab-^{225}Ac cell binding assay.

Figure 7: In vitro cellular cytotoxicity analysis of labeled ^{225}Ac-lintuzumab from the two ^{225}Ac sources



Source A: ^{229}Th "cow" ^{225}Ac
Source B: ^{232}Th accelerator-derived ^{225}Ac

Figure 7: Cytotoxicity analysis of anti-CD33, ^{225}Ac-lintuzumab labeled with ^{225}Ac material from the two sources was performed on two CD33 positive acute myeloid leukemia cell lines, THP1 and U937. Cells were incubated with titrations of labeled antibody for 4 hours at 37 degrees, washed and then incubated in complete media for 72 or 96 hours. Cell viability was measured by XTT assay. In this assay, the results demonstrated comparable levels of anti-leukemia cell killing irrespective of ^{225}Ac source.

SUMMARY

- During production of ^{225}Ac from ^{232}Th using accelerator, the presence of ^{227}Ac and other minor impurities is unavoidable. At 1% activity, molar ratio of ^{227}Ac to ^{225}Ac is 8.01.
- Even though molar ratio of ^{227}Ac is higher than ^{225}Ac in accelerator generated material, labeling efficiency is similar and no free ^{225}Ac was detected after labeling
- Critical quality attributes such as Radiochemical Purity and Immunoreactivity were similar between both ^{225}Ac.
- ^{225}Ac-labeled anti-CD33 lintuzumab showed comparable effectiveness in an in vitro cellular cytotoxicity study irrespective of ^{225}Ac source
- These studies demonstrate the feasibility of using accelerator generated ^{225}Ac for targeted alpha therapy

References:
1. Jiang, Z., Revskaya, E., Fisher, D. and Dadachova, E. Current Radiopharmaceuticals, 2018. 11(3): 215-222
2. Actinium-225 Production via Proton Irradiation of Thorium-232: Justin Reed Griswold

Conflict-of-Interest Disclosure: D.L.L. and V.P. have equity ownership in and are employed by Actinium Pharmaceuticals, Inc.; E.D. receives research funding from Actinium Pharmaceuticals, Inc.; R.G. and K.A. have no disclosures.