Assessment of Long-Lived Contaminants in Zr-89 Labeled Monoclonal Antibodies

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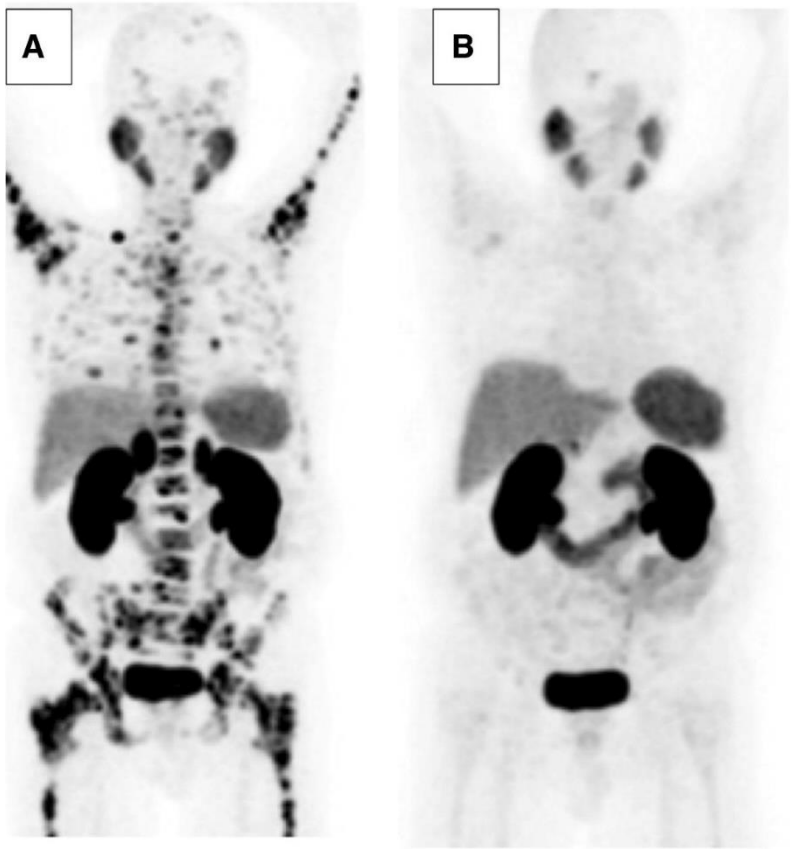
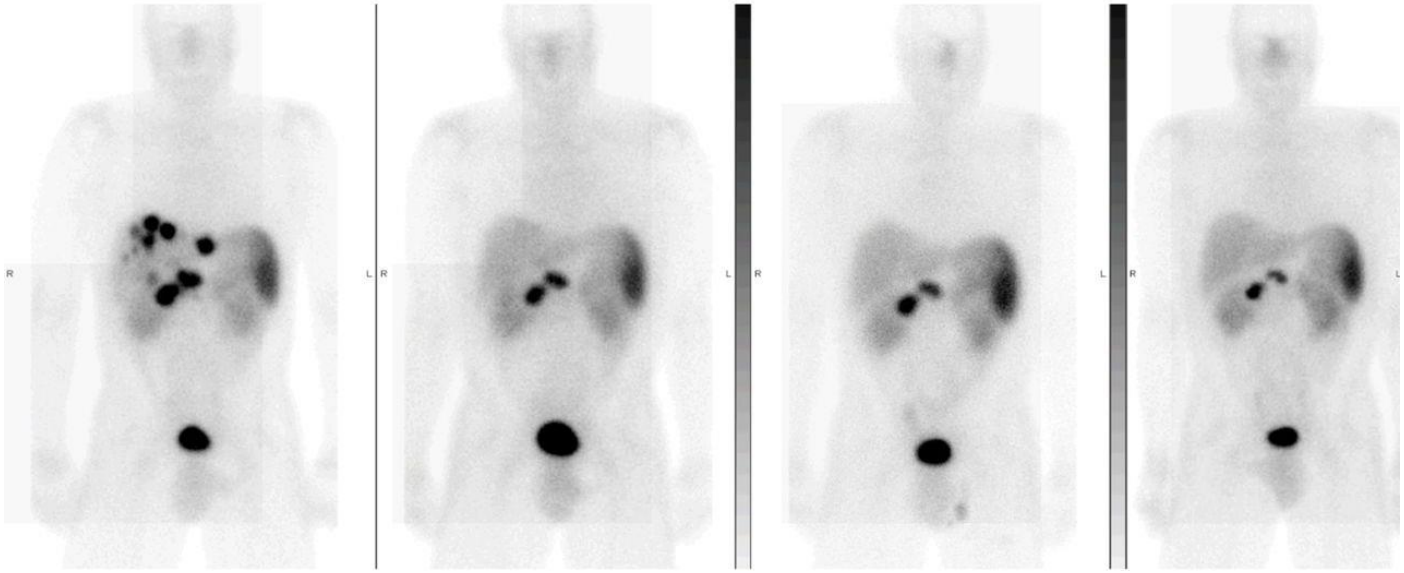
Theranostics

- Theranostics (Therapy and Diagnostics) is sweeping western medicine.
- Major referral centers are establishing dedicated clinics throughout the western world.
- In this new treatment philosophy, an imaging isotope with a short half-life is chelated to a tumor-specific ligand and the patient is imaged on a PET/CT scanner.
- If the tumor uptake of the compound is considered acceptable, the imaging isotope is replaced by a strong beta emitter (e.g. Lu-177) or an alpha emitter (e.g. Ac-225) and the patient is treated with several courses of the therapy compound.



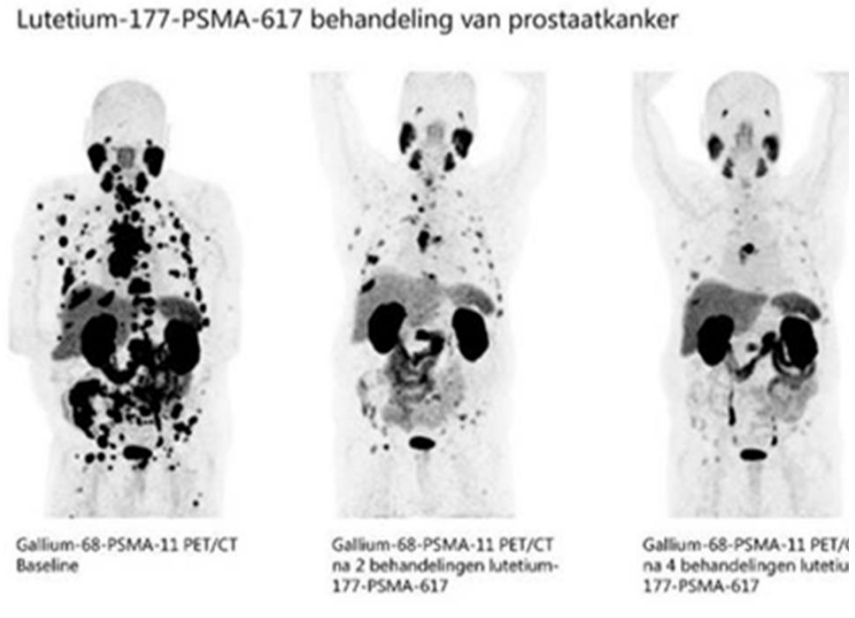
Lutathera ®

- Lutathera (Lu-177 DOTATATE) has been approved for the treatment of Neuroendocrine disease in the pancreas and the gastrointestinal tract. This is a relatively rare tumor.



Pluvicto ® (Lu-177 PSMA)

- Lu-177 tagged to a Prostate Specific Membrane Antigen is now widely used for salvage therapy of advanced Prostate Cancer.
- Efforts are being made to move the therapy earlier in the treatment progression.
- Two other PSMA ligands are in clinical trials (Eclipse and Splash studies).



Theranostic Imaging Isotopes

- The imaging isotope chosen must be a positron emitter (obvious), but also must meet other criteria.
- The isotope must be such that the ligand of interest can be chelated to the isotope and be stable from synthesis through the imaging protocol.
- The isotope must have a half-life that allows production, synthesis, delivery, and imaging to occur within a reasonable time period.
- The isotope must be readily available and capable of meeting FDA purity requirements. Currently, there is insufficient production of both the imaging and treatment isotopes to meet demand.

Theranostics – Imaging Isotopes

Isotope	Half Life
F-18	109.77 m
Ga-68	67.71 m
I-124	4.176 d
Zr-89	78.41 hrs
Cu-61	3.33 hrs
Tc-99m	6.02 hrs

An Explosion of Research

- The success of the early treatments has produced an explosion of research and development in the production of isotopes for both the diagnostic and the therapeutic side of the theranostic equation.
- Many new ligands are under investigation for use in the theranostic protocols. All are targeted agents, in that they localize in a specific lesion rather than the destruction of cells with a high mitotic rate (many chemotherapy regimens).
- Let's consider monoclonal antibodies.

Monoclonal Antibodies

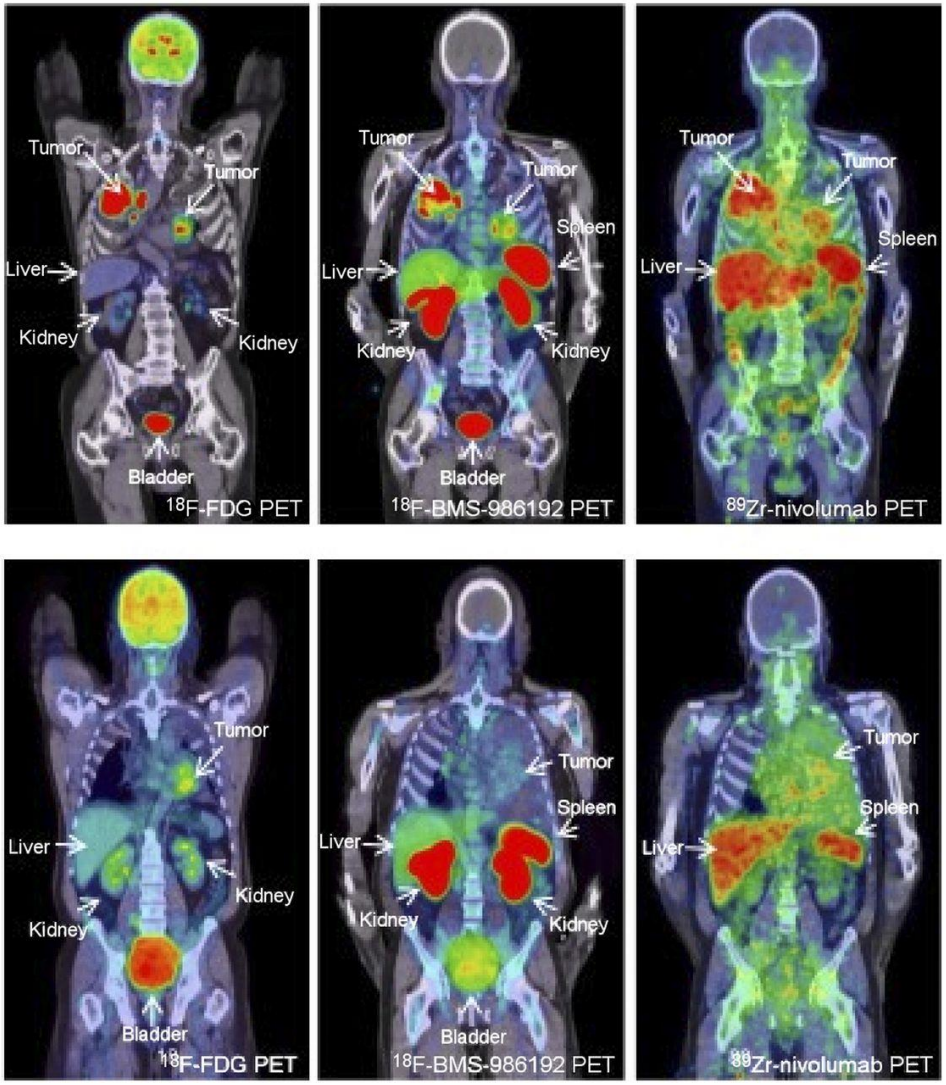
- Monoclonal antibodies (mAbs) are laboratory-made proteins that mimic the immune system's natural antibodies. They are designed to recognize and bind to specific targets, such as antigens on cancer cells or viruses.
- mAbs are produced by cloning a single white blood cell that produces an antibody against a specific target. This ensures that all the antibodies produced are identical and bind to the same target.
- They are used to treat multiple types of cancer, including lymphoma, leukemia, and breast cancer.
- Over 40 mAbs have been approved by the FDA with dozens more in development.

Pharmacokinetics

- The main mechanism by which mAbs distribute from the blood into the tissue is through convective transport. Convection is determined by the flux of fluid from the vascular space to the tissue, which is driven by the blood-tissue hydrostatic gradient, as well as by the sieving effect of the paracellular pores in the vascular epithelium.
- This process is slow compared to the uptake mechanisms used for most PET imaging. mAbs require prolonged circulation times to reach maximum target accumulation.
- Consequently, the short half-life of the most common imaging isotopes (F-18 & Ga-68) makes them impractical for imaging with mAb ligands.
- From Ryman & Melbohm, "Pharmacokinetics of Monoclonal Antibodies", CPT Pharmacometrics Syst. Pharmacol. (2017) 6, 576-588

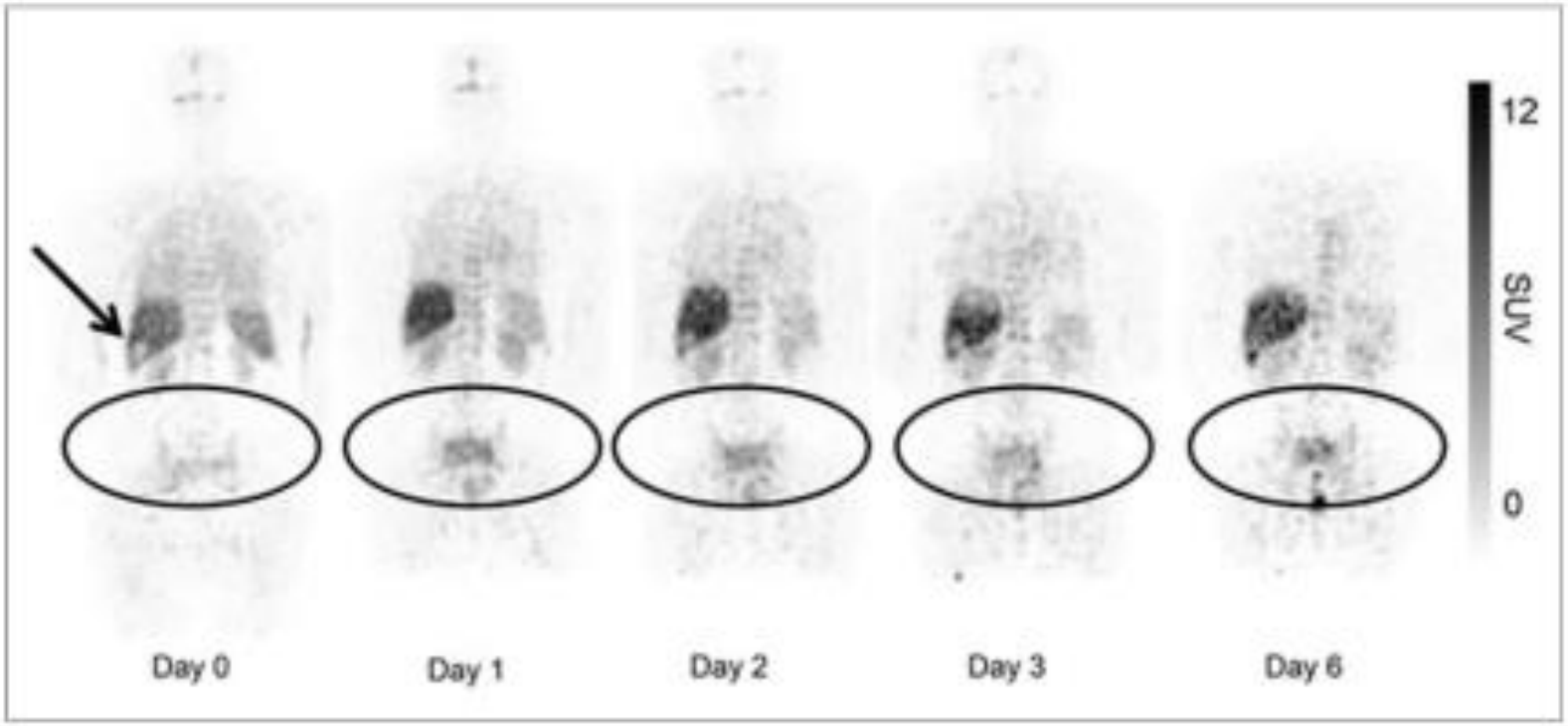
Initial Clinical Trials

- In clinical trials, several mAbs have been tagged to Zr-89 to help measure the efficacy of immunotherapy with mAbs, and to evaluate their potential for Theranostic regimens.
- There are no released Zr-89 imaging agents.
- Guus et.al, The Role of 89Zr-Immuno-PET in Navigating and Dersking the Development of Biopharmaceuticals, J Nucl Med 2021, 62:438-446.



Slow *In Vivo* Kinetics

- A ^{89}Zr-cetuximab image showing uptake of the labelled mAb over time.



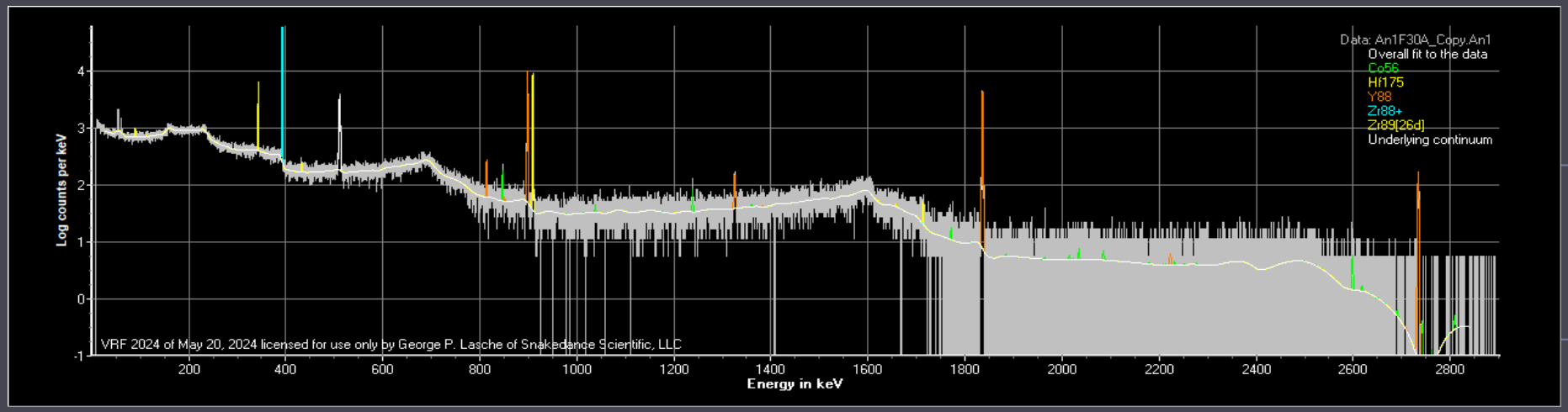
Alternative Isotopes for mAb Imaging

- Zr-89 ($T_{1/2}$ 78.41 hrs), is well suited for the imaging of mAbs.
- The half-life is long enough to allow the tagged mAbs to accumulate in the tumor that needs to be imaged.
- Radiopharmacists describe the process to chelate the mAbs to the Zr-89 as complex but achievable, and the complex is stable for the length of time needed for imaging.
- The production of impurities (described below) can be controlled such that the compound can meet the FDA limit both at EOS and at expiry.

Production of Zr-89

- The most common production method for Zr-89 utilizes a natural abundance Y-89 solid target in a medical cyclotron at beam energies of 13 – 16 MeV.
- The $^{89}Y(p,n)^{89}Zr$ is optimal at low proton beam energies (<13 MeV), so an aluminum degrader is commonly used to lower the incident beam energy.
- The two most common contaminants (^{88}Zr and ^{88}Y) are produced by the $^{89}Y(p,2n)^{88}Zr$ and the $^{89}Y(p,pn)^{88}Y$ reactions.
- The proper choice of proton beam energies and degrader can suppress most of the long-lived contaminants.
- Gaja, et.al, "Production and Semi-Automated Processing of 89Zr Using a Commercially Available TRASIS MiniAiO Module" Molecules 2020, 25, 2626

Zr-89 Contaminant Spectrum



Common Contaminants in Zr-89

Isotope	Half-Life
Y-88	106.65 d
Zr-88	83.4 d
Zr-89m	4.161 m
Zn-65	244.06 d
V-48	15.97 d
Co-56	77.23 d
Tb-156	5.35 d
Hf-175	70 d

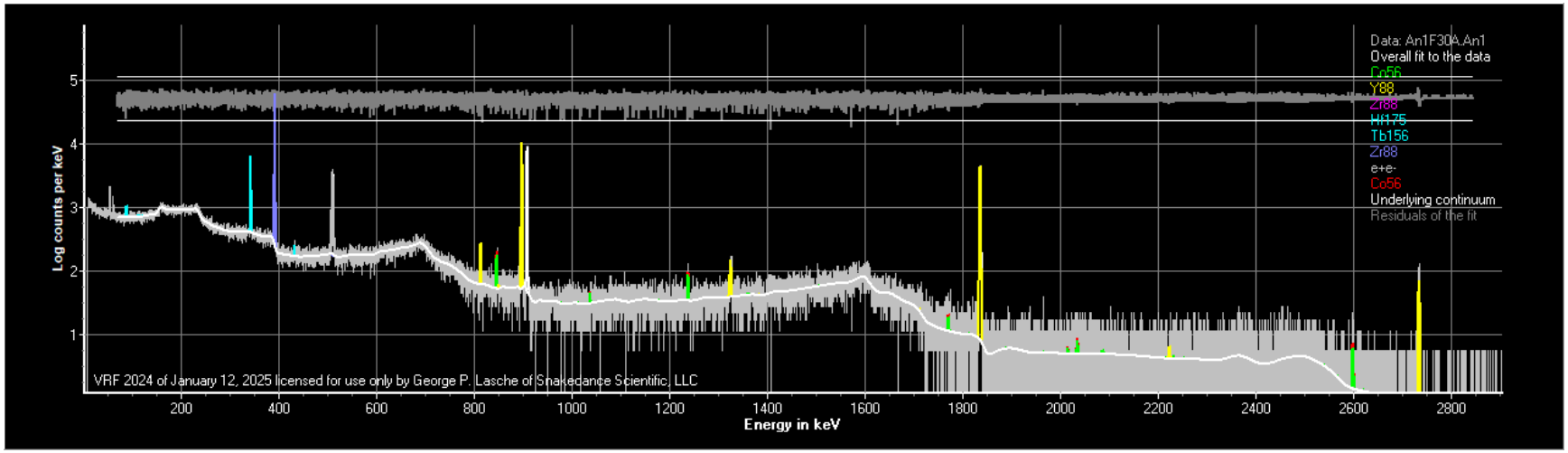
Method Development

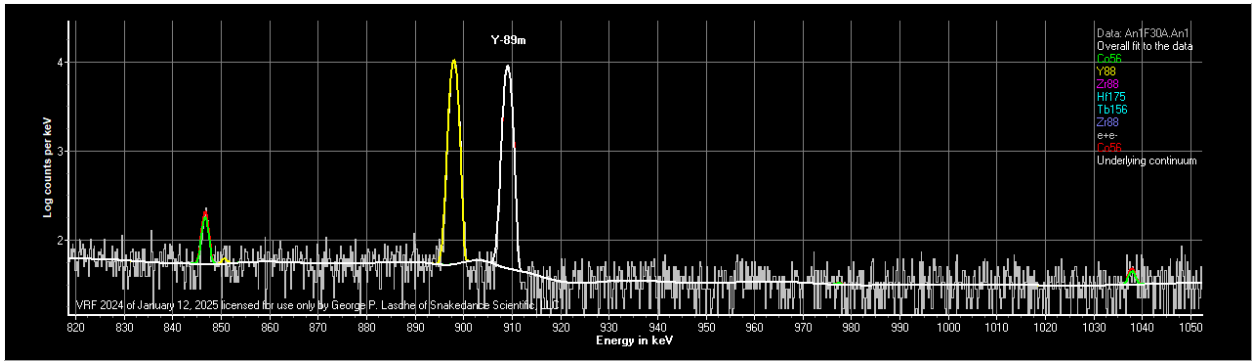
- Samples of Zr-89 labeled mAbs were taken at the End of Synthesis (EOS) and were measured in a medical dose calibrator to determine the initial activity.
- After allowing the Zr-89 to decay for several half-lives, the samples were counted on a high purity gamma spectrometer, and the recorded spectra was analyzed using the Gammavision software package (Ortec). The list of common contaminants noted previously was provided as a library. The analysis was run both for EOS and Expiry as provided by the producer.
- Results were presented at contaminant activity per MBq ^{89}Zr at EOS and expiry.

Method Validation

- The method was validated by analyzing the same spectra collected using VRF, rather than Gammavision.
- VRF uses a completely different algorithm for analysis of HPGe generated spectra than the normal peak-search algorithms used by Gammavision and Genie (Canberra).
- VRF uses a Levenberg/Marquart iterative fitting process to analyze a spectrum.
- Both Gammavision and VRF had been previously calibrated with a multiline standard (Eckert & Ziegler) in the same geometry used for the analyses.

VRF


Data: An1F30A.An1
Overall fit to the data
Co56
Y88
Zr88
Hf175
Tb156
Zr88
e+e-
Co56
Underlying continuum
Residuals of the fit
Log counts per keV
Energy in keV
VRF 2024 of January 12, 2025 licensed for use only by George P. Lasche of Snakedance Scientific, LLC


Y-89m
Data: An1F30A.An1
Overall fit to the data
Co56
Y88
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Hf175
Tb156
Zr88
e+e-
Co56
Underlying continuum
Log counts per keV
Energy in keV
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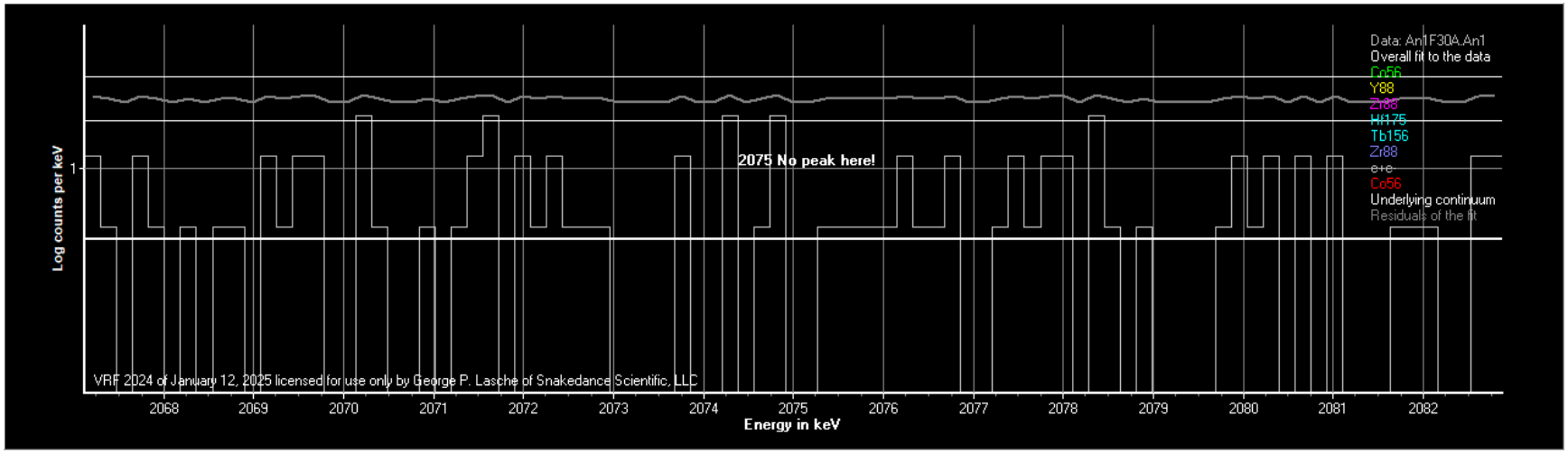
Intercomparison Results

	Y-88	Zr-88	Co-56	Tb-156	Hf-175
GV	2470 ± 16	5280 ± 20	49.6 ± 5.5	<4830	563 ± 10
VRF	2339 ± 9.6	5235 ± 11.3	29.9 ± 1.3	<12295	580 ± 3.9
GV/VRF	1.06	1.01	1.66	NA	0.97

Good agreement was observed for all positive isotopes with the exception of Co-56. Co-56 has 72 photons and a high probability of True Coincidence Summing. The Gammavision and VRF codes use different methods of correcting for TCC. This algorithm used by GV is overly aggressive and caused the observed difference.

No Observed Summing in This Sample



Conclusion

- A straightforward method has been developed to assess the long-lived contaminants in ^{89}Zr labelled mAbs generated from solid ^{89}Y targets irradiated by conventional cyclotrons.
- The method has sufficient sensitivity to measure contaminant loads at and below the FDA 99.5% purity limit both at EOS and Expiry.

Homeland Security Monitors

- Homeland Security Monitors, usually constructed of large plates of plastic scintillator, will alarm for the ^{89}Zr PET patient for several days or longer after the dose is administered.
- Some of the long-lived contaminants are avidly retained by the body and can also cause the patient to alarm the detectors an undetermined length of time after the study. The long-lived contaminants do not show as "medical" isotopes in the Detective and other scanner libraries. This can create problems for the patient.
- A card should be issued to ^{89}Zr PET patients.