Direct visualization of a non-radioactive antibody-based radiopharmaceutical analog in a mouse model of pancreatic cancer using imaging mass cytometry

[1]Felix Salazar, [2]Smriti Kala, [2]Daniel Majonis, [2]Hang Zhou, [1]Bao Ying Chen, [2]Qanber Raza, [2]Christina Loh, [2]James Mansfield, [1]Anna M. Wu

[1]Department of Immunology and Theranostics, AR-DMRI, Beckman Research Institute of City of Hope, Duarte, CA 91010; [2]Standard BioTools, Toronto, Ontario, Canada

BACKGROUND

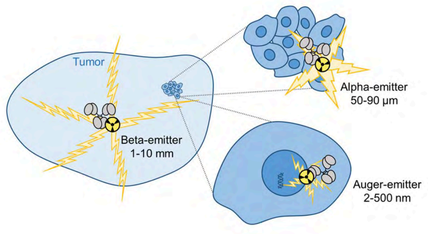
Radiopharmaceutical therapy (RPT)

Targeted delivery of radionuclides can provide specific imaging and effective therapies in cancer

- Xofigo (Ra-223), Lutathera and Pluvicto (Lu-177) are standard of care
- New agents in development and translation based on small molecules, peptides, antibodies and alternative protein scaffolds as targeting agents
- In addition to efforts in academia, pharmaceutical companies have invested heavily in novel agents for RPT

Challenge:

- Biological effects and treatment outcomes using RPT can be highly dependent on localization of radionuclides
- Beta vs alpha vs auger emissions vary greatly in energy and path length
- Understanding the deposition of targeted radionuclides at the tissue and cellular level is essential for assessing effects in tumors and normal tissues



Tsai and Wu, J. Radiolab. Comp. and Radiopharm. 2018

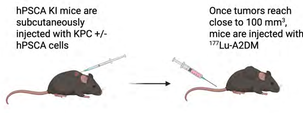
Prostate Stem Cell Antigen (PSCA)

- 123 a.a. GPI-linked cell-surface glycoprotein
- Low expression in normal organs (prostate, bladder, stomach)
- Highly expressed in **prostate** cancer (~95%), **pancreatic** (60-80%) and **bladder** cancers

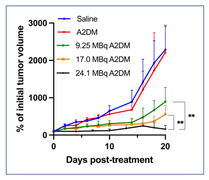
Engineered anti-PSCA antibody fragment for radioimmunotherapy: ^{177}Lu-DTPA-A2DM

- scFv-Fc2 anti-PSCA antibody fragment
- H310A/H435Q mutations ensure rapid clearance
- Conjugated with DTPA for radiolabeling with ^{177}Lu

Radioimmunotherapy (RPT using antibodies)

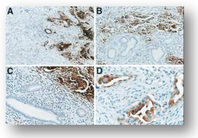


Dose-dependent inhibition of KPC-hPSCA tumors following iv administration of ^{177}Lu-DTPA-A2DM



K. Zettlitz et al., abstract WMIC 2021; B.Chen et al. abstract WMIC 2024

PSCA IHC
Pancreatic cancer



Radiopharmaceutical

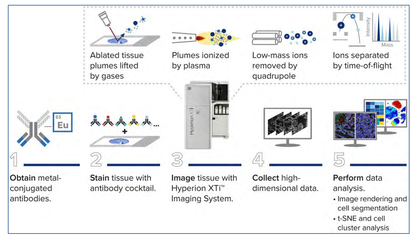
APPROACH

Imaging mass cytometry (IMC) for detection of non-radioactive analog

Radiopharmaceuticals can be challenging to detect following in vivo administration

- Radiopharmaceuticals are frequently produced using “no carrier added” preparations of radionuclides, in order to reach the highest specific activities.
- Radiopharmaceuticals are frequently utilized in preclinical studies and clinical trials at trace levels.
- As a result, standard IMC methods are not sensitive enough for direct detection of RPT agents.

Generation of the corresponding targeting agent labeled with mass amounts of stable metals could enable direct visualization of the spatial distribution of the surrogate RPT agent



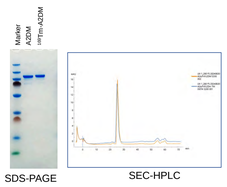
Radiopharmaceutical Cold Surrogate

Nuclides with masses between 89 and 209 have the potential for detection by IMC.

METHODS

Generation of non-radioactive surrogate

- Anti-PSCA A2DM antibody was conjugated with a 10-fold molar excess of Tm-p-SCN-Bn-Dota (Macrocyclics) for 2 h at 37°.
- Unbound ^{169}Tm-DOTA was removed by 2 x spin columns.
- ICP-MS analysis shows 3 - 4 ^{169}Tm atoms per antibody.

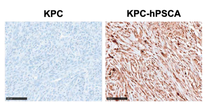


SDS-PAGE

SEC-HPLC

Mouse model of pancreatic cancer

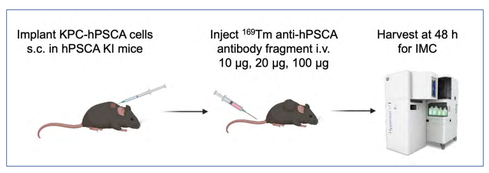
- KPC-hPSCA tumor cells were implanted subcutaneously in syngeneic hPSCA knock-in mice (hPSCA KI) and allowed to reach a volume of approximately 100 mm^2
- ^{169}Tm-A2DM was administered intravenously at protein mass doses of 10, 20, 0r 100 µg.
- 48 h later, mice were euthanized, and tumors and liver removed for fixation and processing by IMC.



PSCA IHC of s.c tumors

Imaging mass cytometry (IMC)

- Tissue sections were stained and detected using the antibody panel shown to the right, except that the RPT surrogate was detected directly.
- Data were acquired using a Hyperion XTi in Tissue and Cell modes.

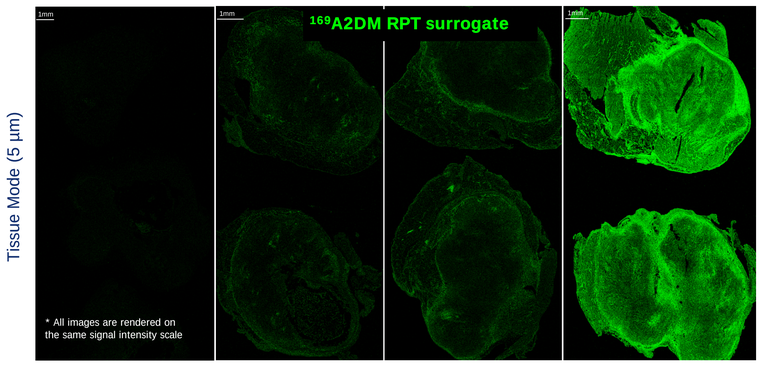


Markerm	Nuclide
RPT Surrogate	^{169}Tm
PSCA	^{168}Er
Pan-cytokeratin	^{142}Nd
E-Cadherin	^{158}Gd
CD44	^{153}Eu
CD45	^{147}Sm
CD31	^{151}Eu
α-SMA	^{141}Pr
Collagen	^{144}Nd
DNA	$^{191/193}$ Ir

IMC marker panel

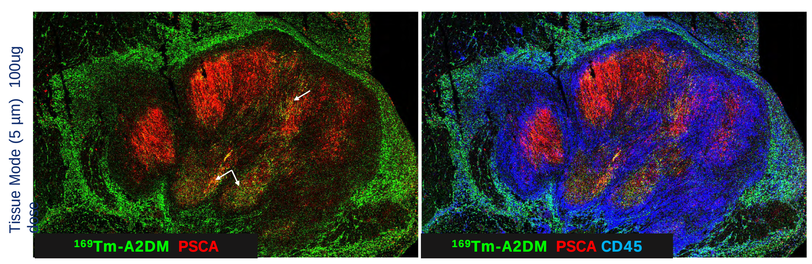
RESULTS

Effect of injected dose of ^{169}Tm-A2DM on detection by IMC



Strength of the ^{169}Tm signal clearly increased as the mass of A2DM administered to the mice was escalated. In previous RPT experiments using ^{177}Lu-A2DM, a 10 µg protein dose was used.

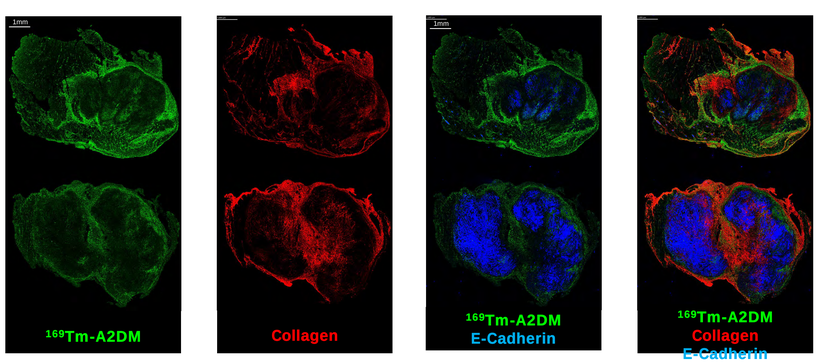
Visualization of overlap between ^{169}Tm-A2DM and PSCA



PSCA-rich regions of the tumors (red) showed low-level accumulation of the 169Tm-A2DM analog (green) at 48 h. However, the majority of the RPT surrogate was confined to the periphery of the tumor. Intense infiltration of the tumor core by CD45+ immune cells (blue) was also observed.

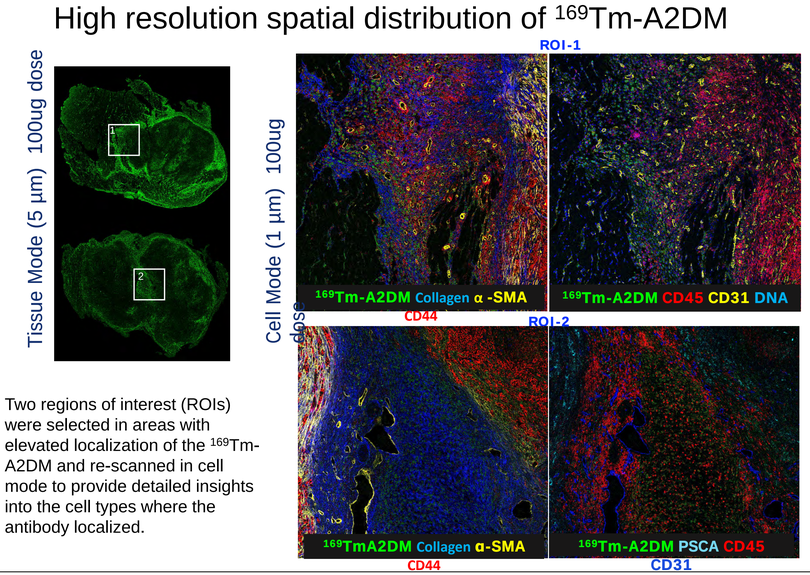
RESULTS (cont.)

Spatial distribution of ^{169}Tm-A2DM within PDAC tumor microenvironment



Overlay images of the ^{169}Tm-A2DM reveals patterns of distribution of the RPT analog with regard to the stromal compartment (collagen) and epithelial cells (E-cadherin).
The RPT analog appears to accumulate in collagen-rich areas in this stromal-rich model of PDAC.

High resolution spatial distribution of ^{169}Tm-A2DM



Two regions of interest (ROIs) were selected in areas with elevated localization of the ^{169}Tm-A2DM and re-scanned in cell mode to provide detailed insights into the cell types where the antibody localized.

CONCLUSIONS

The use of non-radioactive metal-labeled RPT surrogates is a viable approach for understanding the localization and deposition of radionuclides in target and normal tissues using IMC.

- Assessment of the tissue and cellular distribution of targeted radionuclides will be essential for understanding their biological and therapeutic effects.
- Radiopharmaceutical agents labeled with cold metals can be administered to mice and microdistribution in tumors and normal tissue detected by IMC.
- The anti-PSCA A2DM antibody fragment co-localizes with its target in KPC-PSCA tumors, but significant accumulation is seen in stromal regions. This may be a characteristic of pancreatic adenocarcinomas (PDAC) which are notoriously stromal-rich and difficult to treat.

Future directions

- Improve sample handling and detection to enable direct detection of radionuclide decay products.
- Evaluate additional nuclide pairs (e.g. ^{225}Ac/^{209}Bi).
- Employ IMC to track the effects of radiopharmaceutial therapies in the tumor/immune microenvironment.