Targeted Alpha Therapy for the treatment of multiple myeloma using CD38-targeting sdAb 2F8

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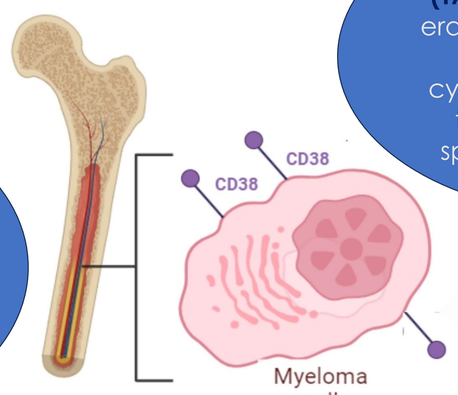
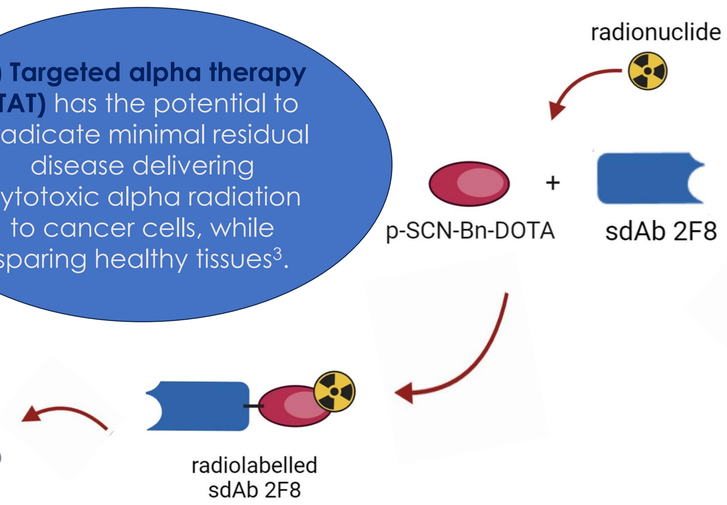
Introduction

This project involves developing a novel therapy for the treatment of multiple myeloma. This therapy, known as Targeted Alpha Therapy (TAT) is based on coupling sdAb 2F8[1] (derived from *Camelidae*), targeting CD38 on MM cells, with therapeutic radiometal Actinium-225 (^{225}Ac; α-particle emitter) and Lutetium-177 (^{177}Lu; β$^-$-particle emitter).

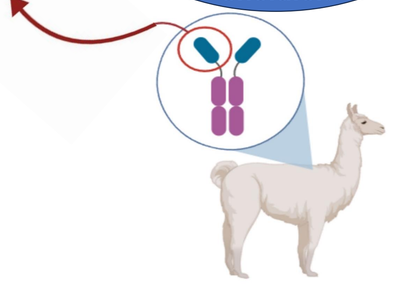
1) Multiple myeloma (MM) is an incurable hematological malignancy , and patients experience relapse despite achieving complete remission[2].



2) Targeted alpha therapy (TAT) has the potential to eradicate minimal residual disease delivering cytotoxic alpha radiation to cancer cells, while sparing healthy tissues[3].



3) TAT is carried out using a vector radiolabelled with a therapeutic radionuclide by means of a **chelator.**



4) Single-domain antibody fragments (sdAbs), derived from Camelidae heavy-chain antibodies, exhibit ideal properties as carrier molecules for TAT.

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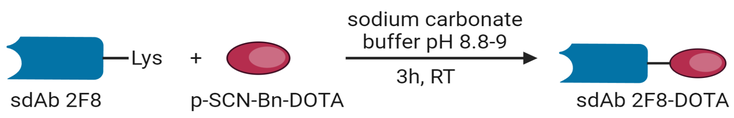
Methods

sdAb 2F8 random DOTA conjugation

Random conjugation of 100 eq of p-SCN-Bn-DOTA on sdAb 2F8's lysines. Evaluated by mass spectrometry.



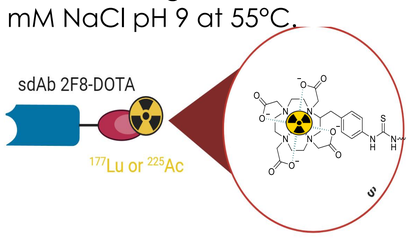
Stability studies in human serum

Evaluation of the stability of the radiolabeled sdAb in human serum at 37°C over the time.

Radiolabeling of sdAb 2F8-DOTA with ^{177}Lu or ^{225}Ac

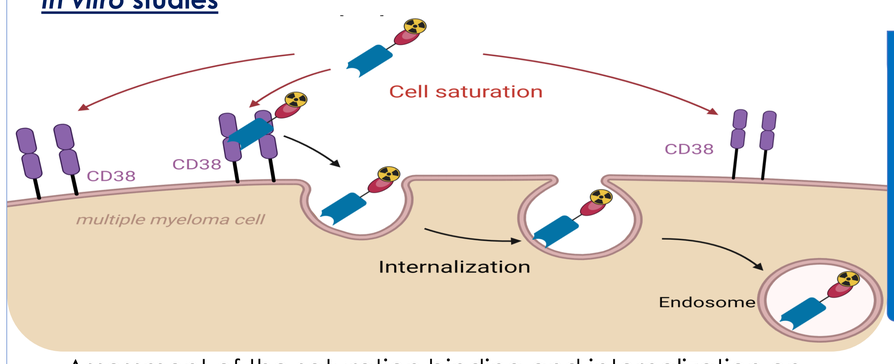
Radiolabeling performed by incubating the sdAb and the radionuclide for 30 minutes:

- ^{177}Lu-labeling was carried out in 0.5 M NH_4OAc pH 5.5 ± 0.1 at 50°C
- ^{225}Ac-labeling was carried out at 75mM TRIS, 225 mM NaCl pH 9 at 55°C.



The quality control after each reaction is performed by radio-HPLC (SEC) and iTLC.

In vitro studies

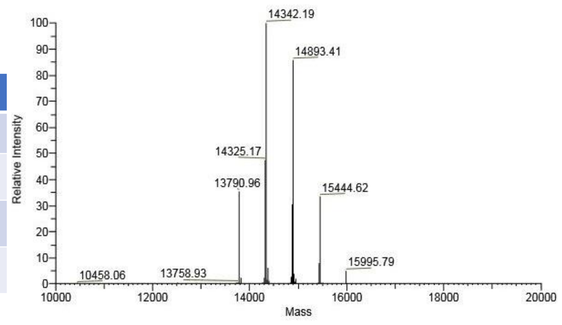


Assessment of the saturation binding and internalization on CD38+ MM cells of the radio conjugate.

Results

DOTA random conjugation on sdAb 2F8

Samples	Expected mass (Da)
Starting sdAb	13790.6
sdAb:DOTA 1:1	14342.19
sdAb:DOTA 1:2	14893.41
sdAb:DOTA 1:3	15444.62

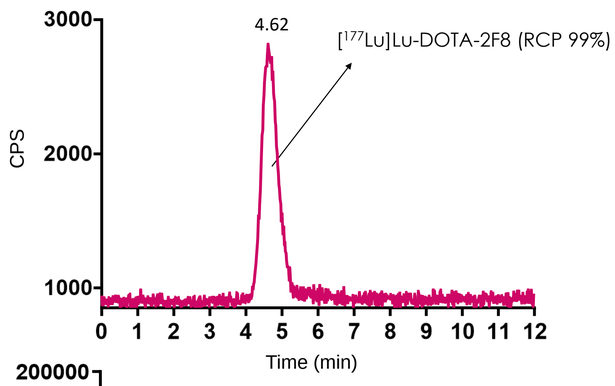


Deconvoluted mass spectra. Mass spectrometry ESI-Q-TOF analysis. Peaks at the expected molecular weight of sdAb adducts. The sdAb is successfully conjugated to p-SCN-Bn-DOTA with an average of 1,4 DOTA per sdAb.

^{177}Lu and ^{225}Ac-labelling of sdAb 2F8-DOTA

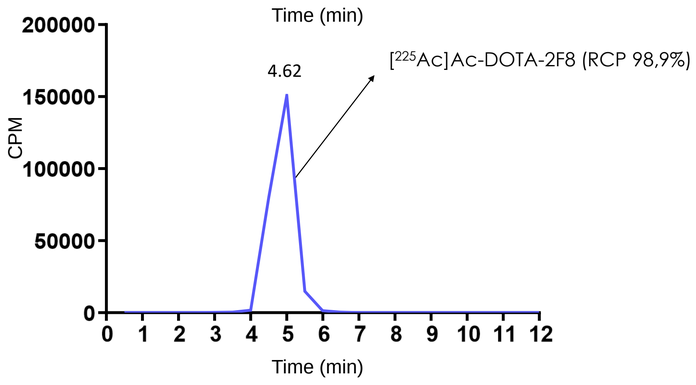
Size exclusion chromatography (SEC-HPLC):

^{177}Lu measurement method.



Size exclusion chromatography (SEC-HPLC):

Reconstitution Radio-SEC chromatogram after decay (6h). Evaluated considering equilibrium with ^{213}Bi and ^{121}Fr (the daughter isotopes of ^{225}Ac – γ emitters)

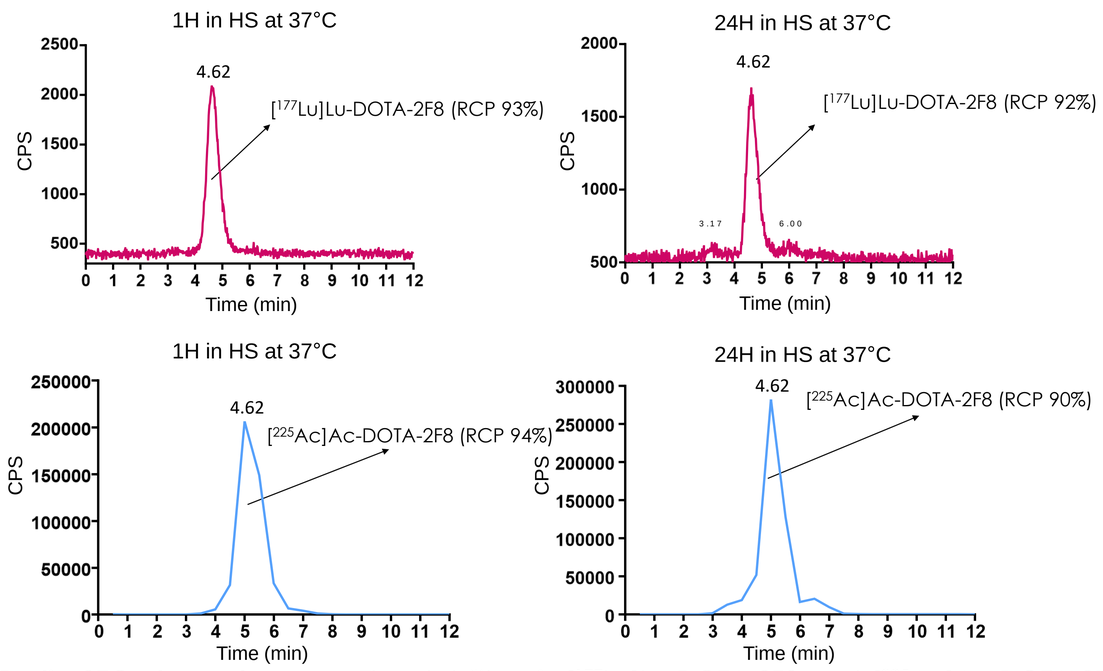


Radio-SEC chromatograms. The radiolabeling is evaluated comparing the intensity of radiations over the time.
The peak of sdAb 2F8-DOTA (at Rt ~ 4 min) present radiochemical purity (RCP)>90% in nearly completely absence of free radionuclide (Rt>6min).

- ^{177}Lu-labeling was realized with 14.81 MBq/nmol (sdAb 20 µM)
- ^{225}Ac-labeling was realized with 100 kBq/nmol (sdAb 20 µM)

The sdAb 2F8-DOTA can be radiolabeled with ^{177}Lu and ^{225}Ac. For the alpha emitter less radioactivity is needed because it is more effective, however the radiolabeling require a high ratio of chelator conjugated compared to ^{177}Lu-labeling.

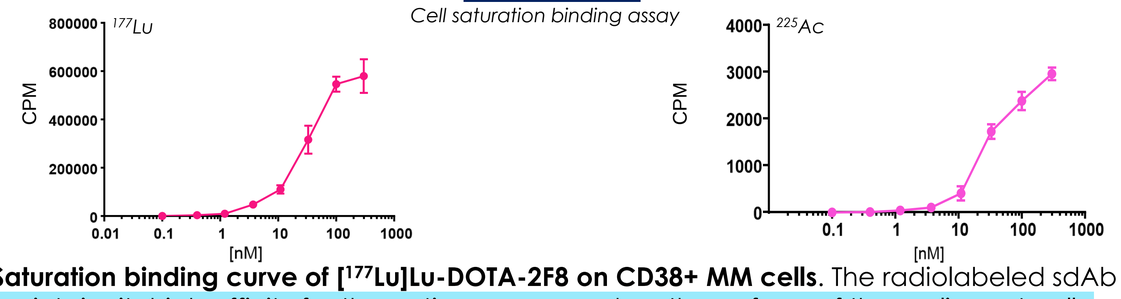
Stability in human serum (HS)



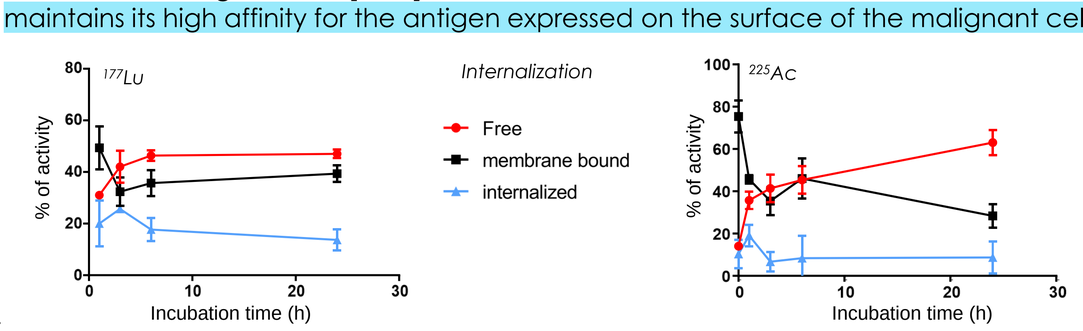
Radio-SEC chromatograms. The stability of [^{177}Lu]Lu-DOTA-2F8 and [^{225}Ac]Ac-DOTA-2F8 in human serum at 37 °C was evaluated at different time points. The peak at ~4,6 min corresponds to the radiolabeled sdAb and remains stable (RCP>90%) in each measurement taken per time point without releasing free radionuclide.

In vitro studies

Cell saturation binding assay



Saturation binding curve of [^{177}Lu]Lu-DOTA-2F8 on CD38+ MM cells. The radiolabeled sdAb maintains its high affinity for the antigen expressed on the surface of the malignant cells.



Plot representing the cell internalization assays. The internalized fraction is evaluated over the time considering the % of activity. It presents a very low and constant activity over time resulting in only 20% of internalization.

Conclusions

TAT using sdAb 2F8 direct against CD38 can be considered an innovative approach for MM treatment. The sdAb can be successfully conjugated to the p-SCN-Bn-DOTA, obtaining a good chelator: sdAb ratio. The radiolabeling with ^{225}Ac allowed to get a pure radioconjugate (RCP>95%) stable up to 24h in similar human's body conditions. The results were compared with [^{177}Lu]Lu-DOTA-2F8 to have an idea of the coherence. Primary in vitro studies guaranteed the interaction of radiolabeled sdAb 2F8 with the antigen CD38 on the surface of cancer cells, however a clean saturation curve must be obtained to calculate the EC_{50} value. Next steps are *in vivo* experiment to ensure that the efficacy is not impacted by the low internalization rate.

References

1. Duray, E., Lejeune, M., Baron, F., Beguin, Y., Devoogdt, N., Krasniqi, A., Lauwers, Y., Zhao, Y. J., D'Huyvetter, M., Dumoulin, M., & Caers, J. (2021). A non-internalised CD38-binding radiolabelled single-domain antibody fragment to monitor and treat multiple myeloma. *Journal of hematology & oncology*, *14*(1), 183. https://doi.org/10.1186/s13045-021-01171-6
2. Ahmed, A., & Killeen, R. B. (2023). Relapsed and Refractory Multiple Myeloma. In *StatPearls*. StatPearls Publishing.
3. Pruszynski, M., D'Huyvetter, M., Bruchertseifer, F., Morgenstern, A., & Lahoutte, T. (2018). Evaluation of an Anti-HER2 Nanobody Labeled with ^{225}Ac for Targeted α-Particle Therapy of Cancer. *Molecular pharmaceutics*, *15*(4), 1457–1466. https://doi.org/10.1021/acs.molpharmaceut.7b00985

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