CD38-based Targeted Alpha Therapy for the treatment of multiple myeloma

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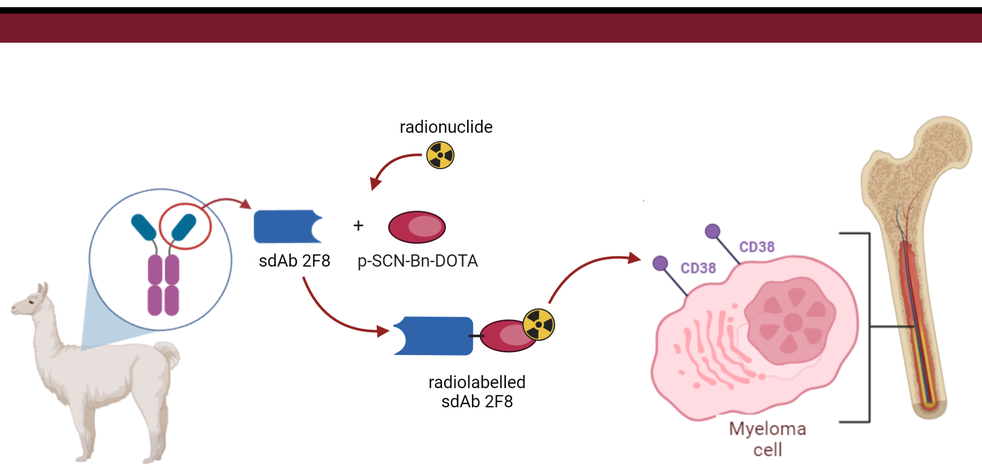
Introduction

Multiple myeloma (MM) is an incurable hematological malignancy , and patients experience relapse despite achieving complete remission[1]. **Targeted alpha therapy (TAT)** has the potential to eradicate minimal residual disease delivering cytotoxic alpha radiation to cancer cells, while sparing healthy tissues[2]. TAT is carried out using a vector radiolabelled with a therapeutic radionuclide by means of a chelator. **Single-domain antibody fragments (sdAbs),** derived from Camelidae heavy-chain antibodies, exhibit ideal properties as carrier molecules for TAT.

Aim of the study

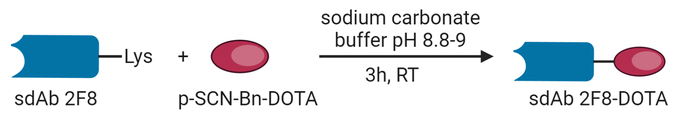
This project aims to develop a sdAb-based radiopharmaceutical for TAT of MM by radiolabelling the CD38-binding 2F8 sdAb[3] with therapeutic radiometals: lutetium-177 (^{177}Lu; β$^-$-particle emitter) or actinium-225 (^{225}Ac; α-particle emitter).



Methods

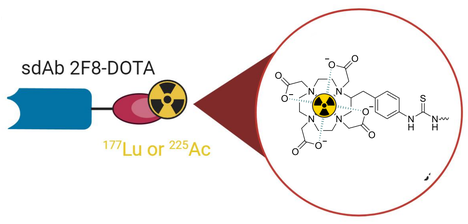
sdAb 2F8 random DOTA conjugation

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



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

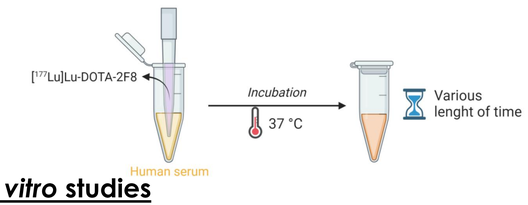
Radiolabelling performed by incubating the sdAb and the radionuclide in 0,5 M NH_4OAc pH 5.5 ± 0.1 for 30 minutes.



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

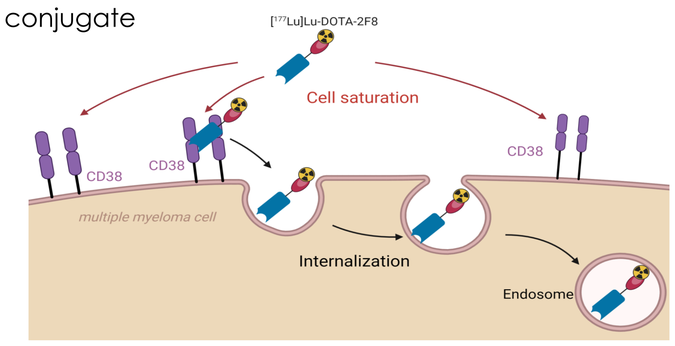
Stability studies in human serum

Evaluation of the stability of the radio-conjugate in human serum at 37°C.



In vitro studies

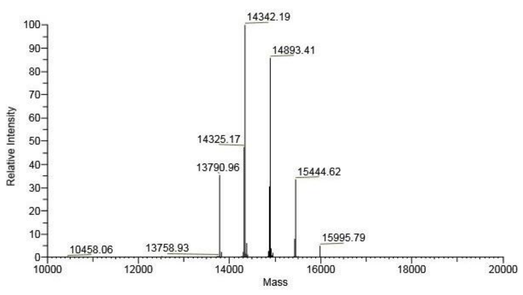
Assessment of the saturation binding (EC_{50}) and internalization on CD38+ MM cells of the radio conjugate



Results

DOTA random conjugation on sdAb 2F8

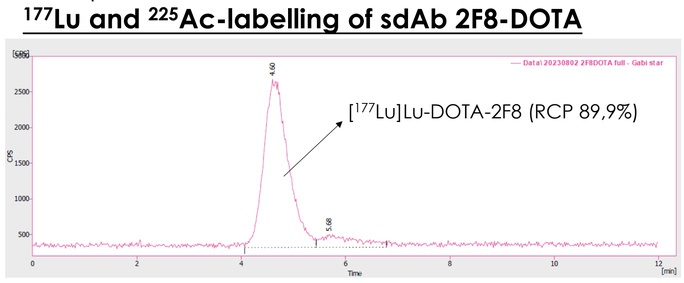
Samples	Expected mass (Da)
Starting sdAb	13790.6
sdAb:DOTA 1:1	14342.25
sdAb:DOTA 1:2	14893.9
sdAb:DOTA 1:3	15445.55



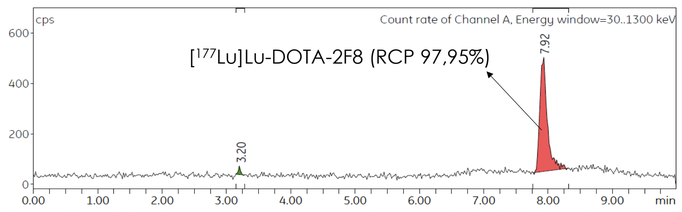
Deconvoluted mass spectra. Mass spectrometry ESI-Q-TOF analysis shows mass peaks at the expected molecular weight of conjugated sdAb adducts with 1, 2 or 3 DOTAs. The sdAb is successfully conjugated to p-SCN-Bn-DOTA with an average of 1,4 DOTA per sdAb.

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

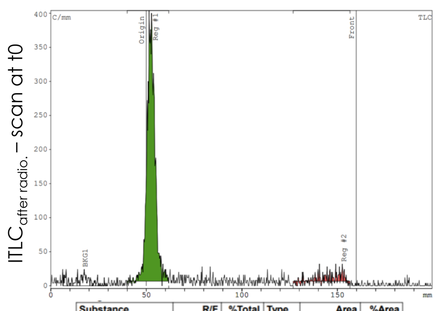
Size exclusion chromatography (SEC-HPLC)



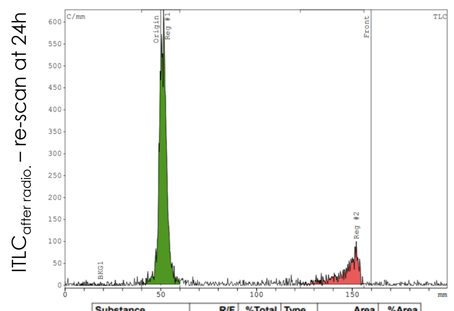
Reverse phase chromatography (RP-HPLC)



Radio-SEC and Radio-RP chromatograms. The radiolabelling is evaluated comparing the intensity of radiations over the time. The peak corresponding to sdAb 2F8-DOTA (at the correct Rt) present radiochemical purity (RCP)>90% in nearly completely absence of free radionuclide. The radiolabelling with 200 MBq ^{177}Lu is therefore achieved.



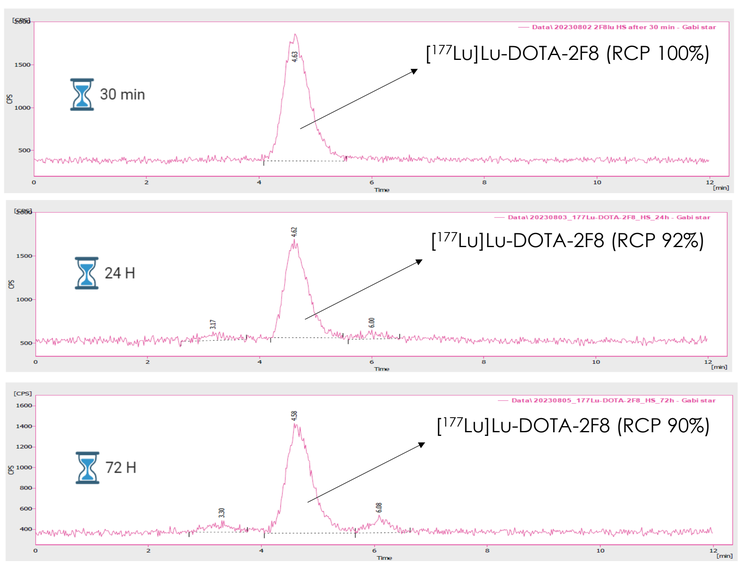
Substance	R/F	%Total %	Type	Area Counts	%Area %
Reg #1	0.023	62.59	DD(M)	1825.065	95.18
Reg #2	0.939	3.17	DD(M)	92.387	4.82
Sum in ROI	-	-	-	1917.452	100.00
Total area	-	-	-	2915.903	-
Area (total) RF	-	-	-	2507.000	-
Remainder (Tot)	-	-	-	998.45	34.24



Substance	R/F	%Total %	Type	Area Counts	%Area %
Reg #1	0.014	67.38	DD(M)	2589.931	80.47
Reg #2	0.927	16.36	DD(M)	628.772	19.53
Sum in ROI	-	-	-	3218.703	100.00
Total area	-	-	-	3843.574	-
Area (total) RF	-	-	-	3012.000	-
Remainder (Tot)	-	-	-	624.87	16.26

iTLC graphs. The sdAb 2F8-DOTA is radiolabeled with ^{225}Ac and not its daughter isotopes because after 24 H the peak is still detectable with a weak presence of free radioactivity on the front of the strip. The radiolabelling of sdAb 2F8-DOTA with ^{225}Ac is feasible (RCP=80%), but the protocol needs optimization.

^{177}Lu]Lu-DOTA-2F8 stability in human serum (HS)

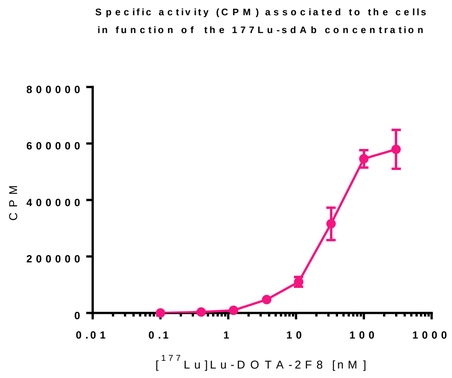


Radio-SEC chromatograms. The stability of 200 MBq [^{177}Lu]Lu-DOTA-2F8 in human serum at 37 °C was evaluated at different time points up to 120h. The peak at ~4,6 min corresponds to [^{177}Lu]Lu-DOTA-2F8 and remains stable (RCP>90%) in each measurement taken per time point without releasing free ^{177}Lu.

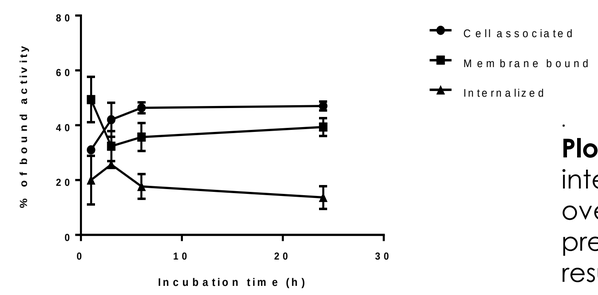
[^{177}Lu]Lu-DOTA-2F8 in HS at 37°C									
	<0.5h	1h	2h	3h	19h	24h	48h	72h	120h
Radio-iTLC	>99%	>99%	>99%	97%	NM	98%	91%	95%	91%
Radio-SEC	>99%	93%	95%	90%	88%	92%	94%	90%	76%

The table summarizes the RCP of [^{177}Lu]Lu-DOTA-2F8 in human serum at 37 °C over the incubation time. The RCP is > 95% in serum for two hours and > 90% for three hours indicating that the radio-conjugate is stable.

[^{177}Lu]Lu-DOTA-2F8 binds on CD38+ MM cells



Saturation binding curve of [^{177}Lu]Lu-DOTA-2F8 on CD38+ MM cells. The maximal effective concentration (EC50) calculated for [^{177}Lu]Lu-DOTA-2F8 is 40 nM meaning that it maintains its high affinity for the antigen expressed on the surface of the cancer cells.



Plot representing the cell internalization assays. The internalized fraction of [^{177}Lu]Lu-DOTA-2F8 is evaluated over the time considering the % of bound activity. It present a very low and constant activity over time resulting in a nearly absence of internalization.

Conclusions

The application of TAT using sdAb 2F8 is a feasible approach for treating MM. The targeting-CD38 sdAb 2F8 can be successfully conjugated to the p-SCN-Bn-DOTA and the radiolabelling with ^{177}Lu allowed to obtain a pure product (RCP>95%) stable for the whole duration of the half-life of the sdAb. Primary studies gave an idea about the EC_{50} the partial internalization of the sdAb guaranteeing the interaction of sdAb 2F8 with the CD38 on the surface of malignant cells. The primary results obtained are encouraging. However, the radiolabelling with ^{225}Ac has to be optimized to achieve higher specific activity and using a batch of 2F8-DOTA presenting more conjugation.

References

1. Ahmed, A., & Killeen, R. B. (2023). Relapsed and Refractory Multiple Myeloma. In *StatPearls*. StatPearls Publishing.
2. 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
3. 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

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