Optimizing Chelator-to-Antibody Ratio Improves Tumor Targeting and Pharmacokinetics of 225Ac-Labeled Antibodies

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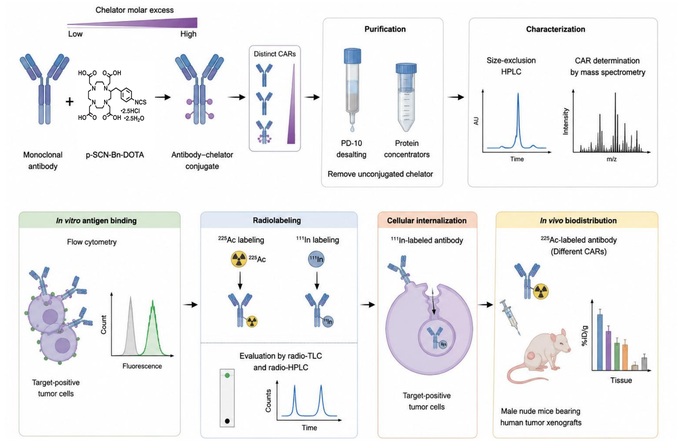
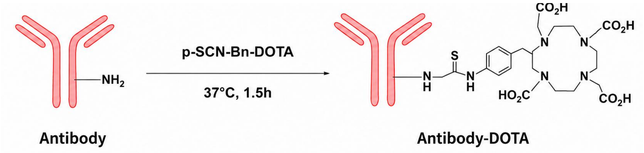
INTRODUCTION

- Monoclonal antibody-based radiopharmaceuticals are a rapidly advancing modality for cancer imaging and therapy, combining the target specificity of antibodies with the diagnostic or cytotoxic potency of radionuclides.
- Radiolabeling antibodies with metallic radionuclides (e.g., 225Ac, 177Lu, 111In) requires conjugation of bifunctional chelators; however, this chemical modification can often perturb antibody structure, charge, and biological function in a chelator-to-antibody ratio (CAR)-dependent manner.
- While increasing CAR can improve radiolabeling efficiency and achievable specific activity, excessive chelator loading may compromise immunoreactivity, promote aggregation, alter pharmacokinetics and tissue biodistribution.
- Systematic evaluation of CAR is therefore critical for the rational optimization of antibody-based targeted radiotherapies, particularly for alpha-emitting payloads with narrow therapeutic windows.

METHODS



Conjugation Scheme of Antibody



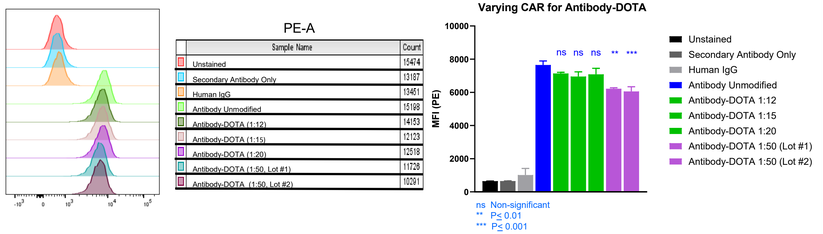
- The bifunctional chelator p-SCN-Bn-DOTA was conjugated to the antibody through reaction of the isothiocyanate group with accessible amine groups on the antibody surface under mild conditions (37°C, 1.5 h).
- This conjugation generated an Antibody-DOTA construct that retains the antibody targeting capability while introducing a metal-chelating moiety for radiolabeling.
- The resulting bifunctional antibody conjugate enables stable coordination of therapeutic or imaging radionuclides such as 225Ac or 111In for targeted radiopharmaceutical applications.

Efficient Radiolabeling was Achieved with Conjugates Exhibiting a CAR Greater than 1.7

Conjugate (Antibody: Chelator)	CAR	^{225}Ac Labeling (0.3 µCi/µg)	^{111}In labeling (15 µCi/µg)
1:5	0.7	Unsuitable for radiolabeling	NA
1:10	1.7	77%	NA
1:12	2.5	95%	100%
1:15	3.2	92%	100%
1:20	3.2	100%	100%
1:50 (lot #1)	7.8	100%	100%
1:50 (lot #2)	8.7	100%	100%

- Antibody-DOTA conjugates spanning CAR values of 0.7 - 9 were successfully prepared.
- A CAR of 0.7 was insufficient to support efficient 225Ac labeling, whereas CAR ≥ 1.7 enabled robust radiolabeling.

Antibody-DOTA with Lower CAR (2.5 - 3.2) Demonstrated Higher Immunoreactivity

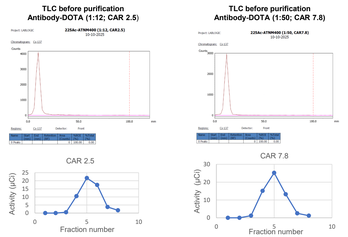


- Highest antigen binding (91 - 98%) was observed at lower CARs (0.7 - 3.2), compared to reduced binding (79 - 85%) at higher CARs (7 - 9).

Lower or Higher CAR Does Not Affect the Stability of the Radiolabeled Antibody

PD10 Purification of 225Ac-Antibody-DOTA

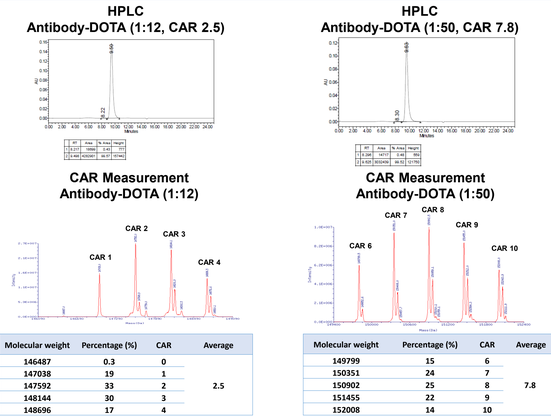
Fraction	Volume (mL)	CAR 2.5 Activity (µCi)	CAR 7.8 Activity (µL)
1	1	0	0
2	1	0	0
3	1	0.49	1.2
4	0.5	10.5	15.1
5	0.5	21.7	25.2
6	0.5	17.4	13.2
7	0.5	3.8	2.5
8	0.5	1.7	1.2
column		7.1	7.5
total		62.69	65.9
Frac 4, 5, 6	1.5	49.6	53.5
yield (%)		79.12	81.18



Radiochemical Purity (%)				
CAR	Before Purification	After Purification	3 Days	7 Days
CAR 2.5	99.32	99.35	98.69	97.37
CAR 7.8	98.96	99.12	98.89	97.81

- Low-CAR (2.5) and high-CAR (7.8) conjugates were radiolabeled with 225Ac at 0.1 µCi/µg. Purification was performed using PD-10 columns, followed by desalting and protein concentrators to remove unconjugated chelator.
- Both high- and low-CAR conjugates were stored at 2 - 8°C for 7 days and demonstrated comparable stability, indicating that CAR value does not affect radiolabeled antibody stability.

Antibody-DOTA with Lower CAR (2.5 - 3.2) Demonstrated Higher Immunoreactivity

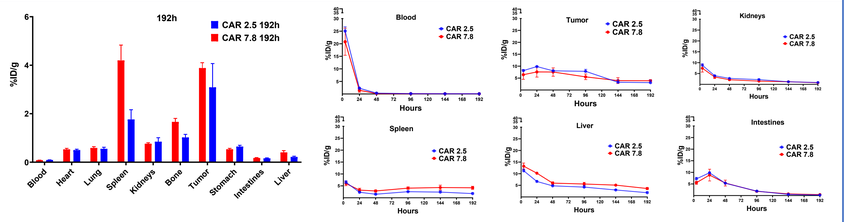


Molecular weight	Percentage (%)	CAR	Average
146487	0.3	0	
147038	19	1	
147592	33	2	2.5
148144	30	3	
148696	17	4	

Molecular weight	Percentage (%)	CAR	Average
149799	15	6	
150351	24	7	
150902	25	8	7.8
151455	22	9	
152008	14	10	

- 111In-labeled-antibody conjugates with lower CARs demonstrated greater cellular internalization, consistent with the cellular binding data.

Antibody with Lower CAR Demonstrated Comparable Tumor Uptake with Lower Liver and Spleen Uptake



- Lower uptake in Liver and Spleen for the lower CAR at endpoint (192 h).
- Tumors demonstrated sustained and comparable retention up to 192h for both CARs.
- Similar trend of uptake and clearance in all other relevant organs for both CAR 2.5 and CAR 7.8 with rapid clearance from blood, kidneys, and intestines across all time points.

CONCLUSIONS

- CAR is a critical determinant of both in vitro immunoreactivity and in vivo pharmacokinetic behavior of radiolabeled antibodies.
- While higher CAR improves radiolabeling feasibility, excessive chelator loading compromises target binding and increases off-target uptake in clearance organs such as liver and spleen.
- These findings underscore the importance of CAR optimization as a key design parameter for preserving the biological integrity and therapeutic index of 225Ac-labeled antibodies, with direct implications for the development of next-generation 225Ac antibody radioconjugates.

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