LEAD OPTIMIZATION OF RADIOPHARMACEUTICALS FOR MOLECULAR RADIOTHERAPY AND PRECLINICAL EVALUATION

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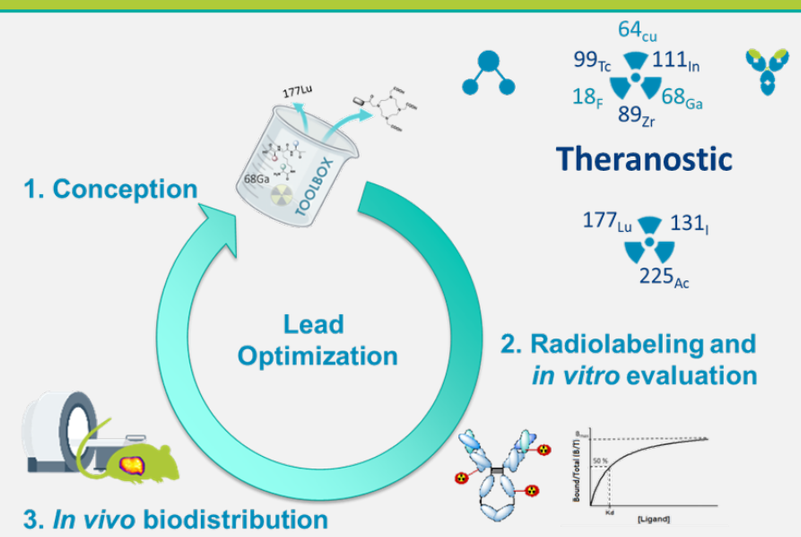
1 CONTEXT & OBJECTIVES

Molecular Radiotherapy (MRT) targeting SSTR2 or PSMA have proven to be highly efficient for treatment of neuroendocrine or metastatic prostate cancer respectively. Beyond the leading radiopharmaceutical molecules ^{177}Lu-DOTATATE or ^{177}Lu-PSMA-617, a variety of vectors (small molecules, peptides, panel of biologics) have been developed on the same targets in order to improve the biodistribution within the tumor, the blood clearance, the route of elimination or the dosimetry.

Labeling of the targeting ligand, whatever its nature, is a crucial step as it may affect significantly the properties of the theranostic conjugate, i.e. its binding affinity, PK and biodistribution. The addition of linkers, such as albumin binding domain or PEG, and choice of chelating agents have a major impact on the chemical and biological properties of the vectors. Random or site-specific bioconjugation, click chemistry, have also to be considered in the early stage as the choice of the selected technology will modify your development plan and manufacturing.

New ligands and biological platforms are now being developed based on this historical knowledge, improved Target Product Profiles are built to conduct optimal lead optimization of MRT. Herein, we will present our lead optimization and preclinical evaluation process to select efficiently good radiolabeled molecules and list the key parameters to be checked. To date, it remains hard to predict the behavior of the modified bioconjugated molecules, and versatile synthesis strategies are needed to screen various combinations of radiometal complexes / linker / conjugation function, in order to converge rapidly to the optimized bioconjugate. For instance, we will present a study case where the conjugation of various bifunctional chelating agents on a small NTS1 receptor antagonist resulted in drastically different in vivo behavior of the resulting 68Ga-labeled compounds.

Once optimal in vivo tumor uptake has been achieved, preclinical evaluation requires the selection of appropriate and relevant models, driven by target expression, radioresistance, and potentially tumor immune infiltrate for combination studies with immunotherapies. The therapeutic evaluation should take into consideration the dose and specific activity, tolerance of the model related to ionizing radiations and the scheduling of treatment (cumulated dose, fractionation).



Vector, Chelate & Radiolabeling

- Choice of vector: small molecules, biologics, nanoparticles?
- Choice of chelating agents? Radioisotopes? Charge?
- Conditions: buffer? heating? pH range?

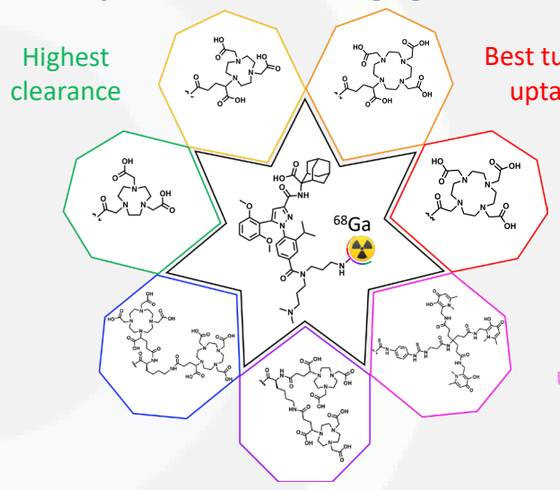
Bioconjugation

- Random vs site specific technology?
- Conjugation conditions?
- Pretargeting, dual labeling & multimodality

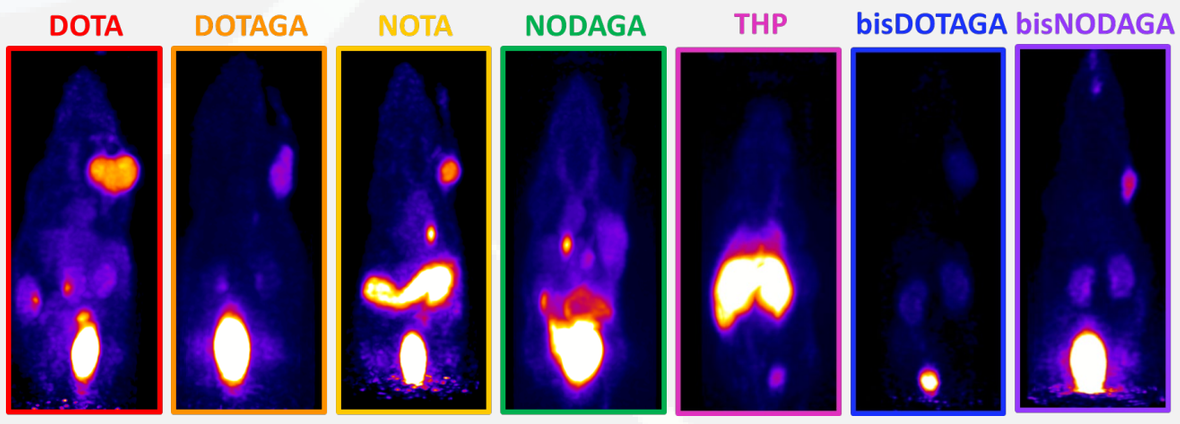
2 RESULTS

Case study: Optimization of a ^{68}Ga-labeled small-molecule antagonist of neurotensin receptors

- **Introduction of a variety of chelators in order to evaluate systematically the impact of the chelating agent on PK properties**



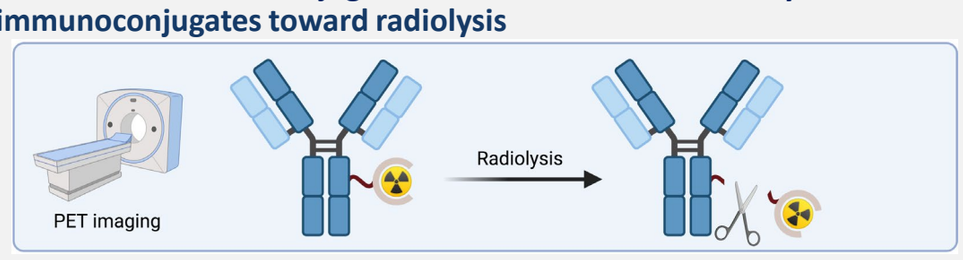
^{68}Ga-labeled neurotensin bioconjugated with a variety of chelating agents injected into *Nude* mice xenografted with HT29 tumor cells, 2 h p.i. (Renard E, et al. J Med Chem 2021)



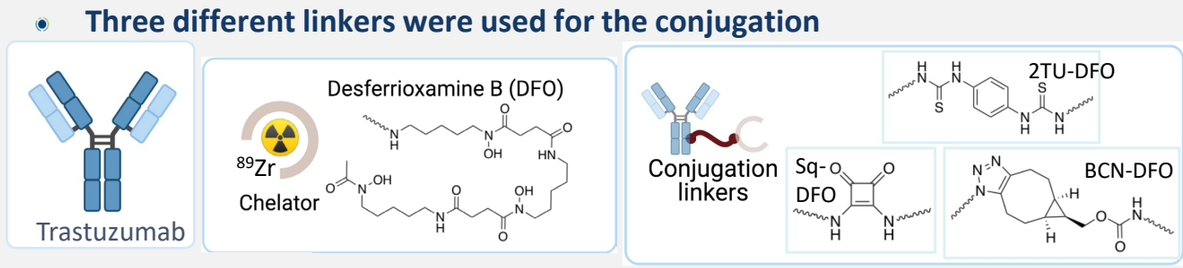
- ➢ **The chelator makes the difference: careful selection of the chelator is critical**
- ➢ **Introduction of an additional chelator is a reliable strategy to speed up renal excretion, provided affinity is retained**

Case study: Optimization of ^{89}Zr-labeled immunoconjugate

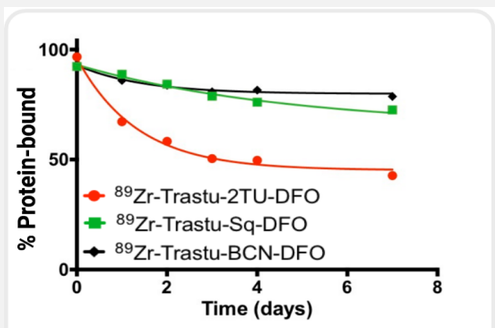
- **Influence of the conjugation linker on the stability of ^{89}Zr-labeled immunoconjugates toward radiolysis**



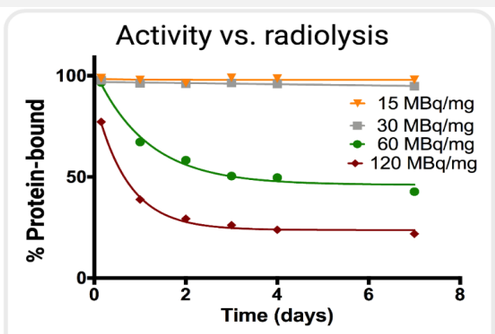
- **Three different linkers were used for the conjugation**



- **Study of the stability of the radioconjugates by SEC-HPLC**



Stability of the three radioconjugates in PBS, n=3



Stability of trastuzumab-2NCS-DFO in PBS with different activities, n=3

- ➢ **The radioconjugate formed through the formation of thiourea bonds (standard method), ^{89}Zr-Trastu-2TU-DFO, shows much lower stability when compared to those obtained via squaramide bond formation or SPAAC click reaction**
- ➢ **The stability of ^{89}Zr-Trastu-2TU-DFO depends on the specific activity**

3 CONCLUSION

- **Multiparameters in the design and lead optimization of radiopharmaceuticals have to be considered: target, vector, isotope, chelating agent, linker, bioconjugation group**
- **It is difficult to predict the road ahead due to the complexity of biology BUT it is possible to anticipate scenario and/or go back to optimization when needed**
- **Select the best vector by taking early into consideration the chemistry**
- **Optimal *in vitro* and *in vivo* preclinical evaluation help to make the right decision in the early stage as the choice of the vector will modify your development plan and manufacturing.**

- **Lead optimization of the radiopharmaceutical - Impact to be considered on:**
  - **Affinity, selectivity, specific activity, purification, radiolysis, stability**
  - ***In vivo* biodistribution: clearance, route of elimination, tumor/tissue uptake ratio, dosimetry**
  - **Therapeutic efficacy: isotope energy, toxicity**

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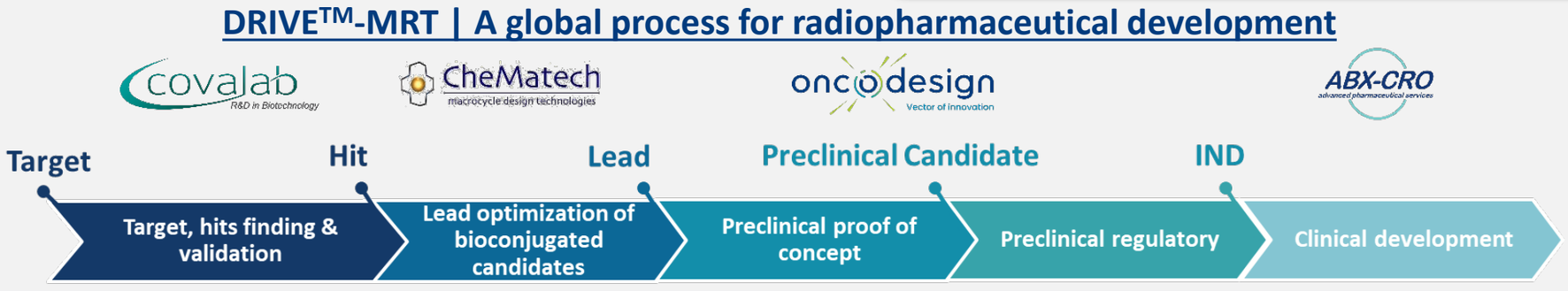

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