New CD8 imaging agents to evaluate T-Cell activity in tumors and predict response to immunotherapy

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CLINICAL CONTEXT

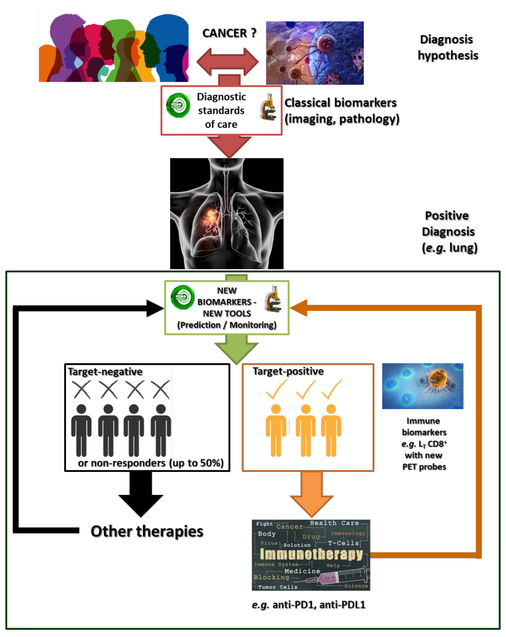
Recently, immunotherapies using monoclonal antibodies targeting checkpoints inhibitors of the immune response, such as PD-1, demonstrated their superiority compared to standard chemotherapies.

However, despite the strong contribution of anti-PD-1 (nivolumab) in some cancer treatment, this immunotherapy is not effective in all patients.

➡ **Predictive biomarkers of treatment efficacy are needed for efficient patient management**

➡ **Recent translational studies suggested that the level of tumour infiltrated CD8 T Lymphocytes is a good biomarker to predict immunotherapy efficacy**

In this context, one of the aim of the BIOCAIR project is t**o obtain a proof of concept of the use of radiolabeled anti-CD8 antibody and fragments as imaging biomarkers of the efficacy of immunotherapies.**

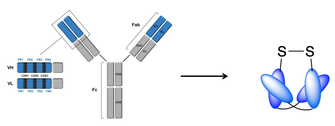


BIOENGINEERING → CHEMISTRY → SCREENING → RADIOCHEMISTRY → IN VITRO → IN VIVO

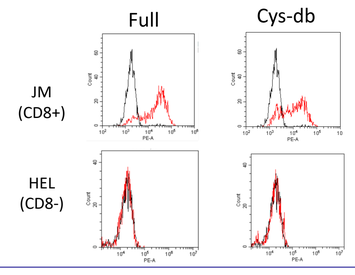
BIOENGINEERING

CD8 FRAGMENTS PRODUCTION

1. Clone sequencing and reformatting in Cys-diabody



2. Validation of recombinant format by flow cytometry



1. Sequencing and reformatting

B-Z31 anti-CD8 antibody was first sequenced to determine the nucleotide variable sequences VH and VL.

B-Z31 candidate was then reformatted by molecular biology to Cys-diabody (Cys-db) with the following construction:

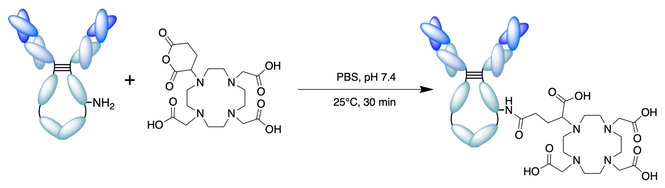
Nter - **VH** + **linker** + **VL** + **Cys** – Cter

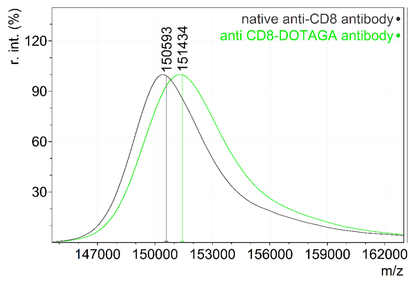
Recombinant Cys-db was produced by transient transfection in CHO cells. Purification was performed by affinity chromatography.

2. Validation of Cys-db recombinant fragment

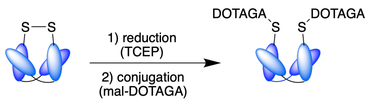
The Cys-db was validated in flow cytometry on CD8 expressing cell line.

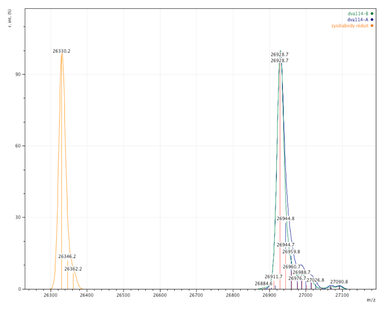
CHEMISTRY





B-Z31 anti-CD8 antibody was randomly conjugated to DOTAGA and the degree of labeling was determined by MALDI-TOF mass spectrometry (1.9 DOTAGA/mAb).

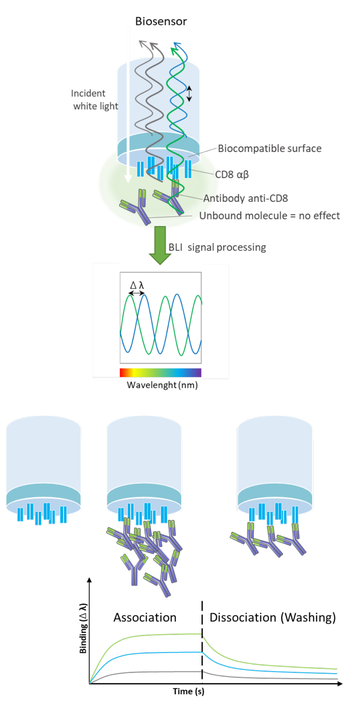




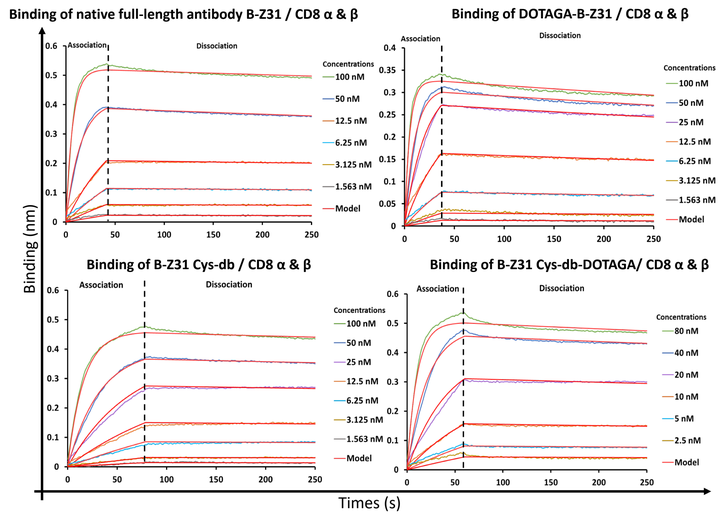
B-Z31Cys-db anti-CD8 Cys-diabody was conjugated to mal-DOTAGA in two steps. First, The disulfide bridge of Cys-diabody was reduced with TCEP. Then DOTAGA-maleimide was conjugated. The degree of labeling was determined by LC-ESI-MS mass spectrometry (2 mal-DOTAGA/Cys-db).

IN VITRO

PRINCIPLE OF BLI

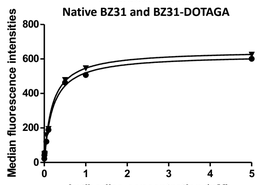


- **AFFINITY DETERMINATION VIA BIOLAYER INTERFEROMETRY (BLI)**



	K_D (nM)	K_a (1/Ms)	K_{dis} (1/s)
Native full-length antibody B-Z31	1.4 x 10^{-1} ± 3.7 x 10^{-3}	1.5 x 10^{+6} ± 1.2 x 10^{+4}	2.0 x 10^{-4} ± 5.1 x 10^{-6}
DOTAGA-B-Z31	2.5 x 10^{-1} ± 3.4 x10^{-3}	1.9 x 10^{+6} ± 4.8 x 10^{+4}	4.8 x 10^{-4} ± 5.3 x 10^{-6}
B-Z31 Cys-db	3.1 x 10^{-1} ± 6.2 x 10^{-2}	6.2 x 10^{+5} ± 3.7 x 10^{+3}	2.0 x 10^{-4} ± 5.0 x 10^{-6}
B-Z31 Cys-db-DOTAGA	2.4 x10^{-1} ± 9.1 x 10^{-3}	1.2 x10^{+6} ± 9.6 x10^{+3}	2.9 x10^{-4} ± 6.4 x10^{-6}

- **AFFINITY DETERMINATION *IN CELLULO* VIA FACS ANALYSIS**

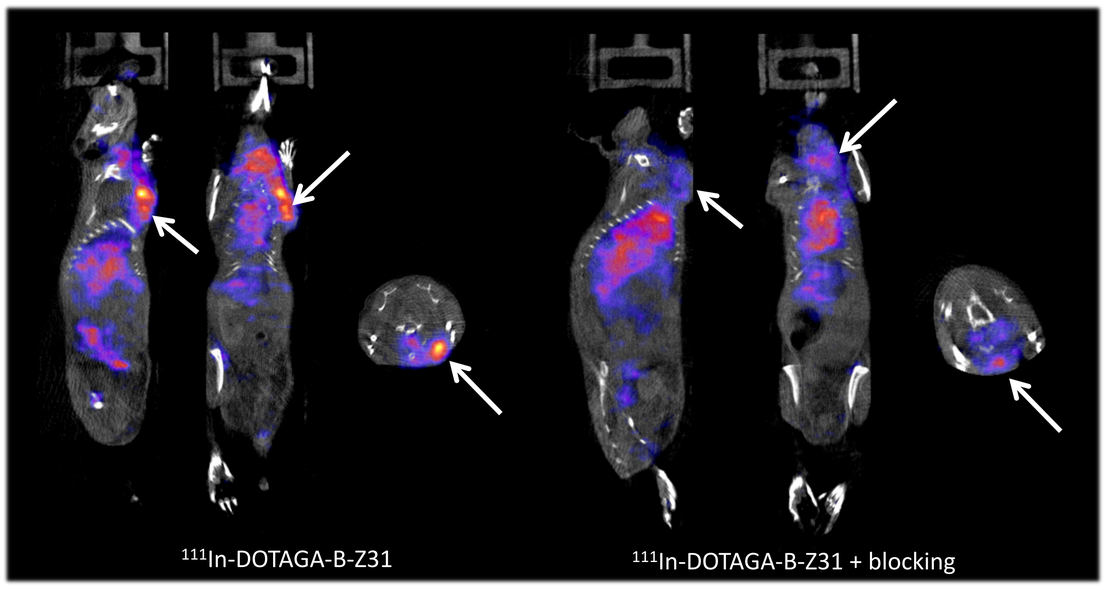


	EC_{50} MOLT4 cells (nM)
Native full-length antibody B-Z31	2.0 x 10^{-1} ± 2.6 x 10^{-2}
DOTAGA-B-Z31	1.9 x 10^{-1} ± 2.7 x10^{-2}

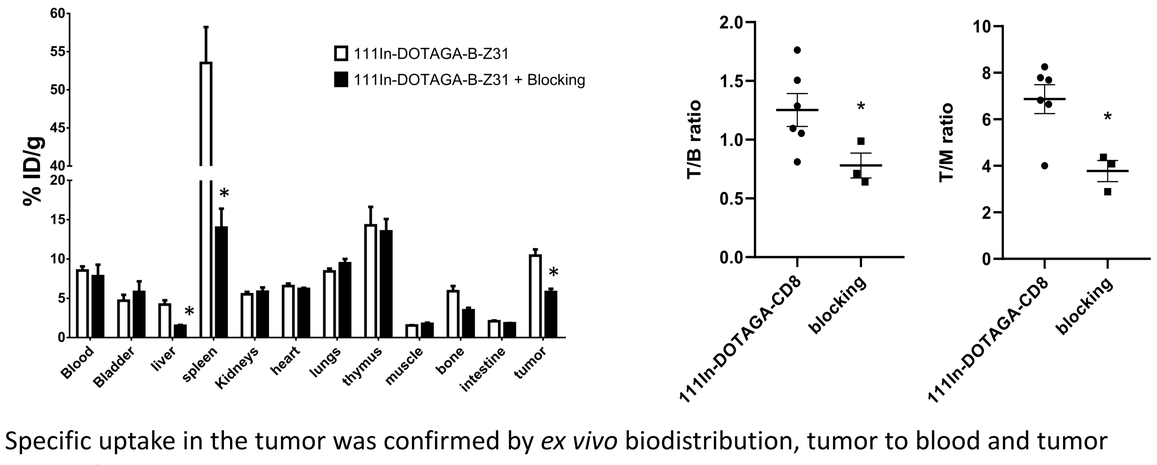
Conjugation of DOTAGA does not affect neither anti-CD8 full-length antibody B-Z31 nor Cys-diabody B-Z31 binding affinity.

RADIOCHEMISTRY – IN VIVO

DOTAGA-B-Z31 was radiolabeled with ^{111}In in ammonium acetate buffer at 37°C for 1h (500 MBq/mg).



SPECT/CT imaging of SCID mouse bearing subcutaneous MOLT4 overexpressing human CD8: representative sagittal, coronal and transversal slices 72h after injection of ^{111}In-DOTAGA-B-Z31. The tumor is indicated by an arrow.



Specific uptake in the tumor was confirmed by *ex vivo* biodistribution, tumor to blood and tumor to muscle ratios.

CONCLUSION

- An antibody targeting human CD8 T Lymphocytes, namely B-Z31, was developped and conjugated to DOTAGA. This conjugate was evaluated *in vitro* and demonstrated high affinity toward CD8-overexpressing MOLT4 cells. The conjugate radiolabeled with ^{111}In was injected in SCID mice bearing MOLT4 subcutaneous tumor and showed high uptake in the tumor, assessing the specificity of this new radioconjugate.
- A Cys-diabody derivative of B-Z31 has been developped in order to increase tumor to background ratio. The *in vivo* experiments are currently under investigation.

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