Antibody anti-PDL1 radiolabelling with ^{89}Zr: feasibility and *in vitro* characterization

***Anis KRACHE*[1,2,3,4], *Charlotte FONTAN*[3], *Pierre PAYOUX*[1,4,5], *Anne-Sophie SALABERT*[1,4,5*], *Mélanie WHITE-KONING*[2,4*]**

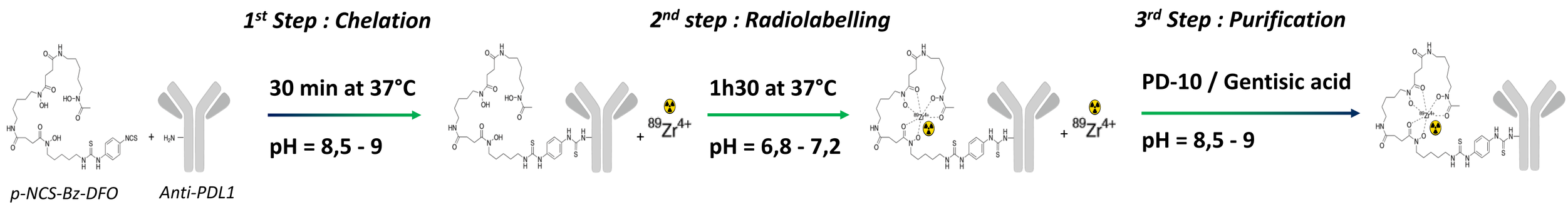
[1] *INSERM – UMR 1214 ToNIC (Toulouse NeuroImaging Center), Toulouse, France.*
[2] *INSERM – UMR1037 Centre de Recherches en Cancérologie de Toulouse (CRCT), Toulouse, France.*
[3] *ZIONEXA, Toulouse, France.*
[4] *Université Paul Sabatier Toulouse III, Toulouse, France.*
[5] *Nuclear medecine unit, University hospital, Toulouse, France.*
**These authors shared senior authorship of this study*
Contact : anis.krache@inserm.fr

Introduction

During immunotherapy treatment of non small cell lung cancer, 15 to 25% of patients respond to the treatment and most of patients develop form of resistance[1]. These resistance phenomena can occur following the expression of co-inhibitory molecules by tumor cells or tumor microenvironment (TME) such as PD-L1 (for tumor) and TIM-3 (for TME). In order to study TME modification, our aim is to visualize, with PET/CT, the evolution of PD-L1 and TIM-3 expression during treatment, by using anti-PDL1 & anti-TIM3 antibodies labelled with zirconium 89 (^{89}Zr). The first objective of our work was to validate *in vitro* parameters of antibodies radiolabelled with Zirconium 89 before *in vivo* studies.

Methodology

Radiolabelling step[2]:



Chemical and radiochemical purity (RCP):

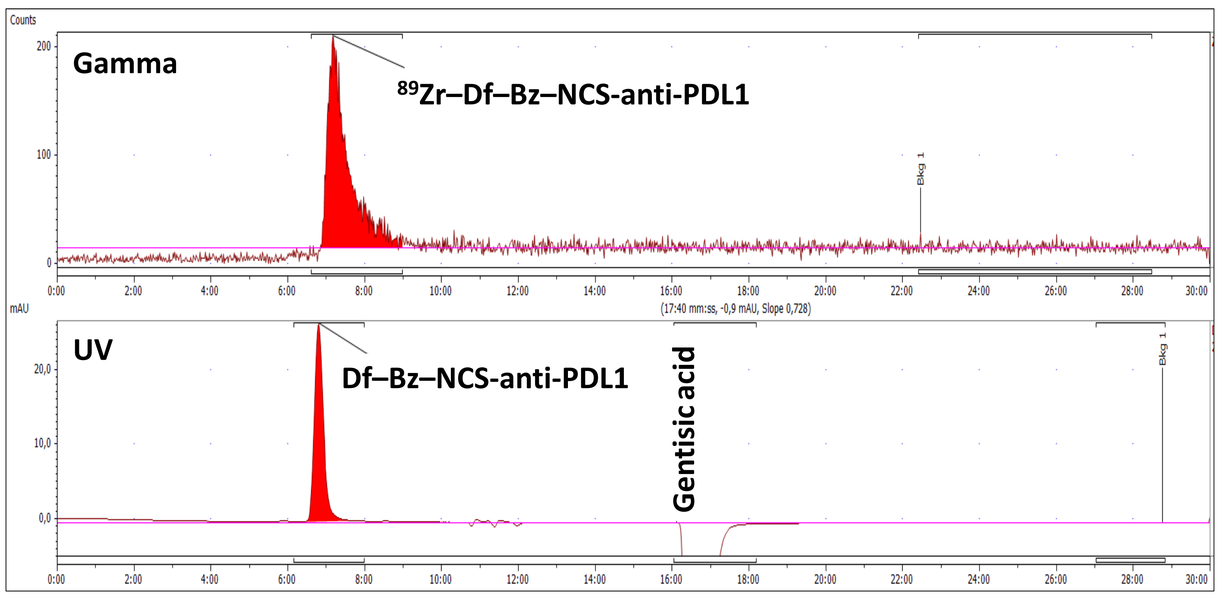
We used size exclusion chromatography (Agilent BioSEC 300 Å) coupled with UV (280nm) and gamma detection to define retention time of anti-PLD1 and free ^{89}Zr-oxalate. We also developed a fast ITLC method to evaluate RCP.

In vitro assays[3]:

Immunoreactivity (IR) assays were performed on CMT167, mice bronchopulmonary cancer cells expressing PD-L1. The cells (0,125.10^6 to 2.10^6) were incubated, in triplicate, for 1 hour with 2 nM Anti-PDL1-DFO-Zr89 for total IR and with presence of 1000-fold unlabelled anti-PDL1 molar excess for non-specific IR. Binding assays were conducted on approximately 2.10^6 cells and were incubated, in triplicate, for 1 hour with 2,5 nM to 100 nM of radiolabelled anti-PDL1 for total binding and with addition of 200-fold unlabelled anti-PDL1 molar excess for non-specific binding.

Results

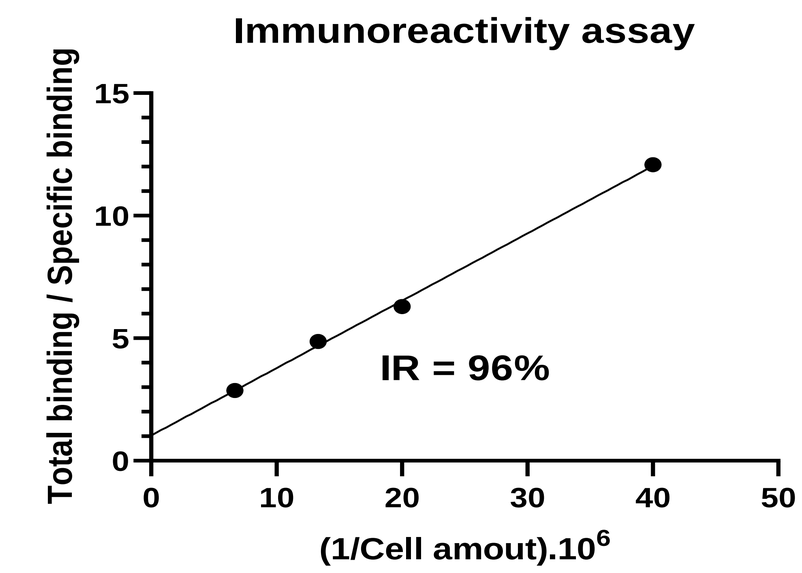
Analytical results



Parameters	Mean (±SD) (n=3)
Radiolabelling yield	67% (22)
RCP	100%
Specific activity	18,1 MBq/mg (4,9)
Volumetric activity	11,7 MBq/mL (4,5)
Concentration	0,935 mg/mL (0,25)
Retention time	6m48s

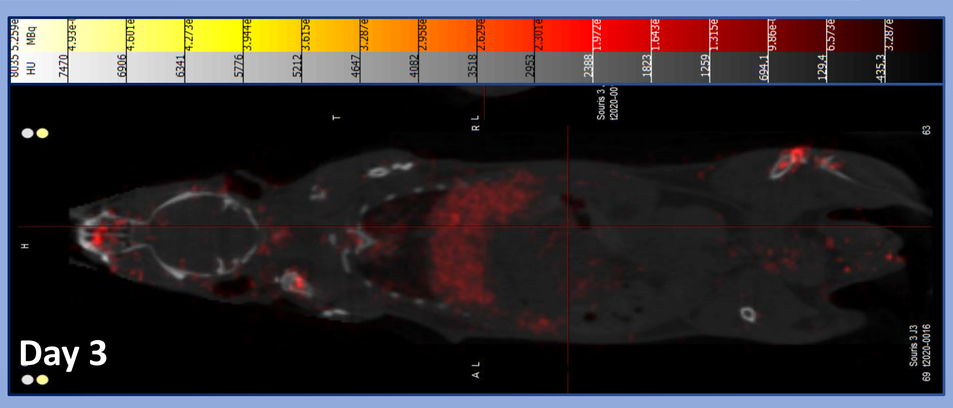
SD : Standard deviation, UV = 280 nm

In vitro results



Conclusion

In vitro tests revealed an IR of 96% compared to 70%[4] found in the literature and Kd of 46nM, indicating that the anti-PDL1 was not damaged by radiolabeling. Radiosynthesis parameters were acceptable except for the volumetric activity of 11,7 MBq/mL (± 4,5 MBq/mL) which needs to be improved. Since *in vitro* tests passed, it allowed us to perform mice injection at 1,2 MBq for biodistribution studies.



[1]Hamilton, Gerhard, et Barbara Rath. « Immunotherapy for Small Cell Lung Cancer: Mechanisms of Resistance ». *Expert Opinion on Biological Therapy*
[2]Vosjan, Maria J W D *et al.* « Conjugation and Radiolabeling of Monoclonal Antibodies with Zirconium-89 for PET Imaging Using the Bifunctional Chelate p-Isothiocyanatobenzyl-Desferrioxamine ». *Nature Protocols*
[3] Lindmo *et al.* « Determination of the Immunoreactive Fraction of Radiolabeled Monoclonal Antibodies by Linear Extrapolation to Binding at Infinite Antigen Excess »
[4]Kikuchi *et al.* « Preclinical ImmunoPET/CT Imaging Using Zr-89-Labeled Anti-PD-L1 Monoclonal Antibody for Assessing Radiation-Induced PD-L1 Upregulation in Head and Neck Cancer and Melanoma ». *OncoImmunology*

4th Nuclear Technologies for Health Symposium 2020, Nantes, France.