Pretargeted imaging of peritoneal carcinomatosis using bioorthogonal chemistry

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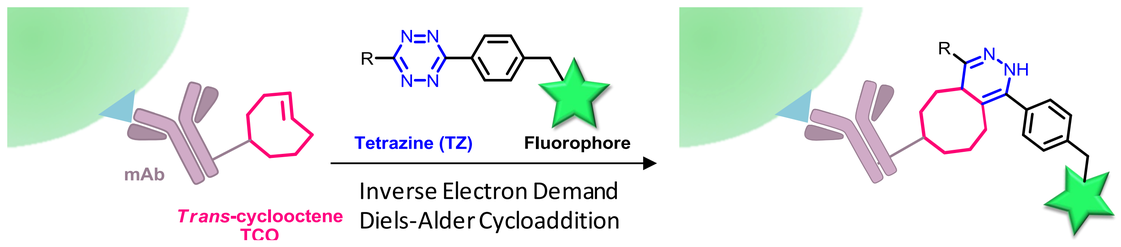


Scheme 1: Bioorthogonal interaction between trans-cyclooctene and tetrazine through IEDDA cycloaddition

Introduction

Bioorthogonal chemistry represents a challenging approach in pretargeted radioimmunotherapy (PRIT) of solid tumors, solving the main radioimmunotherapy drawback, i.e. bone marrow toxicity, through a two-step intervention.

First, monoclonal antibodies (mAbs) conjugated *trans*-cyclooctene (TCO) target the tumor antigen (Ag) . After a delay of 24 or 48 h, a radiolabeled probe linked to a tetrazine (TZ) is injected. The high affinity between TCO and TZ allows a covalent link, in physiological conditions, that is safe towards biological macromolecules and do not required any catalyzer (**Scheme 1**).

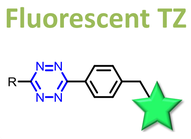
The efficiency of pretargeting (PT) can be influenced by two parameters: the number of TCO grafted on mAbs and the linker length between mAbs and TCO which can be modulated by insertion of several polyethylene glycol (PEG) units. PEGylation is well-known to increase protein solubility without modifying its pharmacokinetics and pharmacodynamics properties.

Thus, in our study we assessed several mAb modifications (number of TCO and PEG linker length) on two models –subcutaneous colorectal cancer and orthotopic peritoneal carcinomatosis (PC)- in both *in vitro* and *in vivo* experiments.

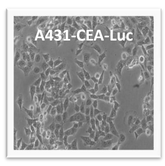
The final aim was to evaluate the influence of PEGylation on PT efficiency and determine the most effective mAb structure for further PRIT on PC.

Material and methods

Fluorescent TZ



- **35A7 mAb:** anti-CEA
  A431-CEA-Luc cells: colon epithelium (transfected for the expression of CEA and luciferase ➔ orthotopic (model of PC)
- **Ts29.2 mAb:** anti-TSPAN8
  HT29 cells: colon adenocarcinoma (**Results not shown**)

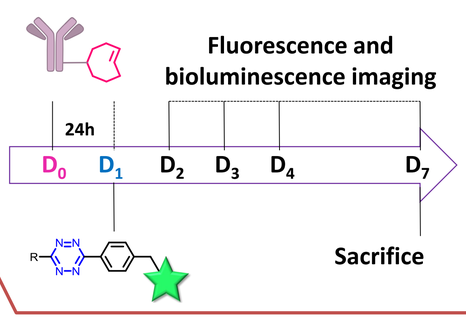


mAbs pharmaco-modulation

Focus 1: mAbs pharmaco-modulation by addition of TCO and insertion of PEG linkers
Focus 2: Determination of the number of PEG_n-TCO_n moieties grafted on mAbs

***In vivo* studies on female Nude mice:**
✓Subcutaneous xenograft (HT29) (n = 24) (**Results not shown**)
✓ Orthotopic model of peritoneal carcinomatosis (A431-CEA-Luc) (n = 12)

***In vivo* pretargeting experimental design:**



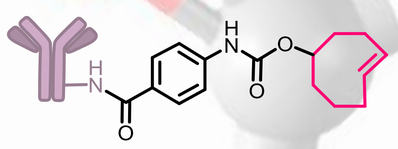
- 0.3 nmol / mouse
- intravenous

- 4 nmol / mouse
- intraperitoneal

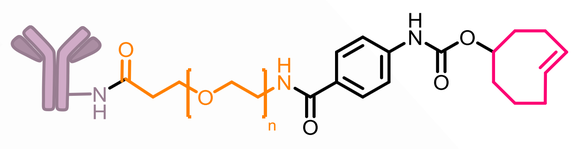
Fluorescence and bioluminescence imaging made on IVIS Spectrum

Syntheses of TCO and TCO-PEG-NHS

Focus 1: mAbs modifications by addition of PEG_n-TCO_n units (PEG_0, PEG_4 and PEG_{12})



Ts29.2-PEG_0-TCO
35A7-PEG_0-TCO



Ts29.2-PEG_n-TCO
35A7-PEG_n-TCO
n = 4
n = 12

Focus 2: Determination of the number of TCO grafted on mAb

MALDI-TOF MS spectra of 35A7-PEG_{12}-TCO mAb.
(A) 35A7 without TCO, (B) 35A7 with an increase amount of TCO (0, 5, 10, 15, 20 and 30 equivalents)

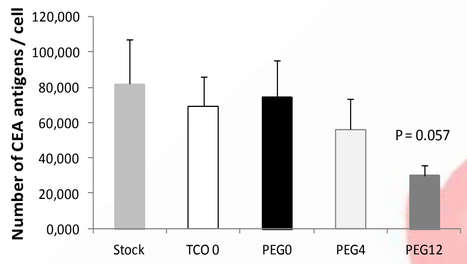
- The number of TCO grafted increases according to the amount of equivalents added in the mixture

10 equivalents of TCO

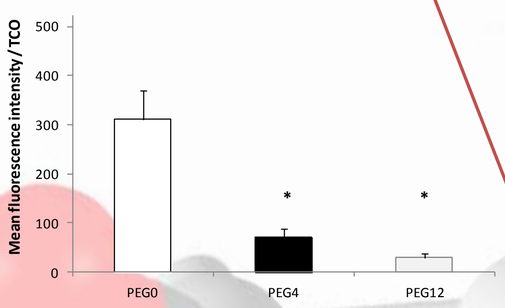
At a same equivalent, addition of PEG linkers increases the number of TCO grafted
➔ Results were then normalized by the number TCO grafted on mAb

Results: *in vitro*

Flow cytometry (CMF):



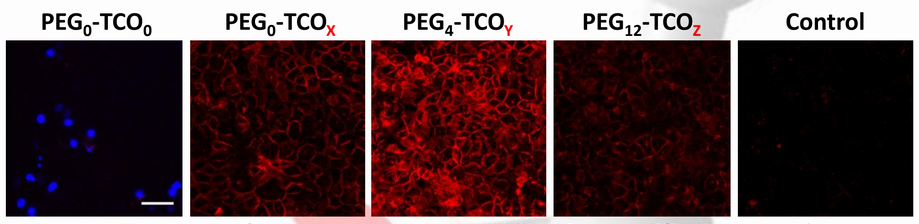
- Modifications did not significantly alter the Ag recognition



- Addition of PEG linkers decreases the TCO/TZ interaction

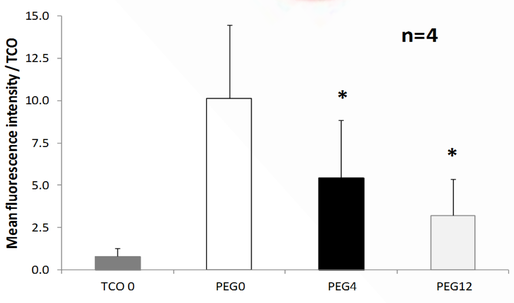
Immunofluorescence assays:

X: 1.2 TCO
Y: 4.9 TCO
Z: 7.2 TCO
Scale bar: 50 µm



Targeting of CEA on A431-CEA-Luc cells using modified 35A7 mAbs

- Specific interaction between TCO and TZ



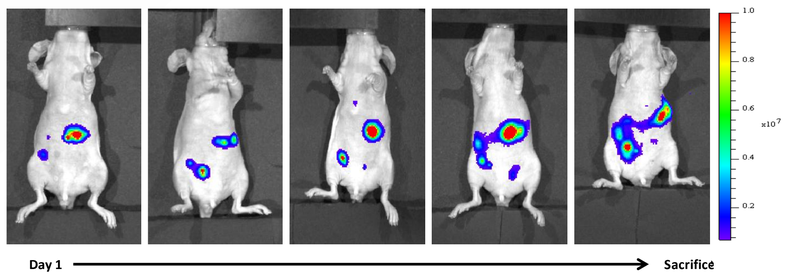
Signal seems higher for PEG_4 but when reported to the number of PEG_n-TCO grafted on mAb it appears that PEGylation significantly decreases the TCO/TZ interaction compared to PEG_0
➔ TZ is less available due to possible isomerization of TCO to unreactive CCO or unfolding of the alkyl chain

TZ-5-FAM

TZ-Cy3

Results: *in vivo*

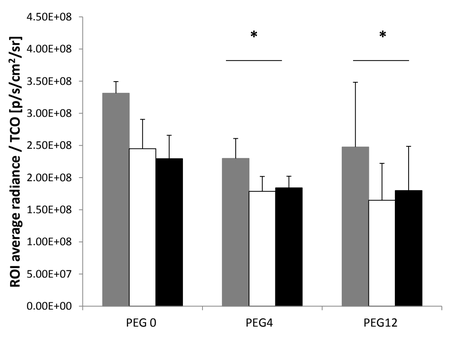
Bioluminescence imaging:



Progressive invasion of A431-CEA-Luc cells in the peritoneal cavity

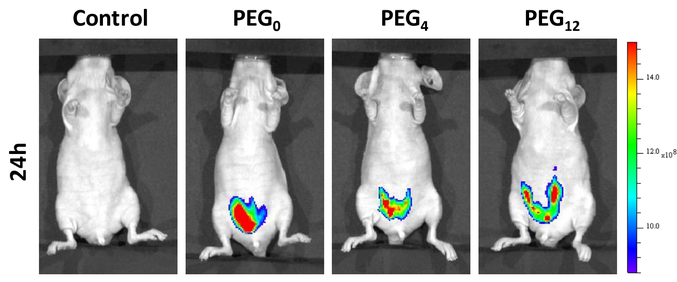
Peritoneal carcinomatosis index (PCI) = 8.9 ± 0.6

Ex vivo quantification of PCI confirms that induction of PC is similar between all mice



Fluorescence imaging:

- Specific signal obtained in PC tumors
- Quantification of the region of interest on the entire peritoneal cavity showed that addition of PEG_4 and PEG_{12} on mAb significantly decreased the interaction between TCO and TZ compared to a non-PEGylated mAb (i.e. PEG_0)

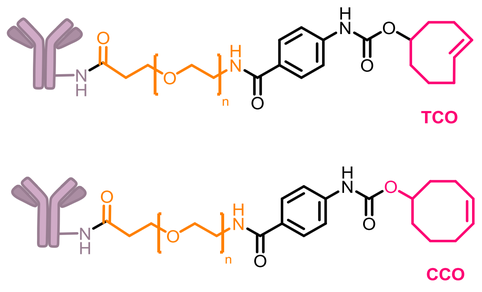


Pretargeting of CEA on A431-CEA-Luc PC tumors with modified 35A7 mAbs

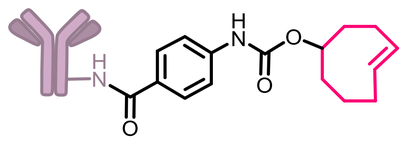
TZ-Cy5

Conclusions

- **Isomerization ?**



- **Most efficient mAb structure for further PRIT on PC tumors:**



mAb-PEG_0-TCO

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