



Targeting cancer cell (nucleus) with ^{111}In -labeled vectors in the context of Targeted Radionuclide Therapy

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PhD student

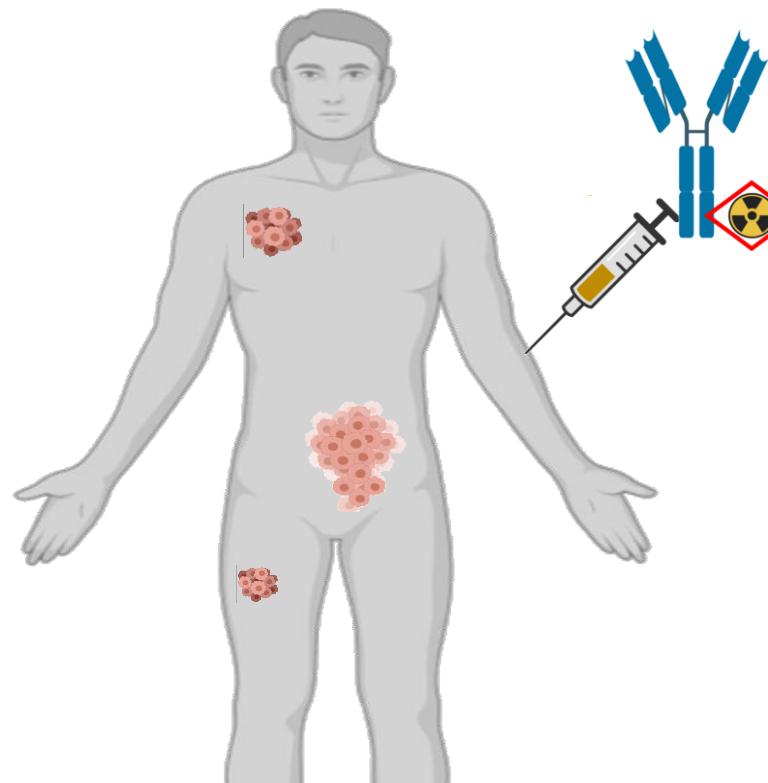
Team: Radiobiology for Targeted and Personalised Radiotherapy

Thesis director: Dr Jean-Pierre POUGET



Targeted Radionuclide therapy (TRT)

- Injection of radiopharmaceuticals directed against cancer cells
- Dedicated to localised, metastatic and disseminated tumors



TRT efficacy

Radionuclide + Vector + Tumor



Auger-electrons, Alpha or Beta emitters

^{125}I : 19 AE/decay, half-life : 59d

^{111}In : 8 AE/decay , half-life : 67h

^{67}Ga : 4 AE/decay, half-life : 13h

Others ?

[Am J Nucl Med Mol Imaging](#). 2014; 4(2): 181–192.
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Phase I trial to evaluate the tumor and normal tissue uptake, radiation dosimetry and safety of ^{111}In -DTPA-human epidermal growth factor in patients with metastatic EGFR-positive breast cancer

Katherine A Vallis,¹ Raymond M Reilly,^{2,3,4} Deborah Scollard,² Pat Merante,⁵ Anthony Brade,⁵ Sobi Velauthapillai,² Curtis Caldwell,^{4,6} Ida Chan,⁴ Marc Freeman,⁴ Gina Lockwood,⁷ Naomi A Miller,⁸ Bart Cornelissen,¹ Jennifer Petronis,⁹ and Kathryn Sabate⁷

Auger-electron emitters (AEEs):

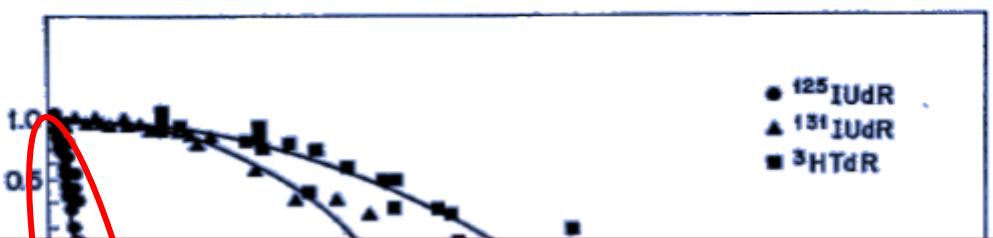
for AE < 1 KeV

Range: 2 – 500 nm

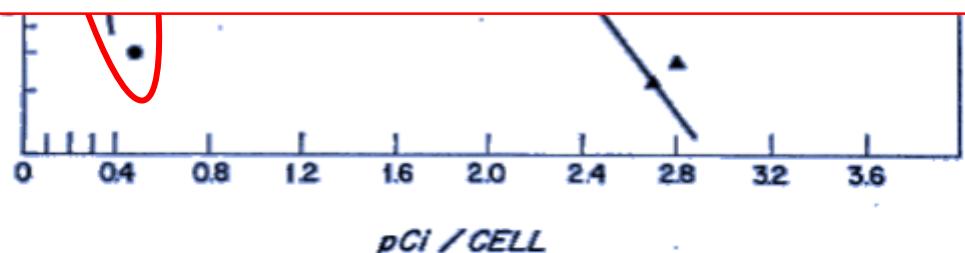
LET: 4 - 26 KeV/ μm

Very localized and densely ionisation

Auger-electron emitters (AEEs)

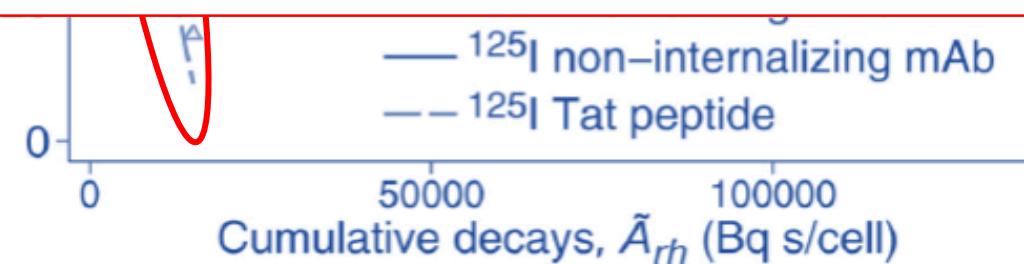


But radiolabeled DNA base analogue (^{125}I -UdR) or radiolabeled TAT (^{125}I -TAT) are not cancer cell specific → Side effects



Chan *et al.* 1975

AEEs are more cytotoxic than beta emitters when incorporated into nuclear DNA



Pouget *et al.* 2008

AEEs are more cytotoxic when delivered into cell nucleus than when delivered at plasma membrane or cytoplasm

Aim

To deliver ^{111}In specifically into the cancer cell nucleus

Scientific question:

Is NLS or TAT peptide a suitable candidate for driving AEEs to the cancer cell nucleus (endosomal escape) ?

Specific objectives:

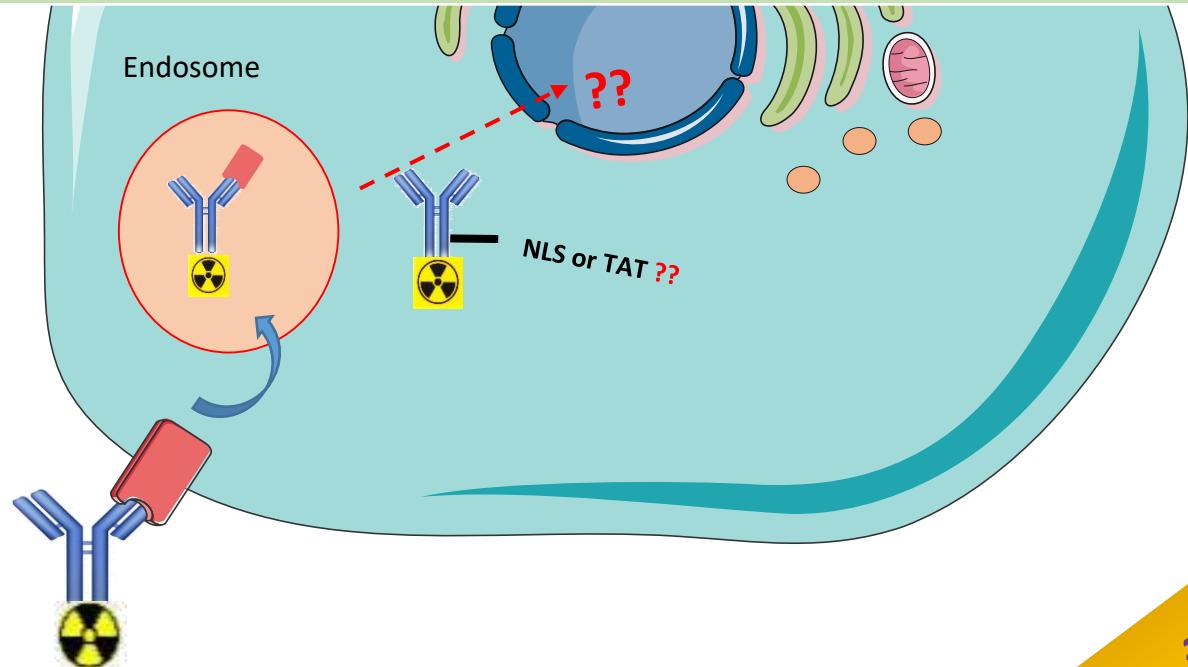
Assessing the cytotoxicity and the subcellular localisation of $[^{111}\text{In}]\text{In}$ -trastuzumab conjugated with nuclear localising sequences



NLS peptide: CGYGPKKRKVGG

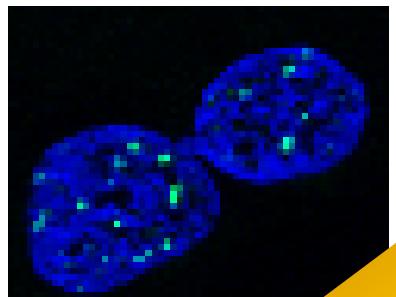
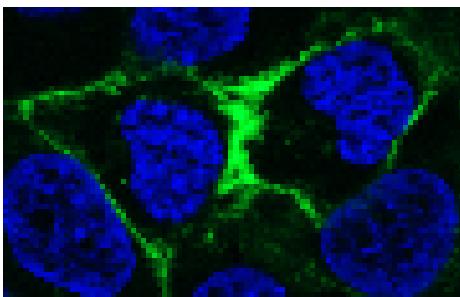
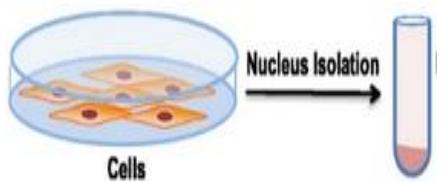
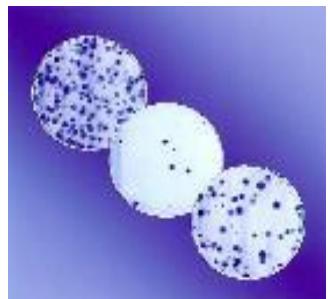
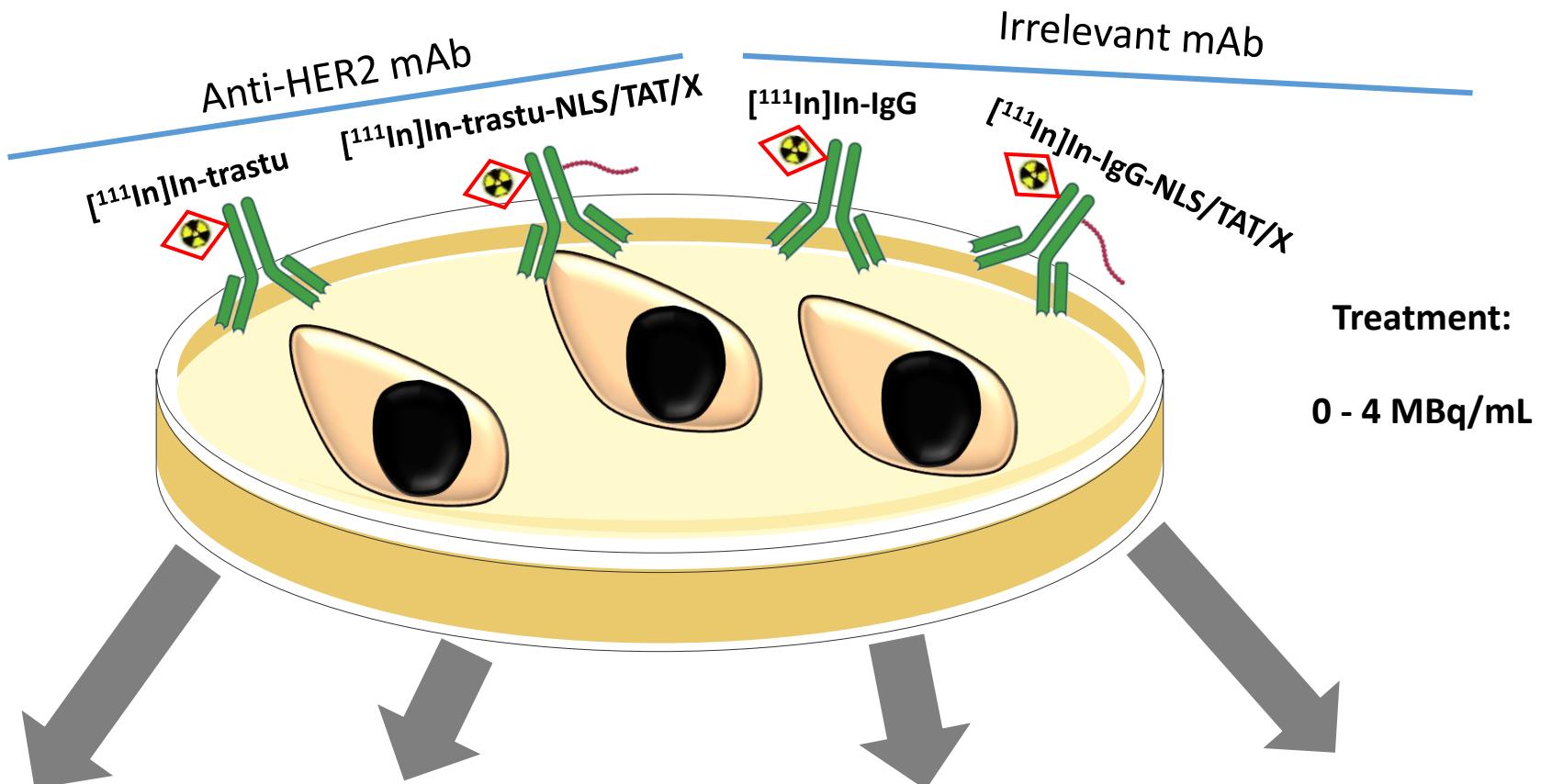
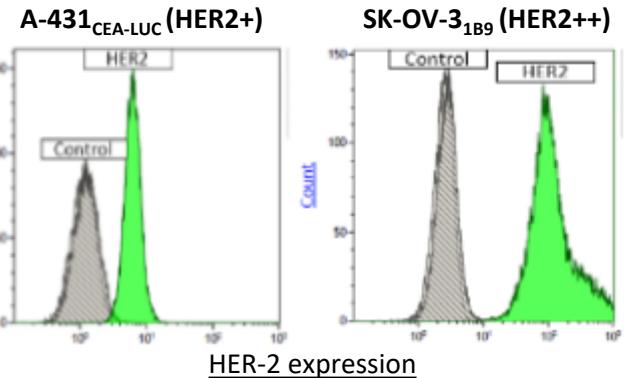
TAT peptide: GRKKRRQRRRPPQGYG

Nuclear addressing compounds



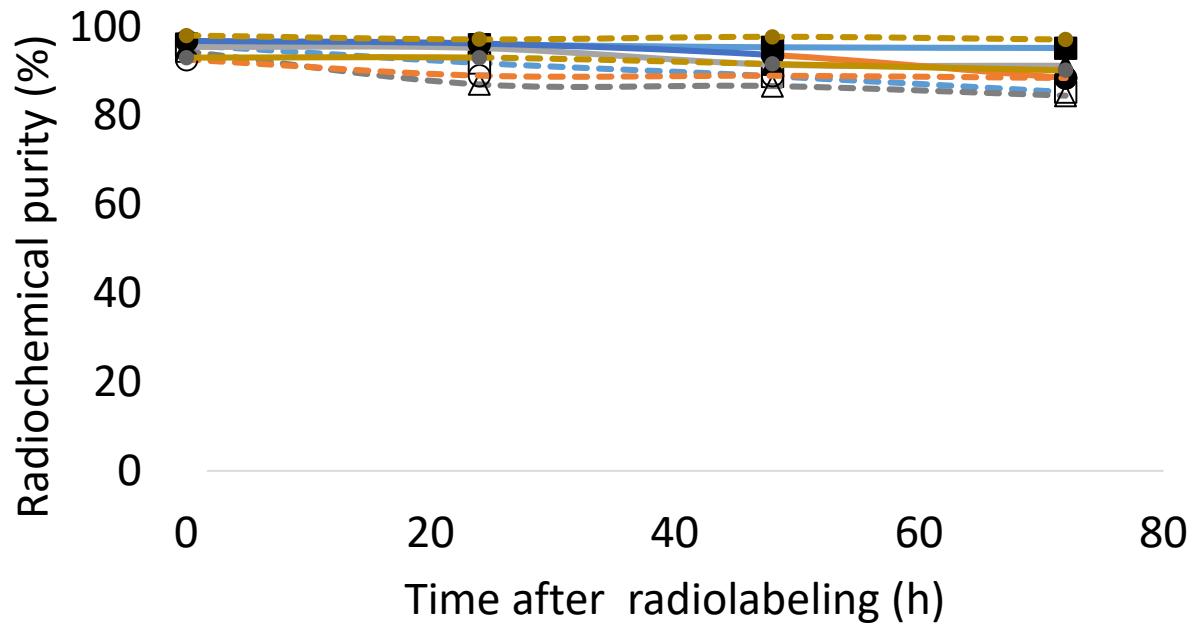
Materials and methods

Cell lines

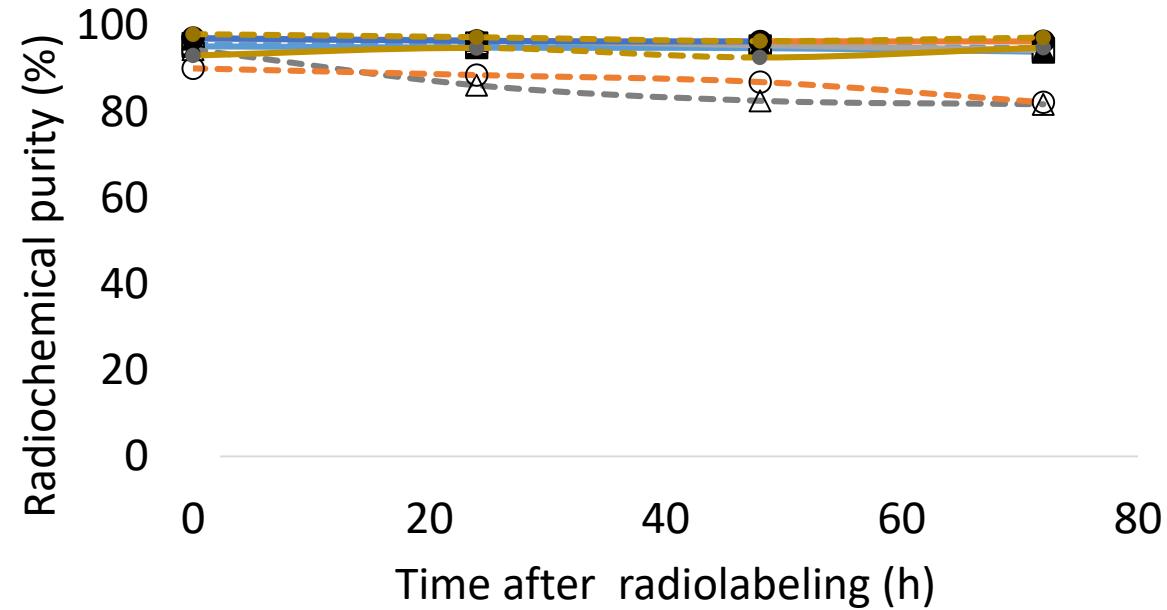


Stability of the radiopharmaceuticals tested

Stability in the culture medium



Stability in human serum



Legend:

- [111In]In-trastuzumab
- [111In]In-trastuzumab-NLS10
- [111In]In-trastuzumab-TAT 1-3
- [111In]In-trastuzumab-X10
- [111In]In-IgG
- [111In]In-IgG-X10

Legend:

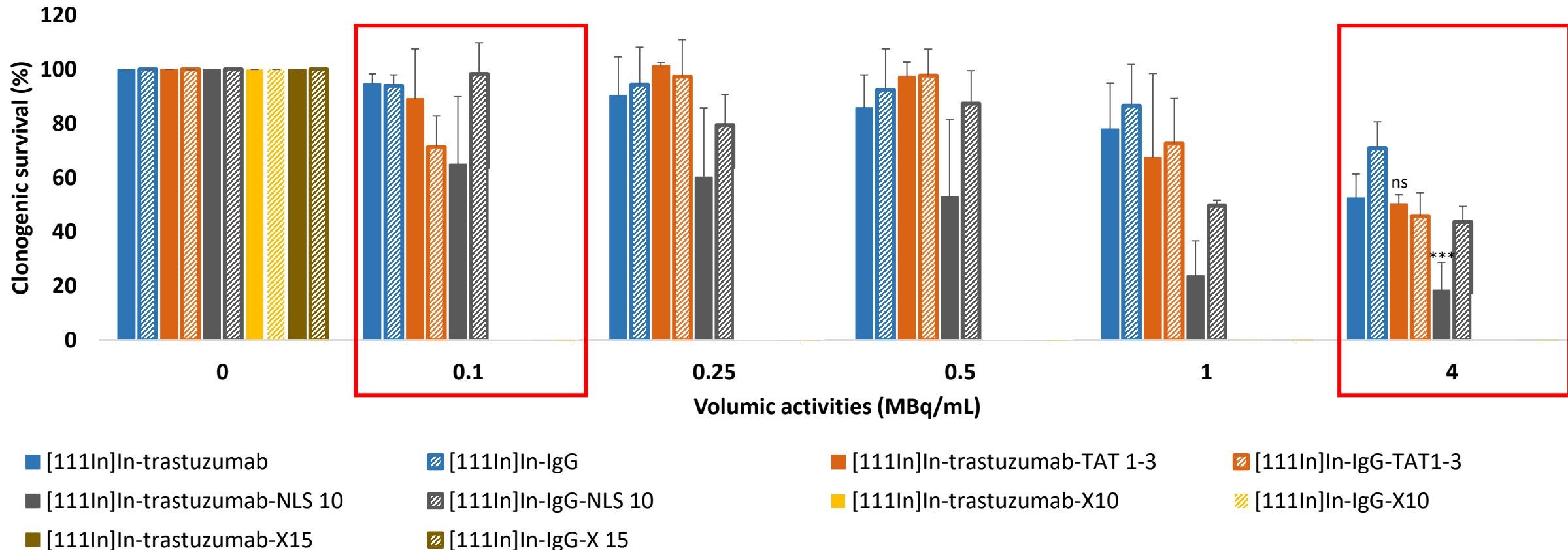
- [111In]In-trastuzumab
- [111In]In-trastuzumab-NLS10
- [111In]In-trastuzumab-TAT 1-3
- [111In]In-trastuzumab-X10
- [111In]In-IgG
- [111In]In-IgG-X10

→ More than 80% of ^{111}In is bound to protein over 72h

Which peptide increases the [¹¹¹In]In-mAb cytotoxicity ?

Clonogenic survival

A-431_{cea-luc}

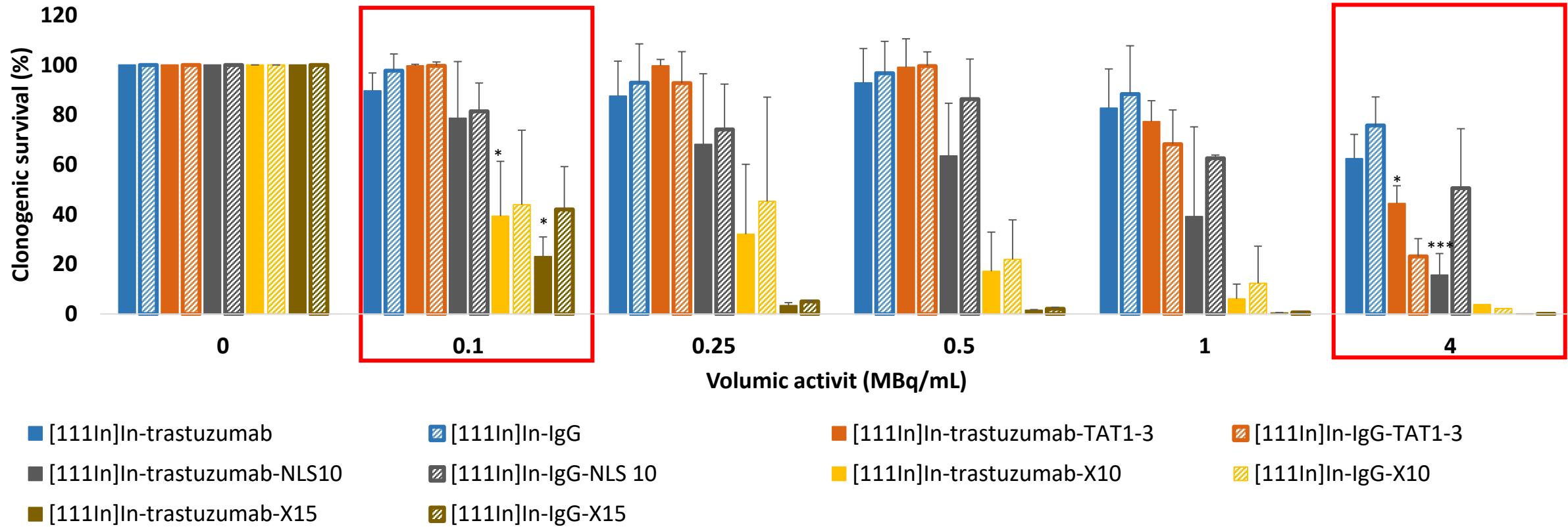


- Functionalisation with TAT₁₋₃ is not associated with cytotoxicity
- Functionalisation with NLS₁₀ is associated with high and specific cytotoxicity
- Functionalisation with compounds X is associated with the highest cytotoxicity

Which peptide increases the [¹¹¹In]In-mAb cytotoxicity ?

Clonogenic survival

SK-OV-3_{1B9}

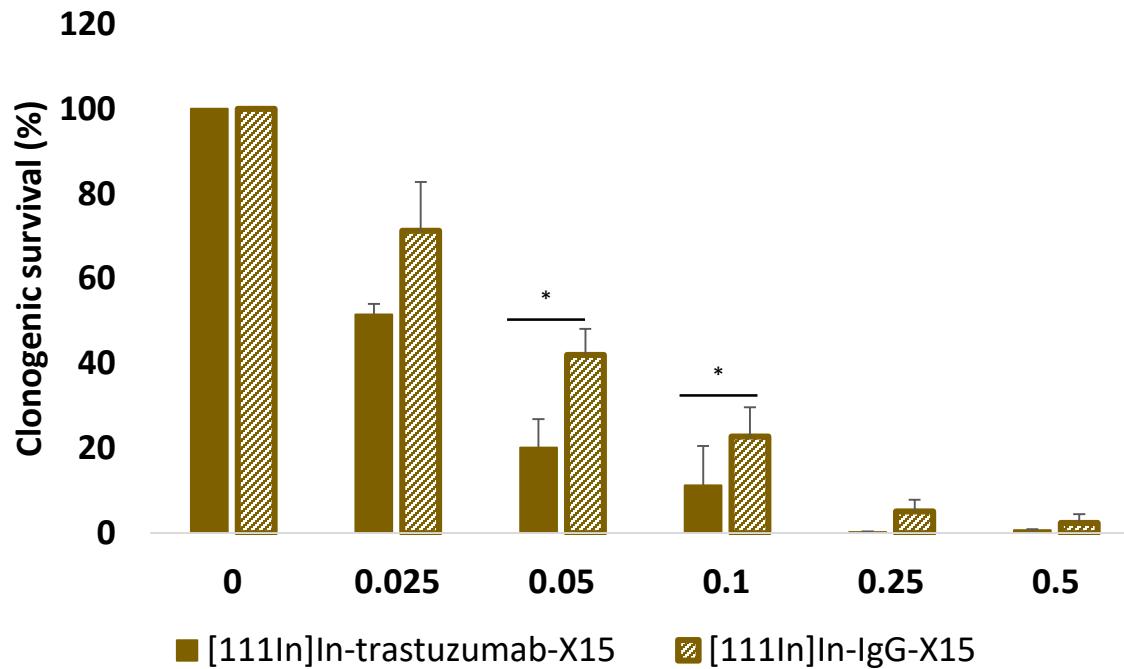


- Functionalisation with TAT₁₋₃ is associated with moderate but non-specific cytotoxicity
- Functionalisation with NLS₅₋₁₀ is associated with high and specific cytotoxicity
- Functionalisation with compound X is associated with the highest cytotoxicity

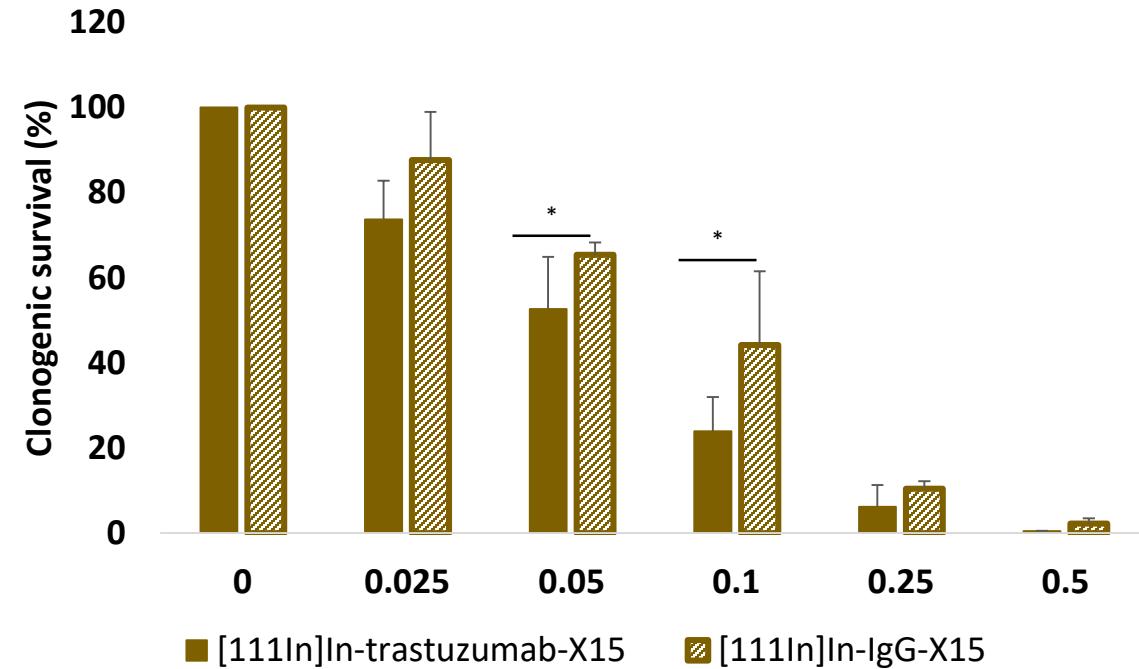
Is [^{111}In]In-mAb-X₁₅ more specific at lower activities ?

Clonogenic survival

A-431_{ceo-luc}



SK-OV-3_{1B9}



→ [^{111}In]In-trastuzumab-X₁₅ is more cytotoxic than [^{111}In]In-IgG-X₁₅ at activities lower than 0.25 MBq/mL

Is the radioimmunoconjugates tested internalised in cells (nucleus)?

Activity uptake measurement

D0: Cell seeding

D1: incubation with 0.1 MBq/mL for 48h

D2, 3 and D6:

Treatment removal

Cell count

Measure of the whole cell activity

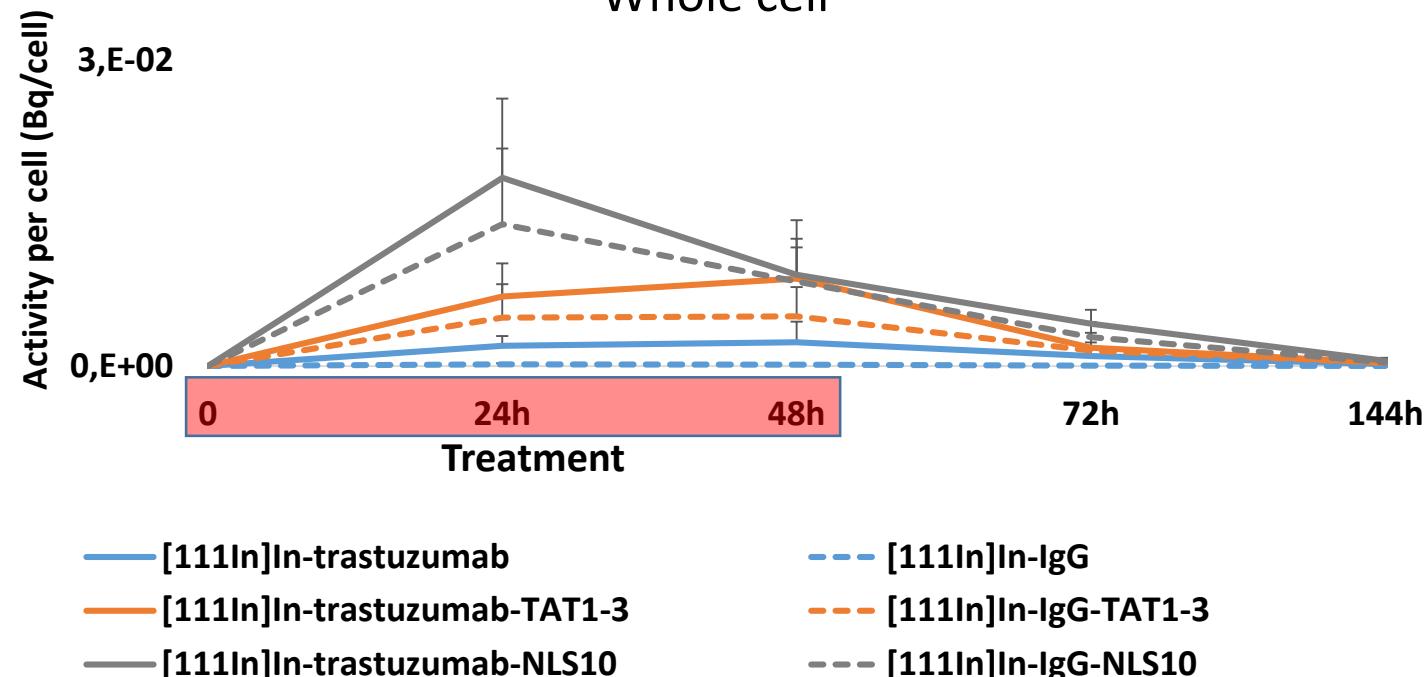
Cell fractionnation

Measurement of the nuclear activity

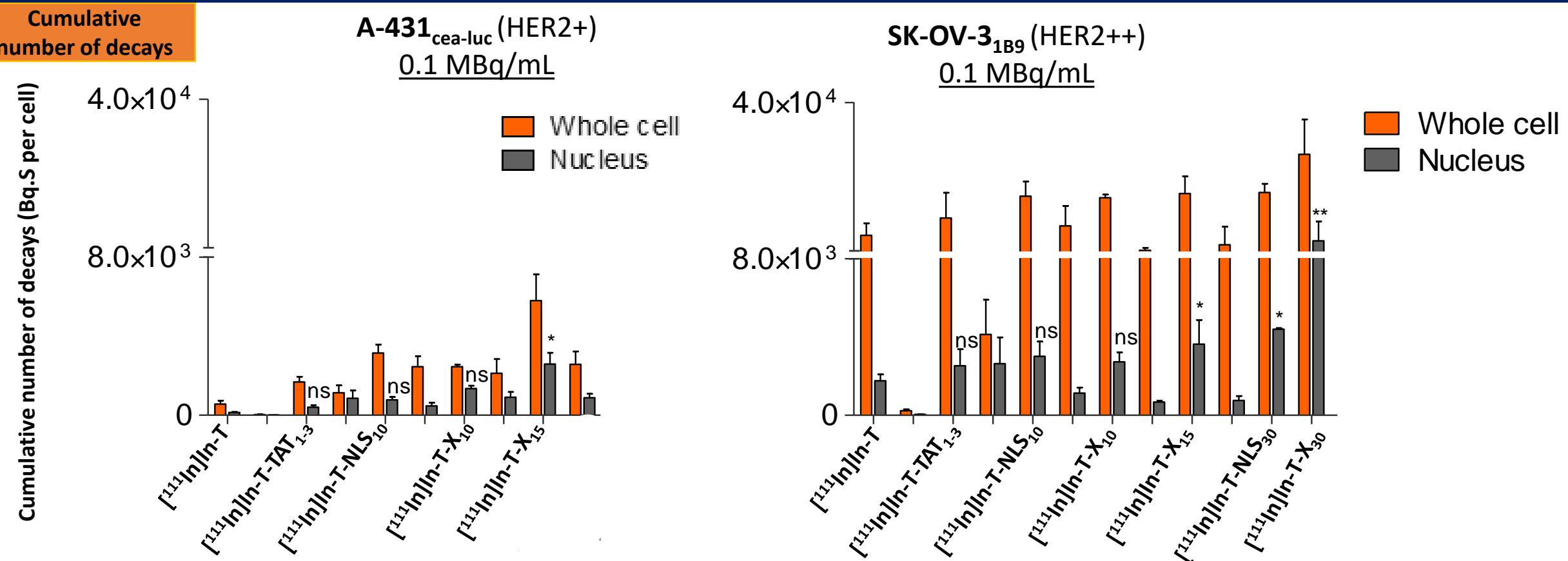


Calculation of cumulative number of decays

A-431_{cea-luc}
Whole cell



Is the radioimmunoconjugates tested internalised in cells (nucleus)?

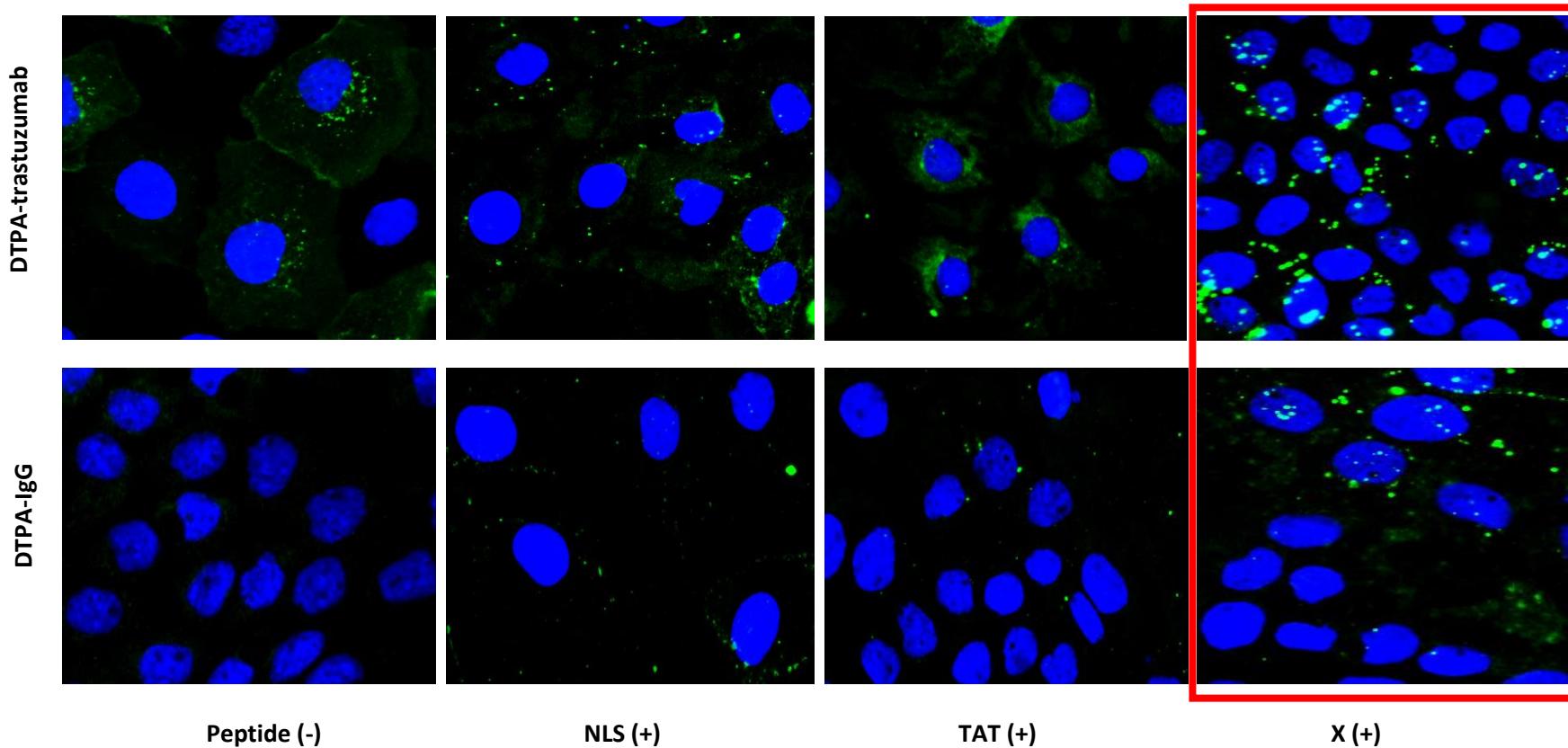


- More activity uptake in SK-OV-3_{1B9} cells than in A-431_{cea-luc} cells
- A-431_{cea-luc}: Higher nuclear uptake of [¹¹¹In]In-T-X₃₀ than [¹¹¹In]In-T-NLS₃₀
- SK-OV-3_{1B9}: Higher nuclear uptake of [¹¹¹In]In-T-X₃₀ than [¹¹¹In]In-T-NLS₃₀

Are the tested immunoconjugates detected in the cell nucleus?

Subcellular localization
of mAb-DTPA

SK-OV-3_{1B9}
48h



- Immunofluorescence shows low nuclear accumulation of NLS- or TAT- immunoconjugates
- X- immunoconjugates are strongly detected in the cell nucleus

CONCLUSIONS

- Limited nuclear uptake of **NLS**- and **TAT**- radioimmunoconjugates
- No drastic cytotoxicity was observed in clonogenic survival Assay with **NLS**- and **TAT**- radioimmunoconjugates
- Strong nuclear internalisation of X- radioimmunoconjugates
- Drastic cytotoxicity was observed in clonogenic survival Assay with X- radioimmunoconjugates

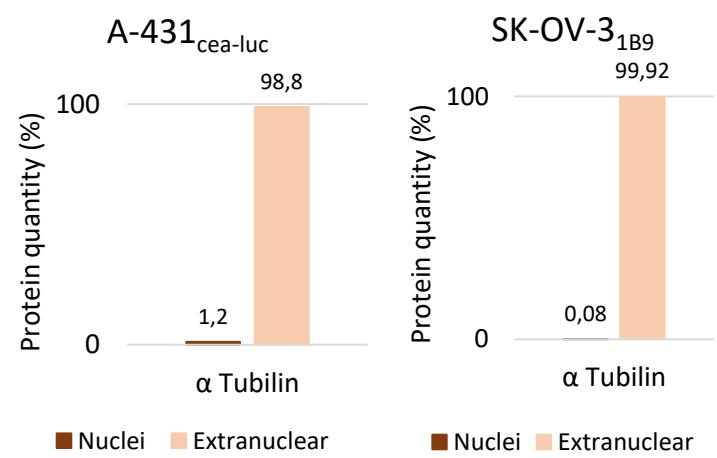
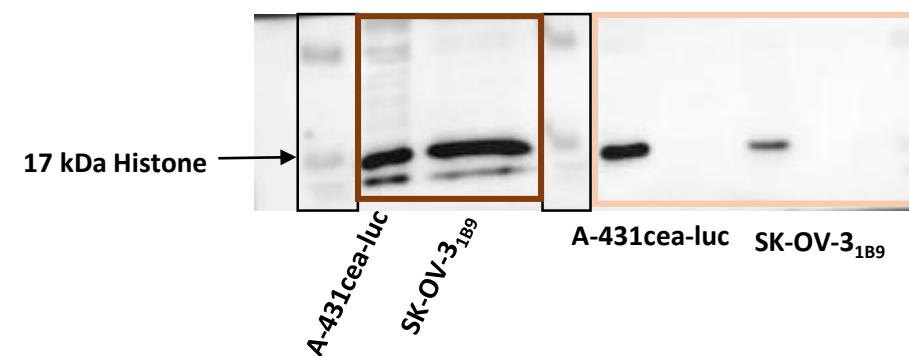
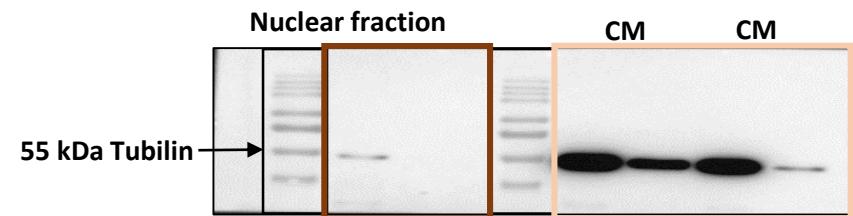
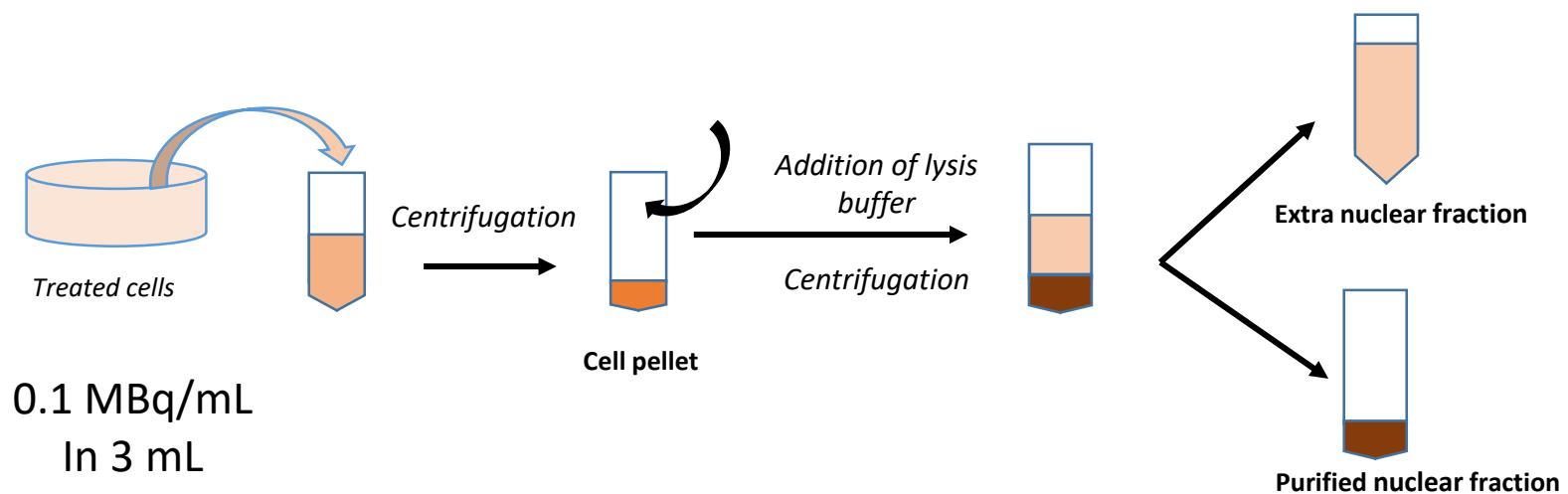
Merci

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- Yasmine
- Julien



Validation of Nucleus-isolation technique

Nucleus isolation method



Design of radiopharmaceuticals

MALDI TOF analysis:

reveals the number of compounds per mAb

ITLC method:

reveals the radiolabeling yield



Nomenclature

