Random versus site-specific conjugation: impact on ADME properties of biologics with different format

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ABSTRACT

Antibody format and conjugation strategy can strongly influence the properties and performance of antibody–drug conjugates. Here, we compare lysine-based and site-selective cysteine conjugation across anti-HER2 formats (IgG, Fab, VHH, and VHH-Fc) to determine how antibody size and conjugation affect stability, affinity, and in vivo behaviour. ^{3}H radiolabelling was performed by lysine acylation or disulfide rebridging followed by click chemistry [2]. Conjugates were characterized by SDS-PAGE, β-imaging, mass spectrometry, and BLI, with in vivo biodistribution assessed by β-imaging, ^{3}H quantification [3], and autoradiography. **Aim: Assess how antibody size and conjugation influence overall biological performance.**

1 Conjugation strategy

Protein size

Small protein (~15-25kDa) — VHH

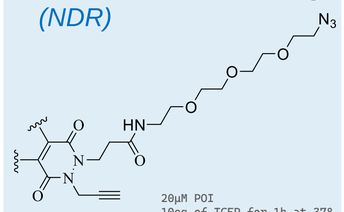
Medium protein (~50-90kDa) — Fab, VHH-Fc

Large protein (~150kDa) — IgG

HER2+ antigen binding site

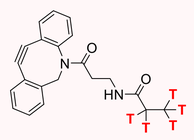
Site-specific conjugation

Step 1 — *Native disulfide rebriding (NDR)*



20µM POI
10eq of TCEP for 1h at 37°
40eq of TAG for 2h at 37°C

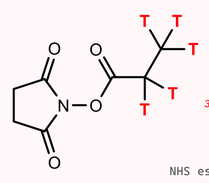
Step 2 — *SPAAC*



20µM of POI
20eq of DBCO-X for 5h at 4°C

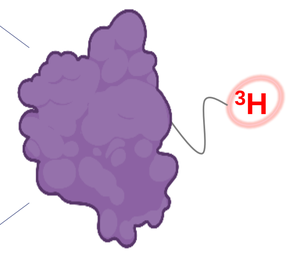
Random conjugation

Step 1 — *Lysine Acylation*

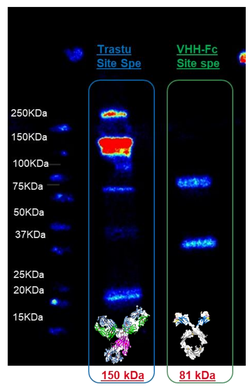


NHS ester chemistry

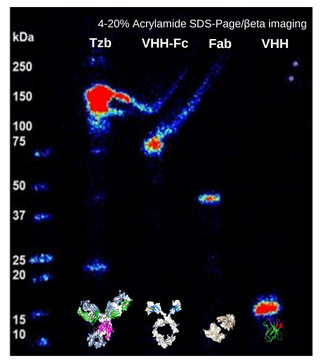
Radiolabelled conjugate



*protein size **x** conjugation strategy comparison*



N° of disulfide bridges:
- IgG → 4
- VHH-Fc → 2
- Fab → 1
- VHH → 1



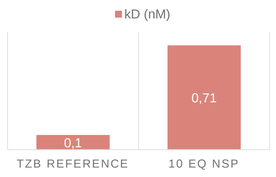
N° of lysines:
- IgG → 45 x 2 = 90
- VHH-Fc → 23 x 2 = 46
- Fab → 26
- VHH → 4

2 *In-vitro* characterization

Binding Affinity
Bio-Layer Interferometry (BLI)

Site specific vs *Random*

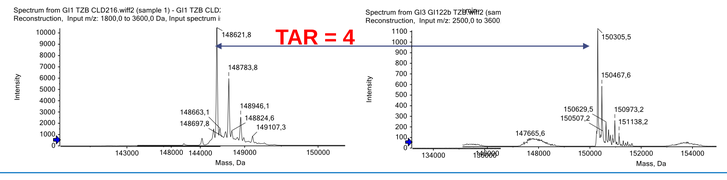
	Ref	NDR	SPAAC
IgG	0,1 nM	0,30 nM	0,33 nM
VHH-Fc	0,62 nM	0,63 nM	0,60 nM



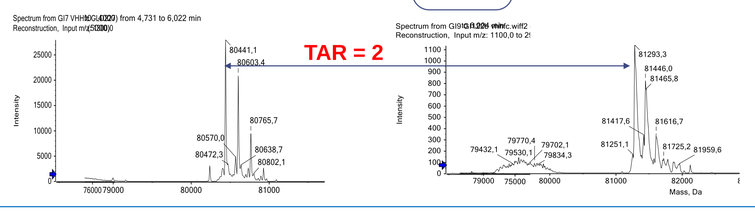
- No evident change of affinity
- Evident change of affinity

TAG to format ratio
HRMS (ZenoTOF) analysis

1) Trastuzumab



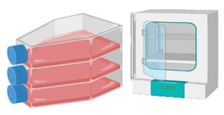
2) VHH-Fc

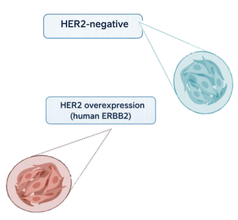


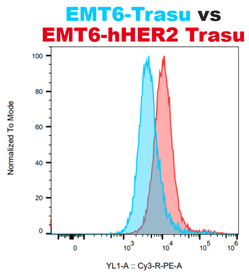
3 *In-vivo / Ex-vivo* characterization

Identification of HER2+ cells
Flow cytometry assay

EMT6/HER2-EMT6 mouse breast cell line culture

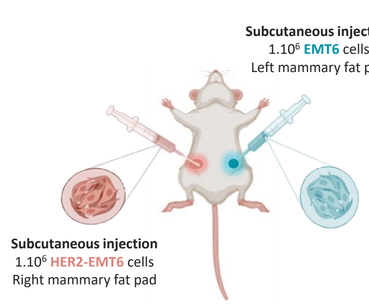






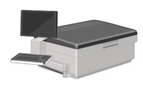
100k cells → 100pmol of protein

IV dose administration

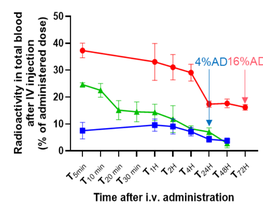


Syngeneic orthotopic mouse model

Pharmacokinetic results



[³H] Quantification by liquid scintillation



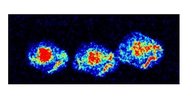
BioDistribution data



Beta imager



whole body imaging



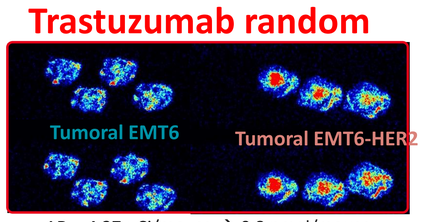
Tumoral sections imaging

3 *In-vivo / Ex-vivo* characterization

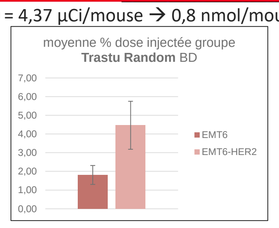
Biodistribution on tumor sections



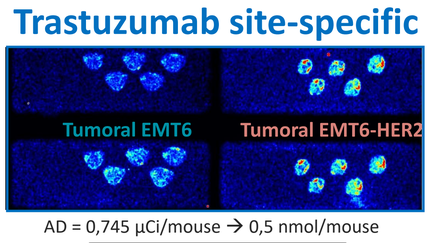
Trastuzumab random



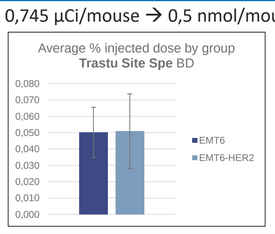
AD = 4,37 µCi/mouse → 0,8 nmol/mouse



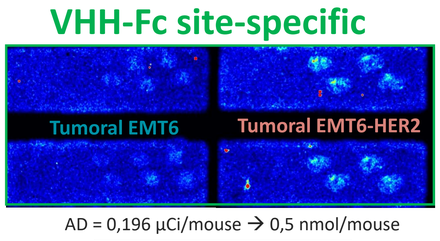
Trastuzumab site-specific



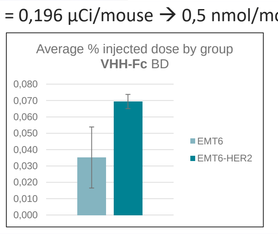
AD = 0,745 µCi/mouse → 0,5 nmol/mouse



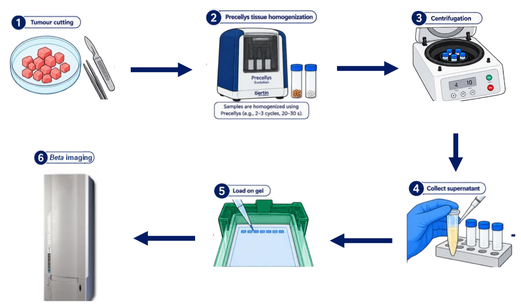
VHH-Fc site-specific

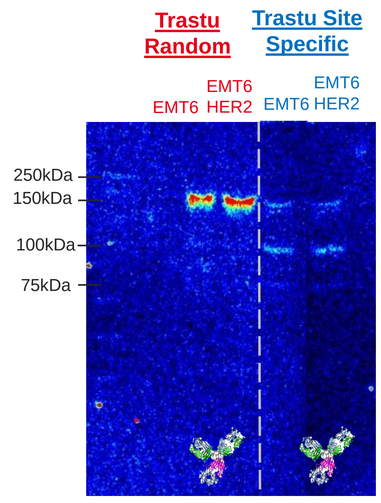


AD = 0,196 µCi/mouse → 0,5 nmol/mouse



Tumor tissue homogenization





Recovery of ^{3}H-labelled biomolecules with the tumor

CONCLUSION

- ✓ **Two bioconjugation strategies were validated**
- ✓ **Determination of TAR of site-specific modification**
- ✓ **Confirmation of HER2 expression**
- ✓ **Affinity tests**
- ✓ **Validation of overall workflow**

✓ ***Next steps:***

The workflow be applied to all of the formats
- **Tissular distribution** : ADME studies and Autoradiography (enables spatial discrimination between the tumor core and peripheral regions)
- **Cellular distribution**: Micropatterning

Our aim with this study is determine the optimal balance between exposure, tumor delivery, stability and safety

[1] N. C. Yoder, C. Bai, D. Tavares, W. C. Widdison, K. R. Whiteman, A. Wilhelm, S. D. Wilhelm, M. A. McShea, E. K. Maloney, O. Ab, L. Wang, S. Jin, H. aeK. Erickson, T. A. Keating, and J. M. Lamber *Mol. Pharmaceutics* **2019**, *16*, 3926−3937.

[2] C. Bahou, D. A. Richards, A. Maruani, E. A. Love, F. Javaid, S. Caddick, J. R. Baker and V. Chudasama *Org. Biomol. Chem.,* **2018**, *16*, 1359

[3] Cahuzac, H.; Sallustrau, A.; Malgorn, C.; Beau, F.; Barbe, P.; Babin, V.; Dubois, S.; Palazzolo, A.; Thai, R.; Correia, I.; Lee, K. B.; Garcia-Argote, S.; Leguin, O.; Keck, M.; Nozach, H.; Feuillastre, S.; Ge, X.; Pieters, G.; Audisio, D.; Devel, L. J. Med. Chem. 2022, 65, 6953–6968.