A New Site Specific Antibody Conjugation Using Bacterial Transglutaminase

ADC Summit, San Francisco

On 15th October, 2013

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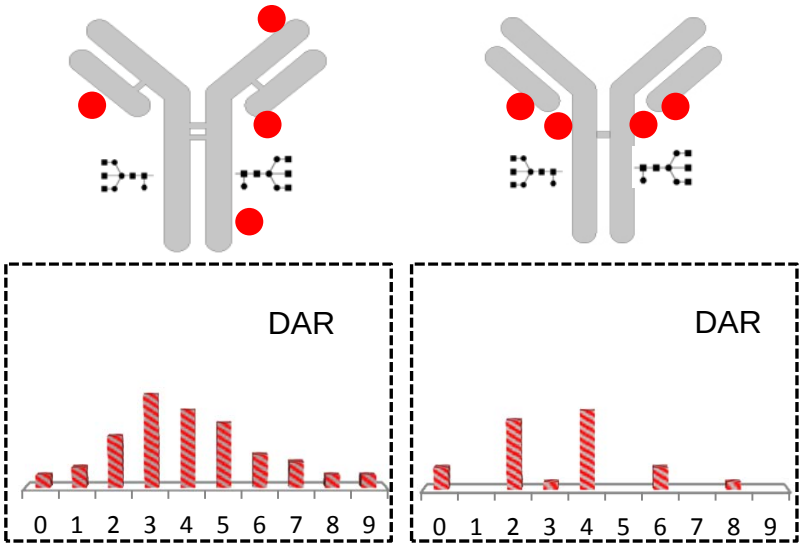
innate pharma

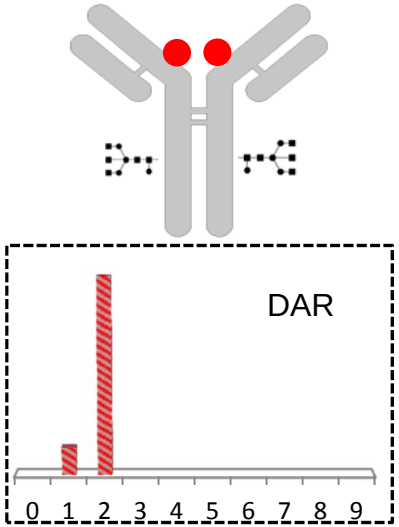
From Chemotherapy to Homogeneous ADCs

Improving the Therapeutic Index



First Generation:
Lysine or Cysteine conjugation

- Increased tumor delivery
- Decreased normal tissue exposure
- Heterogeneous PK



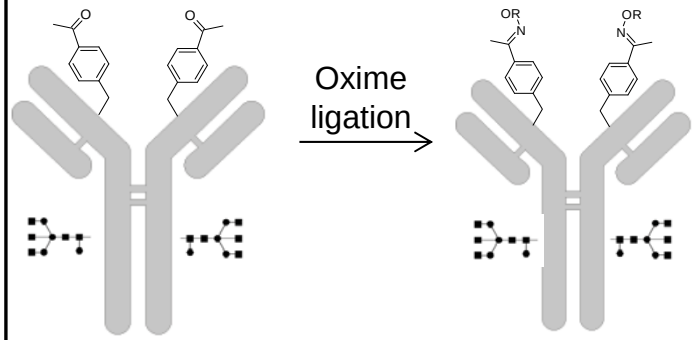
Second Generation:
engineered Cysteine conjugation

- Homogeneous PK
- Unstability questiomark
  - unpaired cystein
  - thiol-maleimide linkage

Next Generation ADCs

Homogeneous and Stable

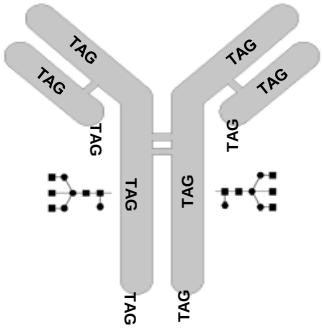
Unnatural Amino Acid



- Expanded genetic code to incorporate orthogonal side chains
- POC (*Axup et al., PNAS, 2012*)
- Specific production system

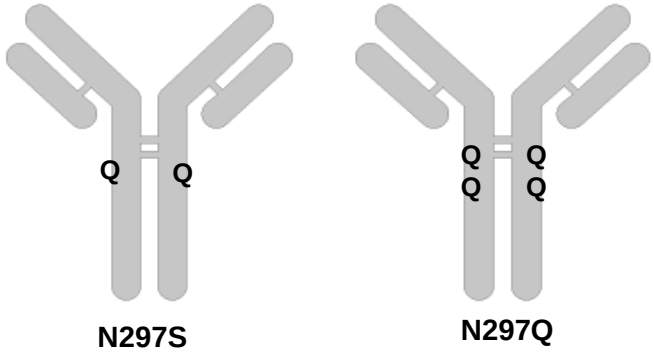
Transglutaminase (TG)

LLQG Tag



- LLQG Tag to create conjugation site
- POC (*Strop et al., Chem. Biol., 2013*)
- Effector function preserved

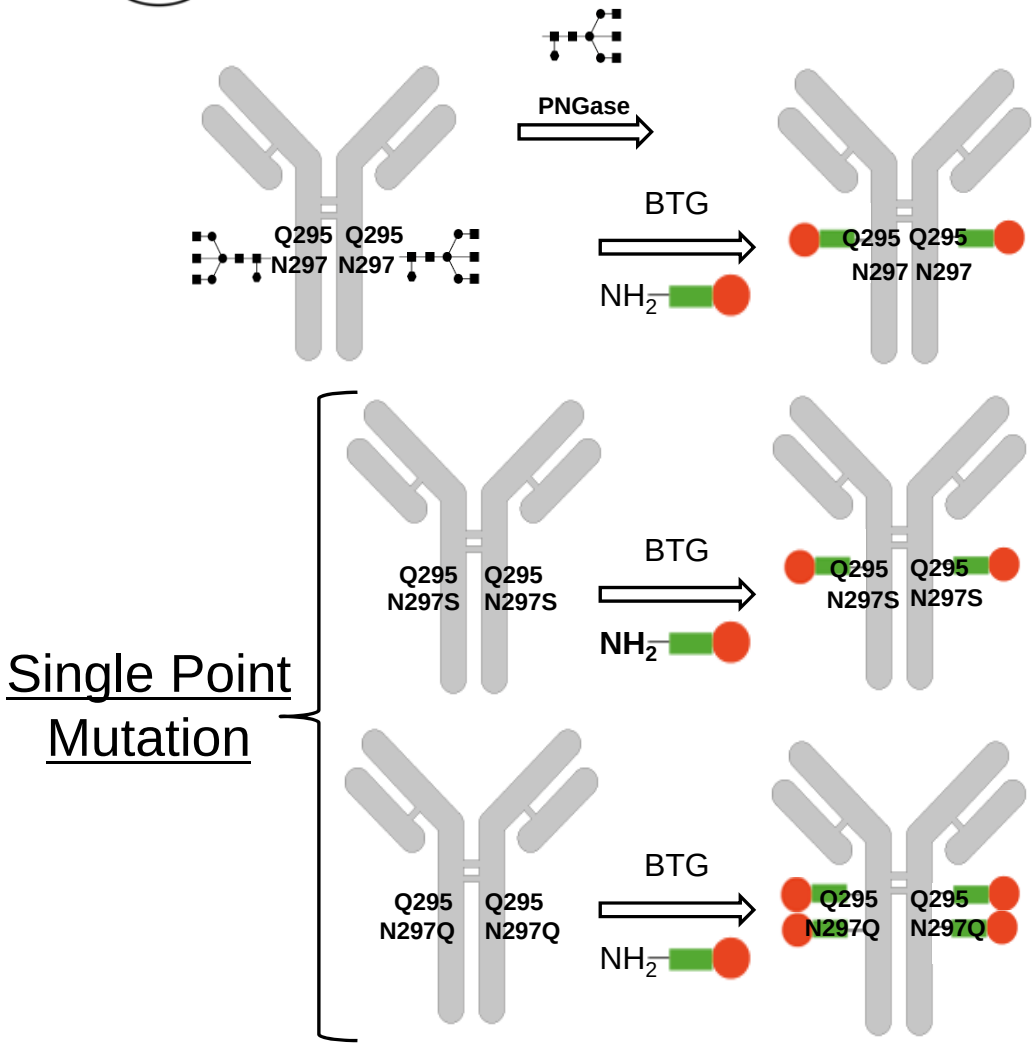
Single point mutation



- Aglycosylated mAb
- Conjugation on endogenous Q295 and possibly on N297Q
- Lower uptake by FcR+ cells might improve tumor-specific targeting and limit off-target toxicity

Bacterial Transglutaminase (BTG)

Site-specific and Stoichiometric Enzymatic Conjugation

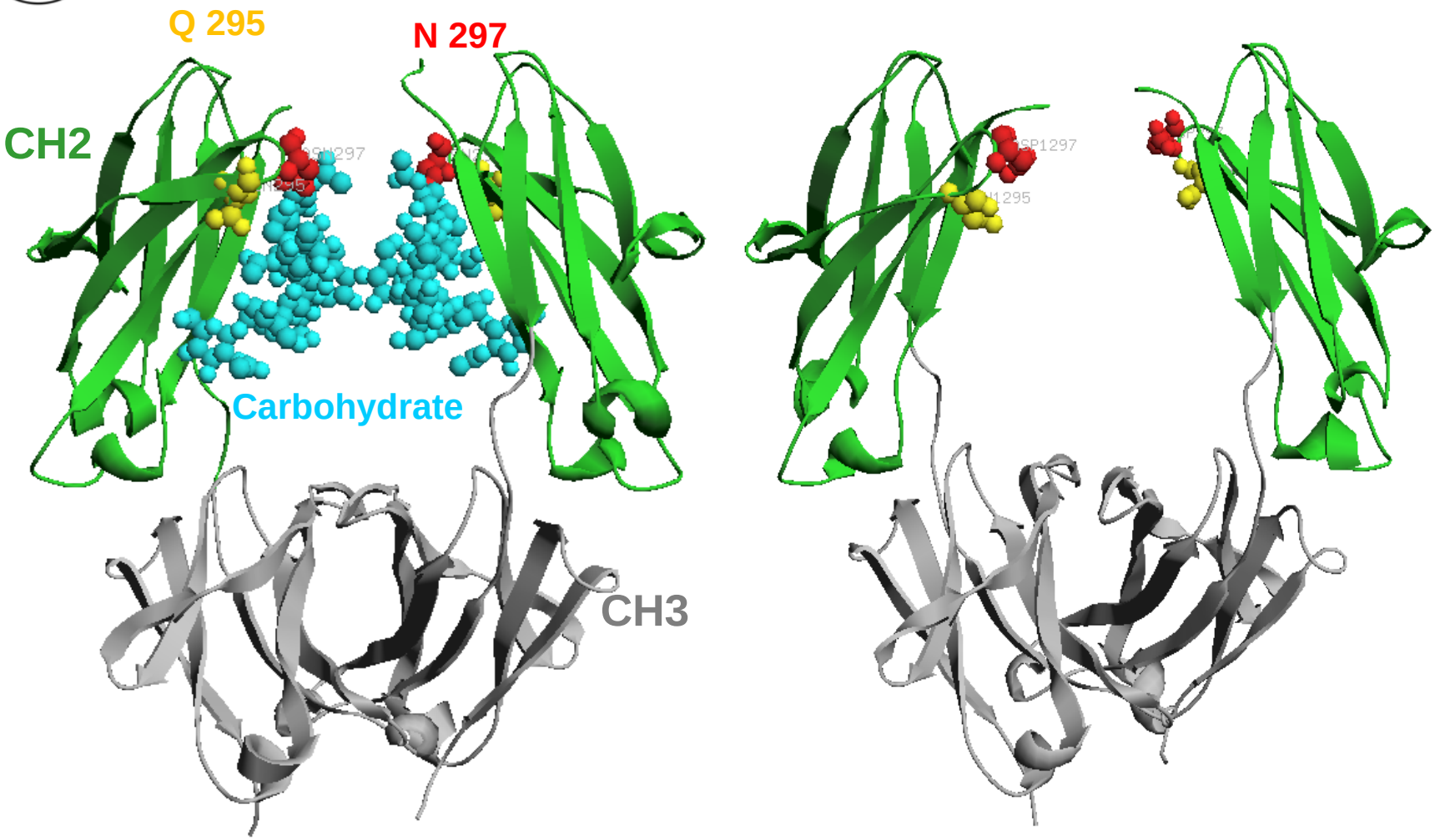


- BTG catalyses reactions between glutamine and lysine
- BTG recognizes exclusively endogenous Q295 located in Fc region of aglycosylated IgG
- N297Q mutation provides 2 additional sites for conjugation

Jeger et al., Angew. Chem. Int. Ed., 2010

BTG Ligation Site in Fc Structure

Before and After Carbohydrate Removal



Q 295 is barely exposed and partially hidden by the carbohydrate

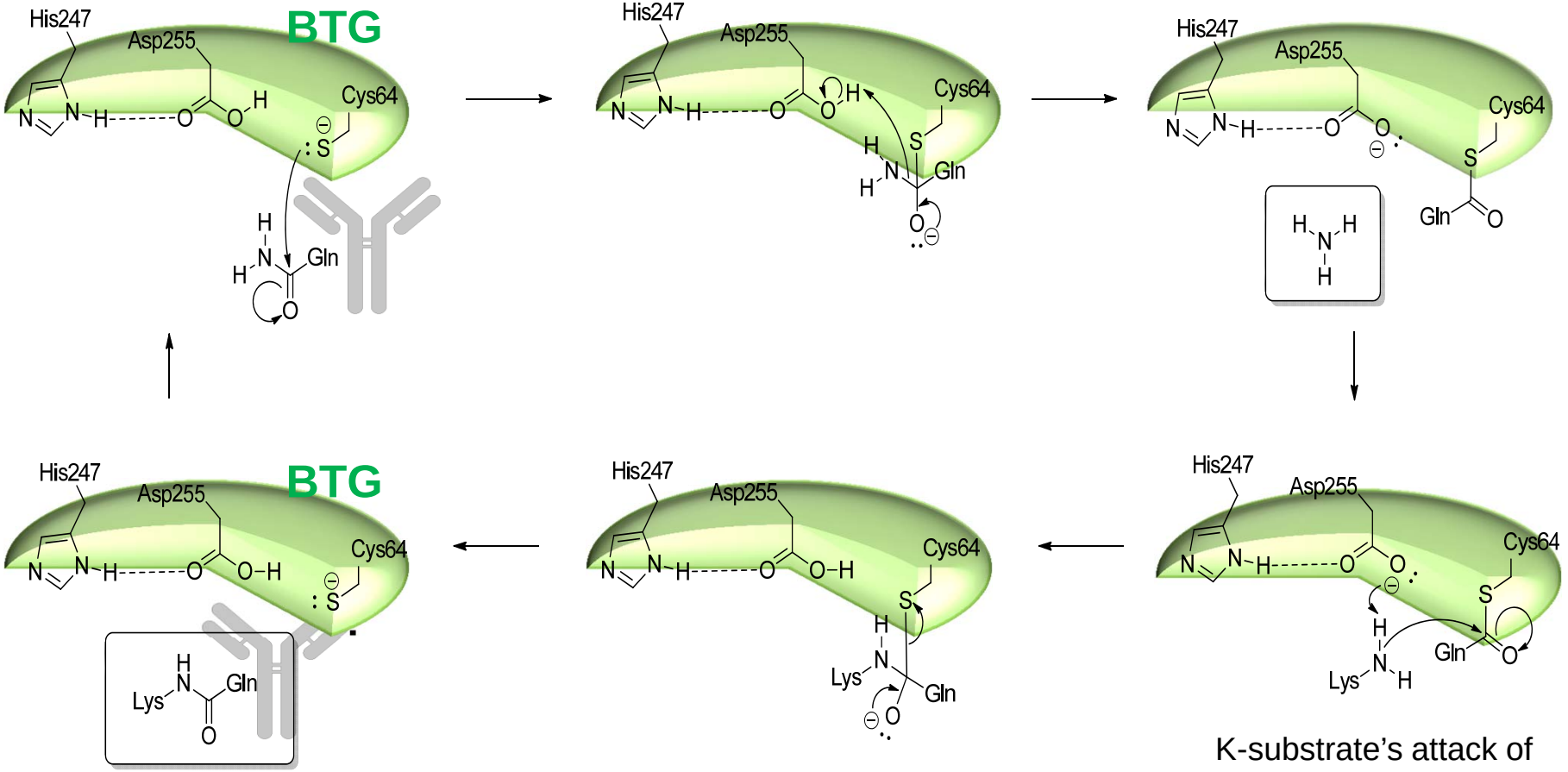
Degree of freedom is improved when carbohydrates are absent

BTG Coupling Reaction

BTG is calcium independant

Acylenzyme intermediate formation

Release of ammonia



Isopeptide bond formation

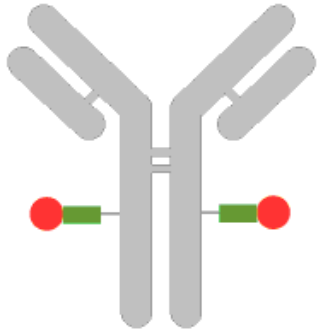
One-step Approach

One-step Approach

BTG

NH_2-step1-●

● = toxin

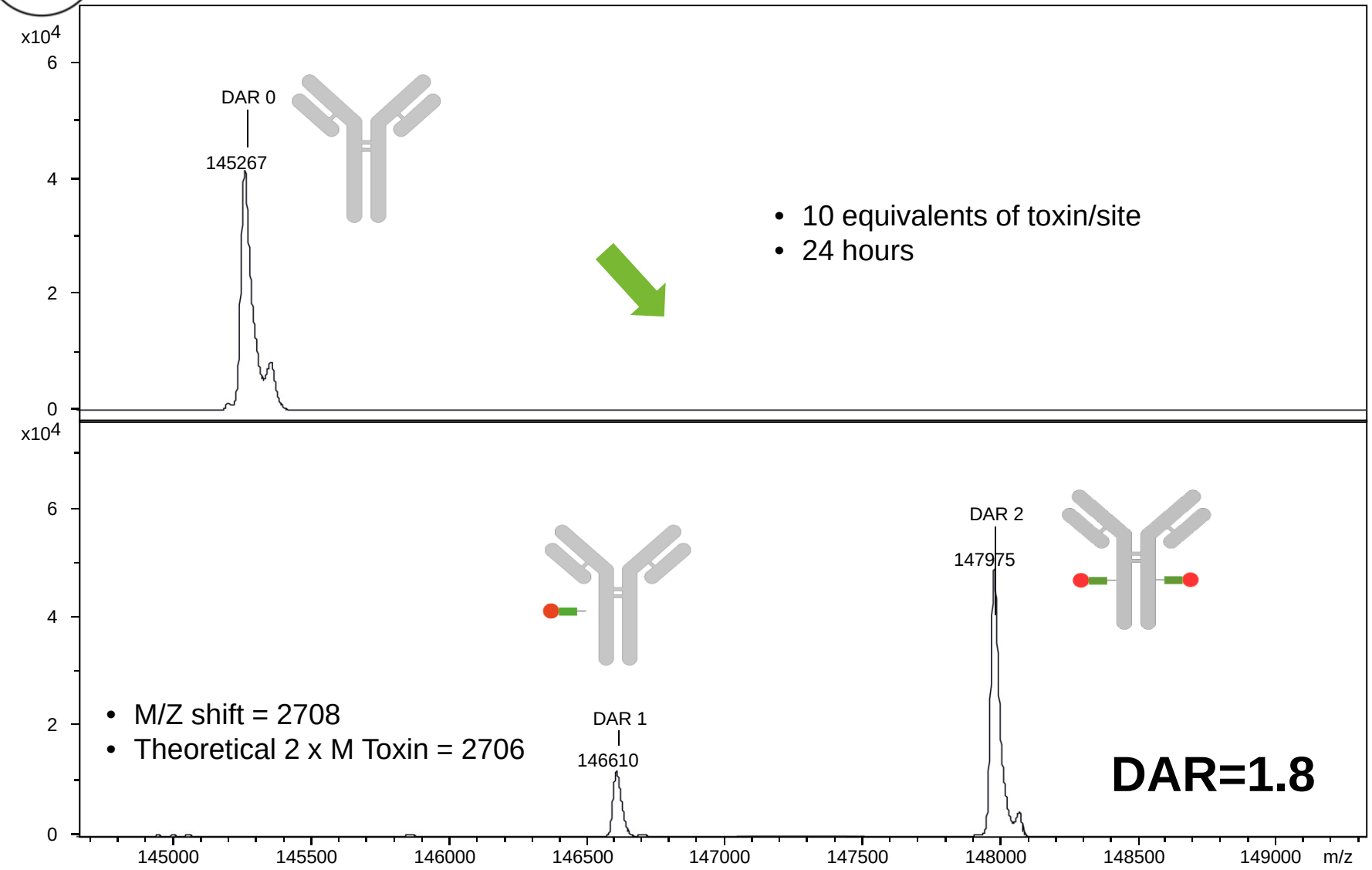


Aglycosylated IgG
Single mutation
N297S or N297Q

x 2 for N297S
x 4 for N297Q

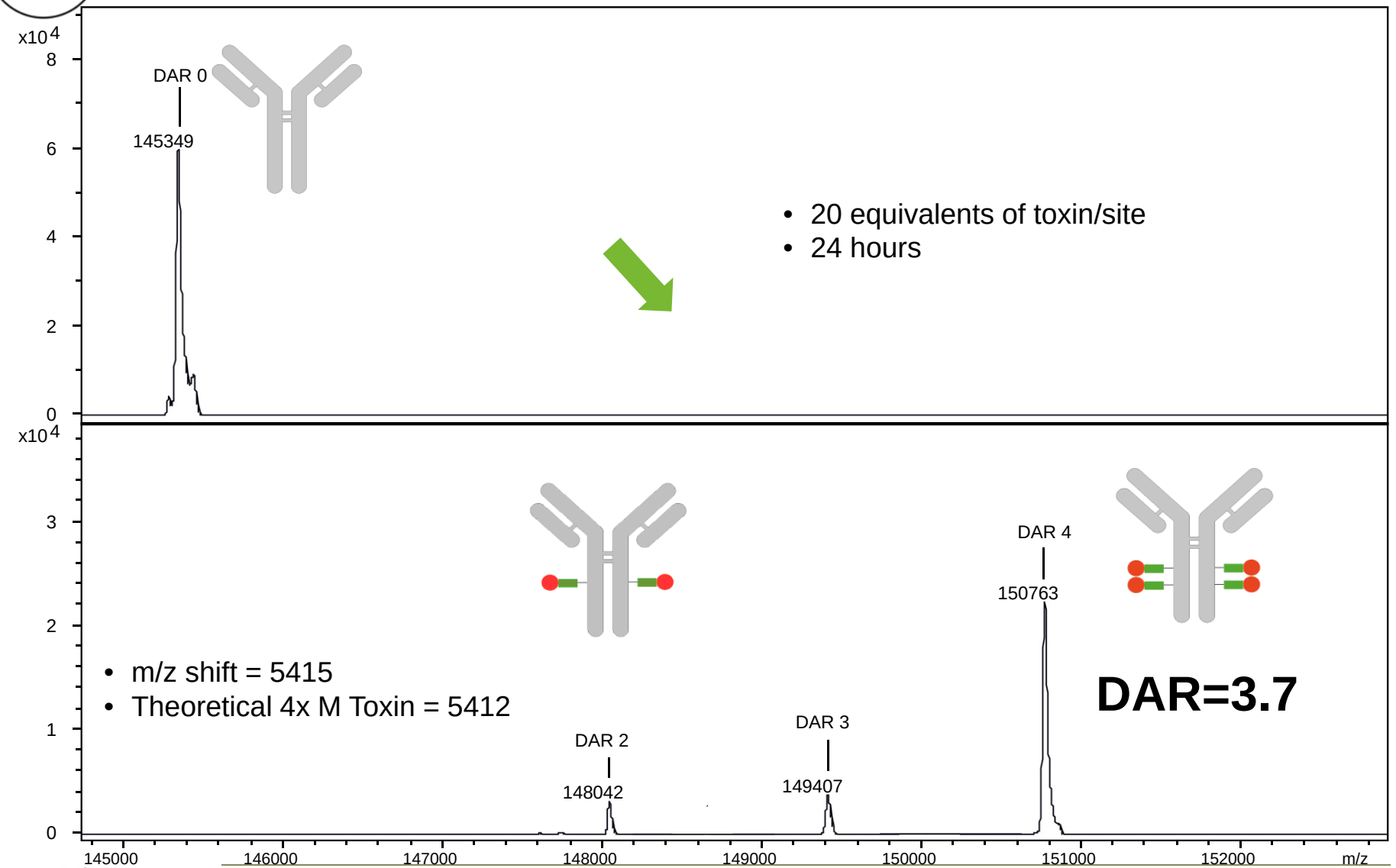
N297S coupling with NH_2-step1a-vc-PAB-MMAE

LC/MS (ESI-qTOF)



N297Q coupling with NH_2-step1a-vc-PAB-MMAE

LC/MS (ESI-qTOF)



Two-step Approach

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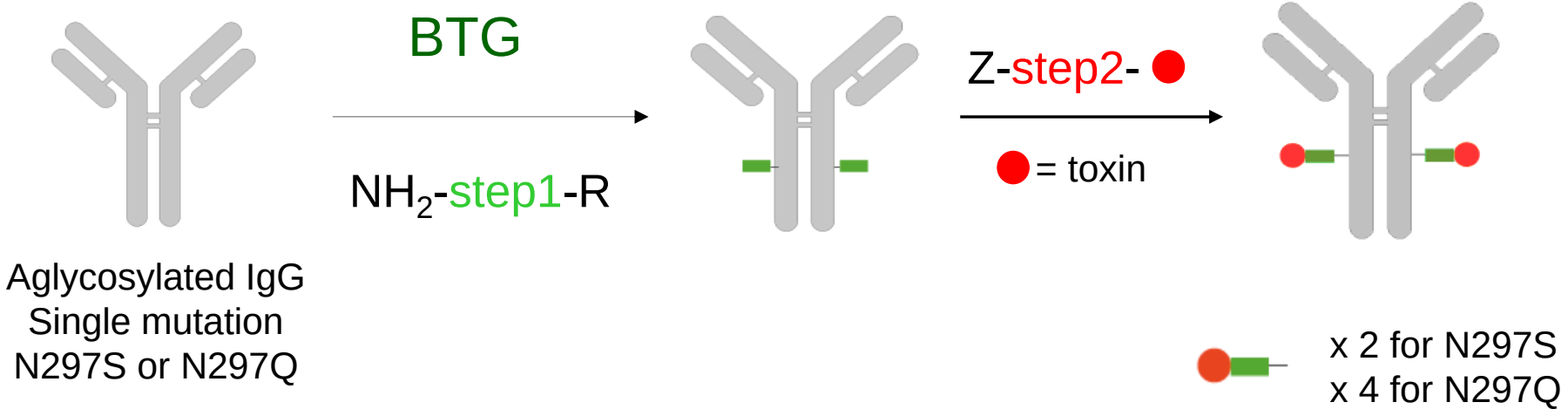
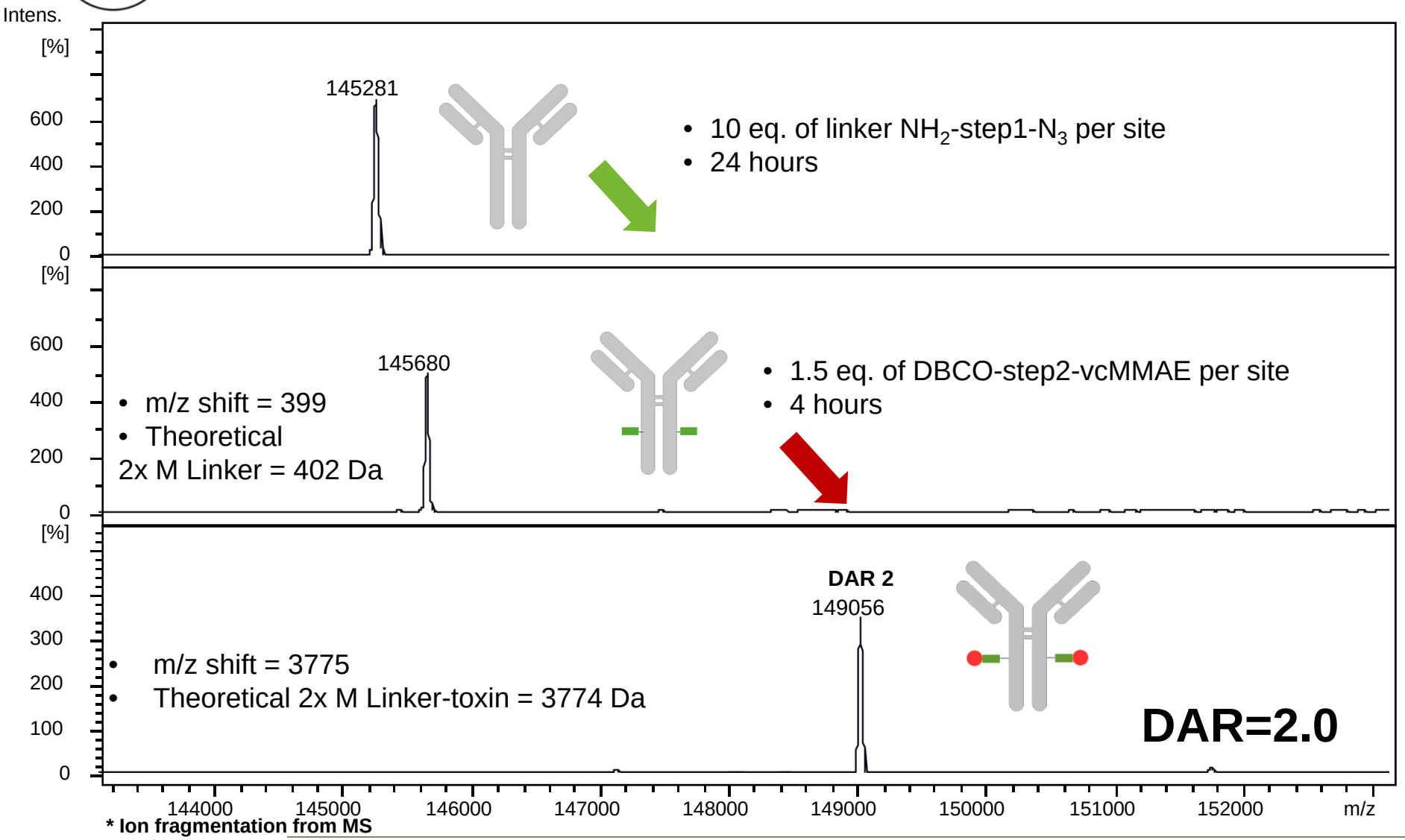


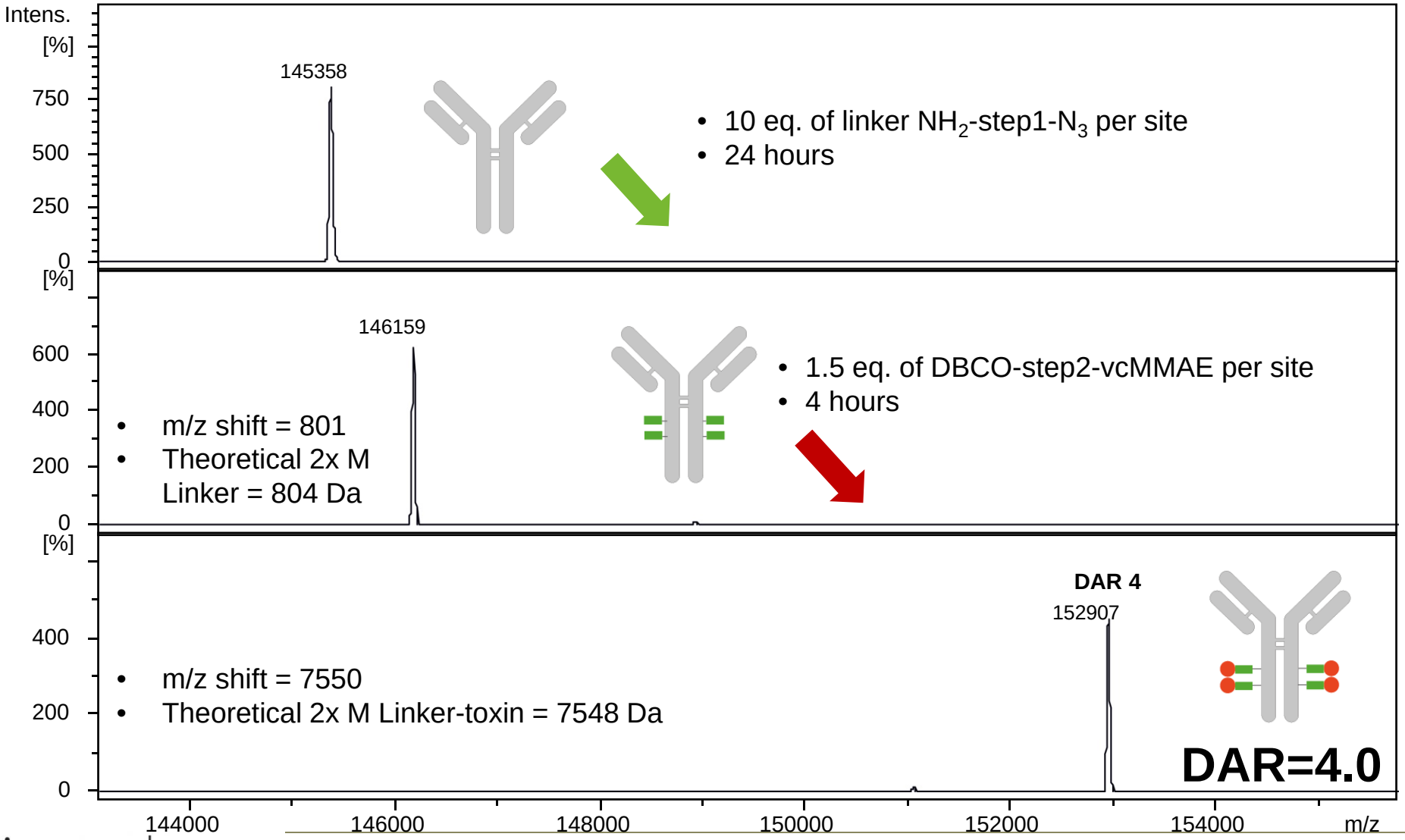
Coupling with Azide Linker and DBCO Toxin

N297S-step1a-click-step2-vcMMAE



Coupling with Azide Linker and DBCO Toxin

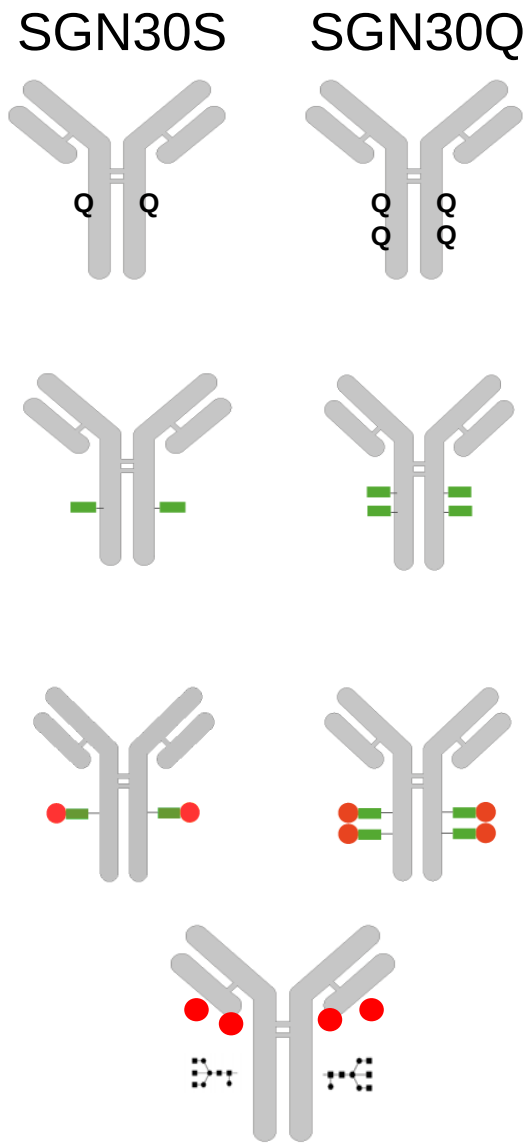
N297Q-step1a-click-step2-vcMMAE



Preclinical POC

Tools for POC

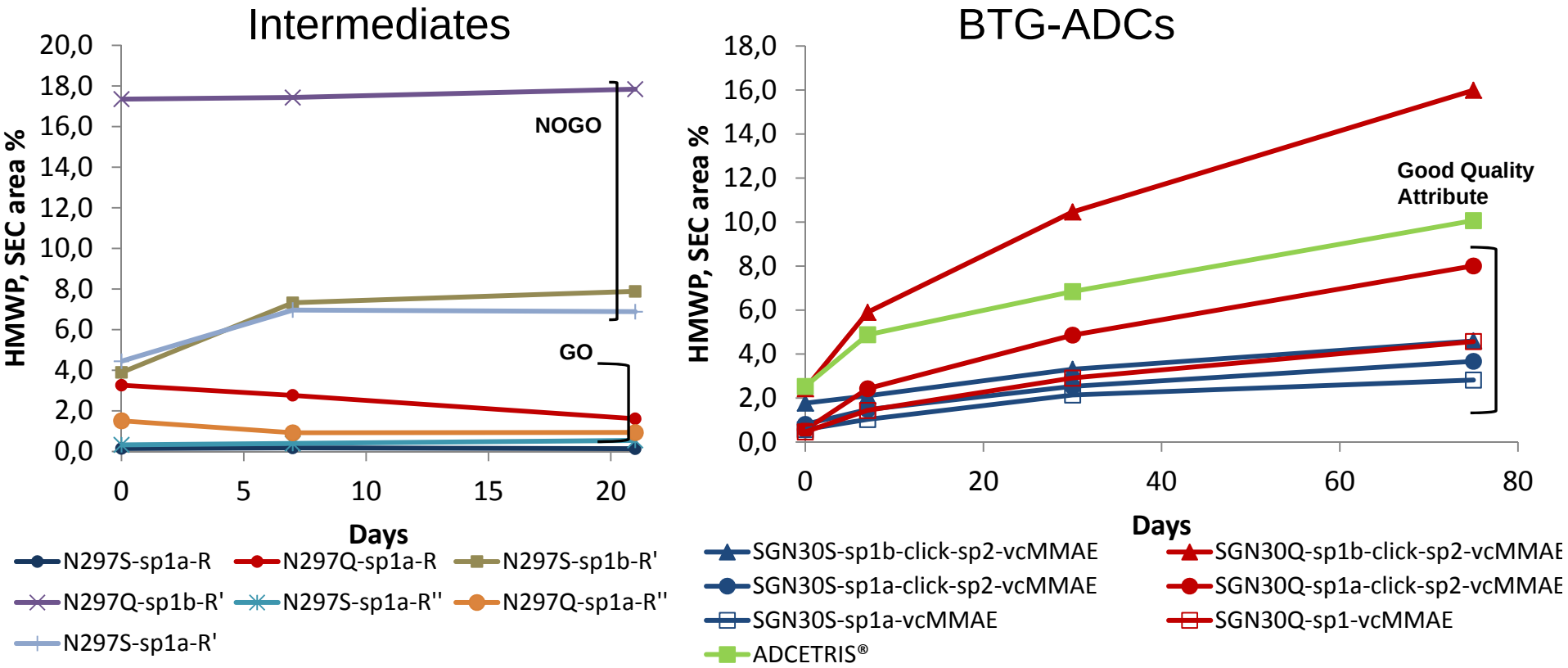
- **Naked antibody**
  - SGN30 (cAC10) targeting CD30
  - SGN30S or SGN30Q with 2 or 4 coupling sites
- **Intermediates: various linkers**
  - Structure of spacer (size, hydrophobicity): step1a, b or c
  - Reactive groups for click chemistry: -R, -R', -R''
- **BTG-ADCs**
  - -vc-PAB-MMAE for all conjugates
  - One-step: NH2-step1-vcMMAE
  - Two-step: DBCO-step2-vcMMAE
- **Comparator**
  - ADCETRIS®, Brentuximab vedotin



Stability in Buffer and in Plasma

BTG-ADCs Stability in Buffer

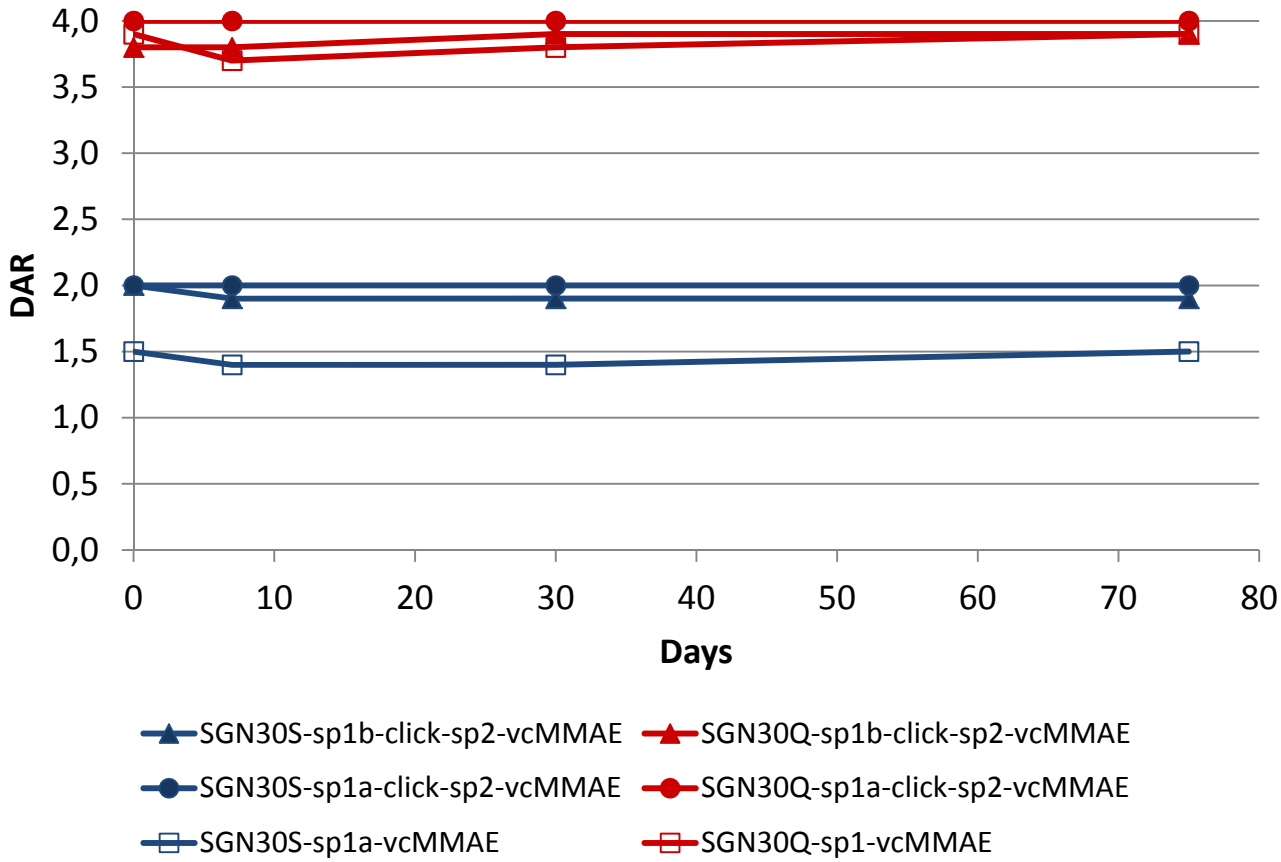
HMWP by SEC at +40°C



- Linker has significant impact on intermediates stability
- R' reactive group abandoned
- All BTG-ADCs (except step1b) showed less soluble aggregates than ADCETRIS®

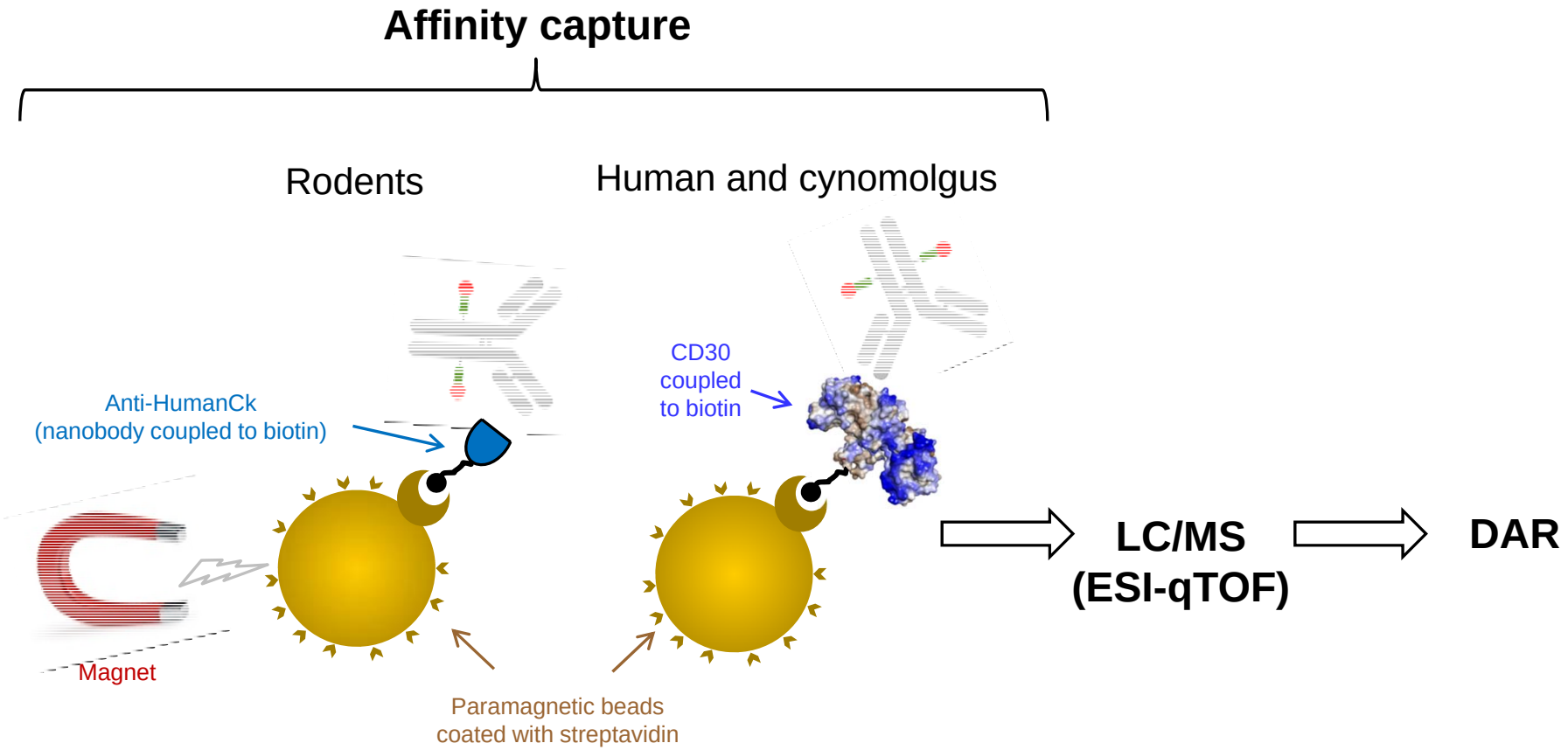
BTG-ADCs Stability in Buffer

DAR at +40°C



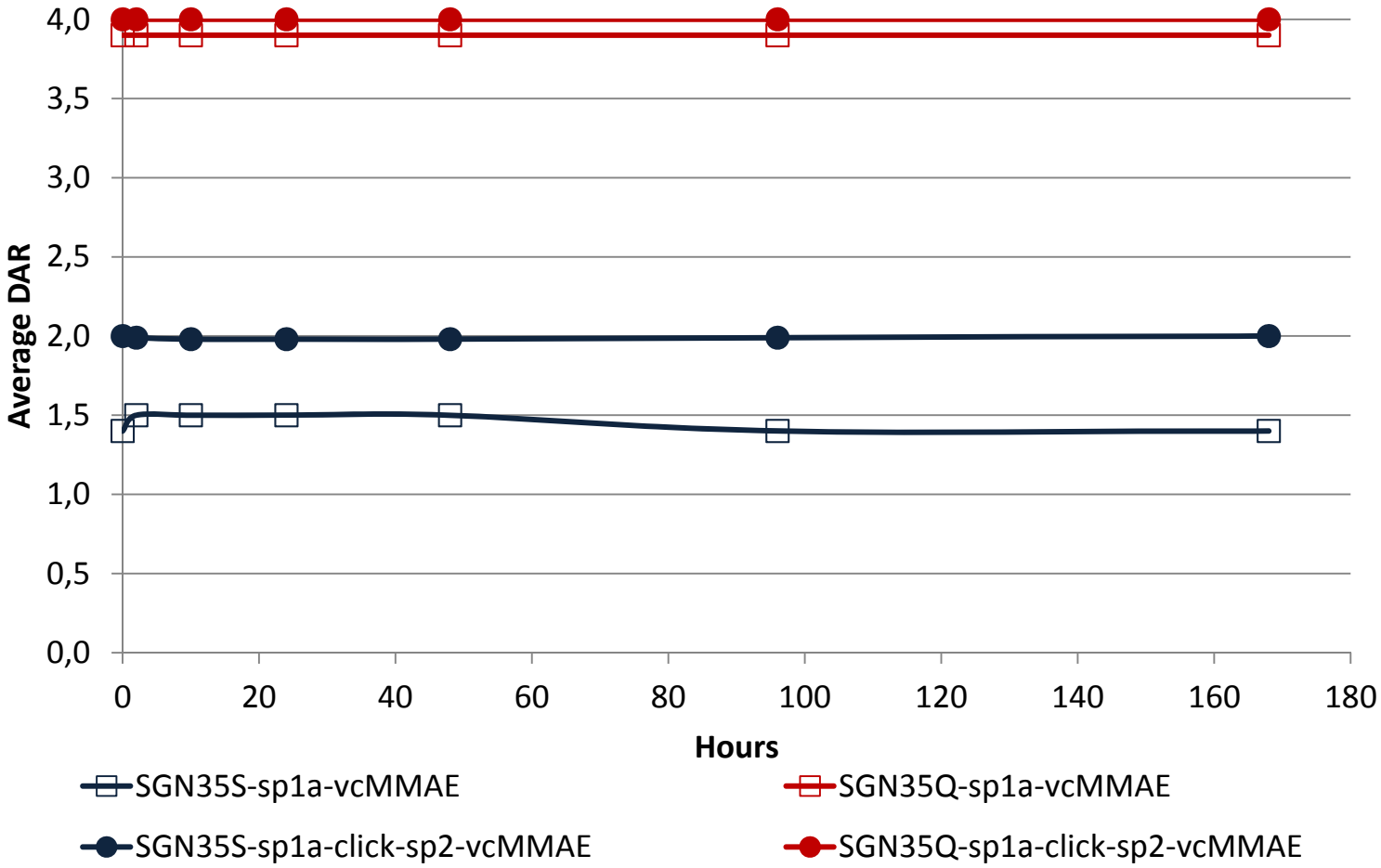
Ex Vivo Plasma Stability

- ADCs spiked in plasma
- Plasma types: rat (Wistar), cynomolgus and human



BTG-ADCs *ex vivo* Stability in Wistar Rat Plasma

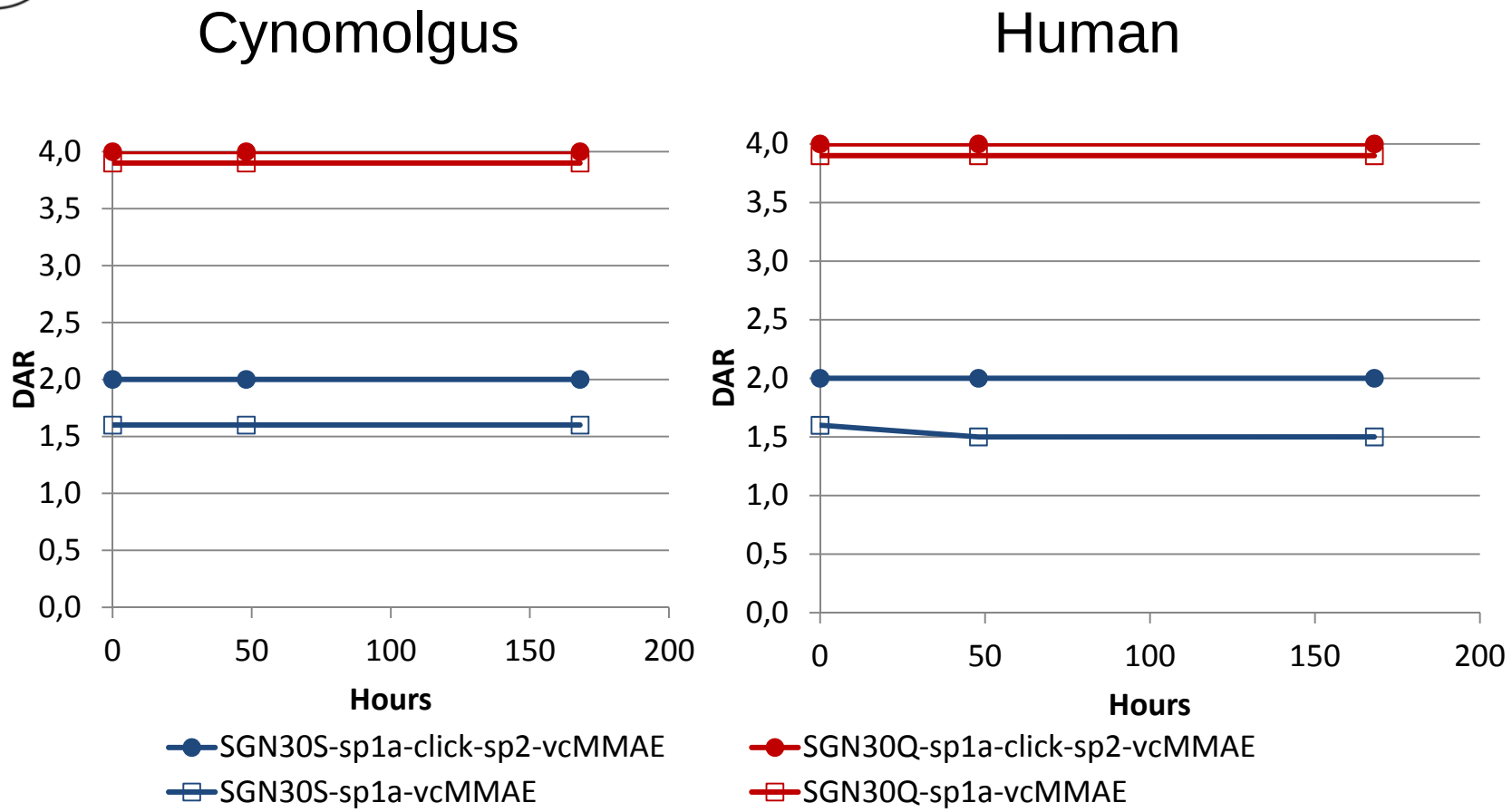
DAR over one week



No DAR variation observed over one week at 37°C

BTG-ADCs *ex vivo* Stability in NHP and Human Plasma

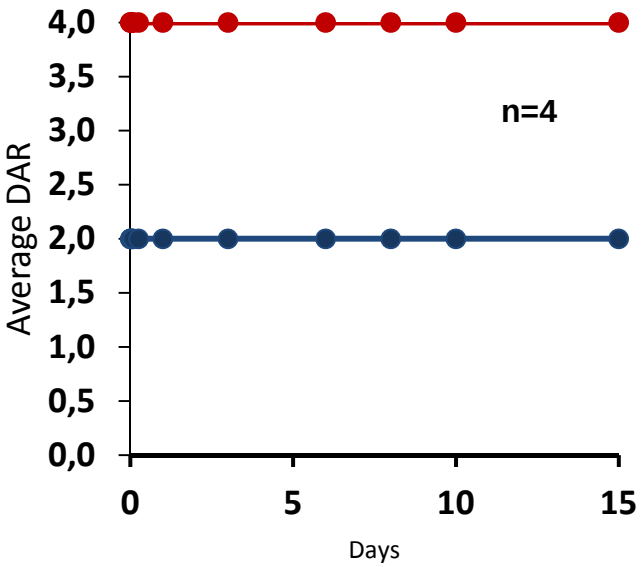
DAR over one week



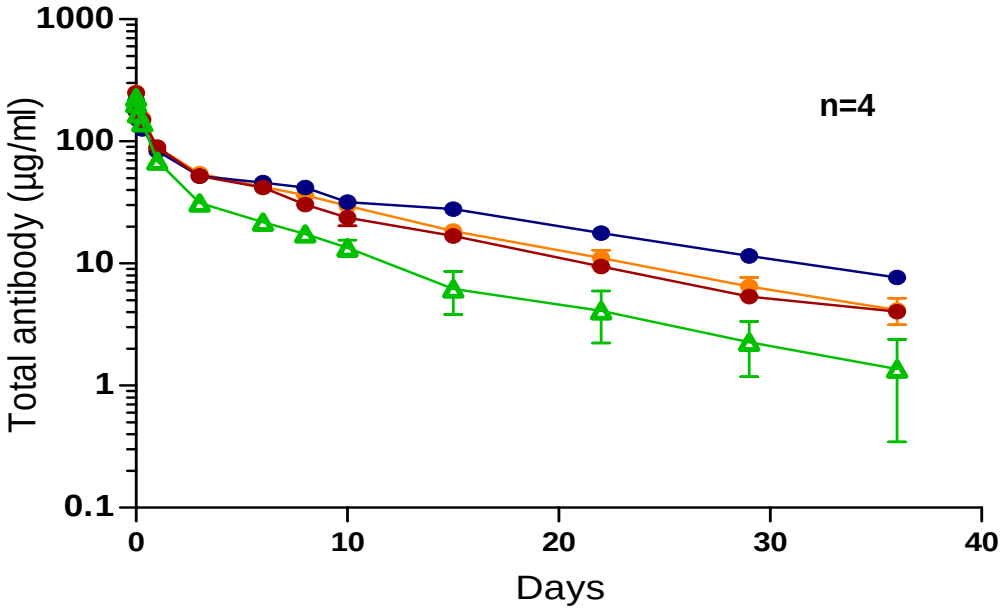
PK Study

BTG-ADCs PK in Wistar Rat

Conjugated antibody

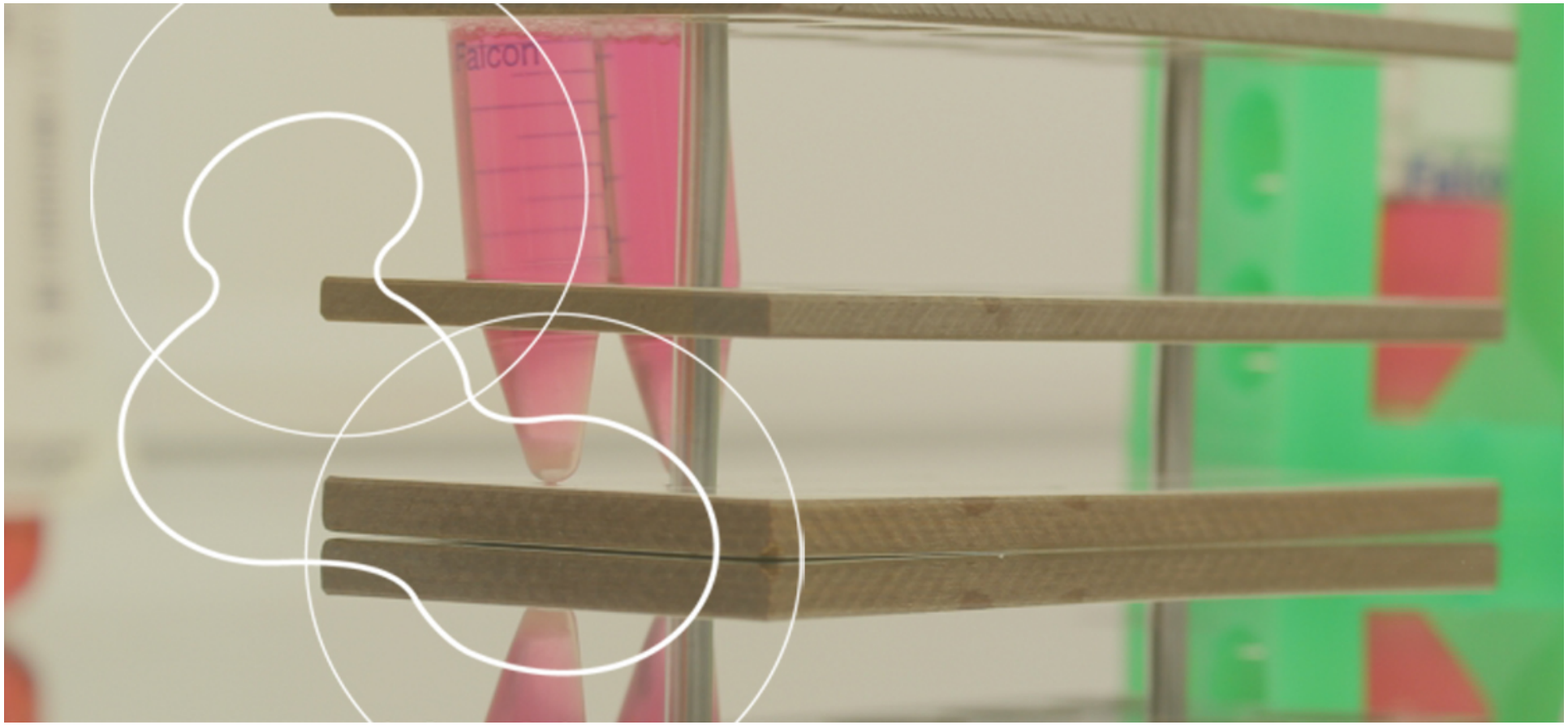


Total antibody



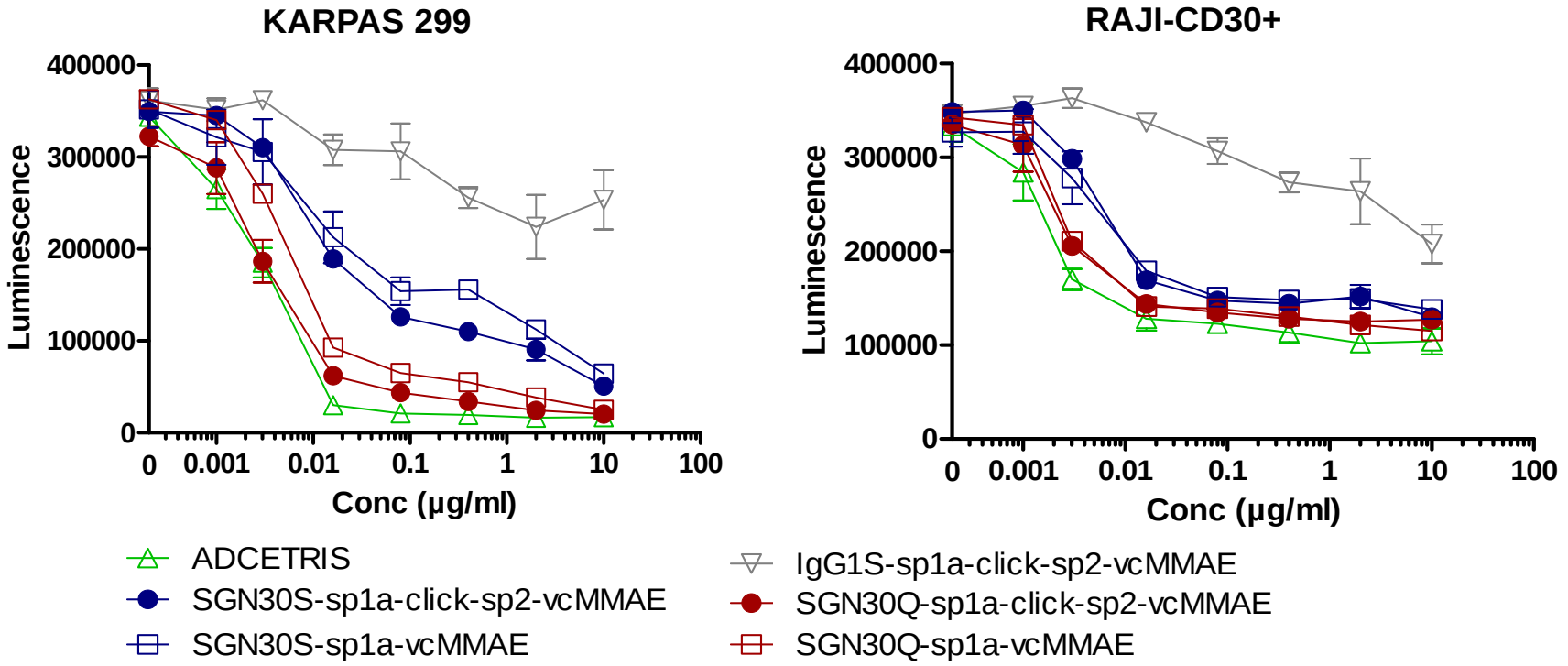
SGN30S-sp1a-click-sp2-vcMMAE
SGN30Q-sp1a-click-sp2-vcMMAE
SGN30S
ADCETRIS

	Unit	SGN30Q-sp1a-click-sp2-vcMMAE	SGN30S-sp1a-click-sp2-vcMMAE	SGN30S	ADCETRIS®
DAR	N/A	4.0	2.0	N/A	~4
Half-Life	days	8.5	12.0	9.6	8.5
Cl	ml/h	0.099	0.071	0.088	0.168



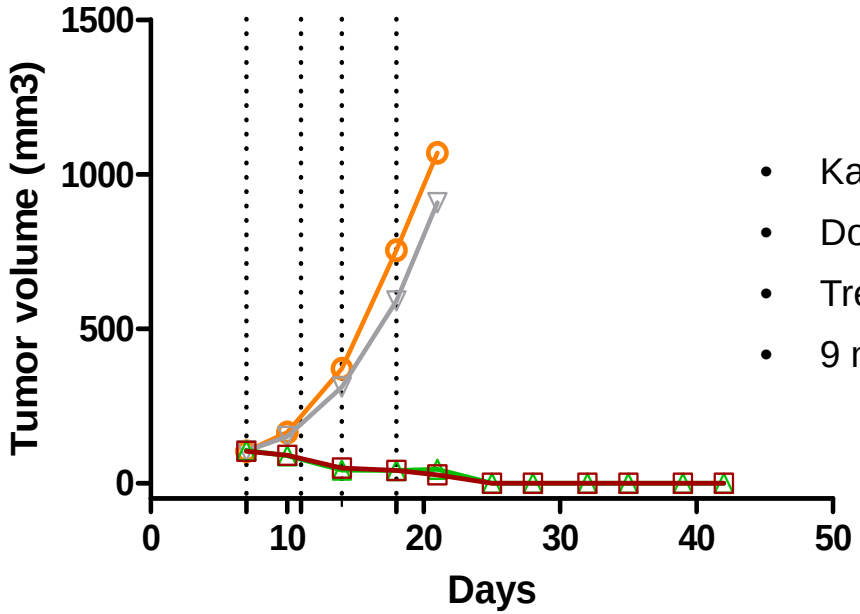
In Vitro and *In Vivo* Efficacy

BTG-ADCs *in vitro* Efficacy



	SGN30S-sp1a-click-sp2-vcMMAE	SGN30Q-sp1a-click-sp2-vcMMAE	SGN30S-sp1a-vcMMAE	SGN30Q-sp1a-vcMMAE	ADCETRIS®
DAR	**2.0**	**4.0**	**2.0**	**4.0**	**~4**
EC_{50} RAJI-CD30+ (ng/ml)	**5.1**	**2.0**	**5.4**	**2.3**	**1.4**
EC_{50} KARPAS 299 (ng/ml)	**11.2**	**3.1**	**14.6**	**4.7**	**2.4**

BTG-ADCs *in vivo* Efficacy



- Karpas 299 (S.C.) in SCID mice
- Dose 0.6mg/kg, I.V., q4d X4,
- Treatment started when tumor ~100mm^3
- 9 mice per group

SGN30Q
IgG1S-sp1a-click-sp2-vcMMAE
ADCETRIS
SGN30Q-sp1a-vcMMAE

Summary

- ADCs with DAR of exactly 2.0 or 4.0 from minimally modified antibody scaffold, i.e. with a single point mutation
- Rapid and versatile process appropriate for testing various linkers and toxins in HTS
- BTG two-step process yields to quantitative coupling using only 1 to 2 molar excess of toxin per site, making it a cost-efficient and scalable process
- BTG-ADCs are stable *ex vivo* in human and cynomolgus plasma and *in vivo* in rat, without DAR variation. In addition, BTG-ADCs clearance is lower compared to Adcetris®
- BTG-ADCs with DAR=4.0 show equivalent *in vitro* and *in vivo* efficacy compared to Adcetris®

Acknowledgment

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