# Site-specific antibody conjugation: TG-ADC Platform

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## Abstract

Bacterial Transglutaminase (TG) allows conjugation of chemical moieties on the endogeneous Q295 from an aglycosylated antibody. We previously demonstrated that TG two-step process leads to ADCs with DAR of 2 or 4 for antibodies with N297S or N297Q single point mutation respectively. Moreover, this two-step process requires a minimal amount of toxin, which can be a crucial argument with regard to large-scale production of ADCs.

In the present study, we describe the *in vitro* and *in vivo* characterization of ADCs obtained using TG site-specific conjugation technology. TG-ADCs were derived from the anti-CD30 antibody cAC10, which is the parental antibody of ADCETRIS®, and were compared to the latter.

Moreover, we demonstrate the versatility and the potential of TG site-specific conjugation for high-throughput screening of ADCs. Indeed, this chemo-enzymatic approach not only allows conversion of virtually any IgG into a functional ADC, but also enables homogeneous coupling of highly hydrophobic toxins such as PBDs.

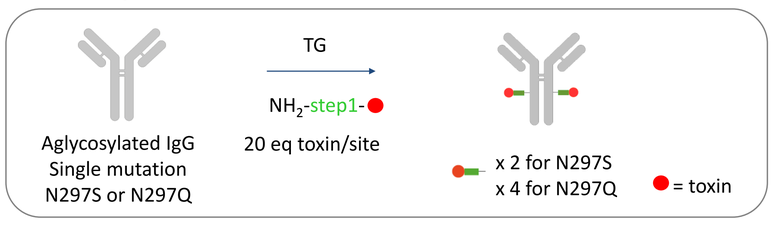
## TG-ADC Platform

### Site-specific conjugation with TG

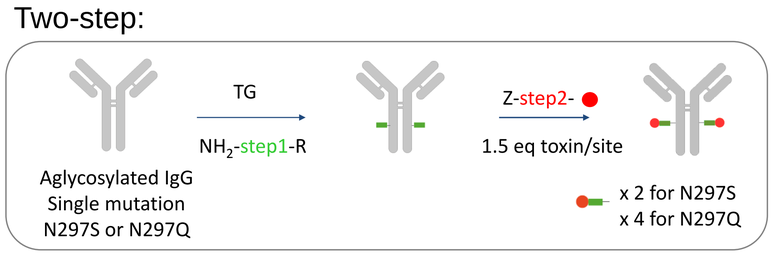
- TG catalyses reactions between glutamine and lysine
- TG recognizes exclusively endogenous Q295 located in Fc region of aglycosylated IgG
- N297Q mutation provides 2 additional sites for conjugation

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

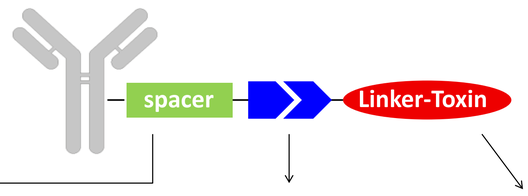
One-step:




Two-step:




### Versatility of the technology




**Spacer:**

- Modulation of length, hydrophilicity, etc…
- Multibranched spacers for ADCs with high drug loading

**Bioorthogonal Chemical Conjugation between 'spacer' and 'linker-toxin':**

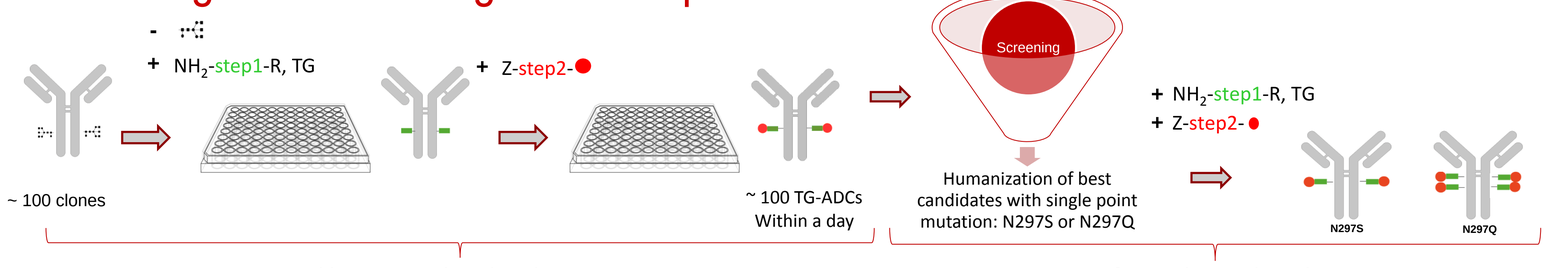
- Click-chemistry
- Staudinger ligation
- Diels-Alder
- Thiol-Maleimide crosslinking, …

**Linker-Toxin:**

- Cleavable/Non-cleavable linker
- Auristatin, PBDs, …

*Dennler et al., Bioconj. Chem., 2014*

### Screening of ADCs using TG-ADC platform



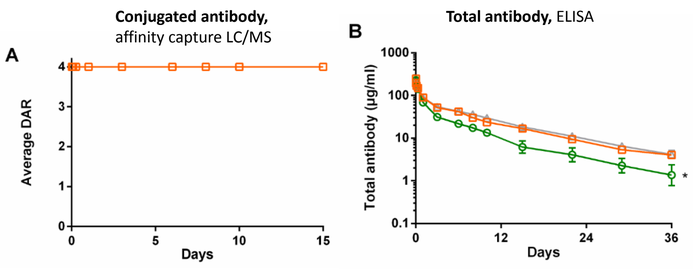

- Yield: 70-80% with mAb scale <5mg
- Monomer SEC profile: 95.0 to 99.0%
- DAR 1.8 to 1.9 with single branched linker
- Various linkers and toxin tested side-by-side

- Accurate DAR of 2.0 and 4.0 with single point mutation mAb N297S or N297Q
- Yield: 75-90% with mAb scale 100mg
- Monomer SEC profile: 95.0 to 99.0%

## Proof of Concept: Comparison of ADCETRIS vs TG-modified anti-CD30 mAb

### Pharmacokinetic in rat / Safety

#### Pharmacokinetic




| | cAC10Q-(4) | ADCETRIS | cAC10S CONTROL |
|---|---|---|---|
| DAR | 4 | ~4 | 0 |
| T1/2 (days) | 8.5 | 8.5 | 9.6 |
| Clearance (ml/hours) | 0.134 | 0.227 | 0.119 |

#### Safety

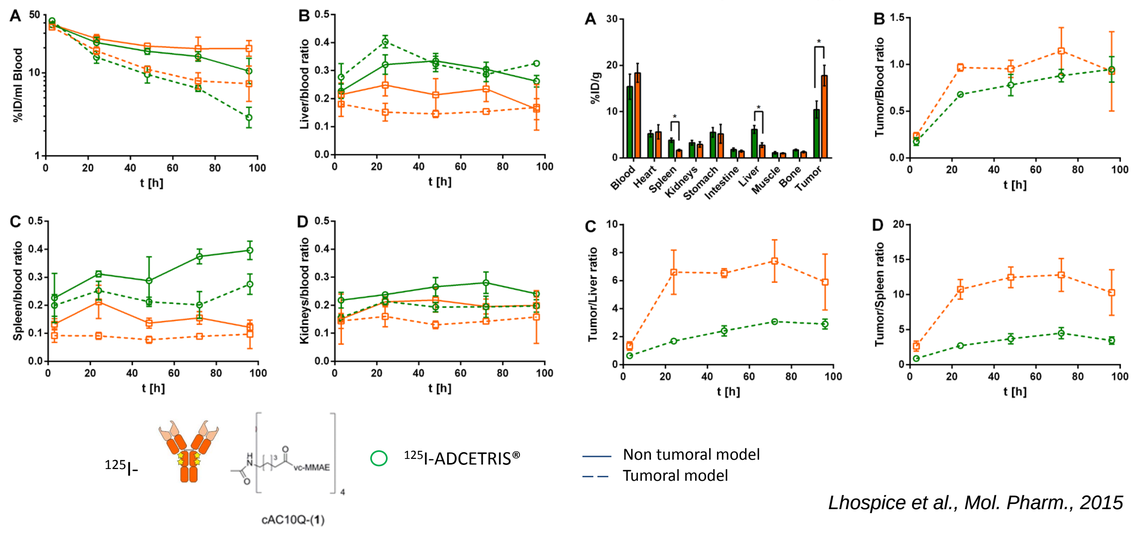
- Protocol: IV injection (bolus) of ADC at D0 in Wistar rat
- Study: C-RIS Pharma (CRO, France)
- Read-out: Daily observation of animals for morbidity, mortality and evident signs of toxicity + twice weekly monitoring for clinical signs and body weights.
- Criteria: The MTD was defined as the highest dose that did not induce >20% weight loss and/or signs of distress.
- Results:

| Product | cAC10Q-(4) | ADCETRIS® |
|---|---|---|
| DAR | 3.8 | 4.1 |
| MTD | >60mg/kg | 18mg/kg[1] |

**1. Consistent with data published by McDonagh *et al* 2006, MTD<20mg/kg in rat**

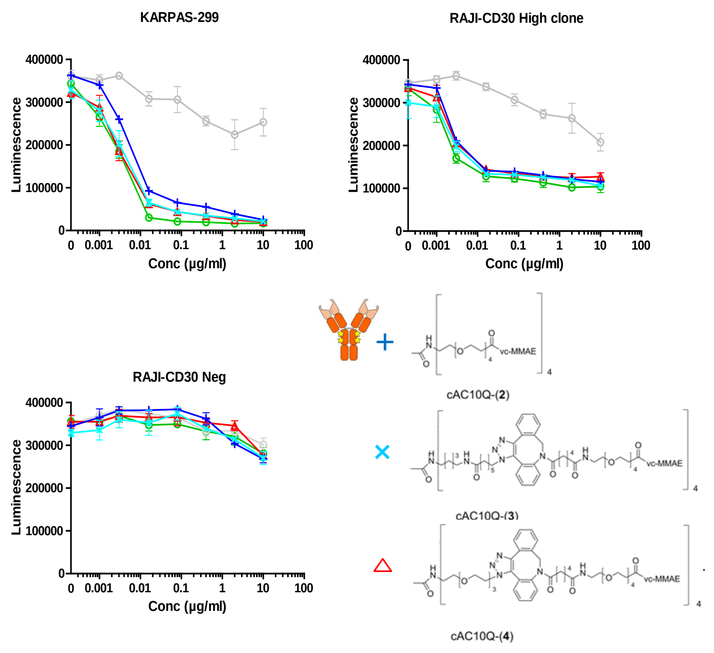
### Biodistribution

- Comparison of the *in vivo* biodistribution of TG-ADC (DAR 4) with ADCETRIS® labeled with $^{125}$I in non-tumoral and tumoral model (scid mice bearing subcutaneous Karpas 299 tumors)
- RESULTS:
- Blood clearance (A) and Time dependent organ-to-blood ratios (B-D) of $^{125}$I-ADCETRIS and $^{125}$I-cAC10Q-AC-vcMMAE (cAC10Q-(1))
- Biodistribution at 24h post injection of radioactivity (A) and time dependent tumor-to-tissue ratios (B-D) of $^{125}$I-ADCETRIS and $^{125}$I-cAC10Q-AC-vcMMAE (cAC10Q-(1))




*Lhospice et al., Mol. Pharm., 2015*

### *In vitro* Efficacy

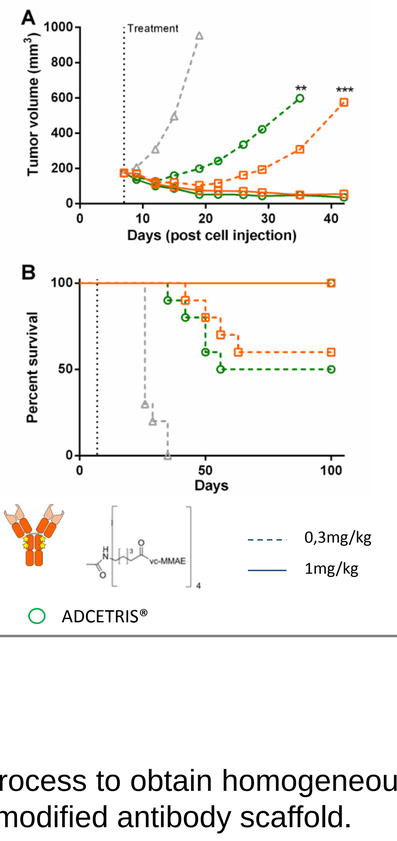



| | cAC10Q-(2) | cAC10Q-(3) | cAC10Q-(4) | ADCETRIS® |
|---|---|---|---|---|
| DAR | 3.9 | 3.9 | 4.0 | ~4 |
| $EC_{50}$ RAJI-CD30+ (ng/ml) | 2.6±0.4 | 2.7±0.4 | 2.0±0.4 | 1.8±0.4 |
| $EC_{50}$ KARPAS 299 (ng/ml) | 4.9±1.0 | 4.7±0.8 | 3.2±0.6 | 3.6±0.6 |

### *In vivo* Efficacy

**KARPAS 299 - Single Dose Injection**

- Karpas 299 (S.C.) in SCID mice
- Single dose IV (0.3 or 1 mg/mL)
- Treatment started when tumor ~200mm$^3$
- 10 mice per group




## Conclusion and Perspectives

- TG site-specific conjugation technology is a scalable process to obtain homogeneous aglycosylated ADCs with DAR 2 or 4 from a minimally modified antibody scaffold.
- TG 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.
- TG-ADCs have an improved pharmacokinetic profile with lower unspecific uptake in spleen and liver, hence a better therapeutic index compared to first generation ADCs.
- Efficacy was demonstrated *in-vitro* and *in-vivo* in CD30-expressing tumoral models.
- TG-ADC rapid and versatile process allows generation of ADCs with various linker-toxin combinations in HTS (~100 TG-ADCs / day) using untouched antibodies and thus accelerating lead identification.

With this work, we point out that TG-ADC platform is a powerful tool for both ADC production and development of next generation ADCs.