Versatility of Site-Specific Conjugation based on Bacterial Transglutaminase

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Abstract

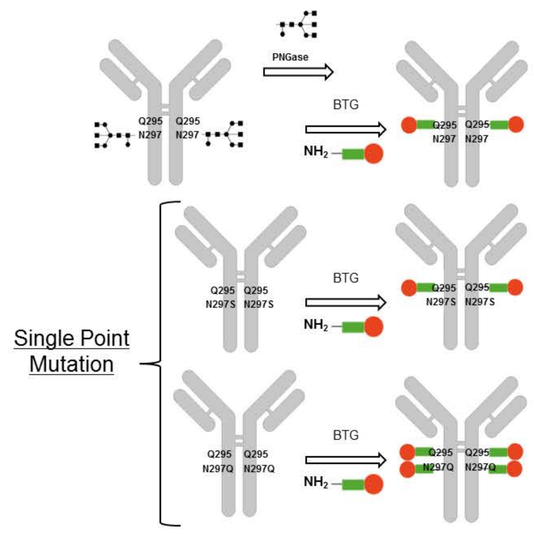
Bacterial Transglutaminase (BTG) allows conjugation of chemical moieties on the endogeneous Q295 from an aglycosylated antibody. We previously demonstrated that BTG two-step process leads to ADCs with DAR of 2 or 4 for antibodies with N297S or N297Q single point mutation respectively. Moreover, this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

Here, we demonstrate the versatility and the potential of BTG site-specific conjugation for high-throughput screening of ADCs. Indeed, this chemo-enzymatic approach not only allows conversion of virtually any IgG1 into a functional ADC, but also enables comparison of various antibodies, linker systems or toxin. As an example, anti-CD30 antibody cAC10 was efficiently conjugated with various drugs, such as MMAE, MMAF or PBD, and evaluated *in vitro*.

BTG coupling: Site-specific and Stoichiometric Enzymatic Conjugation

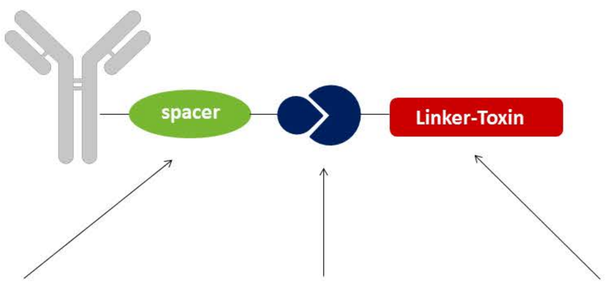
- BTG is a commercially available enzyme
- BTG catalyses reactions between glutamine and lysine
- BTG recognizes exclusively endogenous Q295 located in Fc region of aglycosylated IgG
- N297Q mutation provides 2 additional sites for conjugation

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



Versatility of BTG site-specific conjugation

BTG-ADC scaffold



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
- …

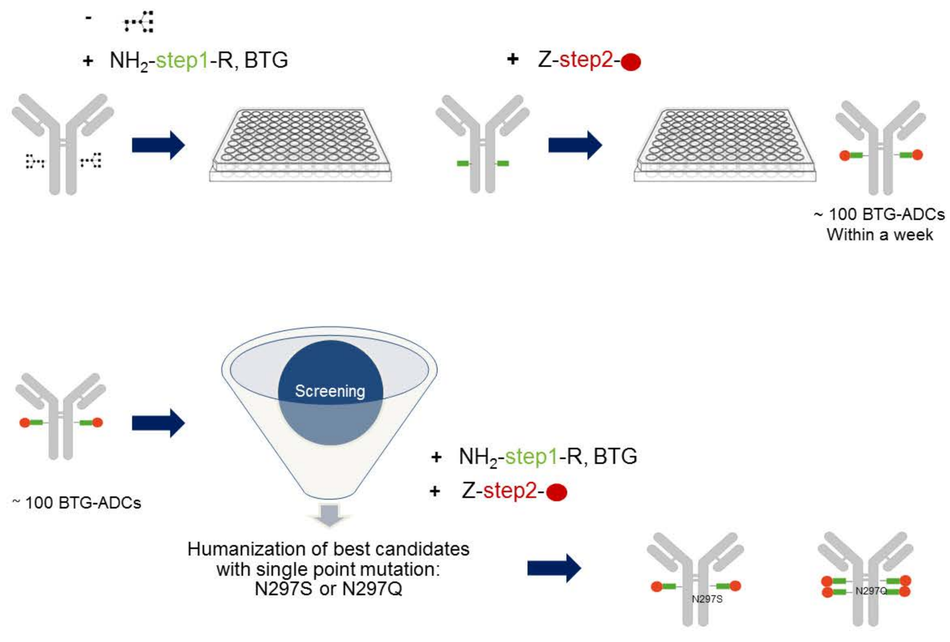
Linker-Toxin:

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

Dennler et al., Bioconj. Chem., 2014

High-throughput screening of ADCs

Site-specific conjugation with BTG: a cost-efficient scalable process



- Yield: 85-90% with mAb scale <5mg
- DAR 1.8 to 1.9 with single branched spacer
- Higher DAR with multibranched spacers homo and heterofunctional
- Possibilty to test various spacers and toxin tested side-by-side

- Accurate DAR of 2.0 and 4.0 with single point mutation mAb N297S or N297Q
- Same yield and purity profile
- Scalable process

Examples: Synthesis and *in vitro* efficacy of new BTG-ADCs

Synthesis

LC/MS of reduced mAb: deconvolution MaxEnt

cAC10Q:

DAR = 4

cAC10Q coupled to **MMAE** + cleavable linker val-cit-PABC:

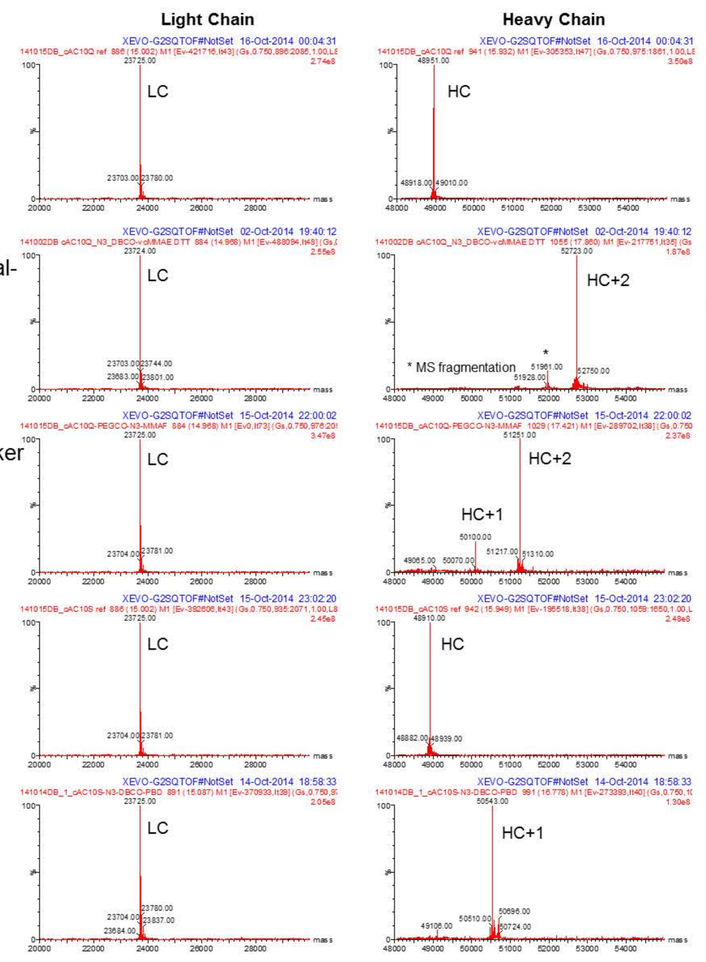
DAR = 3.7

cAC10Q coupled to **MMAF** + non cleavable linker

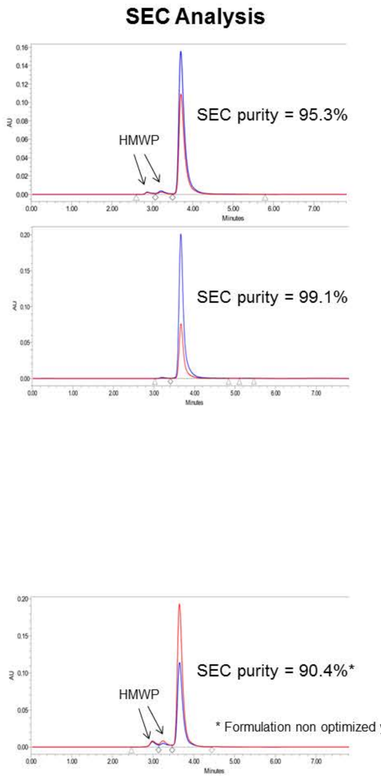
cAC10S:

DAR = 1.9

cAC10S coupled to **PBD** + cleavable linker val-ala-PAB:

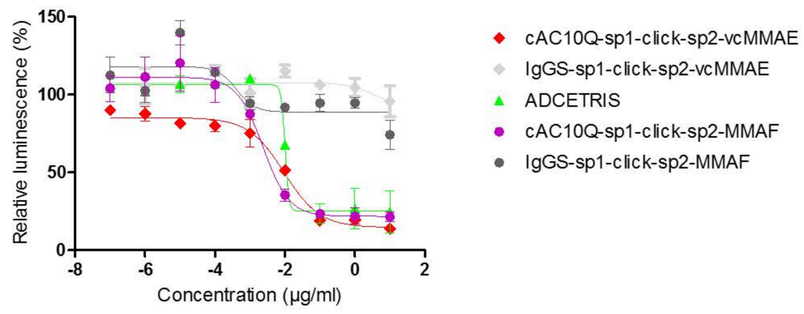


SEC Analysis



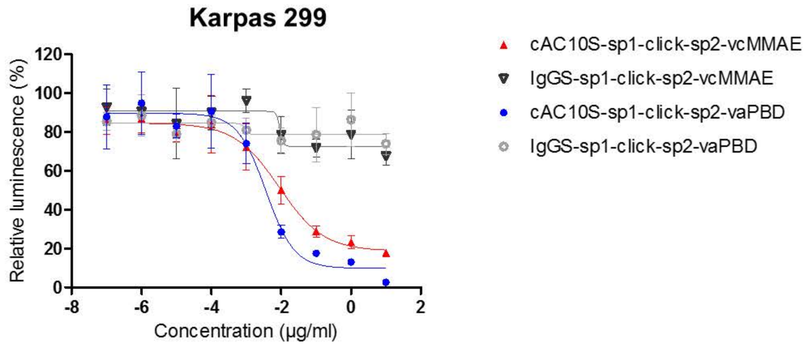
In vitro efficacy

Karpas 299



- Customized MMAF for BTG 2 step ligation is still highly potent upon internalization and release
- EC50 is equivalent to published data with conventional conjugation (Doronina 2006)

Karpas 299



- Site-specific conjugation with BTG enable coupling of PBD toxin

Conclusion and Perspectives

- BTG coupling to a minimally modified antibody scaffold, i.e. with a single point mutation, leads to ADCs with DAR of exactly 2.0 or 4.0
- BTG coupling yields to ADCs within a few hours and is a 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
- With this work, we point out that BTG site-specific conjugation technology is a powerful tool for both ADC production and development of next generation ADCs