New Site-Specific Conjugation using B. Transglutaminase, through Single Point Mutation

Authors

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Abstract

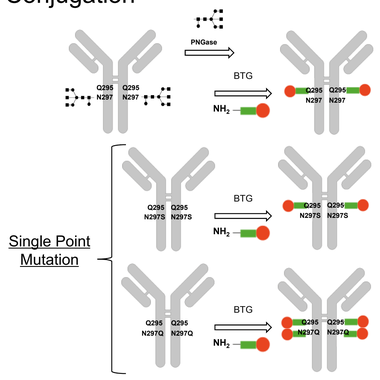
Bacterial Transglutaminase (BTG) allows coupling on endogenous Q295 from an aglycosylated antibody. Here, the work relates to ADCs synthesis through BTG approach on mAbs with single point mutations and the study of stability, pharmacokinetics, as well as *in vitro* and *in vivo* efficacy of resulting BTG-ADCs.

ADCETRIS®, brentuximab vedotin, has been used as comparator, hence for consistency BTG-ADCs have been conjugated with the different linkage strategies (one step and two step) containing distally the same protease sensitive moiety compared to ADCETRIS®, i.e. valine-citrulline-PAB-MMAE.

BTG two-step process leads to ADCs with DAR of exactly 2.0 or 4.0 for antibodies with N297S or N297Q single point mutation respectively. BTG-ADCs were stable in buffer and *ex vivo* in human and cynomolgus plasma. BTG-ADCs showed no DAR variation *in vivo* in rats over 15 days and a lower clearance of the total antibody compared to ADCETRIS®.

Finally, BTG-ADCs with a DAR of 4.0 showed similar *in vitro* potency and better in vivo efficacy compared to ADCETRIS® in a xenogeneic tumoral model.

BTG coupling: Site-specific and Stoichiometric Enzymatic Conjugation

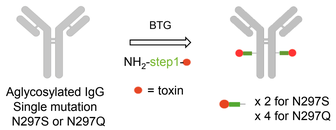


- BTG catalyses reactions between glutamine and lysine
- BTG recognizes exclusively endogenous Q295 located in Fc region of aglycosylated IgG
- N297Q mutation provides 2 additional sites for conjugation

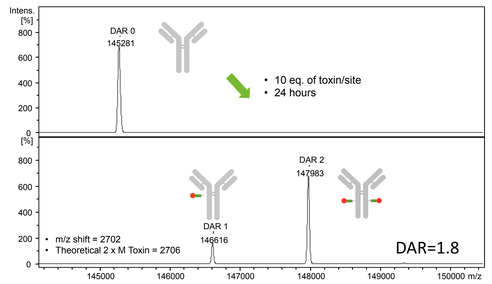
Jeger et al., Angew. Chem. Int. Ed., 2010

ADCs Synthesis

One-Step

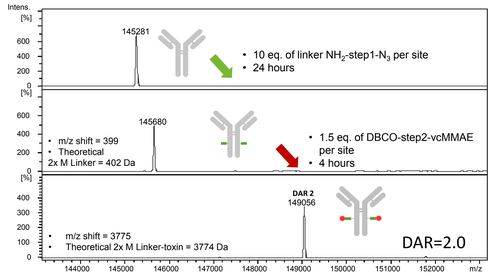


N297S coupled with NH2-step1a-vcMMAE
LC/MS(ESI-qTOF)

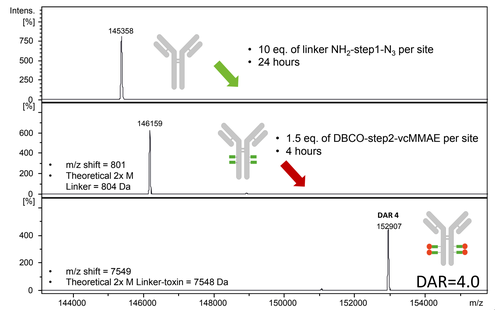


Two-Step

N297S coupled with azide linker and DBCO toxin,
N297S-step1a-click-step2-vcMMAE

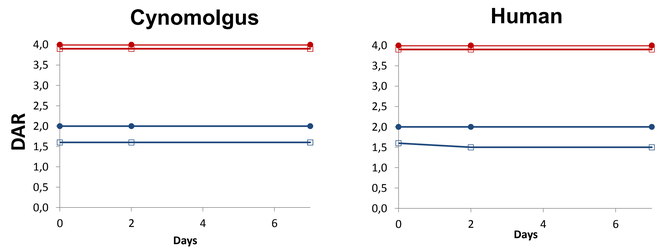


N297Q coupled with azide linker and DBCO toxin,
N297Q-step1a-click-step2-vcMMAE



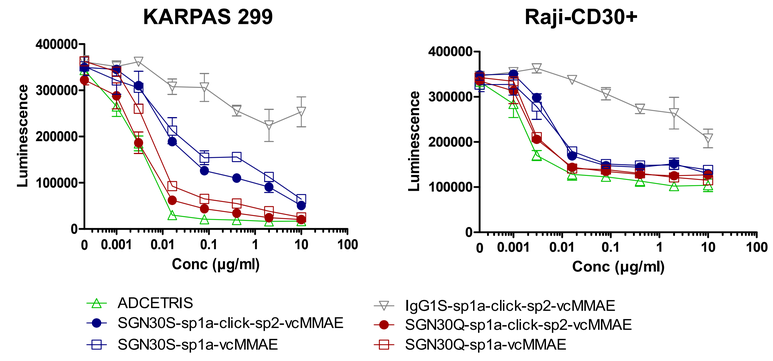
Stability in Plasma, Pharmacokinetics and Efficacy

Stability in Plasma Over One Week



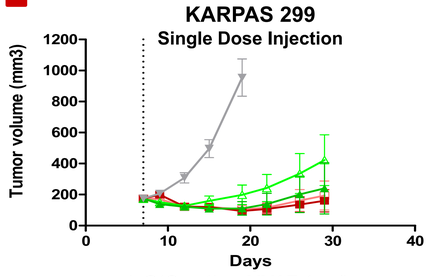
- DAR monitoring by affinity capture LC/MS

In Vitro Efficacy



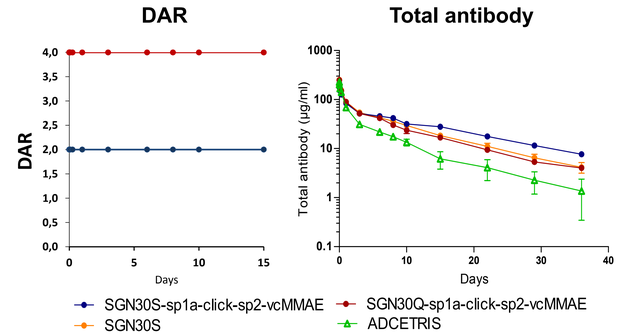
	SGN30S-sp1a-click-sp2-vcMMAE	SGN30Q-sp1a-click-sp2-vcMMAE	SGN30S-sp1a-vcMMAE	SGN30Q-sp1a-vcMMAE	ADCETRIS®
DAR	2.0	4.0	2.0	4.0	~4
EC_{50} RAJI-CD30+ (ng/ml)	5.1	2.0	5.4	2.3	1.4
EC_{50} KARPAS 299 (ng/ml)	11.2	3.1	14.6	4.7	2.4

In Vivo Efficacy



- Karpas 299 (S.C.) in SCID mice
- Single dose IV
- Treatment started when tumor ~170mm^3
- 10 mice per group

Pharmacokinetics in Rat



	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	8.48	11.99	9.58	8.48
Cl	ml/h	0.099	0.071	0.088	0.168

- 4 male Wistar rats per group
- 10mg/kg IV
- Total antibody monitoring by ELISA
- DAR monitoring by affinity capture LC/MS

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
- BTG-ADCs are stable *ex vivo* in human and cynomolgus plasma, without DAR variation over one week
- BTG-ADCs are stable *in vivo* in rat, without DAR variation over 2 weeks. In addition, BTG-ADCs clearance is lower compared to ADCETRIS®
- BTG-ADCs with DAR=4.0 show equivalent *in vitro potency* and better *in vivo* efficacy compared to ADCETRIS®

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