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NextGen Conditionally Active Biologic (CAB) ADCs: Improved Stability and Safety

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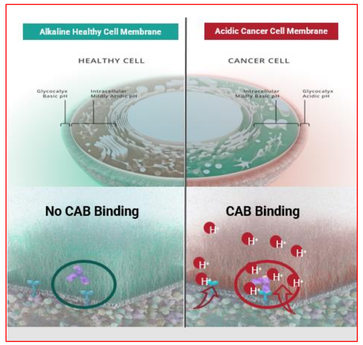
ABSTRACT

Conventional ADCs often show off-tumor, on- and off-target toxicities, thereby limiting the therapeutic window of the drug. BioAtla's Conditional Active Biologics (CAB) technology allows the generation of antibodies that bind to the target antigen only in the acidic tumor microenvironment. To complement CAB technology a newly developed, highly serum stable linker further reduces off-target toxicity while maintaining potency. Toxicology data show highly improved tolerability of a NextGen CAB-ADC targeting Nectin-4.

RATIONALE

Conditionally Active Biologic (CAB) technology[1,2] is a proprietary platform that generates antibodies which have little to no binding to the target antigen in healthy tissue (normal alkaline microenvironment). However, in acidic conditions found on the surface of cancer cells[1-6] the binding of the antibodies to their target molecule is strong. The CAB-ADC's elimination of on-target binding-related toxicity and its reduction of off-target toxicity through the elimination Target Mediated Drug Disposition, enables superior therapeutic index relative to other formats, including non-reversible prodrugs. In this study we explore the potential of combining novel stable linkers for further reducing off-target toxicity with the power of CAB selectivity for generating a **best-in-class ADC system: CAB-ADCmax™**.



CAB ADCs

- Eliminated off-tumor, on-target binding related toxicity
- Reduced off-tumor, off-target toxicity (via elimination of TMDD)
- Increased tolerability due to conditionally active binding
- Dosing limited by off-tumor, off-target toxicity from unstable peptide linker

NextGen CAB ADCs

- Maintains CAB advantages
- Improves serum stability
  - Attachment group inert to Reverse Michael Addition
  - Increased hydrophilicity
- Eliminates the elastase induced neutropenia
  - Cleavage by glycosidases in lysosome instead of protease cleavage
- Adaptable to most payloads

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RESULTS

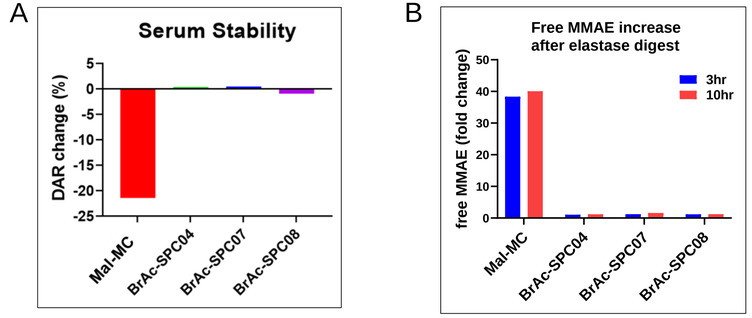


Figure 1. Improved linker stability analyzed by serum stability assay (A) and MMAE release assay (B)

A. Comparison of serum stability of a non-CAB-ADC antibody containing either a peptide linker (Mal-MC) or one of three novel glycosidase linkers (BrAC-SPC04, -07 and -08) with varying spacer molecules. Samples were incubated in IgG-depleted human serum for 14 days, and then the drug-antibody-ratio (DAR) was determined by RP HPLC. There was a 20% reduction in DAR value with maleimide conjugation and no obvious loss of DAR was found in the glyco linker conjugates. Note: Mal-MC is DAR=4 and BrAc-SPC04, -07 and -08 are DAR=4.

B. Samples were incubated with elastase for 3hrs or 10hrs, and the amount of free Monomethyl Auristatin E (MMAE) was analyzed by LC/MS. At time zero, levels of free MMAE were at or below the detection limit (0.02 ng/mL). After enzyme incubation, Mal-MC-ADC showed a 40-50-fold increase of free MMAE, while there was no significant release of free MMAE in the glyco-ADCs (SPC04, SPC07 and SPC08).

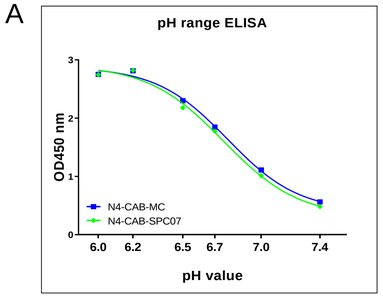
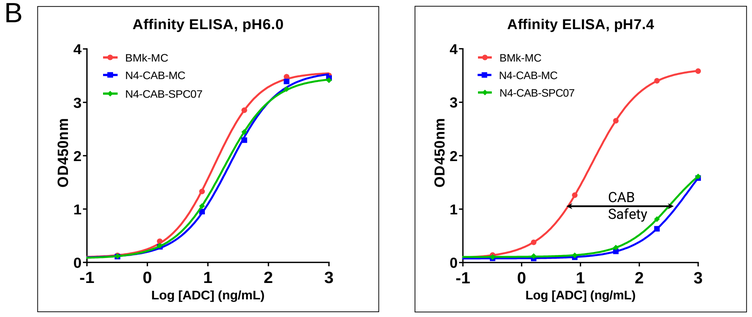


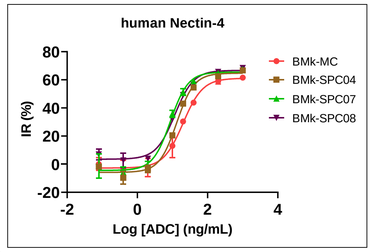
Figure 2. CAB ADCs bind to human Nectin-4 with high affinity in acidic pH

A. Binding of CAB-ADC to human Nectin-4 in different pH conditions was measured by ELISA. The CAB ADC demonstrated differential binding to human Nectin-4 in pH conditions ranging from 6.0-7.4.

B. Binding of CAB to non-CAB ADCs against human Nectin-4 at pH6.0 and pH7.4 was measured by affinity ELISA. CAB Nectin-4 showed higher affinity in acidic cancer cell pH (*e.g.* pH6.0-6.5), but lower binding in alkaline physiological pH (*i.e.*pH7.4).



RESULTS



conjugation	MC	SPC04	SPC07	SPC08
IC50 (ng/mL)	20.08	13.22	8.77	11.67

Figure 3. *In vitro* cell killing potency of peptide and glyco conjugates targeting human Nectin-4 expressing cells. Benchmark (BMk) anti-human Nectin-4 antibody was conjugated with peptide or glyco-linkers. ADCs with glyco-linker induced similar desired cell cytotoxicity compared to peptide conjugate.

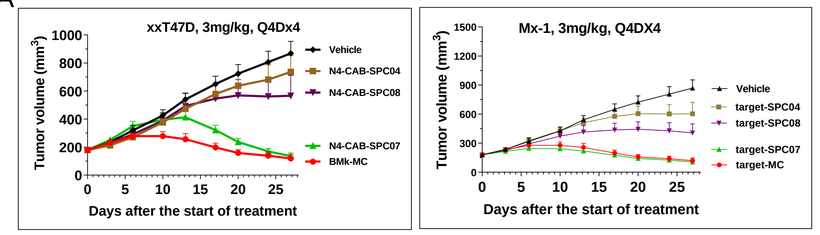


Figure 4. Glyco CAB-ADC conjugates control tumor growth *in vivo*. The efficacy of CAB and Benchmark (BMk) ADCs was evaluated *in vivo* using T47D and MX-1 CDX models of human breast cancer. (A) T47D CDX model and (B) MX-1 CDX model (undisclosed target).

- Differential anti-tumor efficacy of the glyco ADC conjugates against T47D and MX-1 tumors was observed based on the different spacer designs (SPC04, SPC08 and SPC07). The NextGen SPC07 glyco ADC conjugate demonstrated the highest potency *in vivo*.
- CAB Nectin-4 ADC with SPC07 linker induced similar tumor regression as peptide BMk conjugate.

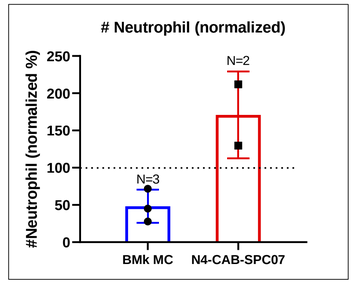


Figure 5. NextGen CAB ADC-SPC07 does not induce neutropenia.
Toxicity of ADCs was evaluated in a non-GLP toxicity study in rats enabled by the antibody species cross-reactivity. Animals were administered with 10mg/kg of ADC conjugates, and the blood neutrophil counts were analyzed on day 8 post treatment. Neutropenia was observed in the groups treated with peptide BMk conjugates (DAR=4), while in the group treated with the NextGen CAB SPC07 conjugate (DAR=6) neutrophil counts remained unchanged despite the higher DAR. NextGen CAB SPC07 is consistent with elimination of both serum instability and elastase-induced neutropenia in the extracellular environment, as its cleavage is effectively restricted to the glycosidases in the lysosome.

CONCLUSIONS

NextGen CAB ADCs

- Eliminates on-target, off-tumor toxicity (CAB)
- Fusion of CAB and NextGen linker eliminates extracellular derived off-target, off-tumor toxicity (*e.g.* off-target toxicities associated with Nectin-4[7]) via a:
  - Highly improved serum stability (NextGen glyco-linker)
  - Increased hydrophilicity for higher DAR (*e.g.* 6) improving potency
- NextGen linker adaptable to most payloads, including those enabling by-stander effects[8], such as MMAE.
- The combination of CABs plus novel linker system provides the opportunity to maximize Therapeutic Index.

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