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

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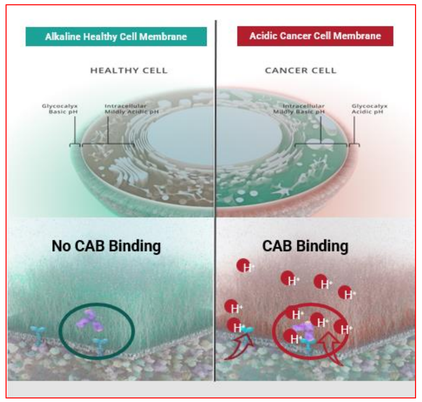
ABSTRACT

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] is a proprietary platform that generates antibodies which have little to no binding to the target antigen in healthy tissue (normal alkaline microenvironment). However, the binding of the antibodies to their target molecule is strong in acidic conditions which are found on the surface of cancer cells[1-6]. The CAB-ADC's elimination of on-target binding-related toxicity and its reduction of off-target toxicity through the elimination of Target Mediated Drug Disposition (TMDD), 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 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

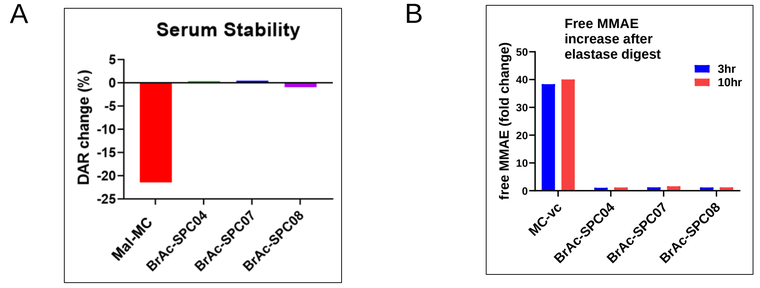


Figure 1: Improved linker stability analyzed by serum stability assay (A) and MMAE release assay (B).

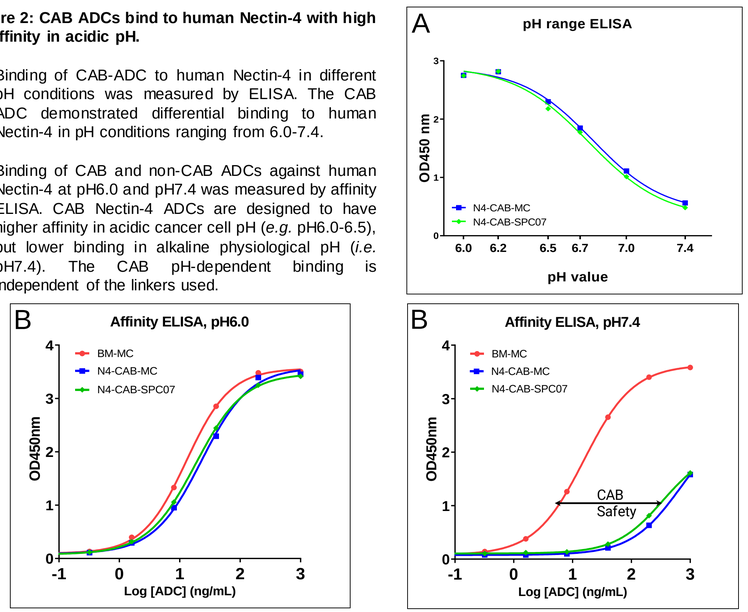
A. Serum stability of three novel glycosidase cleavable linkers (BrAC-SPC04, -07 and -08) with a Bromo-acetamide (BrAc) attachment group compared to a vedotin linker with maleimide coupling. Samples with a drug antibody ratio of 4 (DAR4) were incubated for 14 days in IgG-depleted human serum. DAR before/after incubation was determined by RP HPLC and the %DAR change was calculated. The DAR of ADCs with the novel linkers is unchanged, while it decreases ~20% with the vedotin linker.

B. Samples (DAR4) 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, MC-vc-ADC showed a 40-50-fold increase of free MMAE, while there was no significant release of free MMAE in the ADCs with the novel linkers (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 and non-CAB ADCs against human Nectin-4 at pH6.0 and pH7.4 was measured by affinity ELISA. CAB Nectin-4 ADCs are designed to have higher affinity in acidic cancer cell pH (*e.g.* pH6.0-6.5), but lower binding in alkaline physiological pH (*i.e.* pH7.4). The CAB pH-dependent binding is independent of the linkers used.



RESULTS

Figure 3: Evaluation of *in vitro* cell-killing potency of novel linkers.
Several of the novel linkers and the vedotin-like linker were conjugated to the Benchmark mAb (BM) and the cell-killing activity was tested *in vitro* using a 293F cell line expressing human Nectin-4 on the surface. The novel linkers showed similar killing potency as the vedotin linker used for comparison.

Linker	mc-vc	SPC04	SPC07	SPC08
IC50 (ng/mL)	20.08	13.22	8.77	11.67

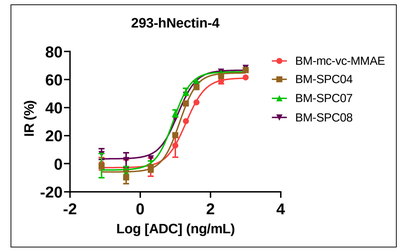
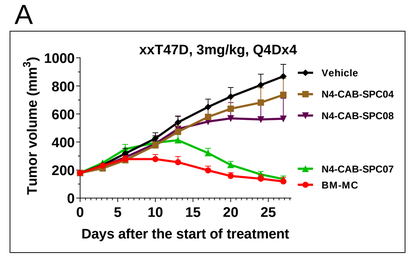
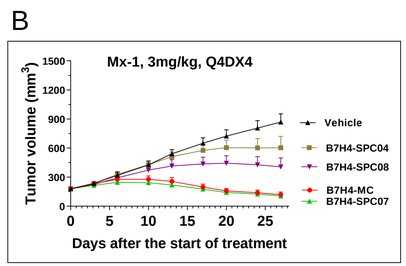
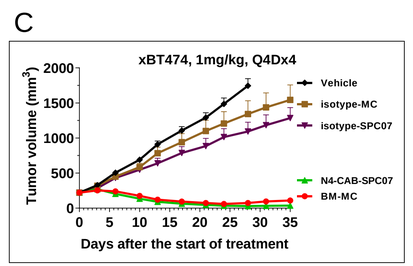


Figure 4: *In vivo* efficacy results of NextGen linkers.
The *in vivo* efficacy of the novel linkers was evaluated using breast cancer xenograft models (A) T47D cells (targeting Nectin-4), and (B) MX-1 cells (targeting B7H4). Linker SPC07 was the most efficacious in both models with similar tumor inhibitions as the vedotin linker. The tumor killing activity mirrors the IC50s measured in vitro: SPC07 (best) > SPC08 > SPC04.
The novel linker SPC07 also showed similar potency as the vedotin linker at 1mg/kg (lowest dose tested) using BT474 cells targeting Nectin-4 (C).







CONCLUSIONS

NextGen CAB ADCs-

- Eliminate 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 glycosidase-linker)
  - Increased hydrophilicity for higher DAR (*e.g.* 6) improving potency
- NextGen linker adaptable to other payloads besides MMAE, with other modes of action (*e.g.* duocarmycin)
- The combination of CABs plus novel linker system provides the opportunity to maximize Therapeutic Index, *i.e.* (CAB-ADCmax™)

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