Conditionally Active Biologic (CAB) ADCs with Novel NextGen Linker Show Improved Efficacy and Safety

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Abstract number 0743

ABSTRACT

Antibody-drug conjugates (ADCs) are a promising treatment for various forms of cancer. However, the efficacy of this treatment is often limited by on-target, off-tumor toxicity caused by antigen expression in healthy tissue and unwanted payload release outside the tumor due to cleavage of the linker via circulating proteases. Our Conditionally Active Biologics (CAB) technology addresses on-target, off-tumor toxicity by reducing binding to the target under normal physiological conditions while maintaining strong binding in the tumor microenvironment (1, 2). To minimize payload release outside the tumor, we developed a novel linker with superior serum stability, solubility, and tumor-specific payload release. Conjugation can be accomplished using regular IgG backbones without the need for sequence modification (3).

Here, we reported our NextGen ADC - CAB anti-Nectin4-ADC (DAR=6), which combines the advantages of CAB antibody pH selectivity with the new linker technology. *In vivo* efficacy data demonstrated complete tumor regression in several cell line derived xenograft models as well as patient-derived cancer models. Additionally, superior efficacy was found in patient-derived pancreatic xenograft model when compared to an enfortumab vedotin analogue, showing the influence of linker technology on specific cancer models and providing a promising tool for developing a tumor selective treatment. A single-dose toxicokinetic study in non-human primates showed good tolerability, long half-life, and good linker stability.

In conclusion, the NextGen Nectin4 CAB ADC represents a potentially more effective treatment with increased safety in the clinic.

1. Chang H.W., et al. PNAS.2021 Mar. 2;118.
2. Frey et al., MABS 16 1), 2322562 (2024).
3. Frey G., et al. AACR 2023, poster 1541.

RESULTS

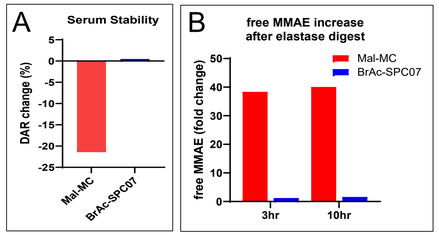

Figure 1: Improved linker stability analyzed by serum stability assay (A) and MMAE release assay (B).

A. Serum stability of benchmark antibody conjugated with the novel glycosidase cleavable linker BrAC-SPC07 with a Bromo-acetamide (BrAc) attachment group (blue) compared to a vedotin linker with maleimide coupling (red).

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.

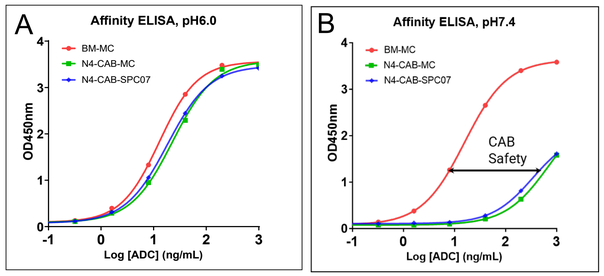

Figure 2: Novel linker maintains the pH selectivity of CAB ADCs.
Binding of CAB and non-CAB (benchmark, BM) ADCs against human Nectin-4 at pH6.0 and pH7.4 was measured by affinity ELISA. The CAB pH-dependent binding is independent of the linkers used.

RESULTS

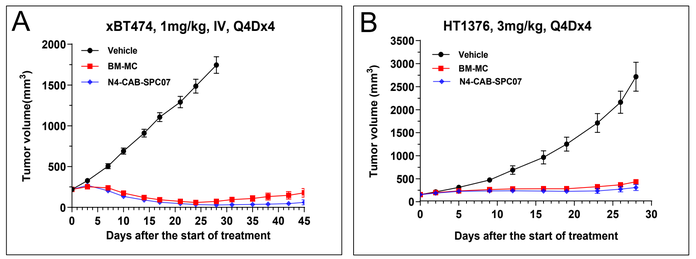

Figure 3: *In vivo* efficacy of CAB anti-Nectin4 ADCs in cell-derived xenograft models.
The *in vivo* efficacy of CAB anti-Nectin4 ADC with the novel linker (N4-CAB-SPC07; DAR6) was evaluated using CDX models: (A) BT474 cells (breast cancer) and (B) HT1376 cells (bladder cancer). N4-CAB-SPC07 demonstrated similar tumor regression to the enfortumab vedotin analogue (BM-MC) in the tested xenografted models.

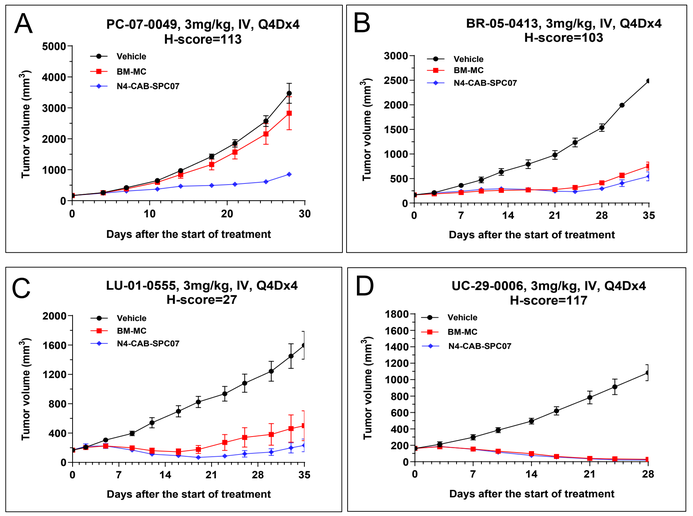

Figure 4: *In vivo* efficacy results of CAB anti-Nectin4 ADCs in patient-derived xenograft models.
The *in vivo* efficacy of CAB anti-Nectin4 ADC with the novel linker (DAR6) was evaluated using Nectin4-expressing PDX models: (A) PC-07-0049 (pancreatic cancer), (B) BR-05-0413 (breast cancer) , (C) LU-01-0555 (lung cancer), and (D) UC-29-0006 (bladder cancer). The Nectin4 expression in tumor tissue was determined by H-scores. N4-CAB-SPC07 showed comparable potency to the enfortumab vedotin analogue (BM-MC) in breast, lung, and bladder cancer models, while presenting enhanced anti-tumor efficacy in the pancreatic cancer model.

RESULTS

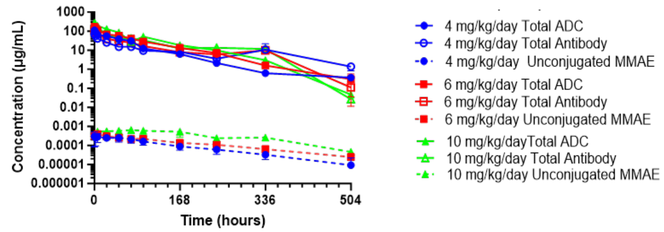

Figure 5: Single dose toxicokinetic study in cynomolgus monkeys.
Monkeys were treated with 4, 6, and 10 mg/kg N4-CAB-SPC07. The overlapping PK profiles of total antibody and total ADC concentrations confirmed good linker stability. The low detectible levels of free MMAE mirrored the profiles of total antibody and total ADC, suggesting slow deconjugation in the absence of a tumor microenvironment and potentially limited associated toxicities. Maximum tolerated dose for N4-CAB-SPC07 was 6 mg/kg, which exceeds the MTD for the benchmark.

Figure 6: The new linker conjugated ADCs against different targets were validated in cell-derived xenograft models.
The *in vivo* efficacy of BM ADCs conjugated with vedotin linker or novel linker SPC07 (all DAR4) was evaluated using CDX models (A) prostate cancer cell LNCap (targeting PSMA) and (B) lung cancer cell NCI-H820 (targeting c-Met). ADCs with the novel linker SPC07 (BM-SPC07) showed enhanced potency compared to the vedotin analogue (BM-MC), indicating the anti-tumor activities of new linker conjugates can be used for a variety of targets.

CONCLUSIONS

- The CAB technology eliminates on-target, off-tumor toxicity.
- The NextGen linker system eliminates extracellular derived off-target, off-tumor toxicity via:
  - Highly improved serum stability (NextGen glycosidase-linker)
  - Increased hydrophilicity for higher DAR (*e.g.* 6), thereby improving potency
- NextGen CAB anti-Nectin4 ADC demonstrates potent anti-tumor activities in *in vivo* models, exhibiting enhanced potency in pancreas cancer models compared to enfortumab vedotin analogue.
- Non-human primate studies validate the excellent stability of NextGen linker system and demonstrate the low toxicity associated with NextGen CAB anti-Nectin4 ADC.
- The combination of the CAB technology with the NextGen linker system offers the opportunity to maximize the therapeutic index.

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