BA3361, A Tumor Selective, Conditionally Active anti-Nectin4 ADC with a Novel NextGen Linker System has improved Efficacy in Pancreatic Cancer Models

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Abstract number 7633

ABSTRACT

Antibody-drug conjugates (ADCs) are a unique therapeutic combination of a tumor-targeting monoclonal antibody, a high-potency cytotoxic drug, and a chemical linker. Together, these components enable the delivery of the highly potent cytotoxin to the target cells. However, treatment efficacy is often limited by on-target, off-tumor toxicity due to antigen expression in healthy tissue, as well as unwanted payload release in normal tissue environments caused by linker cleavage via circulating proteases.

In this study, we present preclinical data for the IND approved BA3361, a NextGen ADC featuring a conditionally active biologics (CAB) anti-Nectin4 DAR6 ADC, optimized with novel CAB and linker technologies. BA3361 demonstrates pH-selective enhanced target binding in the acidic tumor microenvironment (TME), improved serum stability, increased solubility, and tumor-specific payload release. These advancements effectively address critical clinical challenges by reducing off-tumor, on-target and off-target toxicities. *In vivo* efficacy data showed tumor regression in several patient-derived xenograft (PDX) models. Notably, BA3361 when compared to an Enfortumab vedotin (EV) analogue (benchmark), demonstrated superior efficacy in patient-derived pancreatic xenograft models. The *in vivo* potency was tightly correlated with target expression in these models. Furthermore, we discovered that intracellular glycosidase activity, in stark contrast to protease activity, is high in all tested pancreatic tumor tissue samples, correlating efficacy with target expression.

In conclusion, our NextGen Nectin4-CAB-ADC, BA3361, demonstrates superior activity and improved tolerability in lung, breast, and urothelial patient derived cancer models compared to EV analogue and remarkedly outperforms it in pancreatic PDX models. The increased therapeutic index achieved through the conditional binding in the TME is expected to reduce side effects associated with non-CAB-Nectin4-ADCs.

RESULTS

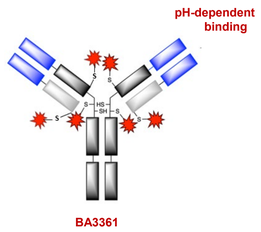

Figure 1: BA3361 Structure Overview.

- Human IgG1/Kappa backbone
  - Conditional binding to Nectin4 in the TME
- Conjugation targeting interchain disulfides
  - Compatible with all molecules in the pipeline
  - Bromo-acetamide attachment group
- Glycosidase cleavage
  - Glycosidases highly expressed in the lysosome
  - Improved payload delivery to the tumor
  - New linkers have increased solubility
  - Improves payload release in target cells
- Payload: MMAE (DAR6)
  - Bystander effect
  - CAB allows for higher/more frequent dosing

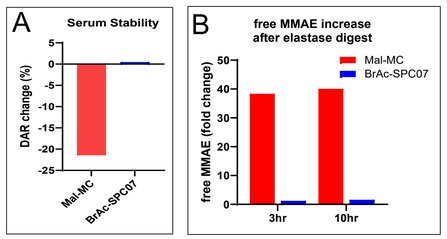

Figure 2: Improved linker stability analyzed by serum stability assay (A) and MMAE release assay (B).

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 (MC) 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.

RESULTS

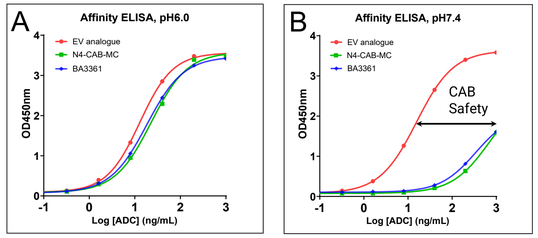

Figure 3: Novel linker maintains the pH selectivity of CAB ADCs.
Binding of CAB (N4-CAB-MC or BA3361) and non-CAB (EV analogue) ADCs to human Nectin-4 at pH6.0 and pH7.4 was measured by affinity ELISA. The CAB pH-dependent binding is independent of the linkers used.

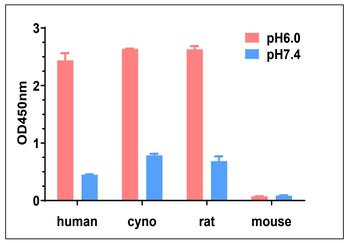

Figure 4: The pH selectivity of CAB ADC in cross-species ELISA.
Affinity of CAB ADC BA3361 binding against target Nectin-4 from different species (human, cyno, rat and mouse) at pH6.0 and pH7.4 conditions was measured by affinity ELISA. BA3361 cross reacts with cyno and rat Nectin4, but not mouse Nectin4.

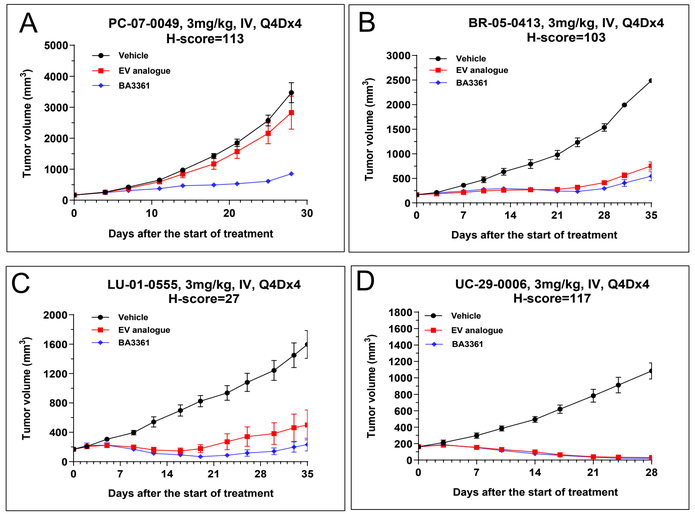

Figure 5: *In vivo* efficacy results of CAB anti-Nectin4 ADCs in PDX models.
The *in vivo* efficacy of CAB anti-Nectin4 ADC, BA3361, with the novel linker was evaluated using Nectin4-expressing PDX models: (A) PC-07-0049 (pancreatic cancer), (B) BR-05-0413 (breast cancer), (C) LU-01-0555 (lung cancer), and (D) UC-29-0006 (bladder cancer). Nectin4 expression in tumor tissue was determined by H-scores. BA3361 showed comparable potency to the enfortumab vedotin analogue (EV analogue) in breast, and bladder cancer models with a modest advantage in lung. However, there was remarkably enhanced anti-tumor activity in the pancreatic cancer model when using BA3361.

RESULTS

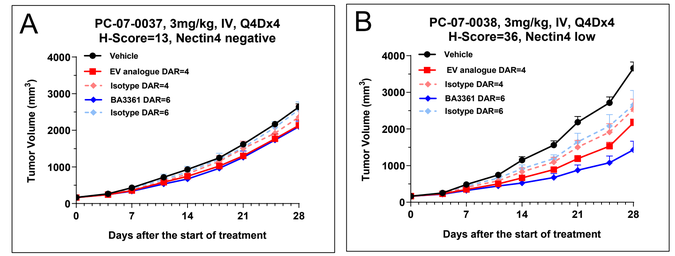

Figure 6: *In vivo* efficacy results of CAB anti-Nectin4 ADCs in pancreatic PDX models.
The *in vivo* efficacy of CAB anti-Nectin4 ADC, BA3361, with the novel linker was evaluated using several pancreatic PDX models with varying Nectin4 expression levels: (A) PC-07-0037, negative, (B) PC-07-0038, low, (C) PC-07-0049, high. Nectin4 expression in tumor tissue was determined by H-scores. The anti-tumor potency of BA3361 is directly correlated with target expression levels in pancreatic PDX models, whereas the non-CAB comparator, EV-analogue, does not show this trend.

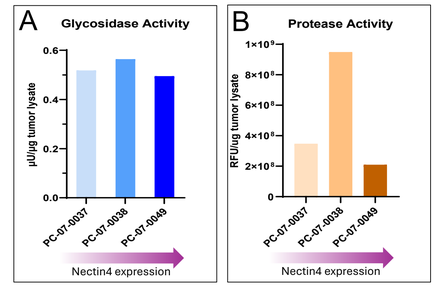

Figure 7: Characterization of enzyme activity in pancreatic PDX models.
Glycosidase (A) and protease (B) activities were measured in the pancreatic PDX models used in the efficacy studies.

Glycosidase activity was high in all samples tested, while protease activity was variable and not correlated with Nectin4 expression.

CONCLUSIONS

- The CAB technology minimizes on-target, off-tumor toxicity.
- The NextGen linker system eliminates extracellular-derived off-target, off-tumor toxicity through:
  - Highly improved serum stability (NextGen glycosidase-linker)
  - Increased hydrophilicity for a higher DAR (*e.g.* 6), thereby improving potency
- BA3361 demonstrates potent anti-tumor activity in *in vivo* models, exhibiting enhanced potency in pancreatic cancer models compared to the enfortumab vedotin benchmark.
- *In vivo* activity of CAB anti-Nectin4 ADC, BA3361, is directly correlated with Nectin4 expression in pancreatic PDX models.
- Variability of protease activity in pancreatic cancer models uncouples EV efficacy from receptor density.
- The combination of the CAB technology with the NextGen linker system offers the opportunity to maximize the therapeutic index.