A novel bispecific BCA356 targeting tumor antigen CAIX conjugated to an attenuated IL-12 demonstrates pre-clinical efficacy with potential for limited systemic toxicity.

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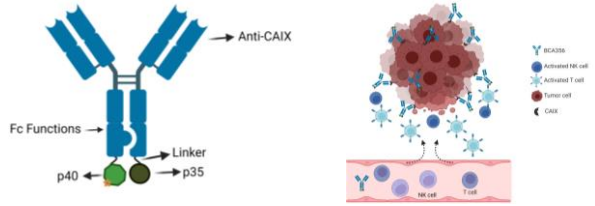
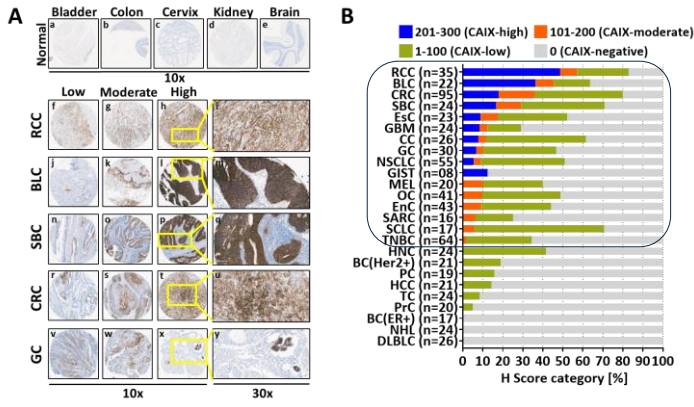
Background



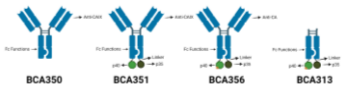
- BCA356 is a bispecific molecule with multifunctional properties: anti-CAIX binding, ADCC conserved humanized IgG1, and attenuated IL-12.
- Mutated p40 and wild type p35 are fused to knob and hole heavy chains Fc, respectively.
- BCA356 is designed to deliver biologically relevant concentrations of IL-12 to the tumor microenvironment that can activate both an innate and adaptive immune cell response.
- By combining both CAIX targeting and attenuating IL-12, BCA356 has the potential to avoid systemic toxicity while delivering clinical efficacy..

Figure 1: CAIX expression across cancer types



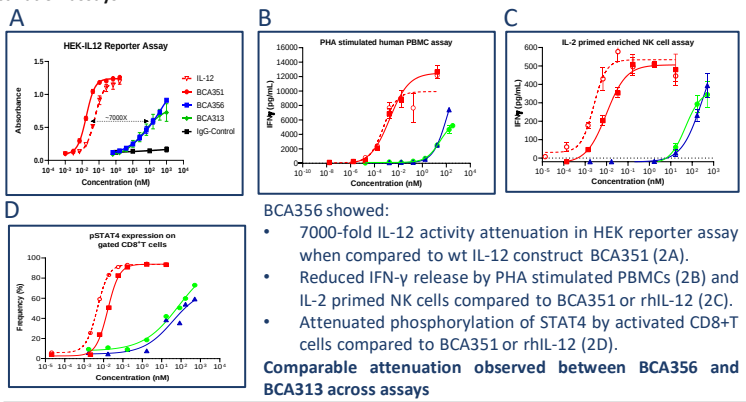
BC(ER+): Breast Cancer(ER+); BC(Her2+): Breast Cancer(Her2+); BLC: Bladder Cancer; CC: Cervical Cancer; CRC: Colorectal Cancer; DLBLC: Diffuse Large B-Cell Lymphoma; EnC: Endometrial Cancer; EsC: Esophageal/GEJ Cancer; GBM: Glioblastoma; GC: Gastric Cancer; GIST: Gastrointestinal Stromal Tumor; HCC: Hepatocellular Carcinoma; HNC: Head & Neck Cancer; MEL: Melanoma; NHL: Non-Hodgkin Lymphoma; NSCLC: Non-Small Cell Lung Cancer; OC: Ovarian Cancer; PC: Pancreatic cancer; PrC: Prostate Cancer; RCC: Renal Cell Carcinoma; SARC: Sarcoma; SBC: Small Bowel Cancer; SCLC: Small Cell Lung Cancer; TC: Thyroid Cancer; TNBC: Triple Negative Breast Cancer.

- CAIX expression is absent in normal tissues (1A, upper) except for its known expression in normal gastric mucosa.
- In different cancers, CAIX expression is membranous (1A, lower).
- High and moderate CAIX-expressing cases are observed in multiple cancer types (1A & B).



BCA350: Anti-CAIX mAb
BCA351: Wt IL-12 fused to anti-CAIX
BCA356*: Attenuated IL-12 fused to anti-CAIX
BCA313: Attenuated IL-12 fused to Fc
***Lead molecule**

Figure 2: BCA356 shows significant attenuation in IL-12 HEK reporter assay and immune cell activation assays



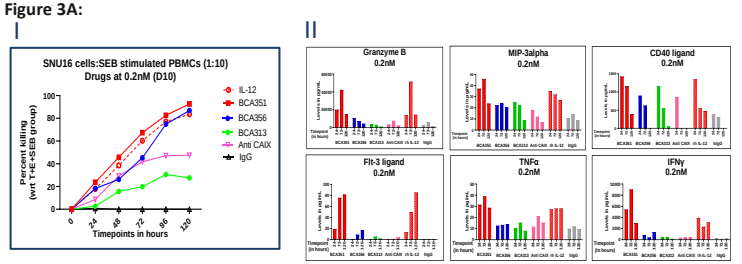
BCA356 showed:

- 7000-fold IL-12 activity attenuation in HEK reporter assay when compared to wt IL-12 construct BCA351 (2A).
- Reduced IFN-γ release by PHA stimulated PBMCs (2B) and IL-2 primed NK cells compared to BCA351 or rhIL-12 (2C).
- Attenuated phosphorylation of STAT4 by activated CD8+T cells compared to BCA351 or rhIL-12 (2D).

Comparable attenuation observed between BCA356 and BCA313 across assays

CAIX targeting by BCA356 has advantages over non-targeted attenuated IL-12 (BCA313)

Figure 3A:



Cytokines were evaluated using Human XL 44-plex luminex assay. Criteria used for shortlisting of key cytokines: 2-fold increase in BCA351 over Human IgG and ≥20% reduction in BCA356 over BCA351 at 24h and were compared across 72h and 120h. 8/44 cytokines/chemokines met the criteria at 24h of which 6 are shown in Fig 3A-II. Other 2, GMCSF and IL-10 are not shown in the figure.

- SNU16 cells were cultured with SEB stimulated hPBMCs at T:E ratio of 1:10 for 5 days.
- Cytotoxicity of SNU16 cells was comparable between BCA356 and rhIL-12/BCA351 (3A-**I**).
- BCA356 showed improved cytotoxicity than BCA313 probably due to CA-IX targeting.
- BCA356 showed comparable inhibition to wild type IL-12 with limited inflammatory cytokine release, suggesting a safe profile (3A-**II**).

Figure 3B

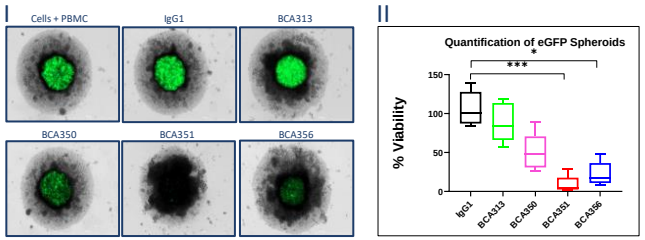
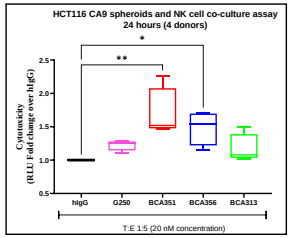
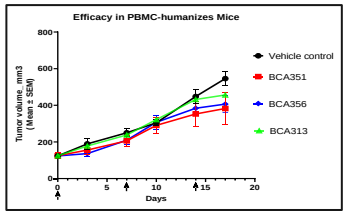


Figure 3C:

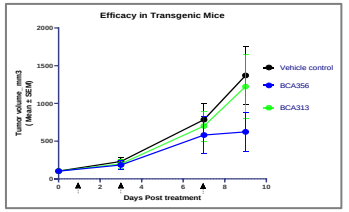


- Figure 3A&B: A549 e-GFP-CAIX spheroids and PBMC co-cultures show comparable cytotoxicity to wt IL-12 (BCA351).
- Figure 3C: CAIX overexpressing HCT116 cells (spheroids) were co-cultured with IL-2 primed enriched NK cells at T:E ratio of 1:5 for 24h.
- BCA356 showed NK cell mediated killing of CAIX overexpressing HCT116 cells which was not observed with BCA313 probably due to CAIX targeting (3C).

Figure 4: BCA356 anti-tumor efficacy is comparable to IL-12 (WT), with reduced toxicity



Group	%TGI	Weight Loss (%)	Deaths Day17
BCA351	30 ± 31	-20	2/6
BCA356	26 ± 21	-14	0/6
BCA313	16 ± 26	-13	0/6



Group	%TGI	Weight Loss (%)	Deaths Day17
BCA356	55 ± 42	4	0/6
BCA313	11 ± 69	9	1/6

- Figure 4A & B: In PBMC-based humanized HCT-116 xenograft model, BCA356 showed similar TGI as BCA351 with reduced toxicity compared to BCA351
- Figure 4C&D: In IL-12 transgenic mice (Biocytogen), BCA356 showed 55% tumor growth inhibition compared to non-targeting IL-12 BCA313
- Unlike BCA351, no deaths were observed in BCA356

Conclusions

- **CAIX is a well-validated tumor-specific antigen with significant expression in tumors beyond RCC with limited expression in normal tissues.**
- **By leveraging CAIX, BCA356 can deliver IL-12 to tumors expressing the tumor-specific antigen with minimal exposure to normal tissue.**
- **BCA356 has the potential for a favorable toxicity profile with efficacy comparable to wild type IL-12.**

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