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A NOVEL BISPECIFIC BCA356 TARGETING TUMOUR ANTIGEN CAIX CONJUGATED TO AN ATTENUATED IL12 DEMONSTRATES PRE-CLINICAL EFFICACY WITH POTENTIAL FOR LIMITED SYSTEMIC TOXICITY

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Background IL-12 is a potent cytokine linked to activation of innate and adaptive immune system for anti-tumour immunity.¹ The clinical development of IL-12 therapy has been constrained with severe toxicity reported from systemic administration of IL-12. Toxicity is attributed to poor pharmacokinetics of IL-12, necessitating frequent dosing, and nonspecific distribution. Recently, there has been a renewed interest in IL-12 therapy with various strategies to improve half-life and reduce toxicity, with lead candidates in preclinical and early clinical stages of development.² Here, we describe novel bispecific BCA356 with an affinity matured humanized anti-CAIX antibody and attenuated IL-12 subunits fused to each of the heavy chains at the C-terminus by a linker in a knob-in-hole format.

Methods *In silico* mutational screening was performed on the p40 and p35 subunits of IL-12 to identify optimally attenuated IL-12 variants (IL-12vs). CAIX as a tumour-targeting antigen was selected based on high expression of CAIX across several solid tumour types. Expression of cellular and soluble CAIX across tumour types was evaluated by immunohistochemistry (IHC) on tissues and ELISA on tumour-matched plasma. BCA356 was identified after IL-12vs were screened in a HEK-Blue™ IL-12 assay (figure 1) followed by phosphorylated STAT4 expression on CD8⁺ T cells and IFN γ release by PHA-stimulated PBMCs/IL-2 primed NK-cells. In *in vitro* 2 and 3D co-culture assays with CAIX-overexpressing cell line and PBMCs, cytotoxicity and cytokine/chemokine release were assessed across days. Efficacy and safety of BCA356 were evaluated in PBMC-based humanized mice models and human IL-12 and IL-12 receptor gene knock-in transgenic mice.

Results IHC studies confirmed high CAIX expression in clear cell renal carcinoma³ and multiple other tumour types, a significant number of samples, expressed high and moderate CAIX expression (figure 2). BCA356 significantly attenuated IFN γ release by stimulated PBMCs, activated NK-cell and pSTAT4 expression in CD8 T cells as compared to IL-12(wt). In co-culture assays, BCA356 showed cytotoxicity of cancer cells comparable to IL12(wt) (figure 3) without significant cytokine release unlike IL12(wt) (figure 4). Finally, efficacy studies in PBMC-based humanized mice models and transgenic mice models confirm that BCA356 is efficacious and safe in CAIX-overexpressing tumour bearing mice.

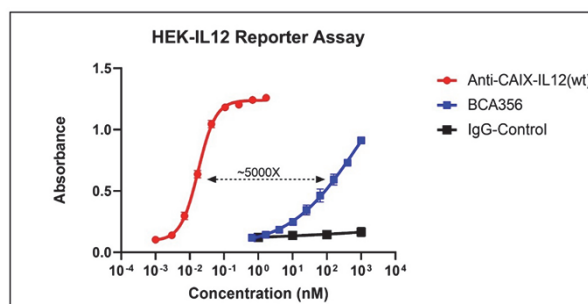
Conclusions BCA356 specifically targets CAIX-expressing tumour cells and similar to wild type IL-12 cytokine, has the potential to reduce tumour proliferation with optimal activation of pro-inflammatory cytokines. Through these *in vitro* and *in vivo* studies, we demonstrate that BCA356 by its CAIX-targeted IL-12v delivery approach is both efficacious and safe.

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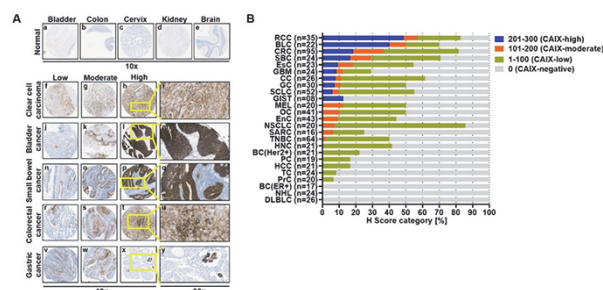
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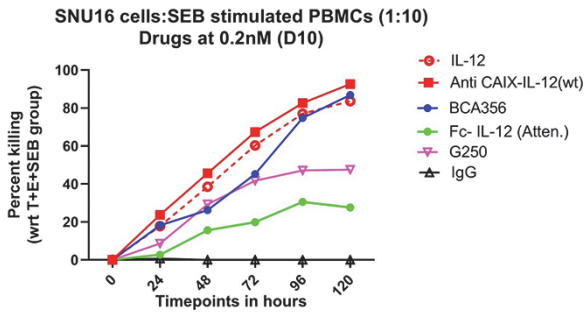
Ethics Approval Mice were maintained as per the regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) guidelines. All animal experiments were approved by institutional ethical committee and performed under approved protocols. All animals were maintained at dedicated Syngene vivarium



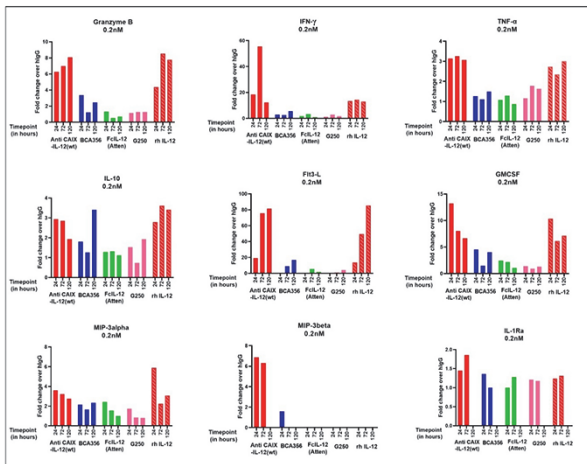
Abstract 1368 Figure 1 Evaluating IL-12 activity by HEK-IL-12 reporter assay. The binding of IL-12 to the IL-12R on the surface of HEK-Blue™ IL-12 cells (Invivogen) triggers a signaling cascade leading to the activation of STAT-4 with the subsequent production of SEAP. HEK-IL-12 cells were incubated with different concentrations of test compounds for 18-24 hours, and IL-12 activity was measured by addition of Quanti-Blue solution. Anti-CAIX-IL-12(wt) showed similar activity as rh-IL-12.



Abstract 1368 Figure 2 CAIX expression in normal and cancer tissues. (A) Upper panel: CAIX expression in normal tissues. (A) Lower panel: Representative images of heterogeneous CAIX expression in different cancer types. (B) For each cancer type (Y axis), percentage of cases corresponding to CAIX-high (201-300), CAIX-moderate (101-200), CAIX-low (1-100), and CAIX-absent (0) H Score category (X axis). BC (Her2+): Breast Cancer(Her2+), BC(ER+): Breast Cancer(ER+), BLC: Bladder Cancer, CC: Cervical Cancer, CRC: Colorectal Cancer, DLBCL: Diffuse Large B-Cell Lymphoma, EnC: Endometrial Cancer, EsC: Esophageal/GEJ Cancer, GBM: Glioblastoma, GC: Gastric Cancer, GIST: Gastrointestinal Stromal Tumour, 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.



Abstract 1368 Figure 3 Real time tumour cell killing by BCA356. SNU16 target cells were seeded with SEB(0.5 pg/mL) stimulated PBMC at T:E ratio of 1:10. Plates were kept in Incucyte and scanned for 120h to measure cytotoxicity over time. Cytotoxicity is a representative graph from two independent experiments.



Abstract 1368 Figure 4 Cytokine release by BCA356. SNU16 target cells were seeded with SEB (0.5 pg/mL) stimulated PBMC at T:E ratio of 1:10. Cytokines were evaluated at 24h, 72h and 120h using Luminex. Criteria used for shortlisting of key cytokines: 2-fold increase in Anti CAIX-IL-12(wt) over Human IgG and $\geq 20\%$ reduction in BCA356 over Anti CAIX-IL-12(wt). Cytokine release is from a single experiment.

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