DEVELOPMENT OF BCA101, A BIFUNCTIONAL ANTIBODY CAPABLE OF SIMULTANEOUSLY DISABLING EGFR AND TGF β SIGNALING, AS NOVEL SINGLE-AGENT IMMUNOTHERAPY

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Background Given the pleiotropic functions of transforming growth factor-beta (TGF β), current approaches to targeting systemic TGF β will likely lead to suboptimal clinical activity and/or undesirable effects. Epidermal growth factor receptor (EGFR) is one of the most extensively validated tumor-associated antigens. Bicara Therapeutics has developed a novel bifunctional fusion protein, composed of a monoclonal antibody against EGFR and an extracellular domain of human TGF β receptor II (TGF β RII). We demonstrate BCA101 has the potential to improve anti-tumor response by leveraging the cooperativity between EGFR and TGF β signaling pathways while restricting TGF β neutralization to EGFR-expressing tissues.

Methods Functional neutralization of TGF β by BCA101 was demonstrated by several in vitro assays which assessed TGF β -dependent epithelial to mesenchymal transition (EMT), cell invasion, inducible Treg differentiation, as well as allogeneic immune responses in tumor cell/immune cell coculture assays. In vivo, the anti-tumor efficacy of BCA101 was determined in tumor xenograft mouse models, using either human tumor cell lines or patient-derived tumor cells (PDX), as well as in a humanized mouse model.

Results In vitro, we showed BCA101 is capable of simultaneously binding EGFR and TGF β 1 with a significantly higher affinity for EGFR. The incorporation of the TGF β RII "trap" did not sterically interfere with the ability of BCA101 to bind EGFR, inhibit cell proliferation or mediate antibody-dependent cellular cytotoxicity (ADCC). Relative to cetuximab, BCA101 showed improved ability to reverse EMT and preserve ADCC activity. In tumor cell/immune cell co-culture assays, BCA101 increased production of proinflammatory cytokines associated with T and NK cell activation and suppressed VEGF release. Further, BCA101 inhibited differentiation of inducible Treg and displayed an immuno-potentiating profile in the BioMAP[®] TME model. In vivo, biodistribution studies showed that BCA101 localized to tumor tissues in xenograft mouse models, with comparable kinetics as cetuximab. TGF β in tissues was neutralized to about 90% at 10 mg/kg of BCA101 while equimolar doses of TGF β RII receptor inhibited TGF β in tumors by around 50%, confirming improved tumor localization with BCA101. In PDX models derived from head and neck cancer squamous cell carcinoma patients, BCA101 exerted sustained antitumor effect and delayed tumor growth compared to cetuximab. Finally, BCA101 improved the anti-tumor activity of PD1 blockade therapy in humanized HuNOG-EXL mice bearing PC-3 xenografts (figure 1).

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BCA101 shows superiority over cetuximab in animal models. (A) & (B). Patient derived xenograft (PDX) models. Patient derived tumors were engrafted into female NOG mice. Once tumor reached about 130 mm³, mice were randomized into control and test groups. Test group mice were treated with either BCA101 (A) or cetuximab (B), thrice a week (i.p), whereas control animals received placebo alone. Mice were treated for 27 days followed by a treatment-free phase until Day 79. Tumor volumes and mice weight were recorded twice a week. (C). BCA101 inhibits FaDu tumor xenograft growth (CDX) in vivo. Nude mice were implanted with FaDu cells on flanks. Once tumor reached about 100 mm³, mice were randomized (n=7) and treated with six doses of test compounds, BCA101 and cetuximab. Tumor volume and mice weight were recorded twice a week. (D). BCA101 and anti-PD1 combination studies in hu-NOG-EXL humanized animal model. PC-3 cells were implanted into flank of Hu-NOG-EXL humanized mice and randomized into control and test groups once tumors reached about 120 mm³. Test group mice were treated with cetuximab or BCA101, intraperitoneally for 6 doses. Anti-PD1 antibody (pembrolizumab) was administered intraperitoneally at a dose of 10 mg/kg with a dosage schedule of every fifth-day for 5 doses (Q5Dx5). Statistical analysis for panel (C) & (D) was performed using repeated measures two-way ANOVA followed by Bonferroni's multiple comparison test. Significance was indicated by * = p value ≤ 0.05 , ** = p value ≤ 0.01 and *** = p value ≤ 0.001 . Tumor volumes are presented as Mean $\pm$ SEM.

Conclusions These results support the clinical development of BCA101 as a targeted immunotherapy with the potential to induce improved anti-tumor response with a wider therapeutic window, either as a monotherapy or in combination with immune checkpoint blockade therapy.

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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. For PDX model, head and neck cancer patient samples were obtained from Mazumdar Shaw Medical Foundation, Bengaluru, India after appropriate approvals were obtained from institutional ethical committee: NHH/MEC-RC2016-404

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