1112 SINGLE-CELL RNA SEQUENCING REVEALS THE POSITIVE FEEDBACK ACTIVATION LOOP BETWEEN T AND DENDRITIC CELLS INDUCED BY PD-L1X4–1BB BISPECIFIC ANTIBODY

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Background Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of multiple cancers. However, only a minority of patients exhibit profound and durable responses, as the majority of patients exhibit innate or acquired resistance to ICI therapy. Although immune costimulatory receptor 4–1BB (CD137, TNFRSF9) targeting agonist antibodies demonstrated potent enhancement of anti-tumor activity of ICIs, the on-target-off-tumor hepatotoxicity have hindered the clinical development of therapeutic 4–1BB agonists.[1] Previously, we have reported a novel engineered tetravalent '2+2' PD-L1x4–1BB bispecific antibody ATG-101 and demonstrated potent antitumor activities in multiple tumor models without inducing hepatotoxicity. In this study, we used single-cell RNA-sequencing (scRNAseq)[2] to better understand the biology of immune responses induced by ATG-101 in the tumor microenvironment (TME).

Methods MC38 tumor-bearing human 4–1BB knock-in mice were randomized and dosed with hIgG (3 mice) or ATG-101 (3 mice). CD45+ immune cells were isolated after removal of dead cells and sequenced using the 10x Genomics Chromium platform. Raw sequencing data was aligned to the custom mouse reference genome mm10 with addition of human 4–1BB to generate a cell-gene count matrix. Certain immune lineages, including T cell and dendritic cells (DCs), were subjected to further functional analysis such as differential expressed genes, pathway activities, gene-set enrichment, and cell-cell communications.

Results ATG-101 treatment increased the proportion of T cells, DCs, B cells, and monocytes in TILs compared to those of IgG treatment (figure 1). ATG-101 also reduced exhausted CD8+ T cells and regulatory T cells proportion. Besides the upregulation of 4–1BB, ATG-101-treated T cells also upregulated Gzmb and downregulated Tox, suggesting improved T cell immunity. ATG-101 increased the proportion of cDC2 and migratory cDC1 subsets while decreasing the proportion of tolerogenic DCs. ATG-101 upregulated DC costimulatory and activation markers such as Cd80 and Cd83, and downregulated immunosuppressive markers such as Apoe and Fn1. T cell activation related ICAM1-mediated interactions between T and DC were identified only in TILs treated with ATG-101. Several tumor promoting interactions such as CCR5/CCL8 were abolished by ATG-101 treatment. ATG-101 also upregulated PD-L1 expression in DC population.

Conclusions Transcriptional profiling of the TME revealed that ATG-101 reversed T cell exhaustion, increased T cell cytotoxicity and reduced tolerogenic DCs. Notably, due to the increased gene expression of PD-L1 in DCs and 4–1BB in T cells following ATG-101 treatment, it is anticipated that ATG-101 will promote greater T cell-Dendritic cell crosslinking over time, further activating 4–1BB signaling, strengthening T cell-DC interaction, and generating a positive feedback loop.

Ethics Approval The protocol and any amendment(s) or procedures involving the care and use of animals in this study were reviewed and approved by the IACUC of CrownBio. All studies were conducted following an approved IACUC protocol. AUP NO.:2004–12-1465, 2004–12-1000; IACUC approval number: IACUC-2021-M-003

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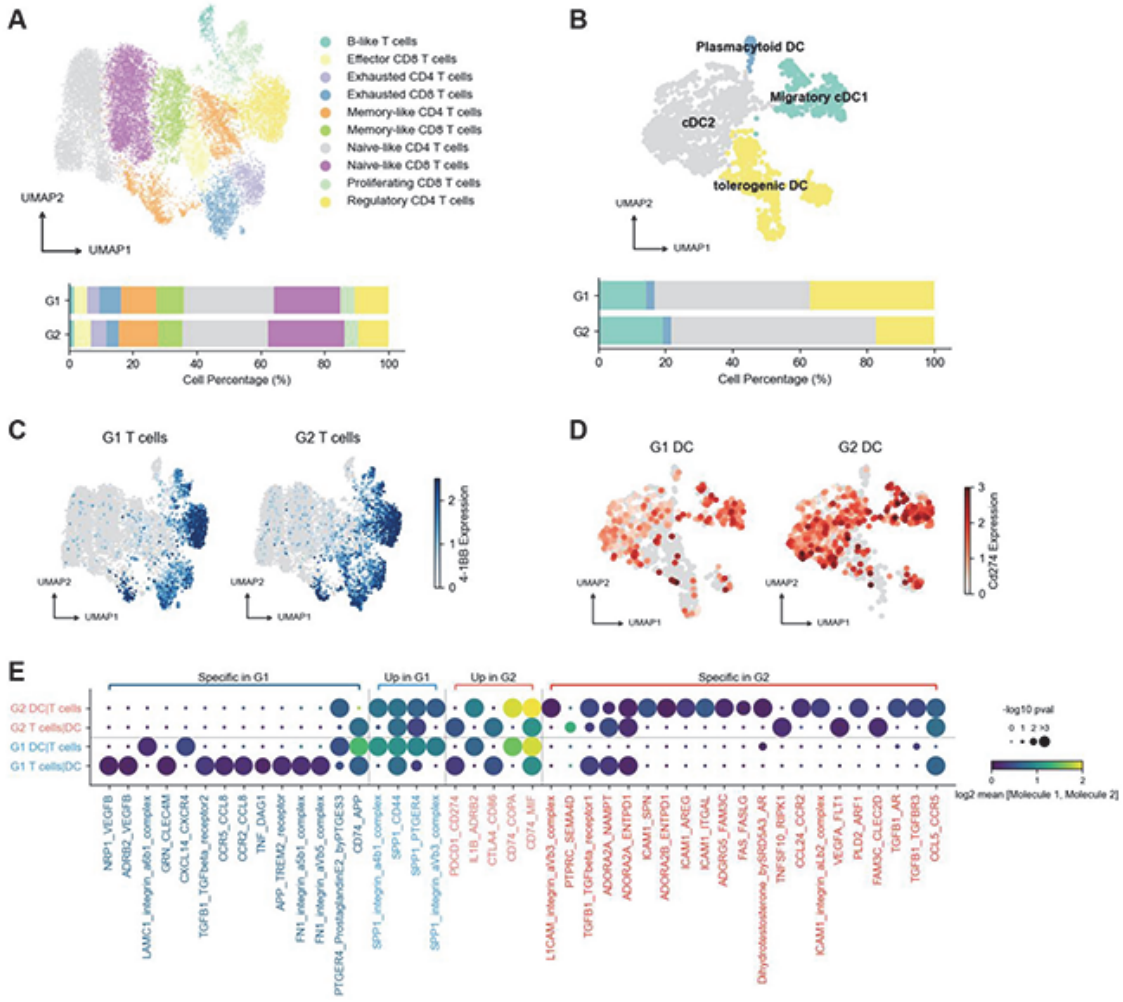

Abstract 1112 Figure 1 Key T and dendritic cells landscape in the TME post ATG-101 treatment. (A) 2D-UMAP embedding plot of T cells colored by T cell subtypes, G1: IgG group, G2: ATG-101 group. (B) 2D-UMPA embedding plot of DC colored by DC subtypes, G1: IgG group, G2: ATG-101 group. (C) Scatter plot showing expression of 4–1BB in T cells across 2 treatment groups. (D) Scatter plot showing expression of PD-L1 (CD274) in DC across 2 treatment groups. (E) Dotplot of significant differential interactions between DC and T cells across 2 treatment groups.

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