Background IL-18 is an inflammasome-induced proinflammatory cytokine that activates T and NK cells and stimulates IFNγ production. The activity of IL-18 is naturally blocked by a high affinity endogenous binding-protein (IL-18BP) induced in response to IFNγ upregulation as a negative feedback mechanism.

Methods By assessing total and free IL-18 we examined whether bound-IL-18 levels in the tumor are above the level required for in-vitro human T-cell activation. To unleash endogenous bound IL-18 activity, COM503, an anti-IL-18BP blocker Ab, was generated and examined in T cell-based assays. In-vivo, IL-18BP blockade was evaluated in multiple mouse tumor models. Tumor microenvironment (TME) modulation was assessed by flow cytometry, scRNA sequencing and cytokine profiling.

Results IL-18 levels were clearly elevated across 75 evaluated tumors compared to serum samples (figure 1A-B). Results show that most of tumor IL-18 was bound to IL-18BP. COM503, an anti-IL-18BP Ab, induced human T-cell responses in-vitro and ex-vivo.

Conclusions IL-18 is upregulated in human tumors and is mostly bound by IL-18BP. COM503, a high-affinity anti-IL-18BP Ab, induces human T-cell responses in-vitro and ex-vivo. An anti-mouse IL-18BP Ab induces potent anti-tumor responses and pronounced TME-constrained immune modulation, this in contrast to systemically administered therapeutic cytokines, which can generate systemic inflammatory responses (figure 7). Taken together, blocking IL-18BP is a promising novel approach to harness cytokine potency for the treatment of cancer. COM503 is currently undergoing IND-enabling studies.
Abstract 550 Figure 1  IL-18 is upregulated in the TME and IL-18BP-bound IL-18 level is above the amount required for T cell activation in vitro. (A) IL-18 levels are significantly higher in the tumor compared to serum. Serum samples and tumor biopsies were collected from cancer patients. Tumor biopsies were dissociated, and supernatants were collected. IL-18 expression was analyzed in serum and tumor derived supernatants (TDS) using ELISA assay. (B) IL-18 expression in TDS samples from individual patients across different indications. (C) Recombinant (r) IL-18 increased IFNγ release by stimulated CD8+ tumor infiltrated lymphocytes (TILs) in TILs-tumor cells co-culture assay in a dose-dependent manner. MEL624 cells and TILs were seeded and treated with rIL-18 (0–100ng/ml). Plates were incubated for 24 hours, after which the supernatant was collected for cytokine secretion evaluation. (D) Levels of bound IL-18 in TDS across indications are above the level required for in vitro T cell activation. Tumor biopsies were dissociated, supernatants were collected and analyzed using free and total IL-18 ELISA assays. IL-18BP bound IL-18 levels were calculated by deducting free IL-18 from total IL-18 measured for each sample by two separate ELISA kits. Dashed red line represent the level required for functional activity (1.5ng/gr).

Abstract 550 Figure 2  COM503, high affinity Ab against IL-18BP, released IL-18 to enhance T cell activity in vitro. (A) COM503 (10ug/ml) displaced IL-18 from a preformed IL-18:IL-18BP complex to increase IFNγ and TNFα release from stimulated human CD8+ TILs (N=3–4) in TILs-tumor cells co-culture assay in the presence of rIL-18BP and rIL-18 (B) COM503 increased IFNγ, TNFα, GZMB, and IL-2 release by human tumor dissociated cells. Resected cancer specimens dissociated to single-cell suspension were cultured with anti-CD3/anti-CD28 mAbs for T-cell stimulation and treated with COM503, anti-PD1 Ab (pembrolizumab) or with combination of COM503+Pembrolizumab (10 ug/ml). After 3 days, cytokines and Granzyme secretion were measured in supernatants. Representative example from a human ovarian TDC sample is shown.
Abstract 550 Figure 3  Anti-mouse IL-18BP Ab inhibited tumor growth across murine syngeneic tumor models as a single agent and in combination with anti-PD-L1. A-C. anti-mouse IL-18BP Ab (15mg/kg) inhibited tumor growth as a single agent in MC38OVA dim (Treatment initiated in 130–260 mm3 tumor volume) (A), E0771 (Treatment initiated in 250- 270 mm3 tumor volume) (B), and B16F10-hmgp100 (Treatment initiated on day 4 post tumor inoculation) (C) mouse tumor models compared to isotype control. Tumors were inoculated in C57BL/6 mice; mice (N=10) were treated twice a week for total of 6 treatments. D. Anti-mouse IL-18BP (15mg/kg) synergized with anti PD-L1 Ab (5mg/kg) to inhibit tumor growth in E0771 tumor model. Treatment initiated in established tumor (330mm3,N=10) and was given twice a week for a total of 6 treatments. Tumor volumes are represented as the Mean volume + SEM.

Abstract 550 Figure 4  IL-18BP blockade increased T cell effector state and clonal expansion in E0771 mouse tumor model. C57BL/6 mice were orthotopically inoculated with E0771 cells and treated with anti-IL-18BP Ab or isotype control (15mg/kg) at tumor volume of 330mm3 twice a week. Tumors were collected 24hr post the third treatment and dissociated. Immune modulation was assessed by flow and scRNA sequencing. (A) Anti IL-18BP Ab increased CD3+, CD8+ and CD4+ T cells infiltration into the tumor. (B) Anti IL-18BP Ab increased T cell polyfunctionality as evident by increase in IFNy+, IL-2+, GrB+ and GrB+IFNy+ CD8+ T cells (C) UMAP projection showing T and NK cells present in E0771 tumors treated with anti-IL-18BP or isotype control. (D) Visualization of the average cell density within the anti-IL-18BP (bottom) and Isotype control (top) group, using embedding density estimation on T/NK UMAP. Darker colors correspond to denser regions. (E) Log2 fold change of T cell subpopulations comparing anti-IL-18BP Ab treatment to the control group. Only populations with significant changes are depicted. (F) Quantification of clonal expansion frequencies in anti-IL-18BP Ab treatment compared to the control group.
IL-18BP blockade increased proinflammatory cytokine secretion and skewed myeloid cells to favor proinflammatory state in E0771 mouse tumor model. C57BL/6 mice were orthotopically inoculated with E0771 cells and treated with anti-IL-18BP Ab or isotype control (15mg/kg) at tumor volume of 330mm³ twice a week. Tumors were collected 24hr post the third treatment and dissociated. Immune modulation was assessed by cytokine profiling and scRNA sequencing. (A) Anti IL-18BP Ab increased IFNγ, TNFα, IL-12p70, CXCL9 and MIP-1α secretion and decreased IL-1β secretion. (B) UMAP projection showing tumor-associated monocyte and macrophage subpopulations present in E0771 tumors treated with anti-IL-18BP or isotype control. (C) Visualization of the average cell density within the anti-IL-18BP (bottom) and Isotype control (top) group, using embedding density estimation on tumor-associated monocyte and macrophage UMAP. Darker colors correspond to denser regions. (D) Log2 fold change of monocyte and macrophage subpopulations comparing anti-IL-18BP Ab treatment to the control group. Only populations with notable changes are depicted.

Anti-mouse IL-18BP Ab alters the immune infiltrate composition of MC38OVA dim tumors without affecting the periphery. MC38OVA dim tumors were inoculated in C57BL/6 mice. At tumor volume of 120 mm³ mice were randomized and treated either with anti-IL-18BP Ab or with isotype control (15mg/kg) twice a week for a total of 4 treatments. Tumors and spleen were harvested 24 hours after the 4th treatment and immune composition was examined. Tumor supernatants and blood serum were collected at the same timepoint and analyzed for cytokine concentrations. A-C. Anti-mouse IL-18BP Ab affected immune composition and cytokine concentrations in the TME (A), but not in the spleen (B) and in the serum (C).
Abstract 550 Figure 7  COM503, a potential first-in-class anti-IL-18BP blocker antibody that unleashes endogenous IL-18 in the TME. IL-18 is an effector cytokine that is upregulated in the TME and secreted upon inflammasome activation. IL-18BP is secreted via an IL-18 negative feedback mechanism, Binds IL-18, and blocks its immune stimulatory activity. COM503, high affinity IL-18BP blocker Ab has the potential to Induce potent anti-tumor responses and pronounced TME-constrained immune modulation.

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