Harnessing natural IL-18 activity through IL-18BP blockade reshapes the tumor microenvironment for potent anti-tumor immune response

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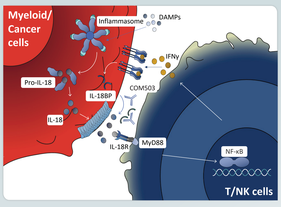
Background

Conventional cytokines have limited anti-cancer efficacy mostly due to narrow therapeutic window and systemic adverse effects. 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), which is induced in response to IFNγ upregulation as a negative feedback mechanism. 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-human IL-18BP blocker Ab, was generated and examined in T and NK 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.

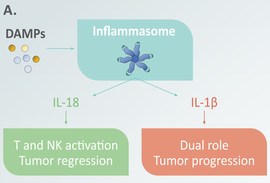


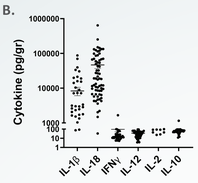
IL-18 is:
- An effector cytokine
- Secreted upon inflammasome activation
- Upregulated in the TME

IL-18BP:
- Binds IL-18 and blocks its immune stimulatory activity
- IL-18BP secretion is increased via an IL-18-mediated feedback mechanism

COM503:
- Has the potential to induce potent anti-tumor responses and pronounced TME-localized immune modulation

Inflammasome induced cytokines such as IL-18 and IL-1β are abundant in the TME

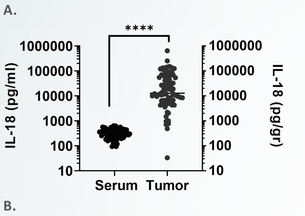


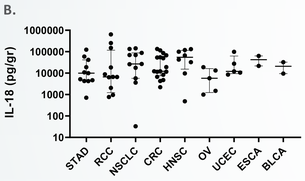


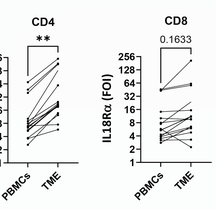
List of indications:
Colorectal
NSCLC
Ovary
Renal
Breast
Endometrial
H&N
Stomach
Pancreas
Esophageal
Bladder
Rectal

A. IL-18 and IL-1β are inflammasome derived cytokines with opposite effects in the TME. **B.** Levels of cytokines in tumor derived supernatants (TDS) across indications.

IL-18 pathway is elevated in the TME across indications

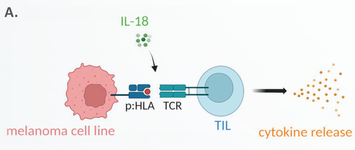


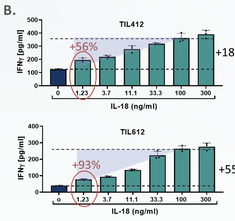


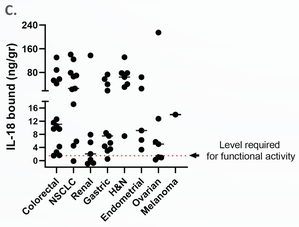


A. IL-18 levels in TDS are elevated compared to levels in serum in cancer patients. **B.** IL-18 levels in TDS across indications. **C.** IL-18 receptor α subunit (IL-18Rα) expression is induced on tumor infiltrating lymphocytes (TILs) in the TME compared to periphery. Statistical analysis was preformed using paired t test (two tailed). P ≤ 0.01**, P ≤ 0.0005****

IL-18BP-bound IL-18 levels in the TME are above the amount required for T cell activation in vitro

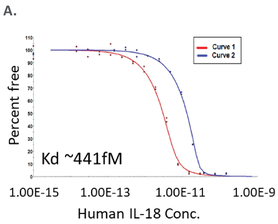


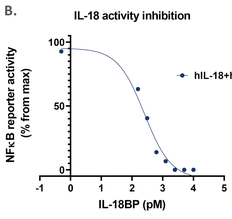




A. Schematic representation of assay setup. **B.** Recombinant (r) human IL-18 increased IFNγ release by stimulated CD8+ TILs in TILs-tumor cells co-culture assay in a dose-dependent manner. Activity was seen with as low as 1ng/ml of IL-18. **C.** Levels of bound IL-18 in TDS across indications are mostly above the level required for in vitro T cell activation. IL-18BP-bound IL-18 levels were calculated by deducting IL-18 free from total IL-18 measured for each sample by two separate ELISA assays. Dashed red line represent the level required for functional activity (1.5ng/gr).

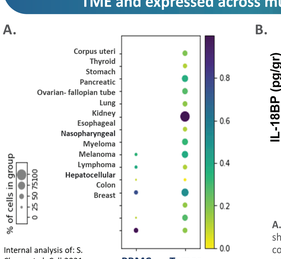
IL-18BP binds IL-18 with high affinity and inhibits its activity in HEK293 reporter system

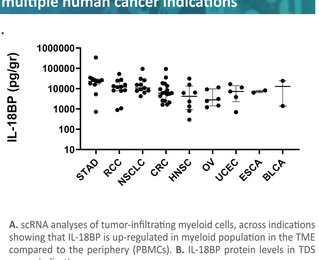




A. Human IL-18-IL-18BP interaction measurements in KinExA. For each run in KinExA, two curves with different column binding protein (CBP) concentrations were run and analyzed using n-curve analysis to determine the Kd. **B.** IL-18BP inhibits IL-18 activity in NFkB-HEK293-IL-18R reporter system

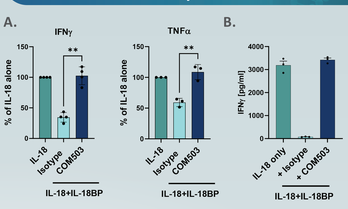
IL-18BP is upregulated in tumor associated macrophages in the TME and expressed across multiple human cancer indications

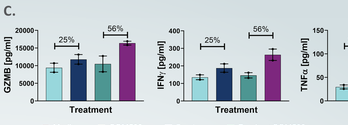




A. scRNA analyses of tumor-infiltrating myeloid cells, across indications showing that IL-18BP is up-regulated in myeloid population in the TME compared to the periphery (PBMCs). **B.** IL-18BP protein levels in TDS across indications.

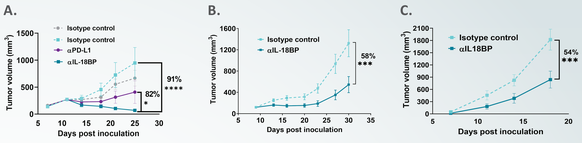
COM503- a fully human high affinity anti-IL-18BP blocker antibody restores human T and NK cell activity





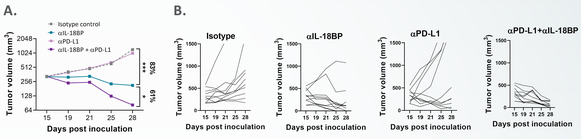
A. COM503 displaced pre-complexed IL-18-IL-18BP to increase IFNγ and TNFα release by stimulated human CD8+ TILs in TILs- tumor cells co-culture assay in the presence of rIL-18BP and rIL-18. **B.** COM503 displaced pre-complexed IL-18-IL-18BP to increase IFNγ release by NK cells in the presence of IL-12. **C.** COM503 increased TNFα, IFNγ, IL-2 and Granzyme B (GZMB) release by human tumor dissociated cells (TDCs). Surgically removed cancer specimens were dissociated into single cell suspension and cultured with anti-CD3/anti-CD28 mAbs in the presence or absence of COM503 (10ug/ml), anti-PD1 (pembrolizumab, 10ug/ml) or both for 72 hours, after which cytokines and granzyme B secretion were measured in supernatants. Representative example from a human ovarian TDC sample is shown.

Anti-mouse IL-18BP antibody demonstrates monotherapy activity across murine syngeneic tumor models



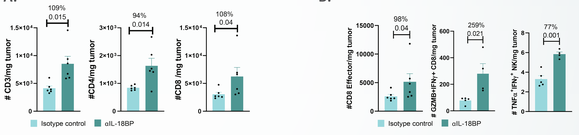
A-C. Anti-mouse IL-18BP Ab inhibited tumor growth in E0771 orthotopic mouse breast tumor model (A) MC38ova mouse CRC tumor model (B) and B16F10-hmgp100 mouse melanoma tumor model (C). Tumor volumes are represented as the Mean volume + SEM. Treatment initiated at tumor volume of 120mm³ and 270mm³ for MC38ova and E0771 respectively, and on day 4 for B16F10-hmgp100, and was injected twice a week for 6 doses.

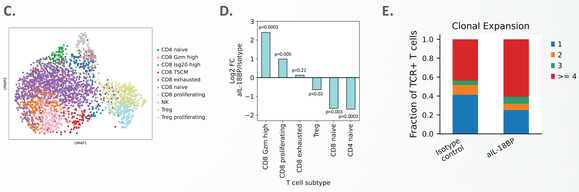
Anti-mouse IL-18BP antibody demonstrates combo activity with anti-PD-L1 Ab in E0771 tumor model



A. Anti-mouse IL-18BP Ab in combination with anti PD-L1 Ab inhibited tumor growth in E0771 mouse tumor model. **B.** Individual E0771 tumors measurements for each mouse are depicted. Tumor volumes are represented as the Mean volume + SEM. Treatment initiated at tumor volume of 270mm³ and was injected twice a week for 6 doses.

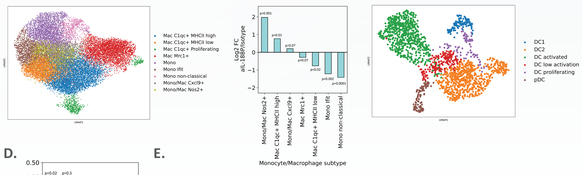
Anti-mouse IL-18BP Ab induces the expansion of polyfunctional non exhausted T cells in the TME

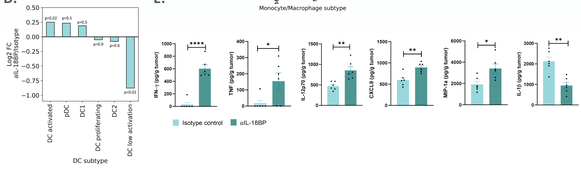




E0771 tumor cells were inoculated in C57Bl/6 mice and treated either with anti-mouse IL-18BP Ab or isotype control. TME modulation was assessed by flow cytometry, scRNA sequencing and cytokines profiling **A.** Anti-IL-18BP Ab increased CD3+, CD8+ and CD4+ T cell numbers in the tumor. **B.** Anti-IL-18BP Ab increased effector CD8+ T cells (CD44+CD62L-), as well as polyfunctional GZMB+IFNγ+CD8+ T cells, and TNFα+IFNγ+-secreting NK cells. **C-D.** Anti-IL-18BP Ab induced polyfunctional non exhausted T cells in the TME. **C.** UMAP projection showing T and NK cells present in E0771 tumors treated with anti-IL-18BP or isotype control. **D.** Enrichment of major T cell subpopulation frequencies in anti-IL-18BP Ab treatment was compared to the control group. **E.** Anti-IL-18BP Ab increased T cell clonal expansion suggesting Ag-specific immune response. Quantification of clonal expansion frequencies in anti-IL-18BP Ab treatment was compared to the control group in TCR+ T cells.

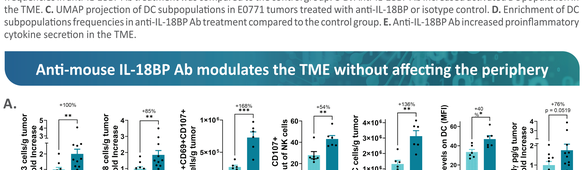
Anti-mouse IL-18BP Ab increases the expansion of proinflammatory myeloid populations and cytokine secretion in the TME

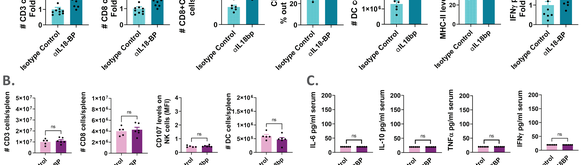




E0771 tumor cells were inoculated in C57Bl/6 mice and treated either with anti-mouse IL-18BP Ab or isotype control. TME modulation was assessed by flow cytometry, scRNA sequencing and cytokine profiling. **A-B.** Anti- IL-18BP Ab increased proinflammatory macrophages in the TME. **A.** UMAP projection showing tumor-associated monocyte and macrophage subpopulations present in E0771 tumors treated with anti-IL-18BP or isotype control. **B.** Enrichment of major tumor-associated monocytes and macrophages frequencies in anti-IL-18BP Ab treatment was compared to the control group. **C-D.** Anti-IL-18BP Ab increased activated DC population in the TME. **C.** UMAP projection of DC subpopulations in E0771 tumors treated with anti-IL-18BP or isotype control. **D.** Enrichment of DC subpopulations frequencies in anti-IL-18BP Ab treatment compared to the control group. **E.** Anti-IL-18BP Ab increased proinflammatory cytokine secretion in the TME.

Anti-mouse IL-18BP Ab modulates the TME without affecting the periphery





A-C. C57BL/6 mice were subcutaneously injected with MC38ova and were treated with anti-mouse IL-18BP Ab. Tumors, spleens and serum were harvested, and immune composition and cytokine concentrations were determined in the tumor microenvironment (A), in the spleen (B) and in the serum (C).

CONCLUSION

• IL-18, upregulated in the TME, is inhibited by IL-18BP, which is induced by IFNg in the TME • Blocking IL-18BP inhibits tumor growth as mono and in combination with anti-PD-L1 in murine cancer models • Immune modulation following treatment with anti-IL-18BP Ab is restricted to the TME suggesting favorable therapeutic window, in contrast to recombinant therapeutic cytokines that are active systemically • COM503, a human IgG4 high affinity anti-IL-18BP blocker Ab, releases IL-18 to activate T and NK cells • IND expected in 2024