Unleashing natural IL-18 activity using an anti-IL-18BP blocker antibody induces potent immune stimulation and anti-tumor effects

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IL-18 binding protein (BP) is a soluble high affinity inhibitor of IL-18 that is induced in response to IFNγ upregulation as part of a negative feedback mechanism to prevent excessive IL-18 immune-stimulatory activity. In the current study we computationally identified IL-18BP as potential immune-resistance mechanism in cancer and demonstrated that blocking IL-18BP is an attractive strategy to leverage natural IL-18 to induce potent anti-tumor activity. Initially we observed an upregulation of IL-18BP in tumor associated macrophages compared to resident macrophages. Moreover, IL-18BP levels were elevated in tumors with increased immune infiltration and were further induced following treatment with immune checkpoint blockers suggesting a potential immune resistance mechanism. Next, by evaluating 88 human tumor specimens and serum samples we were able to show that IL-18 was upregulated in the tumor microenvironment (TME, median 11.2ng/gr) compared to serum (median 0.3ng/ml), and that most of the IL-18 was bound by IL-18BP. Bound IL-18 levels were largely above the amount required for T cell activation *in vitro* (1.2ng/gr), implying that blocking IL-18BP has the potential to release IL-18 in tumors above the minimum range required for immune system stimulation. To assess whether tumor endogenous IL-18 levels released by IL-18BP blockade are sufficient to provoke anti-tumor responses, COM503, a high affinity (<1pM) anti-IL-18BP Ab, was generated and examined in T cell-based assays. In a co-culture assay of tumor cells with *ex vivo* stimulated human tumor infiltrating $CD8^+$ T lymphocytes, COM503 was able to displace IL-18 from a pre-formed complex and enhance IFNγ (197%, $p<0.01$) and TNFα (84% $p<0.01$) secretion. Furthermore, in human *ex vivo* dissociated tumor cells assay, COM503 increased Granzyme B (25%), IFNγ (38%),

TNFα (58%) and IL-12 (50%) production. *In vivo*, IL-18BP blockade resulted in potent anti-tumor responses and increased survival across multiple tumor models. In orthotopic E0771 tumor model, anti-IL-18BP Ab induced significant tumor growth inhibition (91% TGI, $p<0.0001$), as well as pronounced TME-localized immune modulation, including an increase in $CD8^+$ T cells expansion (108.5%, $p=0.015$) and activation, specifically in polyfunctional effector $IFN\gamma^+GrB^+CD8^+$ T cells (259%, $p=0.02$) and $IFN\gamma^+TNF\alpha^+$ NK cells (77%, $p=0.001$). Similarly, anti-tumor effects were shown in $MC38OVA^{dim}$ model (58% TGI, $p<0.001$), accompanied by a robust TME-localized immune modulation including increased $CD8^+$ T cells expansion (85%, $p=0.009$) and IFNγ secretion (76%, $p=0.052$). In contrast to immune modulation in the TME, no increase in inflammatory cytokines and lymphocyte numbers or activation state was observed in serum and spleen. Taken together, our data suggest that IL-18 is upregulated in the TME, mostly bound by IL-18BP, and could be leveraged to induce pronounced TME-localized immune modulation by anti-IL-18BP Ab treatment avoiding peripheral toxicity associated with recombinant cytokine administration. COM503 is currently undergoing IND-enabling studies.