Modulating the Tumor-Targeting Specificity of "Decoy-Resistant" Interleukin-18 by Protein Engineering

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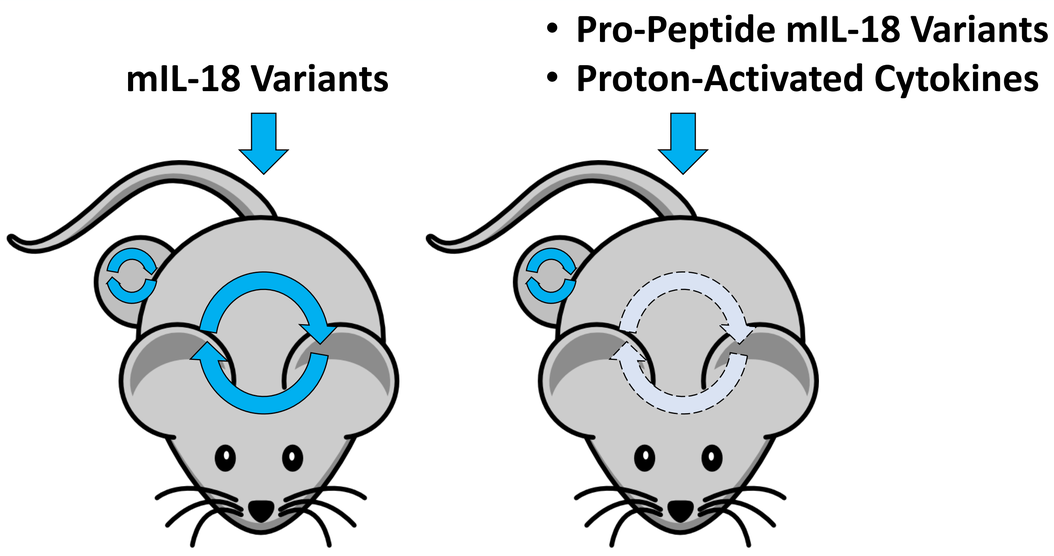
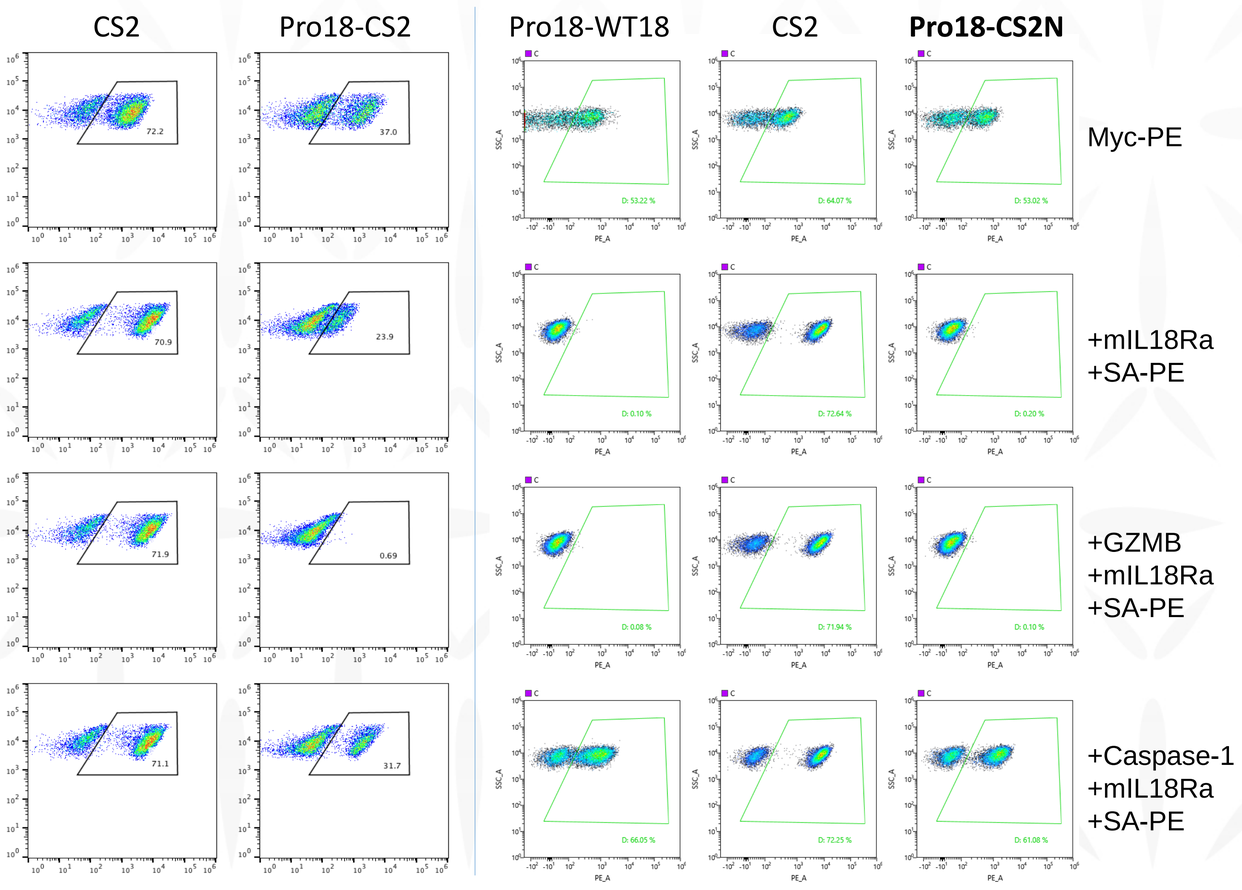
Introduction

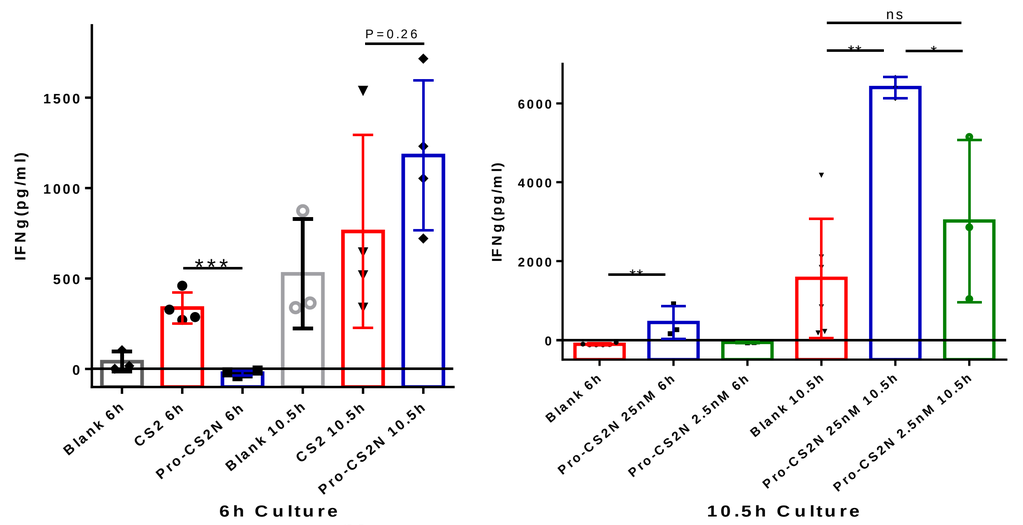
- Interleukin-18 (IL-18) has been taken into consideration as a therapeutic target and its role as a tumor suppressor has been widely identified.
- However, the previous clinical trials showed that IL-18 immunotherapy was not fully effective in the phase 2 evaluation. The upregulation of IL-18 Binding Protein (IL-18BP) should be responsible for this restraint of IL-18 efficacy.
- Our lab has previously utilized directed evolution developing a "Decoy-Resistant" IL-18 (DR-18; also named as CS2) that overcomes the soluble immune checkpoint of IL-18BP to unlock a potent cancer immunotherapeutic cytokine pathway. DR-18 variants are completely independent of IL-18BP and have improved the efficacy in tumor models dramatically.
- Here, we presented two assays, the pro-peptide assay and pH-sensitive assay, to further improve the tumor-targeting specificity of DR-18.

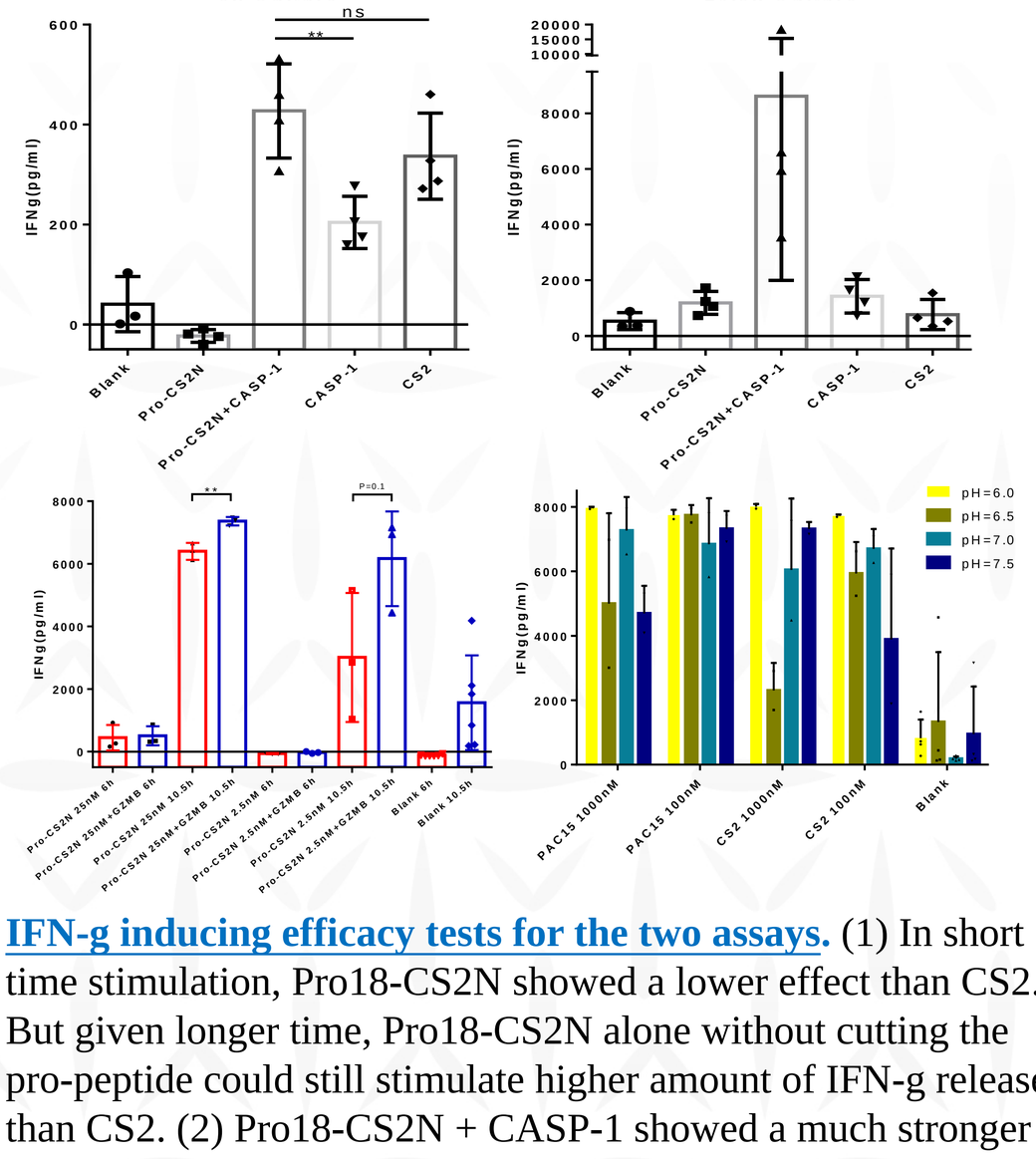


Rationale. (1) We aimed to add a pro-peptide to construct a pro-IL18 variant, given that there are more proteolytic enzymes (CASP-1, GZMB) at tumor sites to mature the inactive form of pro-IL18. (2)We previously constructed a low-pH favorable IL-18 variant, taking advantage of the relatively acidic extracellular environment (pH 6.0) at tumor sites.



Binding tests for the pro-peptide assay. (1) Pro-IL18 peptide partially blocked the interaction between CS2 and mIL18Ra, while totally blocked it between CS2N and mIL18Ra. (2) CASP-1 was effective to cut off the pro-peptide, while GZMB was ineffective in yeast binding tests. (3) Pro1a-mCS2 was as active as the mature form, while pro-IL1b peptide totally blocked the interaction between CS2(N) and mIL18Ra, even adding CASP-1 or GZMB.





IFN-g inducing efficacy tests for the two assays. (1) In short time stimulation, Pro18-CS2N showed a lower effect than CS2. But given longer time, Pro18-CS2N alone without cutting the pro-peptide could still stimulate higher amount of IFN-g release than CS2. (2) Pro18-CS2N + CASP-1 showed a much stronger inducing effect than CS2, especially in a longer-time cell culture. (3) GZMB had no effect in short-time stimulation. But given longer time, GZMB could partially increase IFN-g release. (4) There was no significant alteration between the pH-sensitive variant and mCS2, which also required us to further investigate it in *in vivo* experiments.

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