## 267 MRNAS ENCODING IL-12 AND A DECOY-RESISTANT VARIANT OF IL-18 SYNERGIZE TO ENGINEER T CELLS FOR EFFICACIOUS INTRATUMORAL ADOPTIVE IMMUNOTHERAPY

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**Background** Interleukin-12 (IL-12) is a potent immunotherapeutic cytokine in mouse models which application as a systemic agent in the clinical setting is hampered by IFNγ-dependent toxicity.[1] Engineering T cells with IL-12 is highly efficacious in mouse models[2] but has resulted in serious adverse events in clinical settings.[3] On the other hand, IL-18 is a myeloid-derived cytokine that elicits IFN-γ expression on T and NK lymphocytes.[4]

IL-12 and IL-18 are known to synergize in terms of eliciting massive IFNγ production.[5] For cancer immunotherapy, IL-18 has the caveat of being down-regulated in its function by a decoy receptor termed IL-18BP,[6] which is reportedly abundant in tumor tissues.[7,8] Recently, a mutant sequence of mouse IL-18 termed DRIL18 which preserves its bioactivity but lacks binding to IL-18BP has been reported to exert T-cell-dependent antitumor activity upon systemic delivery.[7]

We previously reported that transient engineering of tumor-specific CD8 T cells with IL-12 mRNA enhanced their systemic therapeutic efficacy when delivered intratumorally.[9] In this study, we sought to improve the therapeutic strategy of intratumoral delivery of T cells transiently engineered to express IL-12 with IL-18 mRNA electroporation.

**Methods** We mixed $CD8^+$ T cells (TCR transgenic, TILs and CAR-T) engineered with mRNAs to transiently express either single-chain IL-12 (scIL-12) or an IL-18 decoy-resistant variant (DRIL18) that is not functionally hampered by IL-18BP. $CD8^+$ T cells were injected repeatedly into mouse tumors for antitumor efficacy experiments. RNA-seq was performed to assess the functional changes induced after mRNA electroporation. Additionally, T-cell metabolic modifications and glycosylation profile functional changes were analyzed using seahorse and cell adhesion assays.

**Results** Pmel-1 TCR-transgenic T cells electroporated with scIL-12 or DRIL18 mRNAs exerted powerful therapeutic effects in local and distant melanoma lesions. These effects were associated with T-cell metabolic fitness, enhanced miR-155 control of immunosuppressive target genes, enhanced expression of various cytokines and unique changes in the glycosylation profile of surface proteins, enabling enhanced adhesiveness to E-selectin. Efficacy of this intratumoral immunotherapeutic strategy was recapitulated using other clinically relevant adoptive T cell therapies as tumor-infiltrating lymphocytes (TILs) and CAR T cells upon IL-12 and DRIL18 mRNA electroporation.

**Conclusions** We report on a substantial improvement of adoptive T-cell therapies strategy based on mRNA transient gene-transfer and repeated intratumoral delivery. The synergistic immunobiology of IL-12 and IL-18, best represented in the form of DRIL18, holds promise for efficacious outcomes in the treatment of metastatic cancer patients.

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### REFERENCES

1. Del Vecchio M, Bajetta E, Canova S, Lotze MT, Wesa A, Parmiani G, *et al*. Interleukin-12: Biological Properties and Clinical Application. *Clinical Cancer Research. American Association for Cancer Research*; 2007;**13**:4677–85.
2. Kerkar SP, Goldszmid RS, Muranski P, Chinnasamy D, Yu Z, Reger RN, *et al*. IL-12 triggers a programmatic change in dysfunctional myeloid-derived cells within mouse tumors. Journal of Clinical Investigation. *American Society for Clinical Investigation*; 2011;**121**:4746–57.
3. Zhang L, Morgan RA, Beane JD, Zheng Z, Dudley ME, Kassim SH, *et al*. Tumor-Infiltrating Lymphocytes Genetically Engineered with an Inducible Gene Encoding Interleukin-12 for the Immunotherapy of Metastatic Melanoma. *Clinical Cancer Research. American Association for Cancer Research Inc.*; 2015;**21**:2278–88.
4. Novick D, Kim S, Kaplanski G, Dinarello CA. Interleukin-18, more than a Th1 cytokine. *Seminars in Immunology*. Academic Press; 2013;**25**:439–48.
5. Chaix J, Tessmer MS, Hoebe K, Fuséri N, Ryffel B, Dalod M, *et al*. Cutting Edge: Priming of NK Cells by IL-18. *The Journal of Immunology. American Association of Immunologists*; 2008;**181**:1627–31.
6. Detry S, Andries J, Bloch Y, Gabay C, Clancy DM, Savvides SN. Structural basis of human IL-18 sequestration by the decoy receptor IL-18 binding protein in inflammation and tumor immunity. *Journal of Biological Chemistry*. Elsevier; 2022;**298**:101908.
7. Zhou T, Damsky W, Weizman O-E, McGeary MK, Hartmann KP, Rosen CE, *et al*. IL-18BP is a secreted immune checkpoint and barrier to IL-18 immunotherapy. *Nature*. Nature Publishing Group; 2020;**583**:609–14.
8. Carbotti G, Barisione G, Orengo AM, Brizzolara A, Airoldi I, Bagnoli M, *et al*. The IL-18 Antagonist IL-18–Binding Protein Is Produced in the Human Ovarian Cancer Microenvironment. *Clinical Cancer Research. American Association for Cancer Research;* 2013;**19**:4611–20.
9. Etxeberria I, Bolaños E, Quetglas JI, Gros A, Villanueva A, Palomero J, *et al*. Intratumor Adoptive Transfer of IL-12 mRNA Transiently Engineered Antitumor CD8+ T Cells. *Cancer Cell*. Cell Press; 2019;**36**:613-629.e7.

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