Background Cytokine-based drugs are being explored as alternative cancer immunotherapies. While the cytokine interleukin-18 (IL-18) has immunostimulatory effects, it is negatively regulated by a secreted high-affinity binding protein, IL-18BP, that functions as an immune checkpoint that limits IL-18’s therapeutic efficacy. A modified version of IL-18, termed ‘decoy-resistant’ or DR-18, that can avoid trapping by IL-18BP while maintaining immune signaling potential, has recently been developed. Here, we aim to test the efficacy and determine the cellular mechanism of action of DR-18 in combination with immune checkpoint inhibitors (ICIs) in immunocompetent preclinical models of renal cell carcinoma (RCC).

Methods We engrafted tumors subcutaneously using two different syngeneic, immunocompetent murine RCC models: Renca and RAG. Mice were treated with single-agent DR-18 and combinations of DR-18 with single- and dual-agent anti-PD-1 and anti-CTLA-4. Tumor growth and survival were monitored. In Renca, plasma was collected post-treatment and cytokine/chemokine levels were profiled using a 31-plex discovery assay. Single-cell RNA and TCR sequencing was also performed, and immune cell depletion studies were conducted.

Results In Renca, DR-18 monotherapy modestly inhibited tumor growth and prolonged survival (figure 1). The effects were comparable to single- and dual-agent ICIs. Adding PD-1 blockade to DR-18 did not enhance efficacy whereas the addition of anti-CTLA-4 to DR-18 significantly increased anti-tumor effects. Triple-therapy (DR-18 plus anti-PD-1 plus anti-CTLA-4) did not further inhibit tumor growth or prolong survival compared to the doublet (DR-18 plus anti-CTLA-4). The RAG model produced similar results, showing modest anti-tumor activity of single-agent DR-18 and enhanced benefit of combining with anti-CTLA-4 but not anti-PD-1. Cytokine/chemokine profiling revealed significantly elevated levels of IP-10 (CXCL10) and MIG (CXCL9) after one cycle of DR-18 plus anti-CTLA-4 compared to controls (figure 2). Single-cell transcriptomics demonstrated changes in intra-tumoral T cells, macrophages, and granulocytes with DR-18 plus anti-CTLA-4 relative to other regimens, including enrichment of CD8+ T cells, NK cells, and interferon-gamma as equally required for efficacy of DR-18 plus anti-CTLA-4 (figure 4).

Conclusions We identify DR-18, a ‘decoy-resistant’ IL-18, in combination with anti-CTLA-4 as having enhanced anti-tumor activity in preclinical models of RCC. This regimen was associated with a more pro-inflammatory immune microenvironment. Investigation is ongoing to further elucidate the cellular mechanism of action of this regimen and lay the groundwork for clinical testing of DR-18-based combination therapy in RCC.

REFERENCES

Ethics Approval This study was approved by the institutional IACUC (protocol #2023–20152) and all institutional guidelines were followed.
Abstract 1070 Figure 2  DR-18 plus anti-CTLA-4 strongly induces inflammatory cytokines/chemokines. (A) Schematic of treatment and sample collection timepoints for cytokine/chemokine profiling and scRNA-seq in the Renca model. (B) Heatmap of the natural logarithm of circulating cytokine/chemokine levels in mice for the indicated treatments and timepoints (n=3 mice/group, with the same mice collected at each timepoint), with unsupervised hierarchical clustering on the y-axis. Data were generated using Eve Technologies’ Murine Cytokine Array/Chemokine Array 31-Plex. (C) Volcano plots of the same data as in B, comparing circulating chemokine/cytokine levels with DR-18 + aCTLA-4 treatment (Combo) to PBS and highlighting the rapid and significant increases in IP-10 and MIG levels.

Abstract 1070 Figure 3  DR-18 alters immune subset composition in Renca tumors. Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction plot of clustering and annotation of (A) all cell populations and (C) T cell subsets isolated from Renca tumors treated for three cycles with PBS, DR-18, anti-CTLA-4, and DR-18 plus anti-CTLA-4 ('Combo') (n=3 mice/group, pooled) based on scRNA-seq analysis. Annotations were performed using SingleR (A) and ProjectTILS (C). Quantification of the percentages of the annotated cell populations for each of the Renca treatment groups, showing changes in granulocytes, macrophages/monocytes, and T cell populations (B) and certain T cell subsets (D). CD8_Tpex = CD8+ precursor exhausted; CD8_Tex = CD8+ terminally exhausted.
CD8+ T and NK cells and IFN-gamma are equally required for DR-18 plus anti-CTLA-4 efficacy. Kaplan-Meier survival curves of mice engrafted with Renca tumors and treated with control PBS or DR-18 plus anti-CTLA-4, either alone (PBS depletion) or with the indicated depleting/neutralizing antibody. Depleting/neutralizing antibodies were given 24 hours prior to every treatment, and twice weekly thereafter for the duration of the experiment. NK cells were depleted using anti-Asialo GM1.

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