includes CD8⁺ T cells driving tumor regression. Our data demonstrate that ATRC-101, bound to its target which is an RNP complex, can activate myeloid cells and are consistent with this activation occurring via FcR and Toll-like receptor (TLR) pathways.

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CD122-SELECTIVE IL-2 COMPLEXES TREAT OVARIAN CARCINOMAS, INDUCE TREG FRAGILITY AND PROMOTE T CELL STEM CELLS

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Background Ovarian cancer (OC) responds poorly to immunotherapies. Regulatory T cells (Treg) engage IL-2 by high-affinity CD25 for differentiation and function,¹ and anti-tumor effector T cells (Teff) use intermediate affinity CD122. We studied IL-2 complexes (IL-2c) that selectively activate CD122 (Teff) over CD25 (Tregs).

Methods Orthotopic ID8agg-luc mouse OC burden was measured by in vivo imaging. Tumor, ascites and draining lymph nodes (TDLN) were analyzed by flow and tSNE. IL-2c was complexed using 1.5 µg/mouse IL-2 and 7.5 µg/mouse aIL-2 (clone JES6-5H4) before i.p. injection every other day x 4 starting at day 7. antiPD-L1 was given at 100ug/mouse every 3 days x 4 starting from Day 11. FIR mice² were used to sort live Tregs.

Results IL-2c but not antiPD-L1 potently inhibits ID8agg (figure 1). IL-2c decreased ascites Treg functional markers (e.g., CD25, granzymeB) while upregulating the same markers on Teffs (figure 2). IL-2c inhibited Treg suppression in ascites while TDLN Tregs were unaffected (figure 3). tSNE showed great similarity of TDLN Tregs treated with isotype and IL-2c while ascites Tregs after IL-2c showed a fragile phenotype (e.g., increased PD-1, T-bet, and IFNγ with maintained FoxP3 expression [figure 4]) which is known to contribute to better response to cancer immunotherapy.³⁻⁴ We observed a complete reduction of tumor bioluminescence with IL-2c and antiPD-L1 combo treatment in nearly all subjects significantly exceeding effect of IL-2c alone (figure 5). A CD8⁺CXCR5

Abstract 690 Figure 1 IL-2c but not aPD-L1 treats ID8agg Luciferase signal of ID8agg-luc tumors treated with isotype, αPD-L1, or IL-2c measured by in vivo imaging. Arrows indicate treatments.

Abstract 690 Figure 2 IL-2c inhibits functional markers on tregs and promotes teff

Expression of CD25 and granzymeB were measured by flow cytometry in indicated population from isotype or IL-2c treated ascites 3 weeks after final IL-2c dose.

Abstract 690 Figure 3 IL-2c reduces ascites Treg suppressive function

+TCF-1+ T cell stem cell (TCSC) population reportedly improves immune checkpoint blockade efficacy.⁵⁻⁶ Since CD122 is regulated by TCF-1,⁷ we explored the effect of IL-2c on these TCSC. IL-2c significantly induced a CD8⁺TCF-1+ TCSC population in ID8agg tumors (figure 6), possibly through a positive feedback loop by further enhancing CD122 expression on TCF-1+, but not TCF-1- cells (figure 7). tSNE analysis of detailed immune phenotype of IL-2c induced TCSC revealed that these TCSC differed from those induced by antiPD-L1. In ID8agg, antiPD-L1-induced TCSC are mostly CXCR5⁺ and PD1⁺, consistent with previous reports in other cancers³⁻⁴ while IL-2c-induced TCSC were PD1- (figure 8), expressed CCR2 and CXCR3, and produced TNFα (figure 9).

Abstract 690 Figure 4 IL-2c induces Treg fragility in ascites but not TDLN tSNE analysis on CD45⁺CD3⁺CD4⁺FoxP3⁺ cells from ascites and TDLN of isotype and IL-2c treated ID8agg-luc challenged mice. Right, representative bar graphs of flow data.

Abstract 690 Figure 5 IL-2c and αPD-L1 combo treatment effectively cures ID8agg

Luciferase signal of ID8agg-luc tumors treated with isotype, αPD-L1, IL-2c or combo measured by in vivo imaging. Arrows indicate treatments.

Abstract 690 Figure 6 IL-2c induces a CD8+TCF-1+ population in ID8agg

ID8agg-luc tumors analyzed by flow cytometry gated on CD45+CD3+CD8+ cells.

Abstract 690 Figure 7 IL-2c induces CD122 on CD8+TCF-1+ but not TCF-1- cells

CD122 expression was measured by flow cytometry in CD8+TCF-1+ and CD8+ TCF-1- cells from tumors treated with isotype or IL-2c.

Conclusions We define two novel IL-2c effects: inducing Treg fragility therefore reducing immunosuppression while

Abstract 690 Figure 8 CD8+TCF1+ cells from ID8agg analyzed by flow cytometry plus tSNE. Cells expressing CXCR5 (blue) and PD1 (red) indicated in overlay

Abstract 690 Figure 9 CD8+TCF1+ cells from ID8agg analyzed by flow cytometry

promoting TCSC that could enhance effective anti-tumor immunity. Current work tests if effects are related and help efficacy, and mechanisms for IL-2c Treg effects. We also show that elicited TCSC differ by treatment and tumor, requiring additional investigations.

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Ethics Approval All mice studies were approved by UT Health San Antonio Institutional Animal Care and Use Committee (IACUC). Approval number 20150093AR, 20140001AR, 20170035AR, 20140039AR, 20140027AR, 20090128AR, 20120071AR, 20180021AR.

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