HIGH DOSE OF RHIL-10 ENHANCES CD8 T CELL AND NK CELL MEDIATED TUMOR CELL KILLING IN VITRO

1Elli Narvi, 2,3Janna Brunell, 1Anu Autio*, 1Aril Thotakura. 1Orion Corporation, Turku, Finland; 2University of Turku and Åbo Akademi University, Turku, Finland; 3University of Turku, Turku, Finland

**Background** Intratumoral activation and expansion of tumor-specific CD8 T cells is essential for successful immunotherapy. Interleukin-10 (IL-10) is known for its anti-inflammatory function, and at higher concentrations IL-10 activates cytotoxicity and enhances proliferation of CD8 T cells.1-4 In this study, we verified the presence of IL-10RA+CD8+ T cells in the tumor microenvironment (TME) and assessed the ability of high dose rhIL-10 to induce CD8+ T cell and NK cell proliferation and cytotoxicity in vitro.

**Methods** Healthy and cancerous tissue sections from various indications were stained for IL-10RA and CD8 co-expression. To study CD8 T and NK cell function, CD8 and NK cells were isolated from healthy donor PBMC. CD8 T cells were activated by anti-CD3+CD28 stimulation, and NK cells activated by rhIL-2+rhIL-15 stimulation, whereafter IL10RA expression was analysed by flow cytometry. After activation, CD8 and NK cells were co-cultured with prostate cancer cell line LNCaP, and imaged with Incucyte to assess functional properties, and IFNγ release was measured.

**Results** Tissue sections from cancer indications, including breast, brain, intestine, kidney, liver, lung, ovary, pancreas, and skin, have high number of CD8+ IL10-RA+ double positive cells compared to respective healthy tissues. However, only prostate cancer reached significantly higher double positive cells. Consistent with the published data, IL10-RA expression was upregulated in activated healthy donor CD8+ T cells (52 +/- 13% IL10-RA+CD8+ cells) in comparison to non-activated CD8+ T cells (7 +/-1% IL10RA+CD8+ cells). rhIL-10 dose-dependently induced CD8+ T cell proliferation and IFNγ release only in activated CD8+ T cells. In addition, GzmB was significantly elevated after 72 h of rhIL-10 treatment in CD8+ T cells. Activation of NK cells didn’t increase IL10RA expression. However, the receptor levels were already detectable at baseline (25% for both non-activated and activated). Addition of rhIL-10 in co-culture settings increased IFNγ release in activated CD8+ T cells (figure 1A). In killing assays, addition of rhIL-10 increased cancer cell killing in activated CD8+ and NK cells (figure 1B,C and 2B,C). Overnight incubation of rhIL-10 induced GzmB and IFNγ production in activated NK cells (figure 2A).

**Conclusions** Followed high dose of rhIL-10 treatment, activated CD8+ T cells and NK cells released cytotoxic cytokines and killed target cancer cells efficiently. This study adds further evidence to pre-existing research of IL-10 cytokine as a potential anti-tumoral treatment.

**REFERENCES**


**Abstract 1044 Figure 1** (A) High dose of rhIL-10 (100 ng/mL) induces further IFNγ release in activated CD8 T cells after 72 h co-culture with cancer cells (LNCaP) measured by ELISA. (B) High dose rhIL-10 (100 ng/mL) further enhances killing of prostate cancer cells (LNCaP stained in red) in IncuCyte assay Caspase Green at 24 h after co-culture. (C) Killing capacity increases over time in rhIL-10 treated CD8 T cell group measured as apoptotic tumor cells over time.

**Abstract 1044 Figure 2** (A) High dose of rhIL-10 (100 ng/mL) induces further Granzyme B activation in activated NK cells measured by flow cytometry. (B) High dose rhIL-10 (100 ng/mL) further enhances killing of prostate cancer cells (LNCaP stained in red) in IncuCyte assay Caspase Green at 24 h after co-culture. (C) Killing capacity increases over time in rhIL-10 treated NK cell group measured as apoptotic tumor cells over time.

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