In an effort to identify effective target combinations, molecules specific for several immuno-oncology targets were combined in vitro using a mixed leukocyte reaction (MLR) assay. Briefly, 20 different healthy human peripheral blood mononuclear cell donor pairs were tested for proliferation and cytokine release in the presence of each molecule alone and in combination. The results from this study are significant (p<0.05) synergy as well as antagonism and additivity were observed. In addition the phenotype and genotype of the MLR donor peripheral blood mononuclear cells (PBMC) were characterized. The results from this study and significant for at least two reasons: 1) In contrast to murine syngeneic models they represent aspects of multiple different human donor "immune responses" and 2) In contrast to purified T-cell assays they represent an assay system in which both the lymphoid and myeloid immune compartments play a crucial role, and so both recapitulate a physiological T-cell response. Furthermore, these results provide information at the human population level, offering a glimpse of "responder" and "non-responder" population profiles. This information is nearly impossible to obtain from the currently available collection of syngeneic mouse models.

**Background**

- CD73, an ecto-5'-nucleotidase, is highly expressed in many human solid tumors and, together with CD90, converts extracellular adenosine tri-phosphate (ATP) to adenosine (reviewed by Linden and Celic, 2012).
- Adenosine enhances polarization of myeloid and T cell subsets to immuno-suppressive phenotypes, promoting tumor growth and survival (Antonioli et al., 2013).
- Targeting CD73 relieves adenosine-mediated immunosuppression within the tumor microenvironment (Antonioli et al., 2013).

**MEDI9447 is a monoclonal antibody that inhibits CD73 enzyme activity (see poster numbers 1538 and 285).**

**Method**

PBMCs from two individual healthy donors were mixed together 1:1 into 96-well sub-plate at 200K cells per donor well in serum-free AIM-V medium. After 72h with the indicated treatments, cells were imaged and supernatants were assayed for cytokine secretion using an ELISA.

**Comparison of Cell Subsets in the MLR**

**Figure 2.** PBMCs from two individual healthy donors were mixed together 1:1 into 96-well sub-plate at 200K cells per donor well in serum-free AIM-V medium. After 72h with indicated treatments, cells were imaged and supernatants were assayed for cytokine secretion using an ELISA.

**Figure 3.** The MLR reflects in vivo data for CD73 + PD-1 combinations.

**Figure 4.** MEDI9447 and antibodies to PD-1, CTLA-4, and CD80 were incubated with naïve T cells and the indicated number of CD80+ and CD80- cells.

**Figure 5.** The MLR reflects in vivo data for CD73 + PD-1 combinations.

**Figure 6.** In a publication by Allard et al. (2013) the authors used an antibody blocking assay with different effector:target ratios using a control IgG or 10 µg/ml isotype control. Using an IFN-γ ELISA it was shown that IgG was not sufficient for 30% (p<0.01) upregulation of IFN-γ. The authors demonstrated that, using an antibody blocking assay, 1 µg/ml MEDI9447 resulted in 60% upregulation of IFN-γ while 10 µg/ml MEDI9447 produced 90% upregulation of IFN-γ. Using an antibody blocking assay, the authors demonstrated that, using an antibody blocking assay, 1 µg/ml MEDI9447 resulted in 60% upregulation of IFN-γ while 10 µg/ml MEDI9447 produced 90% upregulation of IFN-γ.

**Conclusion**

- Targeting CD73 impacts both the lymphoid and the myeloid compartments.
- Combining MEDI9447 and other IFT agents show synergy.
- The MLR provides a model of various components of the tumor microenvironment and reflects aspects of human mouse tumor biology.

**References**

- Allard et al., 2013; Immuno-competent mice were dosed with anti-CD73, anti-PD-1, anti-CTLA-4 and the indicated MEDI9447 concentrations. Using an IFN-γ ELISA it was shown that IgG was not sufficient for 30% (p<0.01) upregulation of IFN-γ. The authors demonstrated that, using an antibody blocking assay, 1 µg/ml MEDI9447 resulted in 60% upregulation of IFN-γ while 10 µg/ml MEDI9447 produced 90% upregulation of IFN-γ.
- The MLR provides a model of various components of the tumor microenvironment and reflects aspects of human tumor biology.