Targeting PD-1, TIM-3 and LAG-3 in Combination for Improved Immunotherapies Combination

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Combination of Anti-LAG-3 and Anti-PD-1 in the MC38 Syngeneic Tumor Model Generates a Durable Anti-Tumor Response

- These studies explore the potential for immunotherapy targeting the immune checkpoints TIM-3 and LAG-3 in combination with anti-PD-1
- Combination studies with antibodies to mouse PD-1, LAG-3 and TIM-3 have been performed in the MC38 syngeneic tumor model
- Chimeric R-mouse antibodies to mouse PD-1 or LAG-3 have been generated and tested as both IgG2a (with effector function) and IgG1 D265A (without effector function) isotypes
- Potential antibodies to human PD-1, LAG-3 and TIM-3 have been generated and evaluated for their ability to activate T cells in vitro both alone in combination

Characterization of Intra-tumoral T cells and PD-1 Expression by IHC (syngeneic Tumor Model)

Activity in Syngeneic MC38 Tumor Model

- Antibodies combining mouse PD-1, IgG2a human PD-1 and mouse TIM-3 in various permutations (mouse PD-1alone, mouse PD-1 + human PD-1 and mouse PD-1 + mouse TIM-3) were evaluated in MC38 tumor-bearing mice. Mice were randomized to receive the antibodies every 4 days for 30 days. PD-1-combining antibodies showed tumor regression and improved survival. Sustained tumor regression and improved survival with mouse PD-1 + human PD-1 alone were observed in subgroups with intratumoral T cell death, I 

Antibodies to Human TIM-3 and LAG-3 Demonstrate Potent Activity in a Dendritic Cell / T Cell Mixture and Have Increased Activity in Combination with Anti-PD-1 and With Each Other

-Tumor responses with antibodies targeting PD-1, LAG-3 and TIM-3 were examined in different permutations of PD-1, LAG-3 and TIM-3. Anti-PD-1 and anti-LAG-3 antibodies showed tumor regression and improved survival in all models. Human TIM-3 antibodies showed increased activity in combination with anti-PD-1 and anti-LAG-3.

- Tumor responses were evaluated across combinations of antibody treatments. Tumor responses were observed with all antibody combinations, with the most potent responses observed with the combination of anti-PD-1, anti-LAG-3 and human TIM-3.

- Combination of antibodies targeting PD-1, LAG-3 and TIM-3 resulted in improved tumor regression and increased survival compared to single antibody treatments. The combination of anti-PD-1, anti-LAG-3 and human TIM-3 showed the most potent activity across all models.

- The results suggest that targeting multiple immune checkpoint molecules simultaneously may offer increased efficacy in immune checkpoint inhibition therapy.

**Introduction**

- Introduction of antibodies to PD-1, LAG-3 and TIM-3 have been performed in the MC38 and CT26 tumors to target the tumor cell's response to anti-PD-1, anti-LAG-3 and anti-TIM-3 antibodies. While each antibody alone showed some degree of efficacy in model systems, combinations of antibodies targeting different immune checkpoints may provide increased therapeutic benefit.

**Combination of Anti-TIM-3 and Anti-PD-1 in the MC38 Syngeneic Tumor Model Shows Improved Anti-Tumor Responses**

- Anti-PD-1 and anti-TIM-3 antibodies alone showed some degree of tumor regression in the MC38 syngeneic tumor model. However, when used in combination, the anti-PD-1 and anti-TIM-3 antibodies showed improved tumor regression compared to each antibody alone.

**Engineering of Chimeric Rat/Mouse Surrogate Antibodies**

- Antibodies from different species, such as rat and mouse, can be engineered to have improved effector function or other desired properties.

**Multiple Immune Checkpoint Molecules are Co-Expressed on Activated T Cells**

- Human donor CD8+ or CD4+ T cells were isolated and activated using plate-bound anti-CD3 and soluble anti-CD28 antibodies. After 48 hours, the T cells were stained for PD-1, LAG-3 and TIM-3. Increased levels of PD-1, LAG-3 and TIM-3 were detected on activated T cells compared to unstimulated T cells. Anti-PD-1, TIM-3 and LAG-3 double-positive cells were observed after activation compared to unstimulated T cells. Anti-TIM-3 double-positive cells were not assessed in this experiment.

**Conclusion**

- Surrogate antibodies to mouse PD-1 in combination with antibodies to mouse TIM-3 or LAG-3 demonstrate potent and durable anti-tumor activity in syngeneic tumor models
- A variant of anti-mouse PD-1 without effector function has improved efficacy over an isotype with effector function, potentially as a result of depletion of effector cells
- Potential antibodies to human PD-1, TIM-3 and LAG-3 have been generated that enhance in vitro T cell activation alone and in combination
- The data suggest that targeting multiple immune checkpoint molecules is a promising approach for improving efficacy of immunotherapy