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Antibodies Targeting Resokine, a Soluble Immune Modulator, Inhibit Tumor Growth in Syngeneic Mouse Models

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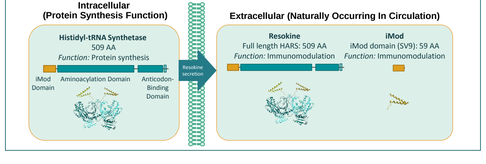
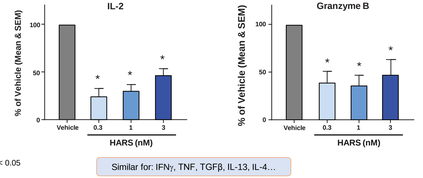
Abstract

A number of non-canonical functions have been established for proteins generated from the tRNA synthetase gene family. One of these, termed Resokine, is derived from histidyl-tRNA synthetase and plays an important role in controlling immune cell activation. Circulating levels are sufficient to down-regulate the extent of T cell activation that can be achieved in vitro. A panel of specific monoclonal antibodies has been generated and tested for their anti-tumor activity in mouse syngeneic tumor models. Antibodies to Resokine demonstrated anti-tumor activity across three different tumor models. Treatment of subcutaneous CT26 tumors resulted in improved efficacy compared to treatment with antibodies that block the PD-1/PD-L1 interaction. Significant efficacy was also observed in the difficult to treat subcutaneous B16F10 melanoma and 4T1 breast tumor models. In addition, anti-Resokine demonstrated significant activity in a tumor seeding model using B16F10 melanoma, which resulted in inhibition of tumor nodules in the lung, and was more efficacious than a combination of antibodies to PD-L1 and CTLA-4. Combinations of anti-Resokine antibody with either anti–PD-1 or anti–PD-L1 demonstrated at least additive, and potentially synergistic activity in these models. Animals with long-term tumor regressions were reimplanted with viable tumor cells, and demonstrated long-term immune memory with rejection of the newly implanted tumors. To understand the mechanism of anti-Resokine antibody therapy, cell depletion studies were carried out in the B16F10 tumor model. In these experiments, the activity of anti-Resokine antibodies was demonstrated to be dependent upon the presence of CD8 T cells and also NK cells, but independent of CD4 T cells. The immune-based mechanism of antibodies to Resokine was further demonstrated by rechallenge of mice that had regressed tumors upon treatment. Tumor regrowth was not observed even in the absence of further treatment whereas control mice grew tumors at the normal rate, suggesting that immune memory had been induced. Antibodies to Resokine offer an exciting new potential option for immunotherapy of cancer, which has significant activity as monotherapy and is compatible with more established modalities. Anti-Resokine antibodies are currently being developed to initiate clinical evaluation.

Resokine Proteins: Extracellular Histidyl-tRNA Synthetase Gene Products With Immune Modulation Activity



Resokine Reduces Cytokine and Granzyme B Release During T Cell Activation



*p < 0.05

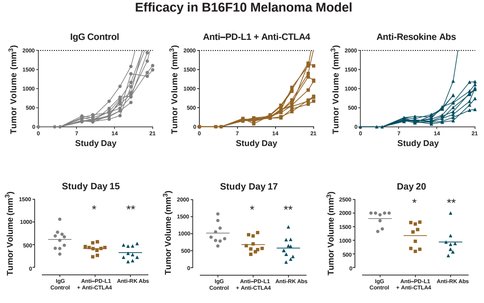
Similar for: IFNγ, TNF, TGFβ, IL-13, IL-4…

- Histidyl-tRNA synthetase is released from cells and is present in systemic circulation (Adams *et al*., AACR 2018).
- Cancer patients have higher serum levels of Resokine compared to healthy subjects.
- Resokine functions to inhibit T cell activation.

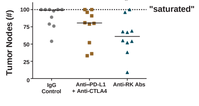
Hypothesis: Resokine restrains immune cell function in cancer and antibodies binding to Resokine will release the inhibition of the immune system leading to therapeutic benefit.

Antibodies to Resokine Have Anti-Tumor Activity in Three Different Syngeneic Tumor Models

Efficacy in B16F10 Melanoma Model



B16F10 Melanoma Tumor Seeding



Antibodies or controls were administered on day -1, 6, and 13

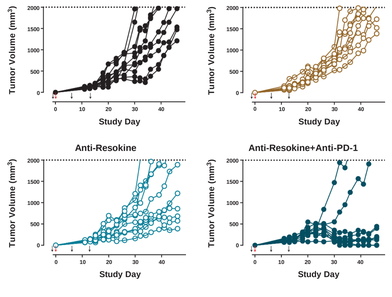
Top panel: Individual tumor volumes (tumor cells implanted subcutaneously on day 0)

Middle panel: Tumor volumes on respective study days (note that tumors exceeding the cutoff of 2,000 mm³ are represented at 2,000 mm³)

Left panel: Tumor nodules counted in lungs harvested 18 days after intravenous tumor cell injection

*p < 0.05; **p < 0.01, 1-way ANOVA followed by Dunnett's post hoc test

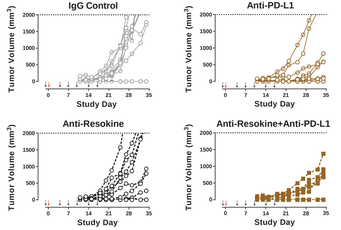
Efficacy in 4T1 Breast Cancer Model



Red arrows indicate tumor cell implantation

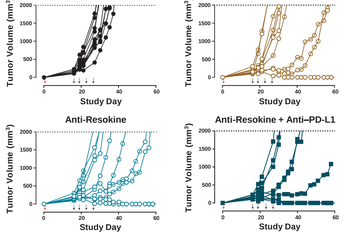
Black arrows indicate antibody administration

Efficacy in Colon Tumor CT26 Model (Prophylactic Dosing)



Antibodies or controls were administered twice weekly beginning on Day -1 (tumor cells implanted subcutaneously on Day 0)

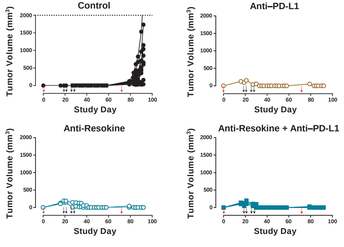
Efficacy in CT26 Colon Tumor Model (Therapeutic Dosing)



Antibody administration was begun when tumors reached a volume of 90-300 mm³. Note the larger number of animals with regressed tumors in groups receiving anti-Resokine antibodies vs. anti–PD-L1 alone.

Complete tumor regressions	
IgG Control	0
Anti–PD-L1	1
Anti-Resokine	2
Anti-Resokine + Anti–PD-L1	4

Tumor Rechallenge With No Therapeutic Agent Administered



On day 72, age-matched previously naive controls or animals with regressed tumors from the therapeutic dosing experiment were challenged with CT26 cells implanted on the opposite (left) flank from the first tumor. Tumors grew in all of the previously naive animals. Tumor growth was observed in several tumor experienced animals, but were subsequently regressed, substantiating that tumor memory had been established during previous treatment.

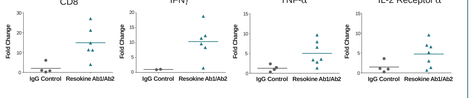
Red arrows indicate tumor cell implantation

Black arrows indicate antibody administration

Anti-Resokine Antibodies Harness an Immune-Based Mechanism

Upregulation of Inflammatory Markers, and Enhanced T Cell Infiltration at the Tumor Site

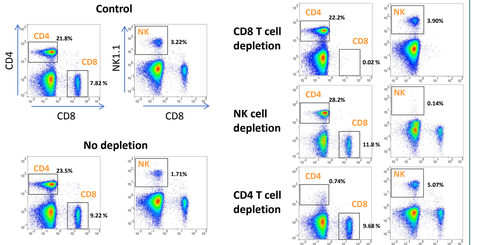
qPCR Analysis of Residual B16F10 Tumors



Gene expression measured in residual tumors from the B16F10 melanoma model experiment shown (at left).

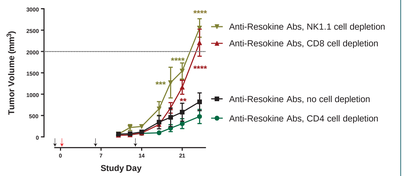
RNA was prepared and gene expression measured using standard methods on a Fluidigm panel. Data from samples with RIN quality scores ≥ 6.9 are reported.

Successful Depletion of Targeted Cell Populations in Tumor-Bearing Animals



Efficacy of Anti-Resokine Abs Dependent on Both CD8 T Cells and NK Cells

B16F10 Melanoma Study



Depletion of CD8+ T cells or NK cells (confirmed by FACS) abolishes activity of anti-Resokine antibodies, suggesting that anti-Resokine–mediated tumor suppression is dependent upon CD8-positive effector T cells and NK cells

Top Panel: Cells in whole blood were stained with labeled antibodies specific to NK1.1, CD3, CD4, or CD8 (clones PK136, 17A2, RM4-5, 53-6.7, respectively). Cell counts were acquired on a MACSQuant 2582.

Bottom Panel: Red arrow indicates tumor cell implantation. Black arrows indicate anti-Resokine antibody administration. Depletion antibodies (anti-CD4, anti-CD8, or anti-NK1.1) were dosed twice weekly

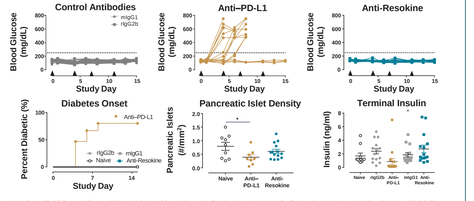
**p < 0.01; ***p < 0.001; ****p < 0.0001, 2-way ANOVA followed by Dunnett's test.

Acknowledgments

Many of the data presented here were generated at Washington Biotechnology (http://www.washingtonbiotech.com). Their efforts and those of Lisa Eide, Angela Gentile, Matt Seikkula, and Erica Wood are greatly appreciated.

The Resokine Pathway Modulates Disease Induction in a Model of Autoimmune Diabetes

Anti-Resokine Antibodies Do Not Provoke Autoimmune Diabetes



Interruption of inhibitory cell-to-cell interactions with a PD-L1 antibody that prevents binding to both PD-1 and B7-1 (clone 10F.9G2) precipitates diabetes in a T cell–specific manner (Paterson et al., 2011, *J Immunol*).

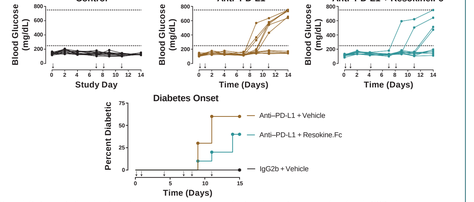
Upper panels: Glucometer readings (AlphaTRAK) measured in female NOD mice receiving Control rIgG2b, Control mIG1, anti–PD-L1, or anti-Resokine.

Lower left: Diabetes was defined as a measurement of > 250 mg/dL. Mice receiving anti–PD-L1 antibodies developed diabetes, whereas mice receiving controls or anti-Resokine antibody did not.

Lower middle: Pancreatic islet density was defined by counting islets on H&E sections and dividing by the 2-dimensional area of the section.

Lower right: Terminal insulin levels were measured by a commercial ELISA. Mice receiving anti–PD-L1 antibodies were insulinopenic compared to animals receiving anti-Resokine antibodies (1-way ANOVA followed by Dunnett's post hoc test).

Exogenous Resokine Delays Anti–PD-L1 Provoked Autoimmune Diabetes



Upper left: Administration of control antibodies plus vehicle have no effect on glucometer readings from female NOD mice.

Upper middle: Administration of anti–PD-L1 antibodies plus vehicle results in hyperglycemia in > 50% of animals, starting 9 days after the initiation of dosing paradigms.

Upper right: Administration of Resokine.Fc, an engineered protein comprised of the immunomodulatory domain of human Resokine fused to human IgG1 Fc and expressed in bacteria, decreased the number of animals that became diabetic when exposed to anti–PD-L1 antibodies.

Lower panel: Survival plot summarizing the data in the other panels of the figure, suggesting that Resokine can slow the onset of type 1 diabetes provoked by anti–PD-L1 administration.

Diabetes Model Conclusions

- Anti-Resokine antibodies did not precipitate autoimmune diabetes in female NOD mice, suggesting a mechanism distinct from blockade of inhibitory cell-to-cell signals.
- Administration of Resokine protein delays and/or inhibits onset of T cell–driven diabetes, confirming the immune inhibitory activity of the pathway in the model.

Conclusions

- Anti-Resokine antibodies slow tumor growth in 3 syngeneic models
  - B16F10 melanoma
  - 4T1 breast cancer
  - CT26 colon cancer
- Anti-Resokine antibodies decrease tumor seeding in lungs after intravenous administration of B16F10 melanoma cells.
- Evidence for an immune-based mechanism
  - Upregulation of inflammatory markers and enhanced T cell infiltration at the tumor site
  - Depletion of CD8-positive effector T cells or NK cells decreases activity
  - Long-term immune memory generated by ORCA ab treatment
- The Resokine pathway plays a role distinct from PD-L1 in a model of autoimmune diabetes.
- Anti-Resokine antibodies are currently being developed to initiate clinical evaluation.

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