Monoclonal Antibodies Targeting the MerTK Receptor De-repress the Innate Immune Response

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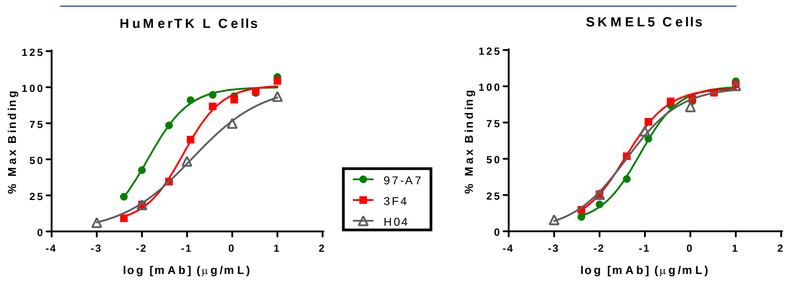
MerTK Background

- MerTK is a member of the TAM (Tyro3/Axl/MerTK) family of receptor tyrosine kinases (RTKs), expressed predominately in innate immune cells (macrophages, dendritic cells and NK cells).
- Activation of MerTK in myeloid cells by its ligands Gas6 or Protein S (PROS) promotes phosphatidylserine-dependent efferocytosis of apoptotic cells, inducing a tolerogenic state and mediating resolution of inflammation.
- MerTK deficient mice exhibit systemic inflammation and auto-immunity, phenotypes consistent with its role as a negative regulator of immune responses.
- MerTK ablation confers tumor immunity in multiple tumor models, increased pro-inflammatory cytokines and tumor lymphocyte infiltration.
- **We describe the discovery and characterization of anti-human and anti-mouse MerTK mAbs that elicit secretion of pro-inflammatory cytokines in vitro and in vivo, and enhance the activity of PD-1 inhibitors.**
- **msMerTK knockout and huMerTK transgenic mice have been generated to establish proof-of-concept with anti-huMerTK mAbs in vivo.**

Anti-MerTK mAb Discovery and Characterization

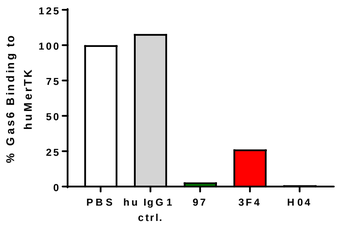
- Phage-display and mouse immunization strategies were utilized to generate >100 monoclonal antibodies against MerTK.
  - Anti-human MerTK mAbs 97-A7 and 3F4 were selected on the basis of their potent binding to huMerTK, and ability to stimulate pro-inflammatory cytokine release from primary human immune cells.
  - H04 was selected as a potent and selective MerTK binding mAb that does not elicit significant cytokine responses.

mAb Binding to Human MerTK Expressing Cells



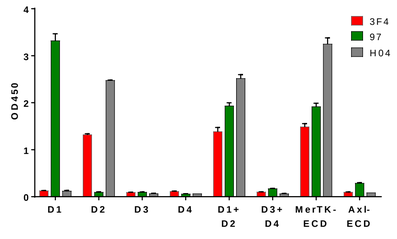
- Anti-MerTK mAbs bind to cell surface-expressed MerTK with picomolar apparent affinity, as measured by flow cytometry.

mAbs Differentially Block MerTK:Gas6 Interactions



- mAbs partially (3F4) or completely (97 and H04) block binding of the human MerTK extracellular domain (huMerTK-ECD) to Gas6 immobilized on ELISA plates.
  - 97 is a lower affinity precursor to mAb 97-A7

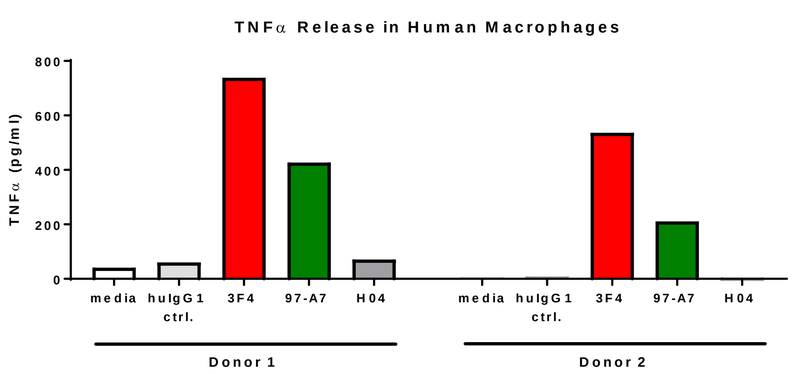
mAb Binding to MerTK Domains



- ELISA-based binding of anti-MerTK mAbs to immobilized purified MerTK domains, MerTK extracellular domain (MerTK-ECD), and Axl-ECD.

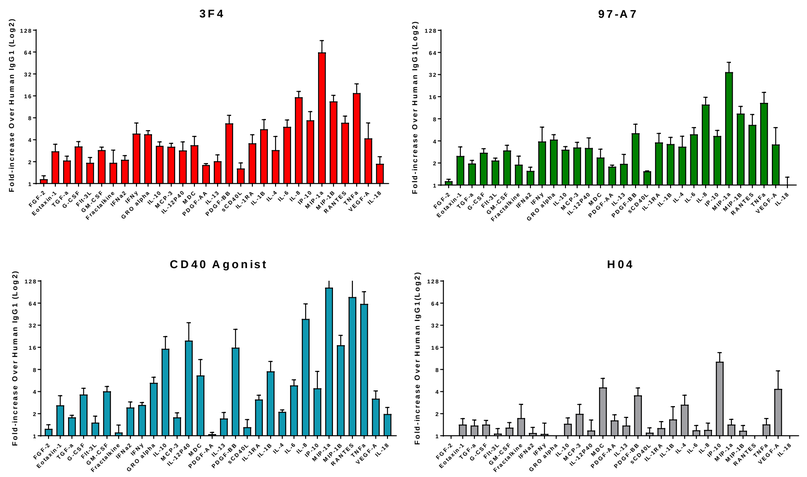
mAb	Origin	Isotype	Affinity			Cell Binding EC50	MerTK Domain	Gas6 Blocking
			K_D (nM)	Kon (1/Ms)	Kdis (1/s)			
3F4	Harbour Mouse	Human IgG1	0.29	5.07 E+05	1.49 E-04	picomolar	2	Partial
97-A7	Phage display	Human IgG1	43	2.45 E+05	1.06 E-02	picomolar	1	Complete
H04	Phage display	Human IgG1	117	1.2 E+05	1.4 E-02	picomolar	2	Complete

Anti-MerTK mAbs Engage MerTK in Human Macrophages to Induce TNF-α Release



- Anti-MerTK mAbs 3F4 and 97-A7, but not H04, induced a robust increase in TNF-α release.
  - MerTK is robustly expressed in monocyte-derived human macrophages
  - Monocyte-derived human macrophages were differentiated from PBMCs with 50 ng/mL of M-CSF, and were treated with 100 nM of mAbs.

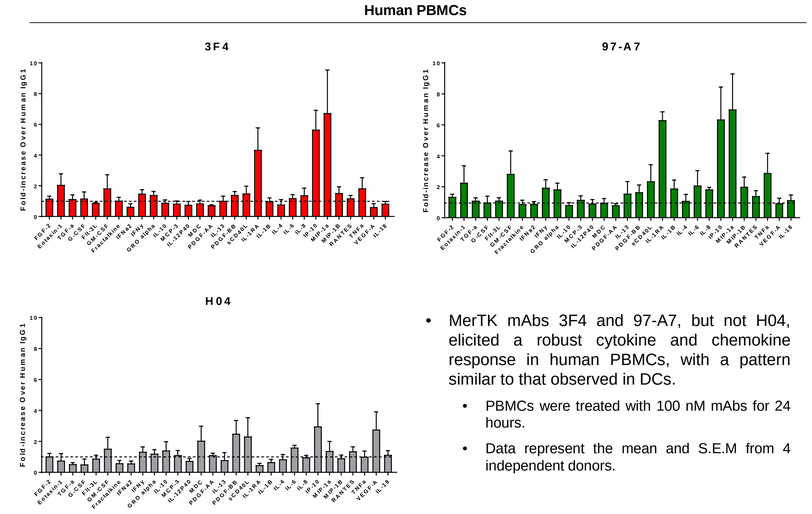
Anti-MerTK mAbs Elicit Potent Cytokine and Chemokine Responses in Human Monocyte-Derived Dendritic Cells



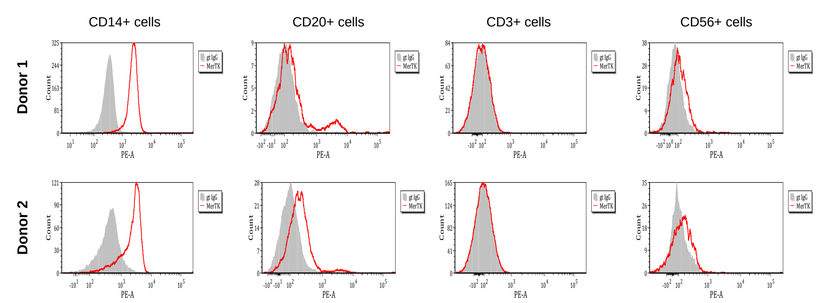
- Anti-MerTK mAbs 3F4 and 97-A7 elicited a strong cytokine and chemokine response similar in pattern and magnitude to an anti-CD40 agonistic mAb.
  - Monocyte-derived dendritic cells were treated with 100 nM of anti-MerTK mAbs, or the CD40 agonistic mAb CDX-1140, for 24 hours.
  - Released cytokine and chemokine levels were measured from conditioned media, and normalized to control human IgG-treated samples.
  - Data represent the mean and S.E.M from 4 independent donors.
- Anti-MerTK mAb H04, which binds strongly to huMerTK, weakly modulated cytokine secretion, suggesting that mAb activity is epitope-dependent.

Anti-MerTK mAbs Modulate Cytokine Responses in CD14+ Monocytes

Human PBMCs

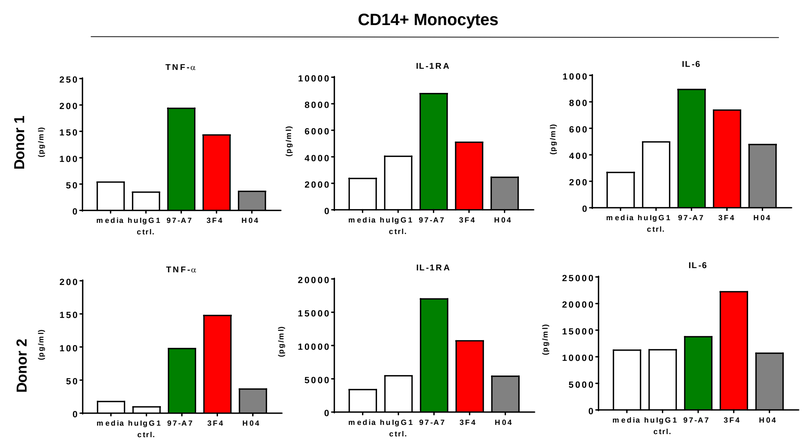


- MerTK mAbs 3F4 and 97-A7, but not H04, elicited a robust cytokine and chemokine response in human PBMCs, with a pattern similar to that observed in DCs.
  - PBMCs were treated with 100 nM mAbs for 24 hours.
  - Data represent the mean and S.E.M from 4 independent donors.



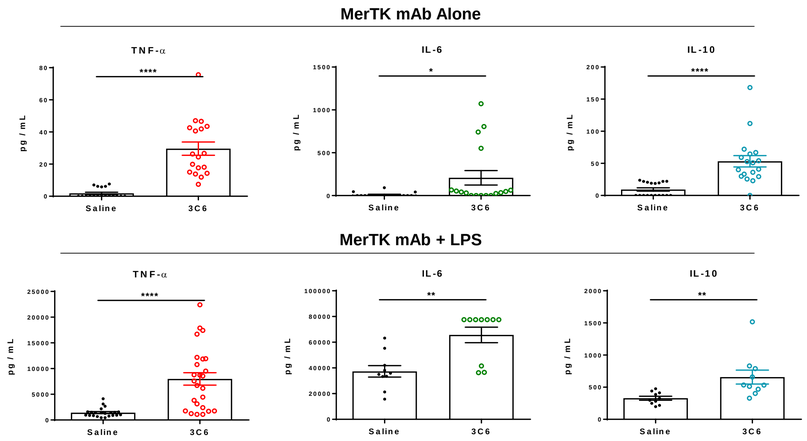
- MerTK is highly expressed in CD14+ monocytes, moderately expressed in CD20+ B cells and CD56+ NK cells, and not detectable in CD3+ T cells.

CD14+ Monocytes



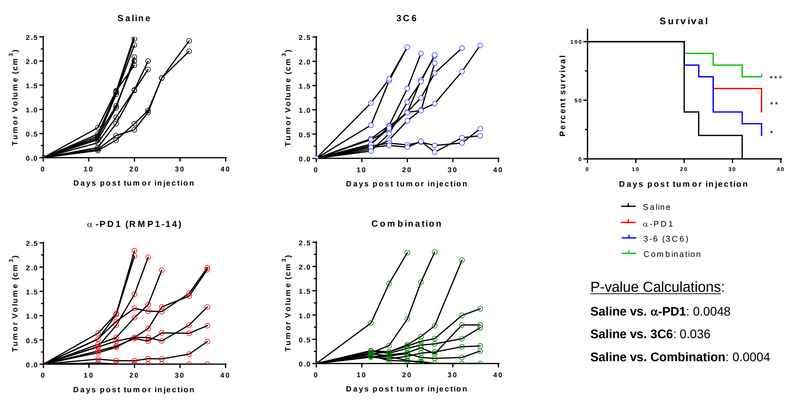
- Anti-huMerTK mAbs modulate MerTK in CD14+ monocytes to induce a pro-inflammatory response.
  - CD14+ monocytes were enriched from donor PBMCs by depleting CD3+, CD19+, and CD56+ cells.
  - mAb-dependent release of TNF-α, IL-1RA , and IL-6 was enhanced in CD14+ monocytes.

A Surrogate Anti-Mouse MerTK mAb Elicits Cytokine Responses In Vivo



- mAb 3C6 induced acute cytokine responses as a single agent, and enhanced LPS-dependent cytokine responses.
  - C57BL/6 mice were dosed i.p. with mAb 3C6, a murine anti-mouse MerTK IgG2b mAb.
  - Mice were dosed with 3C6 alone, or 1 hour prior to administration with 20 μg of LPS.
  - Serum was collected 1 hour (top panel) or 2 hours (bottom panel) after mAb administration, cytokines were measured by Meso Scale Discovery electrochemiluminescence.

A Surrogate Anti-MerTK Mouse mAb Demonstrates Antitumor Activity Alone and in Combination With a PD-1 Inhibitor



P-value Calculations:

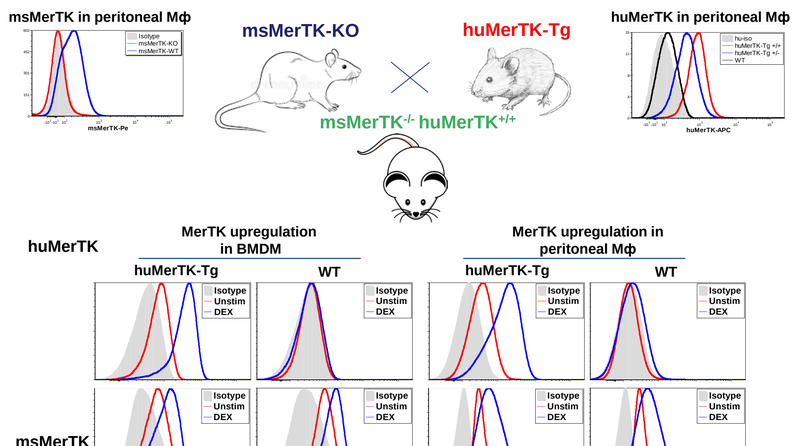
Saline vs. α-PD1: 0.0048

Saline vs. 3C6: 0.036

Saline vs. Combination: 0.0004

- Preliminary data demonstrating anti-tumor activity with mAb 3C6 alone, and potentially enhancing activity of PD-1 inhibition in CT26 tumors.
- CT26 tumor model dosed with anti-mouse MerTK mAb 3C6: 300 μg i.p. twice per week x1, once per week x3 and anti-mouse PD1 mAb (RMP1-14): 100 μg i.p. twice per week x2.

Creation and Characterization of msMerTK knockout and huMerTK Transgenic Mice



- **Goal to establish in vivo proof of concept with human anti-MerTK mAb lead candidates.**
  - Use MerTK-KO mice to benchmark mAb-dependent effects and validate approach.
  - Identify tumor models sensitive to MerTK ablation and phenocopy effect with mAbs.
  - Establish in vivo proof of concept with anti-huMerTK mAb lead candidates in huMerTK-Tg/msMerTK-KO animals.

Conclusions

- Targeting negative regulators of the innate immune response with monoclonal antibodies may be a useful approach to intervene in early steps of the cancer immunity cycle.
- Modulation of MerTK with monoclonal antibodies induces pro-inflammatory responses consistent with de-repression of the innate immune response and MerTK loss-of-function phenotypes.
  - Anti-human and anti-mouse MerTK mAbs modulate cytokines and chemokines in vitro and in vivo
- These promising data support further exploration of antibody-based approaches to target MerTK as a negative regulator of innate immunity.