#1555 Monoclonal Antibodies Targeting the TAM Family of Receptor Tyrosine Kinases

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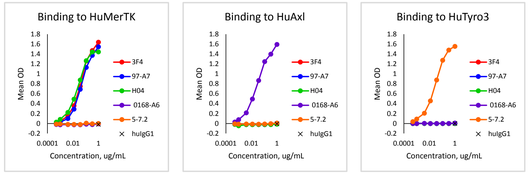
Introduction

- TAM receptors (Tyro3, Axl, MerTK) are receptor tyrosine kinases (RTKs) expressed in innate immune cells.
- TAM activation by their cognate ligands, Gas6 and Protein S, promote efferocytosis of apoptotic cells to induce a tolerogenic state and promote resolution of inflammation.
- Genetic ablation of single or multiple TAM receptors leads to phenotypes consistent with auto-immunity and inflammation.
- MerTK deficiency induces immune activation and provides tumor protection in tumor-bearing mice.
- We describe the characterization of select mAbs targeting each one of the TAM receptors, which induce secretion of pro-inflammatory cytokines, and activate innate and adaptive immune responses.
- A surrogate mAb directed to mouse MerTK demonstrates anti-tumor activity alone and enhances the activity of checkpoint inhibitors.

Anti-TAM mAb Discovery and Characterization

- Anti-TAM mAbs were generated through a diversity of methods to maximize epitope coverage and functional diversity.
- Functional assay screens uncovered mAbs of interest, which are characterized below.



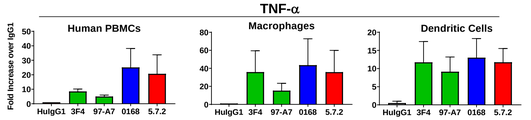
- TAM mAbs bind their respective human target with high specificity.
  - Each anti-TAM mAb was titrated onto ELISA plates coated with the purified extracellular domain of human MerTK, Axl, or Tyro3.

mAb	Target	Origin	Isotype	Affinity (Monovalent) K_D (nM)	Affinity (Monovalent) Kon (1/Ms)	Affinity (Monovalent) Kdis (1/s)	Gas6 Blocking	TAM Phosphorylation Stimulate pY-TAM	TAM Phosphorylation Inhibit Gas6-dep. pY TAM	Human Cell Type
3F4	MerTK	Harbour Mouse	Human IgG1	0.29	5.07 E+05	1.49 E-04	Partial	Weak	Weak	Monocyt. MΦ
97-A7		Phage display*		43	2.45 E+05	1.06 E-02	Complete	No	Yes	
H04		Phage display		117	1.2 E+05	1.4 E-02	Complete	Yes	Weak	
0168	Axl	Rat	Humanized IgG1	0.46	6.8 E+05	3.2 E-04	Complete	Weak	Yes	H1299
5.7.2	Tyro3	Phage display	Human IgG1	0.76	2.03 E+05	1.54 E-04	Complete	No	Yes	K562

* Affinity-matured.

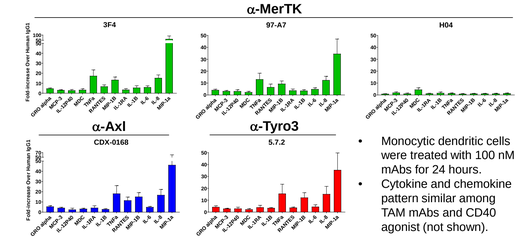
- Anti-TAM mAbs were derived from orthogonal antibody discovery campaigns.
- mAbs were selected for potent binding to cell surface receptors, selectivity, reactivity with cynomolgus TAMs, and activity in functional assays (see below).
- mAbs described herein (except mAb H04) demonstrated significant activity in functional assays.

TAM mAbs Induce an Inflammatory Response in Human Myeloid Cells



- PBMCs, macrophages and dendritic cells were treated with 100 nM mAbs for 24 hours.
- TAM mAbs elicit TNF-α responses in innate immune cells.
- Similar effect observed in human monocytes isolated from PBMCs.z

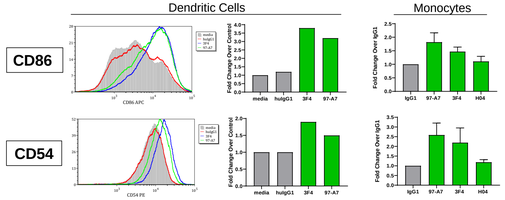
α–MerTK
α-Axl
α-Tyro3



- Monocytic dendritic cells were treated with 100 nM mAbs for 24 hours.
- Cytokine and chemokine pattern similar among TAM mAbs and CD40 agonist (not shown).

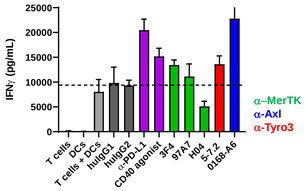
MerTK mAbs Activate Innate and Adaptive Immune Responses

MerTK mAbs Induce Expression of Co-stimulatory Molecules



- Dendritic cells or human PBMC-isolated monocytes were treated with 100 nM of each mAb for 24 hours.
- CD86 or CD54 cell surface protein (dendritic cells) or mRNA levels (monocytes) were measured by flow cytometry or q-RT-PCR, respectively.

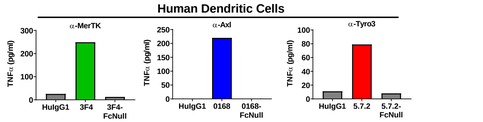
TAM mAbs Induce T cell Activation



- CD4 T cells were incubated with allogeneic DCs and the mAbs for 72 hours and IFNγ production was measured by ELISA.
- Preliminary data indicate active TAM mAbs induce T cell activation, consistent with the observed increase in pro-inflammatory and co-stimulatory molecules.

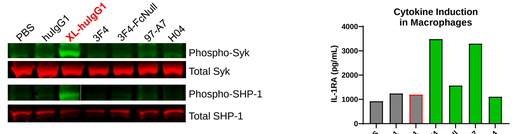
FcγR Binding, but not Signaling is Required for mAb Activity

Fc Mutations that Abolish FcγR Binding (FcNull) Inhibit mAb Activity



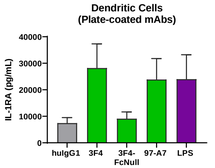
- Monocytic dendritic cells were treated with 100 nM mAbs for 24 hours.
- FcNull point mutations in the Fc region abolish FcγR binding

Cytokine Responses are not Elicited Via FcγR Activation



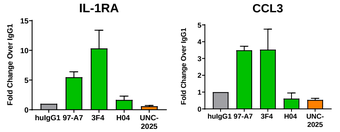
- Macrophages were treated with mAbs for 10 minutes with 100 nM mAbs.
- XL-huIgG1 (a chemically cross-linked aggregated IgG1), but not MerTK mAbs induced Syk and SHP-1 phosphorylation.

- Macrophages were treated with mAbs for 24 hours with 100 nM mAbs.
- XL-huIgG1 does not elicit secretion of IL-1RA or CCL3 (not shown).



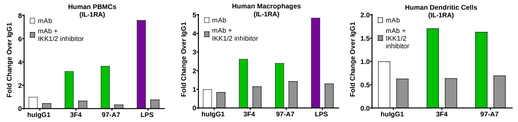
- Artificial mAb aggregation induced by direct plate coating does not promote cytokine release by human IgG1 or 3F4-FcNull.
- mAb aggregation is insufficient to confer activity.
- Data suggest mAbs modulation of receptor activity at the cell surface is necessary and sufficient to induce cytokine release.

MerTK mAbs Function Through a Different Mechanism than MerTK Kinase Inhibitors



- Human PBMCs were treated with 100 nM mAbs and 1 μM UNC-2025 for 24 hours.
- Data suggest mAbs function via a mechanism that is independent of MerTK kinase inhibition.

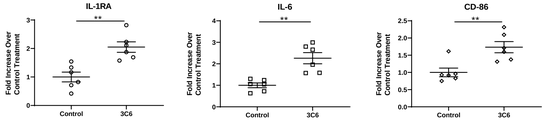
MerTK mAbs Promote NFκB Signaling



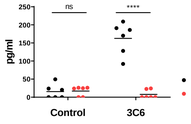
- The IKK1/2 inhibitor BMS345541 (10 μM) inhibits cytokine secretion by TAM mAbs in different myeloid cell types, indicating that NFκB signaling is required for mAb activity.

A Surrogate MerTK mAb (3C6) Induces Immune Activation in Vivo

Immune Activation in Spleens of Tumor-bearing Mice



- Tumor (CT26)-bearing mice were dosed i.p. with 500 μg of anti-mouse MerTK mAb 3C6 or a vehicle control.
- Spleens were harvested 4 hours post-dosing, and target mRNA levels measured by q-RT-PCR.

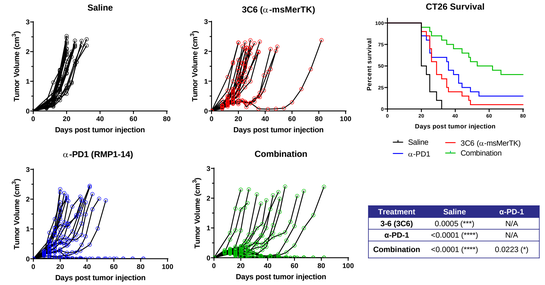


- Wild-type
- MerTK^{KO}

The in Vivo Activity of mAb 3C6 is MerTK-Dependent

- MerTK knockout (MerTK^{KO}) and C57BL/6 wild type mice were injected i.p. with 0.5 mg of 3C6.
- Serum was collected 3 days before (control) and 1 hour post 3C6 injection.
- TNF-α levels from plasma samples were measured by MSD.

MerTK mAb 3C6 Demonstrates Anti-tumor Activity in a CT26 Tumor Model



Treatment	Saline	α-PD-1
3-6 (3C6)	0.0005 (***)	N/A
α-PD-1	<0.0001 (****)	N/A
Combination	<0.0001 (****)	0.0223 (*)

Conclusions and Next Steps

- Select mAbs targeting the TAM family of receptors uniquely induce a similar pattern of pro-inflammatory responses and immune cell activation consistent with TAM knockout phenotypes.
- TAM mAb activity requires binding, but not activation of FcγRs, suggesting potential cooperation between both families of receptors.
- Anti-tumor efficacy with a surrogate MerTK mAb supports combination with checkpoint therapies.
- Data support development of anti-TAM mAbs as therapies to activate innate immune responses with a potential for systemic dosing.

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