Abstract #10117
Poster #LB-090

In vivo efficacy and safety of RGX-019, a MerTK targeting monoclonal antibody

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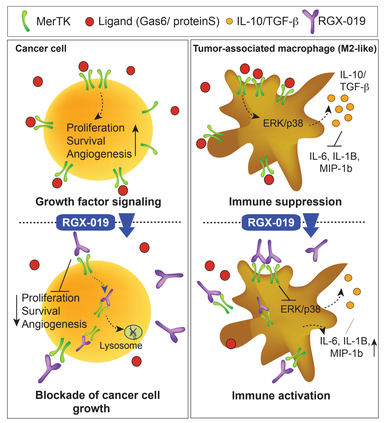
1. Introduction

- MerTK, a member of the TYRO3/AXL/MerTK (TAM) family, is expressed on innate immune cells and overexpressed in various solid and hematologic cancers[1].
- MerTK signaling mediates immunologic tolerance in immune cells (macrophages) via suppression of pro-inflammatory cytokines[2-7] and activation of pro-oncogenic processes in cancer cells including cell survival, invasion, migration, angiogenesis and drug resistance[8-10].
- Efforts to develop MerTK inhibitors, including small molecule approaches, have been hampered by insufficient selectivity, potential for hematologic (platelet) toxicity, as well as retinal toxicity[11,12].
- RGX-019 is a high affinity monoclonal humanized IgG1 antibody with a novel mechanism of action designed to overcome potential limitations of MerTK inhibition.



2. RGX-019 Properties and Mechanism of Action

A

Assay	Antigen	Affinity (KD)	Avidity (KD)
SPR	human Mer-Fc	3.0 nM	5.7 pM
ELISA	human Mer-Fc	0.62 nM	N/A
ELISA	cynomolgus monkey Mer-Fc	0.3 nM	N/A

- Selective and high affinity binding to human and cynomolgus monkey MerTK
- No binding to human Axl, human Tyro3 or mouse MerTK
- Low in vitro immunogenicity was observed in EpiScreen DC:T cell assay (Abzena)
- In silico analysis demonstrated low risk of aggregation and no potential sites for modification including N-linked glycosylation and deamidation

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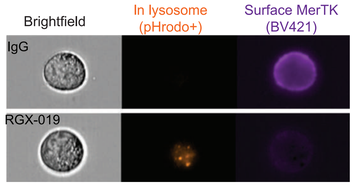

SK-Mel5 melanoma cells were treated with 6.7 nM pHrodo-RGX-019 or IgG for 24 hr. MerTK was stained with BV421 antibody for analysis by ImageStream

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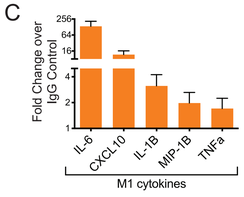

Human cytokine analysis. In vitro differentiated M2 macrophages were treated with 6.7 nM RGX-019 or IgG for 48h; n=4

- RGX-019 has a novel mechanism that drives MerTK degradation in cancer cells and M2 macrophages[13]
- RGX-019 induces degradation of MerTK through receptor internalization and trafficking to the lysosome (Fig. 2B)
- RGX-019 reduces Gas6-induced activation of pAKT in MerTK-expressing cancer cell lines[13]
- RGX-019 promotes a pro-inflammatory cytokine response in human M2 macrophages (Fig. 2C)
- MerTK blockade on macrophages mediates tumor immunogenicity and potentiates anti-tumor efficacy[6,7]

3. RGX-019 Inhibits Viability and Colony Formation of MerTK+ Cancer Cells

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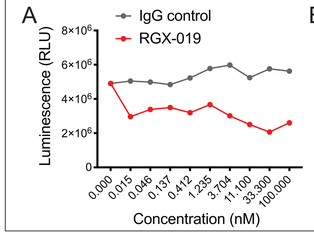

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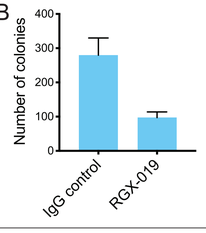

(A- viability assay)
RPMI8226 (AML) cells were plated on 96-well plates and treated with RGX-019 or control human IgG for 5 days. Viability at 96 hrs was quantified as RLU (Relative Luminescence Unit) using CellTiter Glo reagent (Promega).

(B- colony formation assay)
RPMI8226 cells were seeded at a density of 1,000 cells per well and cultured in methylcellulose-based medium with 6.7 nM RGX-019 or human IgG control for 14 days. Colonies of more than 50 cells were counted.

4. RGX-019 Inhibits Lung Colonization of Triple Negative Breast Cancer Cells

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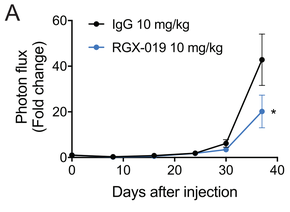

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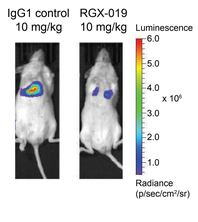
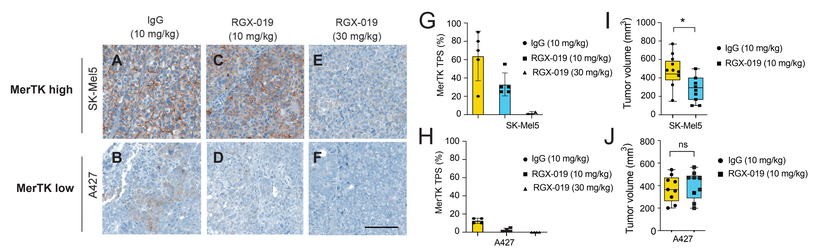


Fifty thousand MDA-MB-231-LM2 TNBC cells were injected into the tail vein of NSG mice. Treatment started on the day of tumor cell inoculation, and mice were dosed with 10 mg/kg IgG1 isotype control or RGX-019 twice per week for 37 days. Tumor metastasis to the lung were quantified by in vivo bioluminescence imaging (A). Representative images of measurements on Day 37 (B). N=7. *p<0.05, Mann-Whitney test.

5. RGX-019 Depletes MerTK in Tumors and Inhibits in Vivo Tumor Growth

In vivo anti-tumor activity is associated with MerTK expression



SK-Mel5 (2.5 million cells) and A427 (5 million cells) were injected subcutaneously into athymic nude mice. Following tumor growth to 80 mm^3, mice were treated with control IgG (10 mg/kg), RGX-019 (10 mg/kg) or RGX-019 (30 mg/kg) for 52 days (SK-Mel5) or 21 days (A427). Four to five tumors were dissected, sectioned and stained for MerTK (dilution 1:400). Three sections from each tumor per condition were analyzed for MerTK tumor proportion score (TPS). Scale bar = 200 µm

6. TAM Receptor Expression in Healthy Human Tissues

In human immune cells MerTK is predominantly expressed in M2 macrophages

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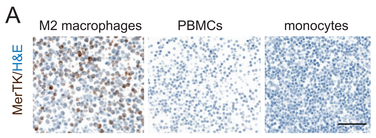
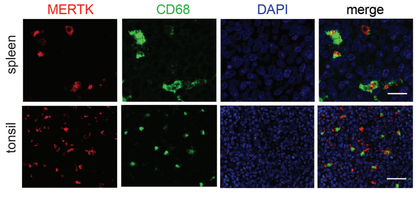


- (A) Monocytes were isolated from human PBMCs by classical monocyte isolation kit (Miltenyi), and M2 macrophages were differentiated in a medium supplemented with M-CSF. The cells were embedded in paraffin, immunostained with MerTK antibody and counterstained with H&E using a Ventana automated system. Scale bar = 50 µm
- MerTK expression was evaluated in various immune cells (PBMCs, monocytes, T-cells, dendritic cells, NK cells, M1 and M2 macrophages) by western-blot, flow cytometry and IHC. Expression was highest in M2 macrophages

MerTK expression in human tissue is primarily restricted to macrophages

B



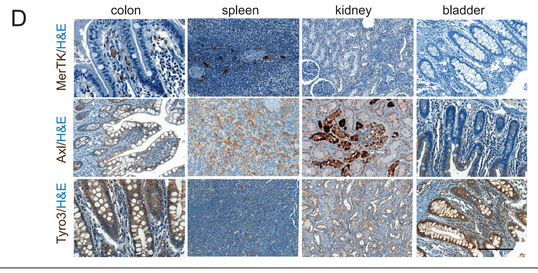
Double immunohistochemistry staining using specific antibodies for the detection of MerTK and macrophage marker CD68 (dilutions 1:400 and 1:1000, respectively) and DAPI to stain nuclei were used in human spleen and tonsil. Scale bar = 20 µm, 50 µm respectively

C

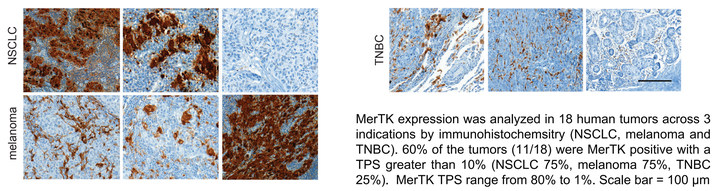
	MerTK	Axl	Tyro3
Spleen, Lymph node, Tonsil, Lung, Esophagus, Heart, Stomach, Small intestine, Colon, Gallbladder, Placenta, Skin	+	+	+
Pancreas, Liver, Bladder, Skeletal Muscle, Aorta, Kidney	-	+	+
Salivary Gland, Thyroid, Prostate, Adrenal gland, Breast	+	-	+
Thymus, Cerebellum	-	-	+

- Human tissue microarrays containing 28 healthy organs were stained with MerTK, Axl and Tyro3 antibodies
- Table shows expression of TAM receptors in the indicated tissues. Unlike MerTK, extensive expression of Axl and Tyro3 was observed in some stromal and epithelial tissue
- Examples of tissues stained with the indicated antibodies. Scale bar = 100 µm

D



7. MerTK Expression in Patient Tumor Specimen



MerTK expression was analyzed in 18 human tumors across 3 indications by immunohistochemsitry (NSCLC, melanoma and TNBC). 60% of the tumors (11/18) were MerTK positive with a TPS greater than 10% (NSCLC 75%, melanoma 75%, TNBC 25%). MerTK TPS range from 80% to 1%. Scale bar = 100 µm

8. RGX-019 is Well Tolerated in Cynomolgus Monkey; no Retinal Toxicity is observed

- MerTK is expressed at the apical membrane of the retinal pigment epithelium; MerTK deficiency or inhibition is associated with retinal degeneration in human, cynomolgus monkey and rodents[11].
- To assess any risk for retinal toxicity, a 28-day dose range finding non-GLP toxicology study was conducted in cynomolgus monkeys
- No retinal toxicity was observed across all dose groups

A 28-day non-GLP toxicology study

Study design:
- Dose groups: Control, 10 mg/kg, 30 mg/kg, 100 mg/kg reduced to 60 mg/kg; n=3 males/cohort
- Dosing paradigm: weekly by slow IV bolus

Summary of the results:
- A dose proportional increase in exposure to the antibody was observed (Fig. B, C)
- Eye, heart, kidney, liver, lung, pancreas, spleen and thymus were examined by histopathology
- Decrease in thymic lymphocytes in animals given 10 mg/kg/dose and 30 mg/kg/dose were the only microscopic findings; no other test article related microscopic findings, including in the retina, were reported

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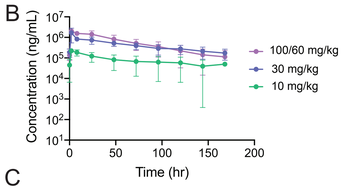

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Dose	Model	Mean T1/2 (hr)	Mean AUCinf_obs (µg*h/mL)
10 mg/kg	Monkey	51	16,579
30 mg/kg	Monkey	77	96,362
100/60 mg/kg	Monkey	40	114,737
10 mg/kg	Mouse	83	21,568

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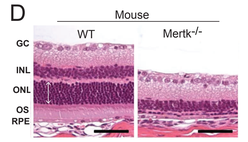

Adapted from Grazyna Palczewska et al. J Biol. Chem. 2016;291:26937-26949

- (D) Representative retinal morphology of 6-month-old wild-type (WT) and MerTK deficient (Mertk$^{-/-}$) mice. Mertk$^{-/-}$ mice lack the outer nuclear layer (ONL) in the retina. Scale bar, 50 µm. WT, wild type; GC, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; OS, outer segment
- (E) Representative image sections of monkey retinas from the toxicology study showing no defects (necrosis or decrease in the width of the ONL layer) at any of the RGX-019 doses administered. Scale bar, 30 µm

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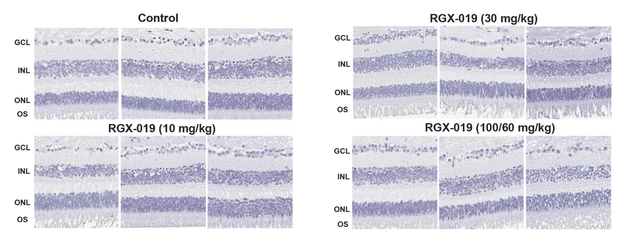

9. Conclusions

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- RGX-019 is a novel MerTK selective monoclonal antibody with a unique mechanism of action and high affinity binding to human and cynomolgus monkey MerTK
- RGX-019 treatment leads to degradation of MerTK expression on cancer cells and M2 macrophages
- RGX-019 can drive in vivo anti-tumor efficacy via direct cancer cell targeting even in the absence of its immune modulating effects
- MerTK is highly expressed in tissue macrophages and tumors of melanoma, NSCLC, and breast cancers. Healthy tissue epithelia does not display MerTK expression
- RGX-019 is well tolerated in cynomolgus monkey at doses up to 60 mg/kg with no major microscopic findings
- The lack of retinal toxicity and any other major histophatological findings at substantial systemic exposures to RGX-019 along with its unique mechanism of action offer a wide therapeutic index
- RGX-019 represents a novel MerTK targeting agent with favorable safety and developability characteristics, making it suitable for development in a variety of MerTK over-expressing cancers

10. References

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