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Characterization of the anti-cancer and immunologic activity of RGX-019, a novel pre-clinical stage humanized monoclonal antibody targeting the MERTK receptor

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Shugaku Takeda[1], Celia Andreu-Agullo[1], Subhasree Sridhar[1], Nils Halberg[2], Ivo Lorenz[1], Robert Busby[3], Sohail Tavazoie[4], Masoud Tavazoie[1], Isabel Kurth[1]
[1]Rgenix Inc., New York, NY; [2]University of Bergen, Department of Biomedicine, Bergen, Norway; [3]Busby Pharma Consulting, Boston, MA, [4]The Rockefeller University, New York, NY

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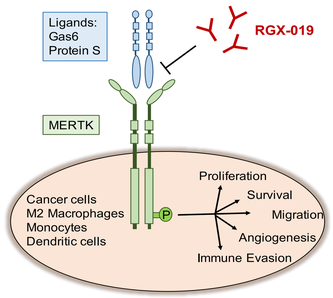
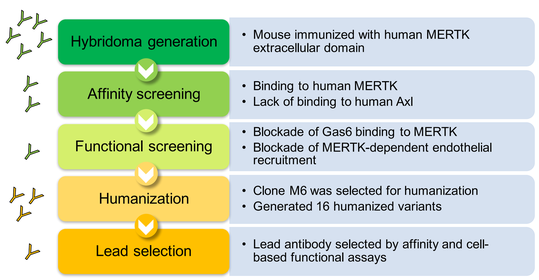
INTRODUCTION

MERTK, a receptor tyrosine kinase of the TYRO3/AXL/MERTK (TAM) family, is overexpressed in a wide variety of cancers, including leukemia and many solid cancers (1-4). Activation of MERTK on cancer cells including melanoma, breast cancer, lung cancer, gastric cancer, and AML results in activation of multiple tumor-promoting signaling pathways including pathways promoting proliferation, survival, migration, cell invasion, and angiogenesis (5-9). Additionally, MERTK contributes to the immune-suppressive environment within tumors. MERTK is predominantly expressed in immuno-suppressive M2 macrophages, where activation of MERTK triggers the release of anti-inflammatory cytokines as a means to maintain immune tolerance (10, 11). Recent pre-clinical and clinical studies have shown promising anti-tumor efficacy upon modulation of TAM receptor signaling with small-molecules. However, current small-molecule approaches to target MERTK are hampered by off-target binding to related TAM receptors as well as other tyrosine kinases, leading to increased potential for toxicity and emergence of therapy resistance associated with small-molecule tyrosine kinase blockade. Therefore, a monoclonal antibody with MERTK-specific activity could suppress the growth of MERTK expressing cancers and enhance anti-tumor immunity without the disadvantages associated with blockade of related kinases. Herein, we report the pre-clinical characterization of RGX-019, a humanized monoclonal IgG1 antibody with high affinity and specificity for human MERTK and a unique molecular mechanism-of-action.



RGX-019: Generation of first-in-class therapeutic monoclonal antibody

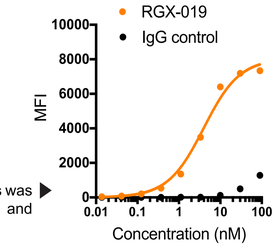


RGX-019 binds human and monkey MERTK with high affinity and specificity

A MERTK binding by SPR and flow cytometry

Antibody	Isotype	Avidity by SPR (K_D)	Affinity by SPR (K_D)	Cell binding (EC50)
M6	Mouse IgG1	6.4 - 9.4 pM	N/A	N/A
RGX-019	Humanized IgG1	5.7 pM	3.0 nM	6.7 nM

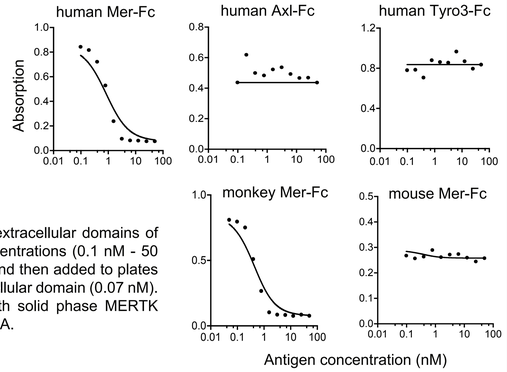
Binding of RGX-019 to surface MERTK on SKMEL5 melanoma cells was quantified by flow cytometry using APC-labeled RGX-019 or IgG and represented as the median fluorescence intensity (MFI).



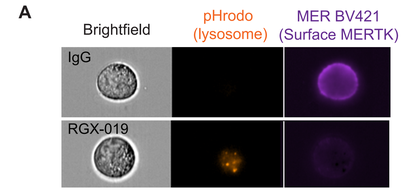
B Competitive ELISA

Antigen	Species	K_D
Mer-Fc	human	1.46 nM
Axl-Fc	human	no binding (> 10 mM)
Tyro3-Fc	human	no binding (> 10 mM)
Mer-Fc	cynomolgus monkey	0.63 nM
Mer-Fc	mouse	no binding (> 10 mM)

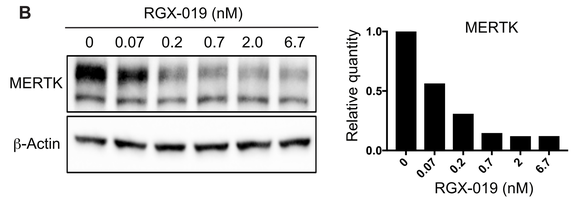
RGX-019 (6 nM) was incubated with extracellular domains of the indicated proteins at different concentrations (0.1 nM - 50 nM) allowing formation of conjugates and then added to plates pre-coated with human MERTK extracellular domain (0.07 nM). Binding of unconjugated RGX-019 with solid phase MERTK was quantified by anti-human IgG ELISA.



RGX-019 induces MERTK degradation through internalization

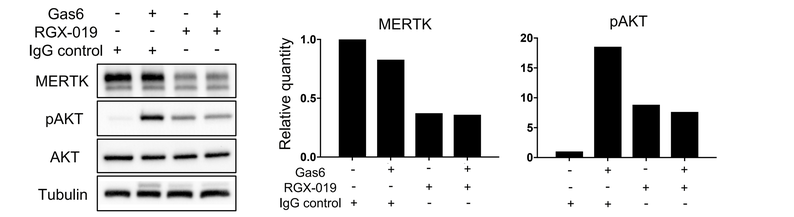


RGX-019 and control human IgG were labeled with pHrodo, a pH-sensitive fluorophore, whose signal becomes measurable only in lysosomes. SKMEL5 cells were incubated with 6.7 nM of pHrodo-labeled RGX-019 or IgG before analysis by flow cytometry. Surface MERTK was stained with a BV421-conjugated MERTK antibody. Images were taken with ImageStream flow cytometer.



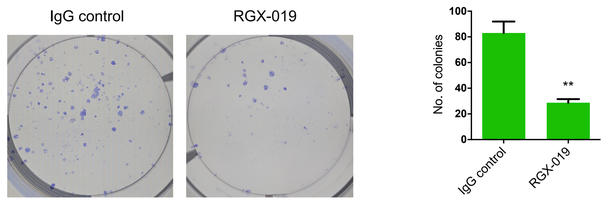
SKMEL5 cells were cultured with RGX-019 for 24 hr. Levels of MERTK were determined by Western blot. The relative quantity of MERTK was normalized to β-actin.

RGX-019 inhibits Gas6-induced activation of downstream signaling



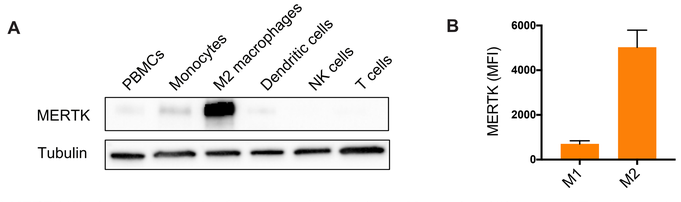
SKMEL5 cells were treated with 2 nM RGX-019 or human IgG control for 2 hr, then stimulated with 200 nM Gas6 for 10 min. Levels of MERTK and downstream phosphorylation of AKT (pAKT) were determined by Western blot. The relative quantity of MERTK and pAKT was normalized to tubulin.

RGX-019 inhibits colony formation of cancer cells



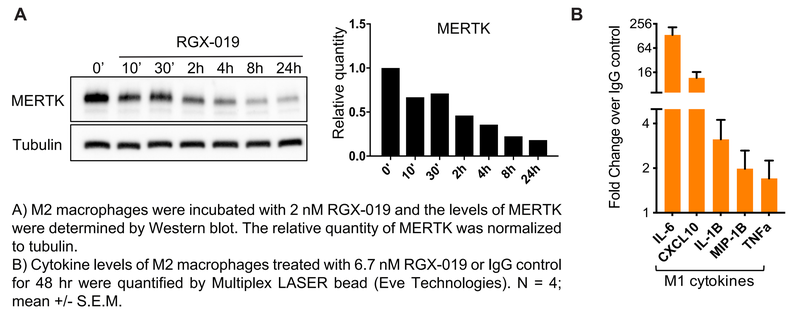
SKMEL5 cells were seeded at a density of 500 cells per well and cultured with 6.7 nM RGX-019 or human IgG control for 12 days. Colonies of more than 50 cells were counted. N = 3; mean +/- S.E.M. **p < 0.01.

MERTK is predominantly expressed in M2 macrophages



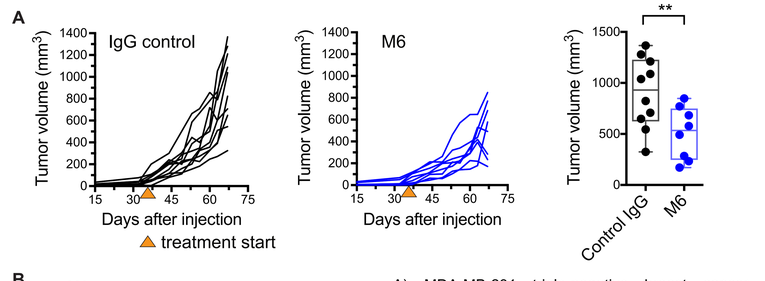
A) MERTK Western blot analysis of immune cell populations. Monocytes, NK cells and T cells were isolated from human PBMCs from healthy donors by magnetic bead isolation. Monocytes were differentiated into M2 macrophages by culturing with M-CSF for 7 days or into dendritic cells with GM-CSF and IL-4 for 6 days, followed by 24 hr stimulation with IL-6, IL-1β, TNFα, and PGE2.
B) Surface MERTK levels on M1 and M2 macrophages were quantified by flow cytometry using a BV421-conjugated MERTK antibody and represented as MFI. M1 macrophages were differentiated from monocytes by culturing with GM-CSF for 7 days. N = 3; mean +/- S.D.

RGX-019 induces proinflammatory M1 cytokines in M2 macrophages



A) M2 macrophages were incubated with 2 nM RGX-019 and the levels of MERTK were determined by Western blot. The relative quantity of MERTK was normalized to tubulin.
B) Cytokine levels of M2 macrophages treated with 6.7 nM RGX-019 or IgG control for 48 hr were quantified by Multiplex LASER bead (Eve Technologies). N = 4; mean +/- S.E.M.

In vivo efficacy of anti-MERTK antibody in xenograft models



A) MDA-MB-231 triple-negative breast cancer tumors, implanted in the mammary fat pad, received 5 mg/kg IgG control or M6, the parental RGX-019 murine antibody, 3 times a week by i.p. injection. Tumor volumes on Day 67 are plotted on the right panel. **p < 0.01.
B) Pharmacokinetics in mouse plasma. RGX-019 was administered (i.p.) to non-tumor bearing NOD SCID mice every two days for a total of seven doses. Plasma samples at nine time points counting from the last dose were analyzed by human IgG ELISA. RGX-019 was well tolerated at both dose levels. N=3; mean +/- S.E.M.

CONCLUSIONS

- RGX-019 is a first-in-class MERTK selective monoclonal antibody with high affinity/avidity to human and cynomolgus monkey MERTK
- RGX-019 blocks Gas6 ligand binding and induces degradation of MERTK through receptor internalization
- Mechanism of action results in inhibition of colony formation of MERTK-expressing cancer cells and induction of a proinflammatory M1 cytokine response in macrophages
- In vivo antitumor activity has been demonstrated with M6 (parental RGX-019 murine antibody) in TNBC xenografts
- Therefore, RGX-019 represents a novel therapeutic agent with a unique mechanism of action for the treatment of a variety of MERTK over-expressing cancers

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