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

- 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 production of pro-inflammatory cytokines, survival, migration, cell invasion, and angiogenesis (5-9).
- MERTK contributes to the immunosuppressive environment within tumors. MERTK is predominately 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).

Binding of RGX-019 to surface MERTK on SKMEL5 melanoma cells was quantified by flow cytometry using APC-labeled RGX-019 or IgG and Binding of RGX-019 to surface MERTK on SKMEL5 melanoma cells was quantified by anti-human IgG ELISA.

RGX-019 inhibits colony formation of cancer cells

- SKMELS cells were treated with 2 nM RGX-019 or human IgG 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 Gas6-induced activation of downstream signaling

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

RGX-019 induces MERTK degradation through internalization

- A) Brightfield pHrodo-labeled RGX-019 or IgG before analysis signal becomes measurable only in lysosomes. B) SKMEL5 cells were cultured with 6.7 nM RGX-019 or IgG for 24 hr. Levels of MERTK were determined by Western blot. The relative quantity of MERTK was normalized to p-Akt.

RGX-019 inhibits MERTK expression 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 actin.

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 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 at a dose level of 1 mg/kg daily for 7 days. Plasma samples were collected at four time points counting from the last dose. The last time point was analyzed by human IgG ELISA. RGX-019 was well tolerated at both dose levels. N=3, mean +/- S.E.M.

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 actin.

REFERENCES


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