2 for 1: Targeting tumor-associated macrophages and cancer cells with a novel MERTK-targeting antibody-drug conjugate (ADC)

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Introduction

- Our RNA-DRIVEr™ platform identified MERTK as a key driver of metastatic progression[1]
- MERTK is a member of the TAM family of receptor tyrosine kinases
- MERTK Is overexpressed in various solid and hematologic cancers and expression is associated with worse outcomes[2-11]
- Binding of its ligands (Gas6, protein S) drives cancer cell proliferation, survival, invasion and drug resistance[4, 12]
- MERTK is expressed on M2 tumor-associated macrophages (TAMs), playing a role in the production of immunosuppressive cytokines and efferocytosis[12]
- MERTK expression within retinal pigment epithelium (RPE) has resulted in retinal degeneration in animal models exposed to MERTK complete antagonists[13]

Background

RGX-019

- Humanized mAb with high affinity and specificity to human MERTK (Kd = 3.0 nM) with partial agonist activity[14]
- Efficiently internalizes MERTK leading to degradation of surface MERTK
- As a partial agonist, blocks binding of Gas6 ligand and diminishes signal transduction
- Induces expression of pro-inflammatory cytokines in M2 macrophages

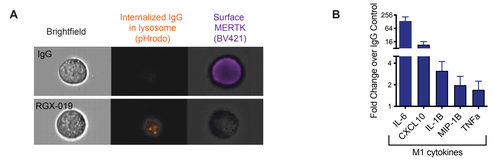
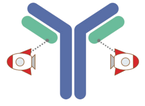


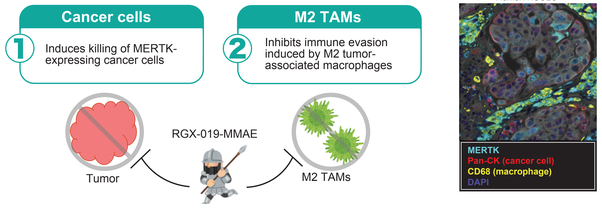
Figure 1. (A) RGX-019 Internalization. RGX-019 and IgG control were labeled with a pH-sensitive dye, pHrodo, that becomes fluorescent in low pH (eg. lysosomes).SK-Mel5 melanoma cells were treated with 6.7 nM pHrodo-labeled RGX-019 or IgG control for 24 hr before imaging with Image-Stream flow cytometer. **(B) Human cytokine array analysis.** M2 macrophages were differentiated from human monocytes by culturing with M-CSF for 7 days, and treated with 6.7 nM RGX-019 or IgG for 48hr. n=4; mean +/- SEM.

RGX-019-MMAE



- RGX-019 MMAE-conjugated ADC
- MMAE payload: direct killing of mitotic cells & bystander efficacy
- Site-specific conjugation
- DAR 4
- Cleavable linker
- Additional spacer moiety to increase solubility

2 for 1 approach



Results

MERTK expression is observed in human solid cancers and AML

Expression of MERTK is seen in the majority of ovarian, urothelial, pancreas, and non-small cell lung cancers (NSCLC)

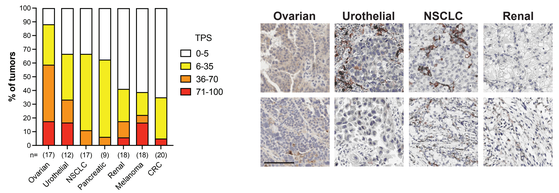


Figure 2. MERTK immunohistochemistry (IHC) in human tumors. MERTK expression in human tumors across seven indications was analyzed by IHC and quantified as Tumor Proportion Score (TPS). Scale bar 100 µm.

Higher MERTK expression is found in M3 and M5 subtypes of AML as well as AML with t(9;11) translocations or PTPN11 mutations

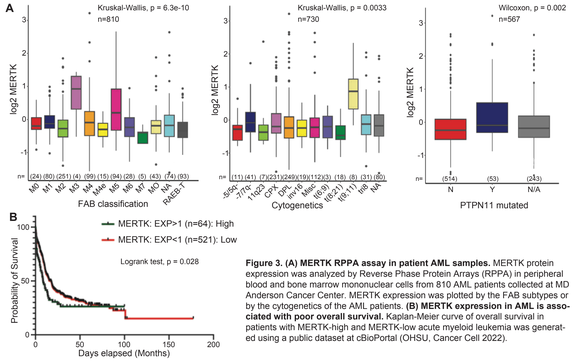


Figure 3. (A) MERTK RPPA assay in patient AML samples. MERTK protein expression was analyzed by Reverse Phase Protein Arrays (RPPA) in peripheral blood and bone marrow mononuclear cells from 810 AML patients collected at MD Anderson Cancer Center. MERTK expression was plotted by the FAB subtypes or by the cytogenetics of the AML patients. **(B) MERTK expression in AML is associated with poor overall survival.** Kaplan-Meier curve of overall survival in patients with MERTK-high and MERTK-low acute myeloid leukemia was generated using a public dataset at cBioPortal (OHSU, Cancer Cell 2022).

RGX-019-MMAE demonstrates efficient internalization and target-dependent in vitro cytotoxicity

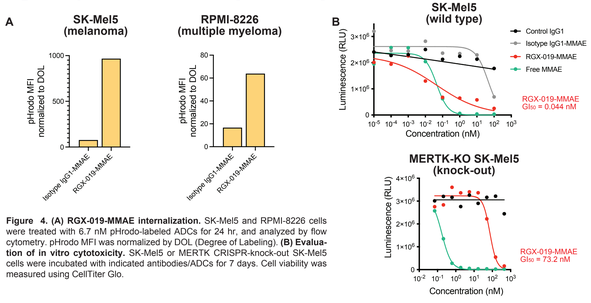


Figure 4. (A) RGX-019-MMAE internalization. SK-Mel5 and RPMI-8226 cells were treated with 6.7 nM pHrodo-labeled ADCs for 24 hr, and analyzed by flow cytometry. pHrodo MFI was normalized by DOL (Degree of Labeling). **(B) Evaluation of in vitro cytotoxicity.** SK-Mel5 or MERTK CRISPR-knock-out SK-Mel5 cells were incubated with indicated antibodies/ADCs for 7 days. Cell viability was measured using CellTiter Glo.

RGX-019-MMAE exhibits robust in vivo anti-tumor efficacy

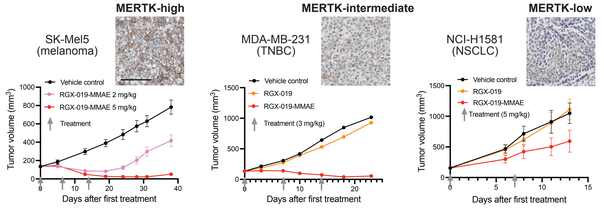


Figure 5. In vivo anti-tumor efficacy in xenograft models. SK-Mel5 or NCI-H1581 cells were injected subcutaneously into NSG or athymic mice, respectively. MDA-MB-231 cells were injected into the mammary fat pad of athymic nude mice. n=8-10, mean +/- SEM. Images represent MERTK IHC staining of control tumors. Scale bar 100 µm.

RGX-019-MMAE shows bystander activity via M2 macrophages

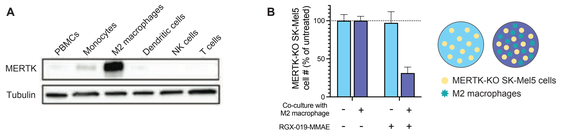


Figure 6. (A) MERTK expression in human immune cells. In vitro differentiated M2 macrophages and other immune cells isolated from human PBMCs were analyzed by anti-MERTK Western blot. **(B) Evaluation of bystander-killing by M2 macrophages.** M2 macrophages were co-cultured with CFSE-labeled MERTK-KO SK-Mel5 cells in the presence of RGX-019-MMAE for 6 days. Cells were stained with anti-CD14 and anti-MERTK antibodies, and numbers of MERTK-KO SK-Mel5 cells were quantified by flow cytometry. n=3-4; mean +/- SD.

RGX-019-MMAE exhibits anti-tumor immune modulation

To address the contribution to tumor inhibition from the host immune system, we injected MMAE resistant tumor cells (MC38-HM) into either wild type mice or mice that express a humanized version of MERTK

- **Tumor growth was suppressed only in animals expressing humanized MERTK**
- **RGX-019-MMAE depletes tumoral M2 macrophages**

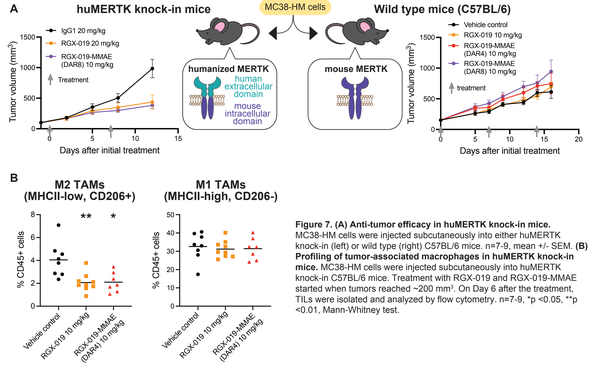


Figure 7. (A) Anti-tumor efficacy in huMERTK knock-in mice. MC38-HM cells were injected subcutaneously into either huMERTK knock-in (left) or wild type (right) C57BL/6 mice. n=7-9, mean +/- SEM. **(B) Profiling of tumor-associated macrophages in huMERTK knock-in mice.** MC38-HM cells were injected subcutaneously into huMERTK knock-in C57BL/6 mice. Treatment with RGX-019 and RGX-019-MMAE started when tumors reached ~200 mm³. On Day 6 after the treatment, TILs were isolated and analyzed by flow cytometry. n=7-9, *p <0.05, **p <0.01, Mann-Whitney test.

RGX-019-MMAE treatment is not associated with retinal degeneration in mice

Partial agonist activity of RGX-019 prevents complete inhibition of RPE phagocytosis, which is needed for maintenance of retinal function, as compared to a full MERTK inhibitor

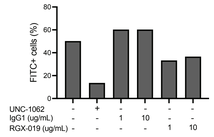


Figure 8. Partial inhibition of phagocytosis by RPE cells. Primary human RPE (retinal pigmented epithelial) cells were differentiated in vitro. The cells were treated with IgG1, RGX-019 or a small molecule MERTK inhibitor UNC-1062 (10 uM) for 2 hr, then incubated with FITC-labeled bovine POS (photoreceptor outer segment) for 2 hr. The proportion of FITC-positive RPE cells was analyzed by flow cytometry.

RGX-019-MMAE treated mice have an intact retina

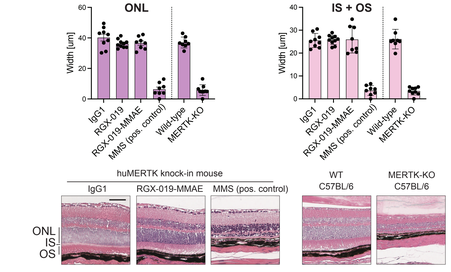


Figure 9. Assessment of retinal degeneration in mice. huMERTK-KI mice were treated with 5 mg/kg IgG1, RGX-019, or RGX-019-MMAE by weekly IV administration for 28 days and eyes were collected for H&E staining. As a positive control, an alkylating agent MMS (methyl methanesulfonate) was administered at 75 mg/kg by a single IP. The thickness of ONL (outer nuclear layer) and IS+OS (photoreceptor inner segment, outer segment) were quantified as measures of retinal degeneration (n=8-10). Scale bar = 50 µm.

Summary

- MERTK is overexpressed in multiple solid and hematologic cancers with expression in healthy tissues generally limited to the retinal pigment epithelium and immune cells, mainly macrophages
- RGX-019 triggers MERTK receptor internalization and partial agonist activity enabling selective delivery of toxic payload to MERTK expressing cancer cells
- Inspirna has developed an RGX-019 based MMAE ADC to enhance cancer cell killing and activate anti-tumor immunity
- Robust in vitro and in vivo efficacy of RGX-019-MMAE is seen in solid and hematologic cancers
- Lack of retinal degeneration observed in cynomolgus monkey[14] and mice expressing huMERTK, after treatment with RGX-019 and RGX-019-MMAE, differentiates RGX-019-MMAE from complete antagonist inhibitors of MERTK

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