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Inhibition of KIT *In Vivo Mo*difies Immune Cell Populations to Improve The Efficacy of Checkpoint Inhibitors in Syngeneic Mouse Tumor Models

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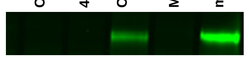
Introduction

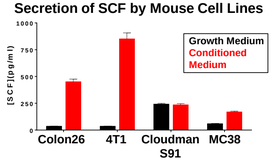
- KTN0158 is a humanized anti-KIT IgG1 monoclonal antibody that binds to the extracellular domain of KIT, and is being developed as a potential therapy for cancer (Phase 1 study: NCT02642016) and other mast cell-related diseases such as neurofibromatosis type 1.
- Expression of KIT in immune cell types, including mast cells, suggests the potential for additional roles of KIT in indirect modulation of tumor progression.
- KIT may be involved in modulating the activity of mast cells and MDSC's in tumors (Danelli et al, Cancer Immunol Res. 2015; Saleem et al, J Immunol. 2012; Pan et al, Blood 2008).
- In melanoma patients, prolonged overall survival is associated with lower numbers of monocytic myeloid-derived supressor cells in peripheral blood prior to treatment with ipilimumab or nivolumab (Kitano et al, Cancer Immunol Res. 2014; Weber et al, Cancer Immunol Res. 2016).
- The ability of anti-KIT mAb treatment to relieve immune suppression and enhance anti-tumor activity of immune checkpoint inhibitors was evaluated in a panel of preclinical tumor models.

KIT and SCF Expression in Mouse Tumor Cell Lines

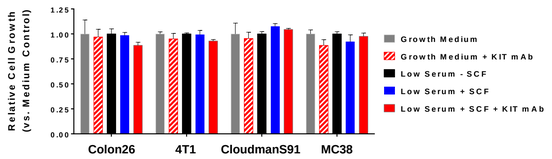
KIT Protein Expression in Mouse Cell Lines



Secretion of SCF by Mouse Cell Lines



Lack of Effect of SCF and KIT Inhibition on Cell Growth *In Vitro*



- No evidence for KIT-dependent proliferation in mouse tumor cell lines *in vitro*, regardless of KIT or SCF expression levels

Methods

Antibodies
- Anti-human KIT mAb (KTN0158, Kolltan Pharmaceuticals)
- Anti-mouse KIT mAb (ACK2, Biolegend)
- Anti-CTLA-4 (UC10-4F10-11, BioXCell)
- Anti-PD-1 (RMP1-14, BioXCell)
- Anti-mast cell tryptase (AA1, Dako)

Immune Cell Flow Cytometry
T-Cell Panel: CD45, CD3, CD4, CD8, CD25, FoxP3.

Myeloid Cell Panel: CD45, CD3, CD11b, Ly6G (g-MDSC), Ly6C (m-MDSC).

Dosing and Sampling Schedules (Flow Cytometry Pharmacodynamic Studies)
Tumors were staged to 150-250 mm^3, dosed q3d x2, then sampled for flow cytometry at day 7.

Dosing Schedules (Efficacy Studies)

ACK2: 15 or 3 mg/kg, biweekly x2 (from day 3).

Anti-CTLA-4: 5 mg/kg x1 (day 8), 2.5 mg/kg (days 11, 14).

Anti-PD-1: 5 mg/kg biweekly x2 (from day 3).

Colon26 (cell line) and Pan02 (tumor fragments) dosed from day 3 of implant.

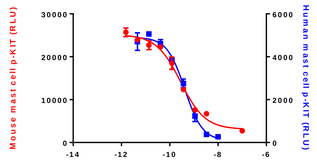
CloudmanS91 (cell line) dosed from day 3 after growth to 140-160 mm^3.

CR indicates number of animals exhibiting complete responses (no measurable tumor mass) within a treatment group.

Results

Anti-KIT Treatment Inhibits KIT Phosphorylation in Mast Cells and Mast Cells are Present in the Tumor Microenvironment

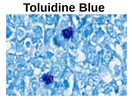
Inhibition of Human and Mouse KIT in SCF-Treated Mast Cells



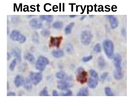
KTN0158 (human KIT) IC_{50} 285 pM
ACK2 (mouse KIT) IC_{50} 418 pM

Mast Cell Staining of Colon26 Tumors

Toluidine Blue

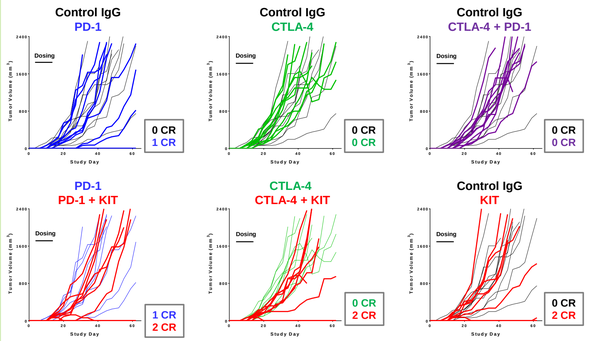


Mast Cell Tryptase



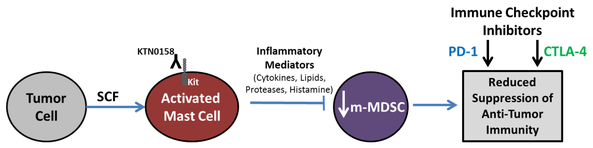
- The potency of KIT inhibition in mouse mast cells by ACK2 was comparable to KTN0158 inhibition of KIT in human mast cells.
- The immune cell content of Colon26 tumors included mast cells identified by *ex vivo* staining of tumor tissue with toluidine blue and an anti-mast cell tryptase antibody.

The Combination of Anti-KIT and Anti-CTLA-4 Exhibits Anti-Tumor Activity in the Pan02 Mouse Pancreatic Tumor Model

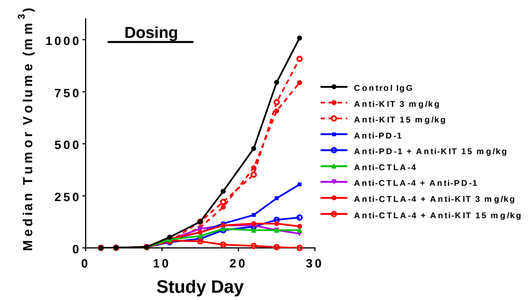


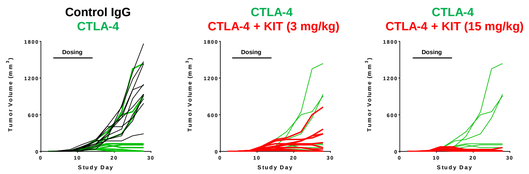
- The combination of anti-KIT and anti-CTLA-4 treatment yielded anti-tumor activity in the Pan02 pancreatic tumor model.
- Anti-CTLA-4 and anti-PD-1 did not exhibit strong anti-tumor activity in the Pan02 model when dosed as single agents or in combination.

Potential Mechanism for Enhanced Efficacy of Immune Checkpoint Inhibitors in Combination with a KIT mAb



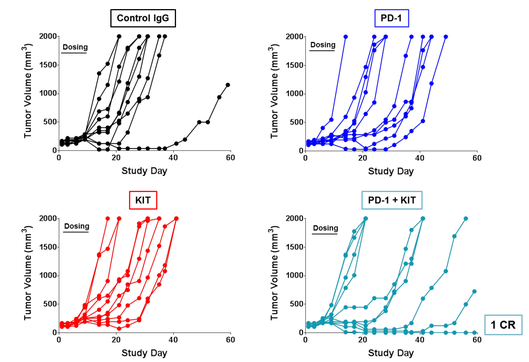
Anti-KIT Treatment Enhances the Anti-Tumor Activity of Immune Checkpoint Inhibitors in the Colon26 Tumor Model





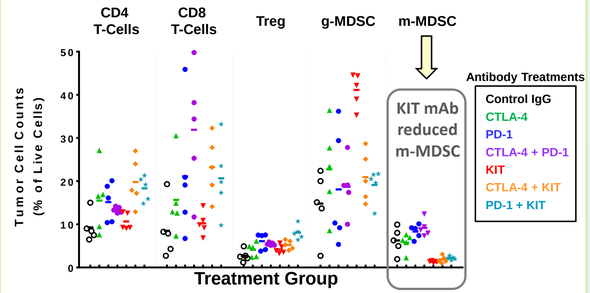
- Anti-CTLA-4 and anti-PD-1 were both efficacious as single agents in the Colon26 model.
- Anti-KIT enhanced activity of both anti-CTLA-4 and anti-PD-1, but had no activity as a single agent in the Colon26 model.

Anti-KIT Treatment Enhances the Anti-Tumor Activity of Anti-PD-1 in the CloudmanS91 Mouse Melanoma Tumor Model



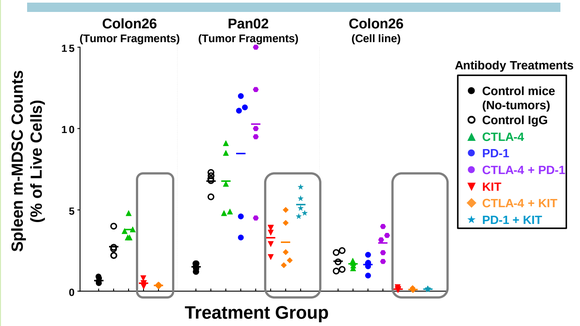
- Combination of anti-KIT and anti-PD-1, or anti-CTLA-4 and anti-PD-1, yielded additional anti-tumor activity in the CloudmanS91 melanoma model compared to single agent treatments.

Immune Cell Profiling of Colon26 Tumors Following Dosing with Antibodies Targeting KIT, PD-1 and CTLA-4



- Anti-KIT treatment reduced monocytic myeloid-derived suppressor cell (m-MDSC) counts in Colon26 tumors.
- Anti-KIT treatment did not further enhance CD8+ T-cell infiltrates induced by anti-CTLA-4 or anti-PD-1 treatments.

Anti-KIT Treatment Reduces Monocytic Myeloid-Derived Suppressor Cell Counts In Multiple Mouse Tumor Models



- m-MDSC numbers were reduced in the spleens of tumor-bearing mice following dosing with an anti-KIT mAb, both in the absence and presence of anti-CTLA-4 or anti-PD-1.

Conclusions

- KTN0158 is a potent humanized anti-KIT mAb in clinical development. Tumor-infiltrating mast cells represent a potential target for anti-KIT antibodies within the tumor microenvironment.
- The combination of an anti-KIT antibody with immune checkpoint inhibitors showed enhanced anti-tumor activity in the Colon26, Pan02 and CloudmanS91 models.
- High pre-treatment m-MDSC counts are associated with reduced survival in melanoma patients treated with checkpoint inhibitors.
- Anti-KIT treatment *in vivo* reduced m-MDSC numbers, which may result in reduced suppression of anti-tumor immunity.
- The data support clinical evaluation of KTN0158 in combination with anti-PD-(L)1 and/or anti-CTLA-4 for the treatment of cancer.