

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

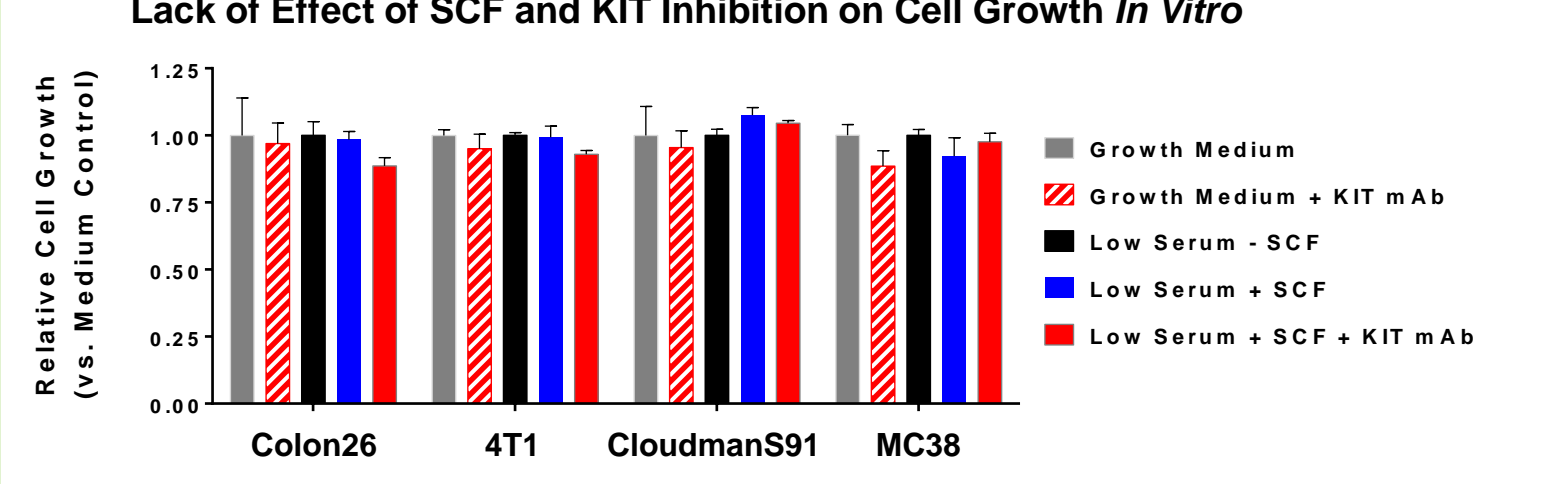
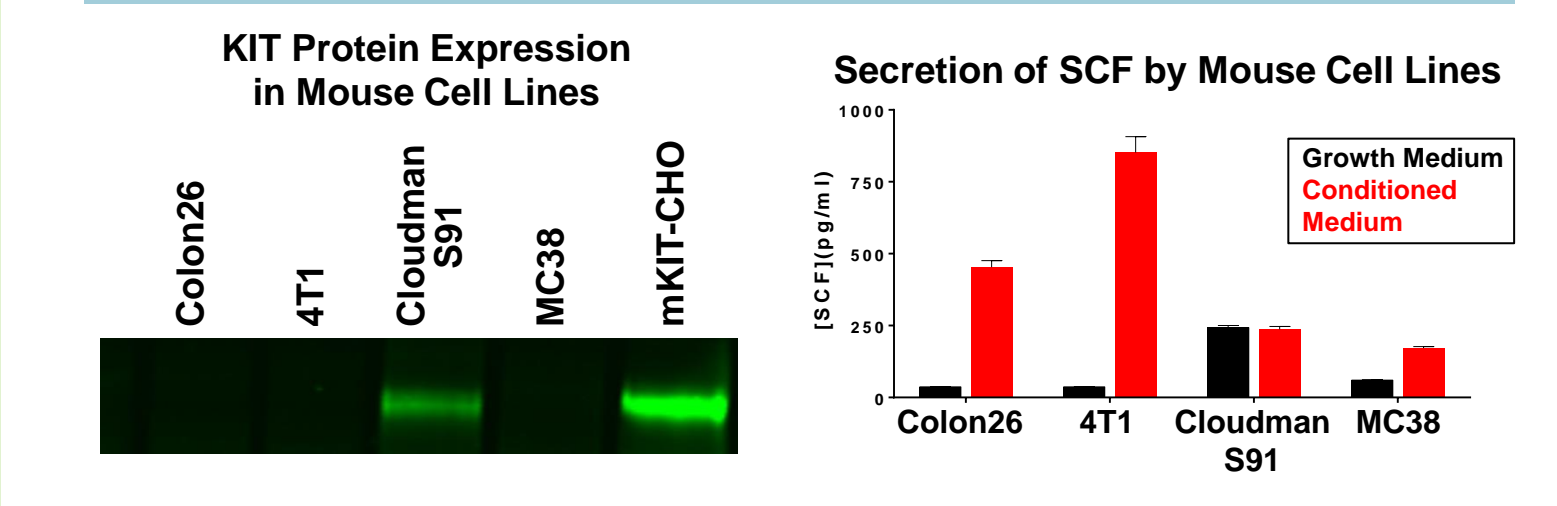
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Introduction

- 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 suppressor 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



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, Biologend)
- 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.

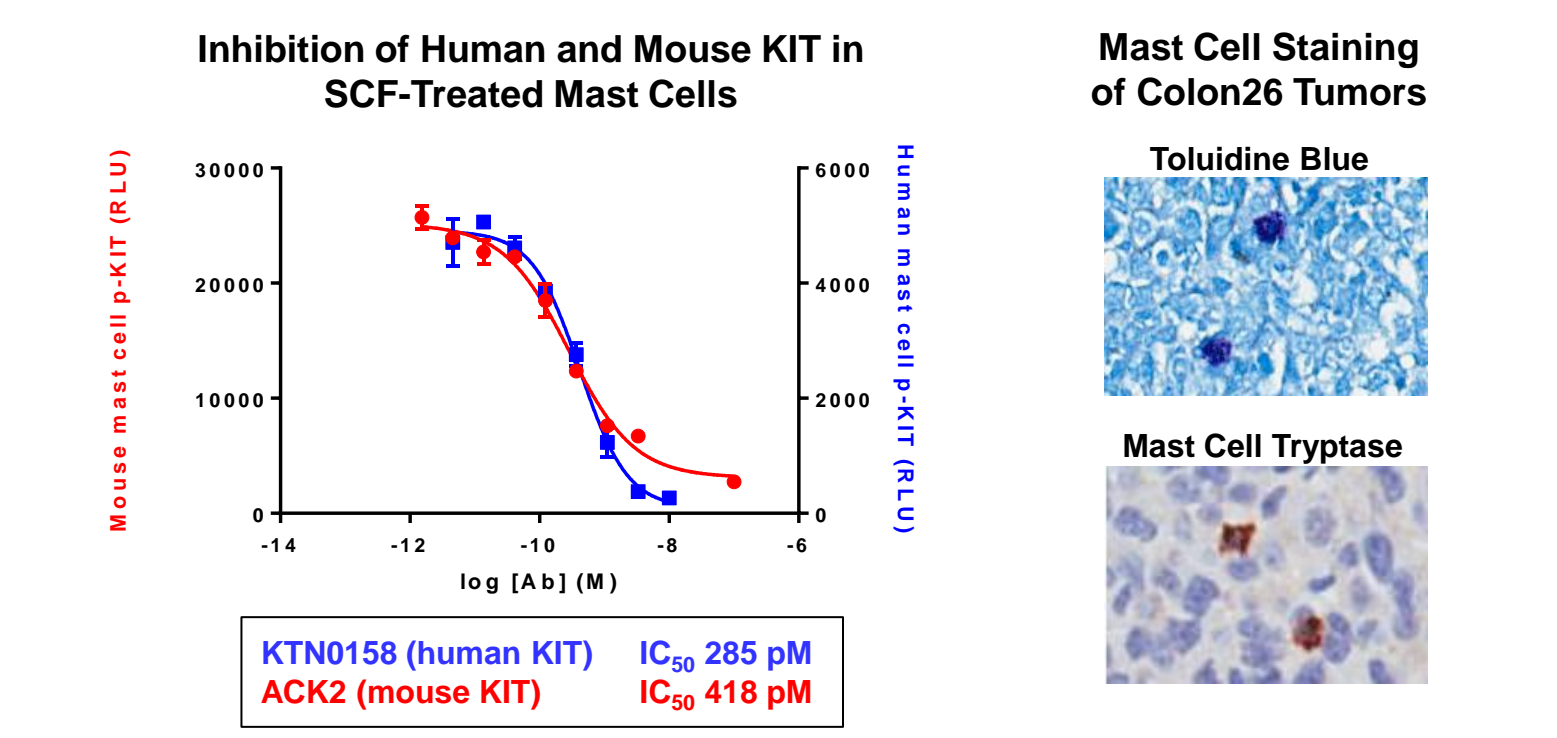
Myeloid Cell Panel: CD45, CD3, CD11b, Ly6G (g-MDSC), Ly6C (m-MDSC).

Dosing and Sampling Schedules (Flow Cytometry Pharmacodynamic Studies)

Tumors were staged to 150-250 mm³, dosed q3d x2, then sampled for flow cytometry at day 7.

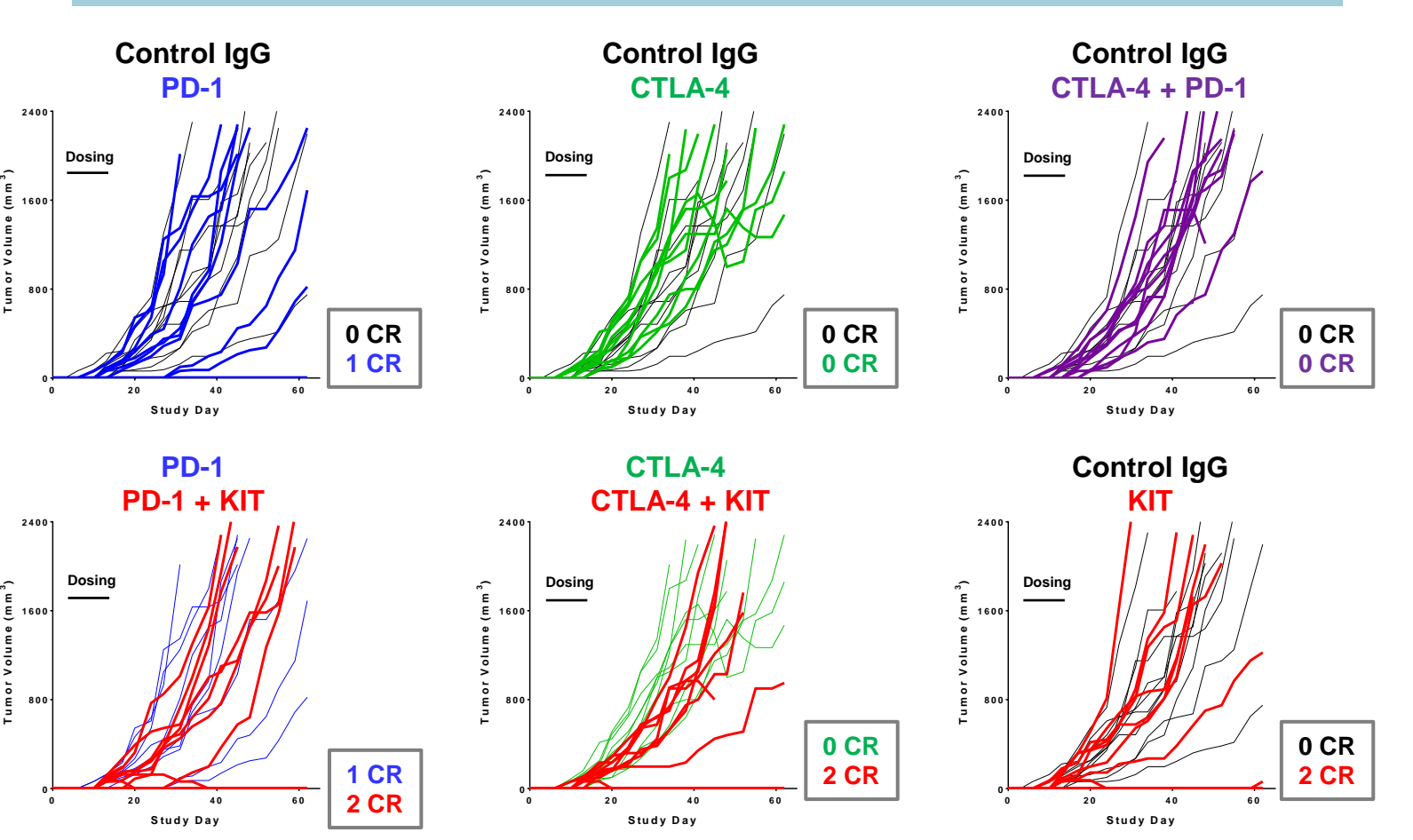
Results

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



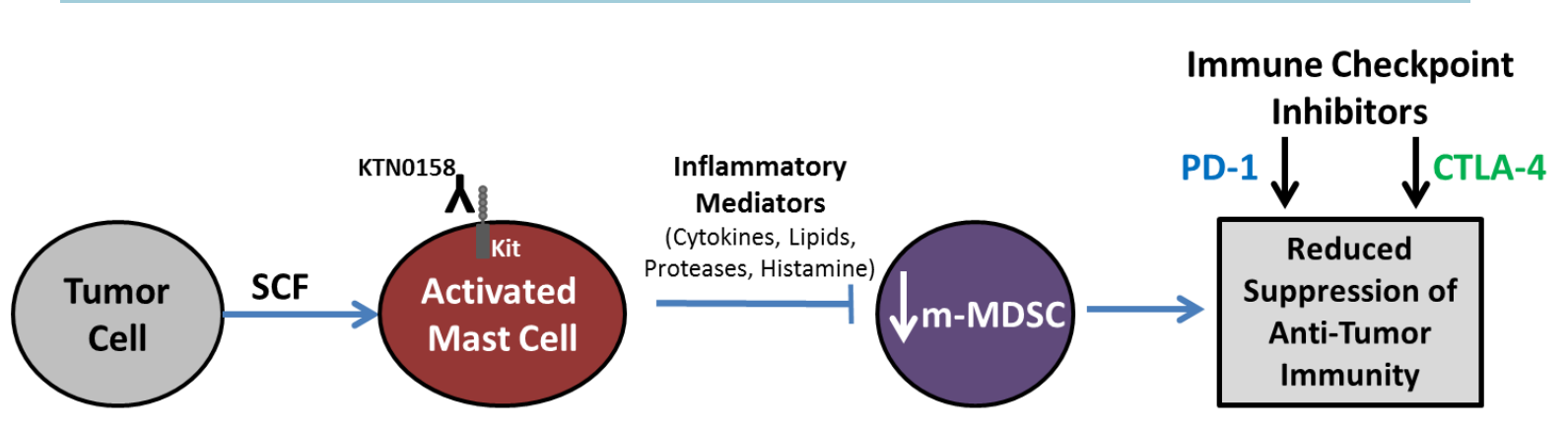
- The potency of KIT inhibition in mouse mast cells by ACK2 was comparable to KTN0158 inhibition of KIT in human mast cells.
- The immune cell content of Colon26 tumors included mast cells identified by *ex vivo* staining of tumor tissue with toluidine blue and an anti-mast cell tryptase antibody.

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



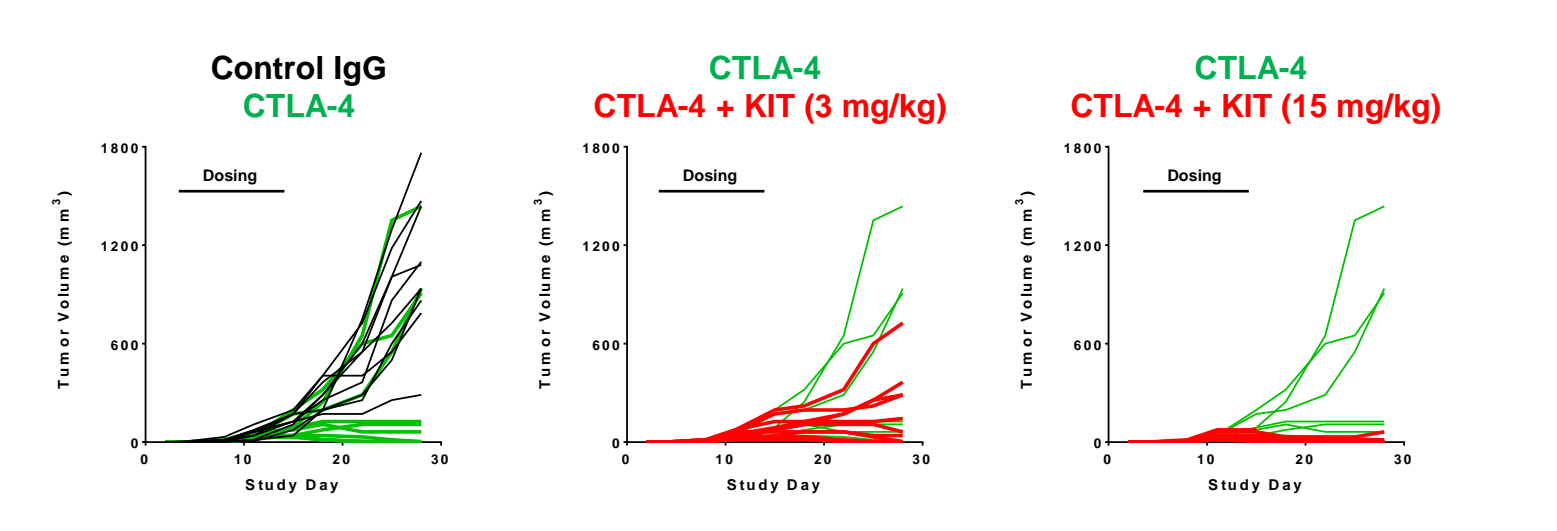
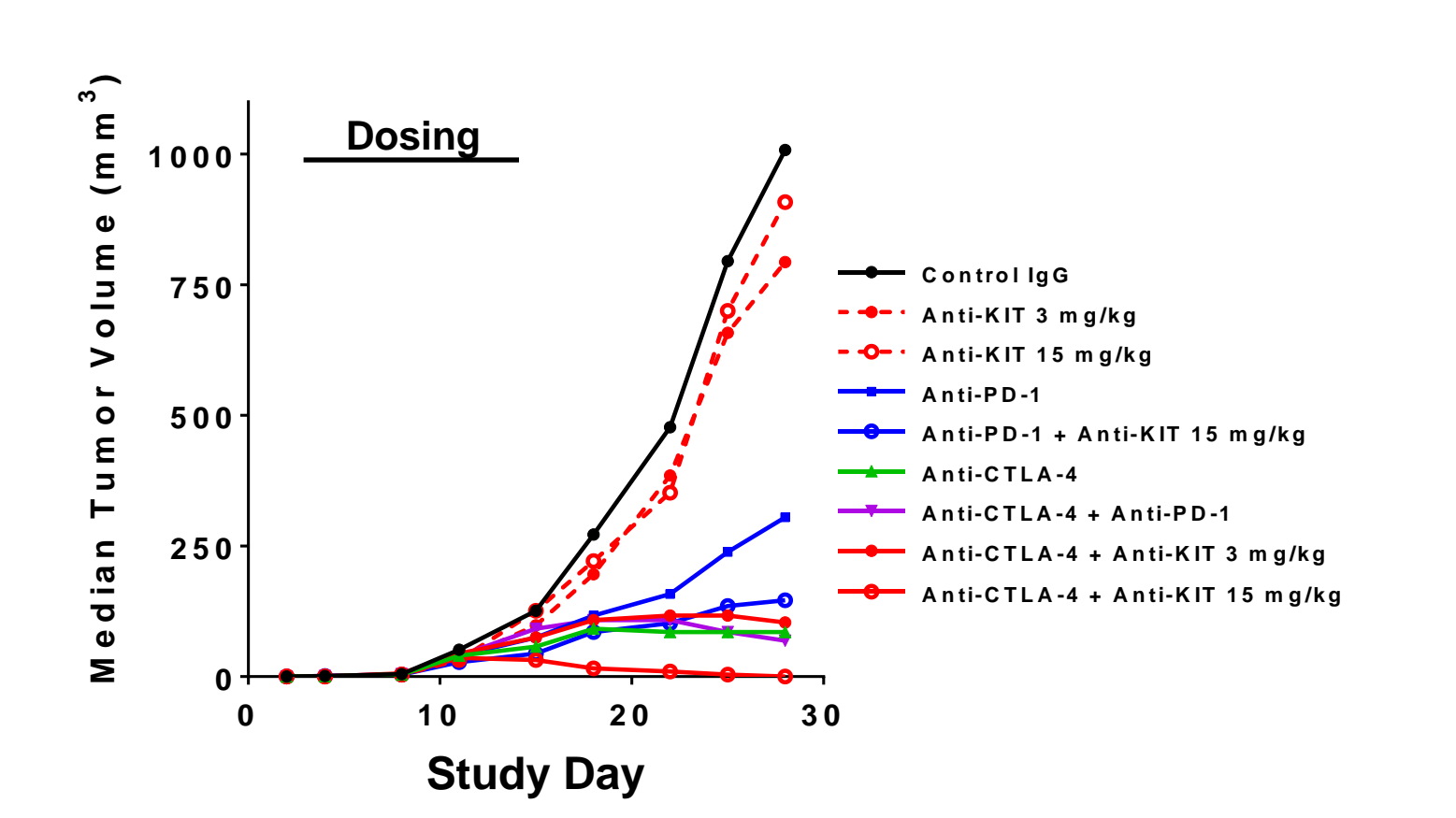
- The combination of anti-KIT and anti-CTLA-4 treatment yielded anti-tumor activity in the Pan02 pancreatic tumor model.
- Anti-CTLA-4 and anti-PD-1 did not exhibit strong anti-tumor activity in the Pan02 model when dosed as single agents or in combination.

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



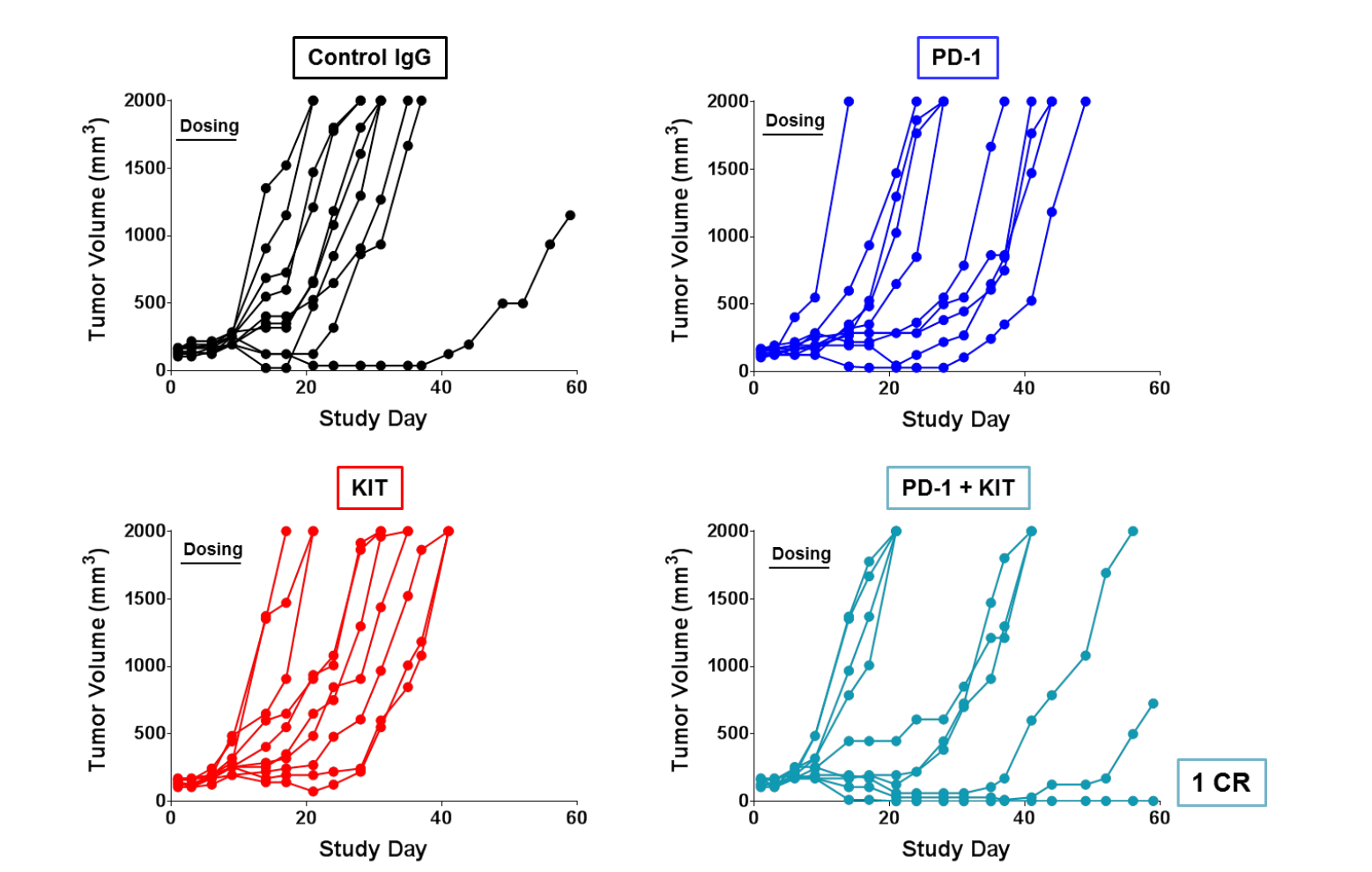
Results

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



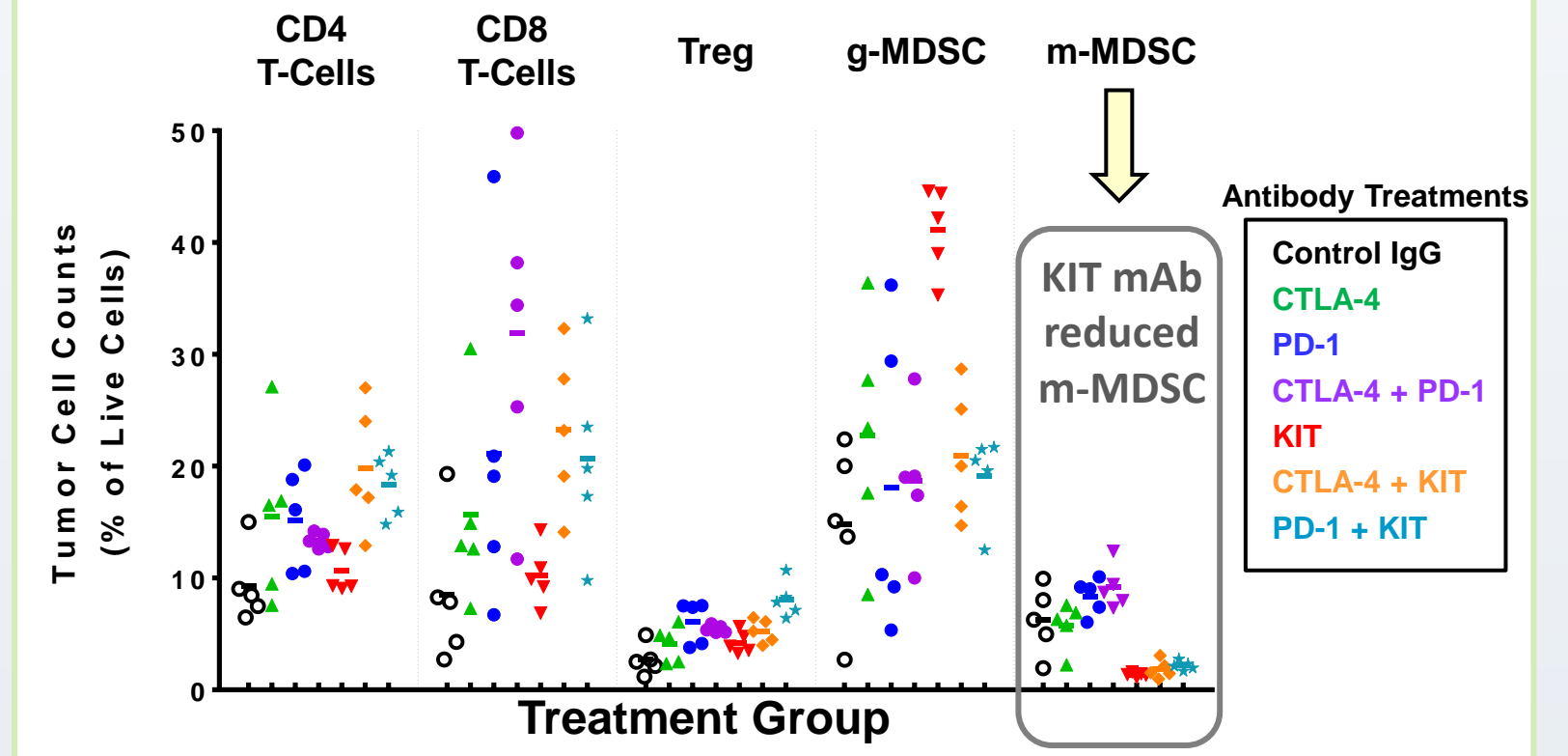
- Anti-CTLA-4 and anti-PD-1 were both efficacious as single agents in the Colon26 model.
- Anti-KIT enhanced activity of both anti-CTLA-4 and anti-PD-1, but had no activity as a single agent in the Colon26 model.

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



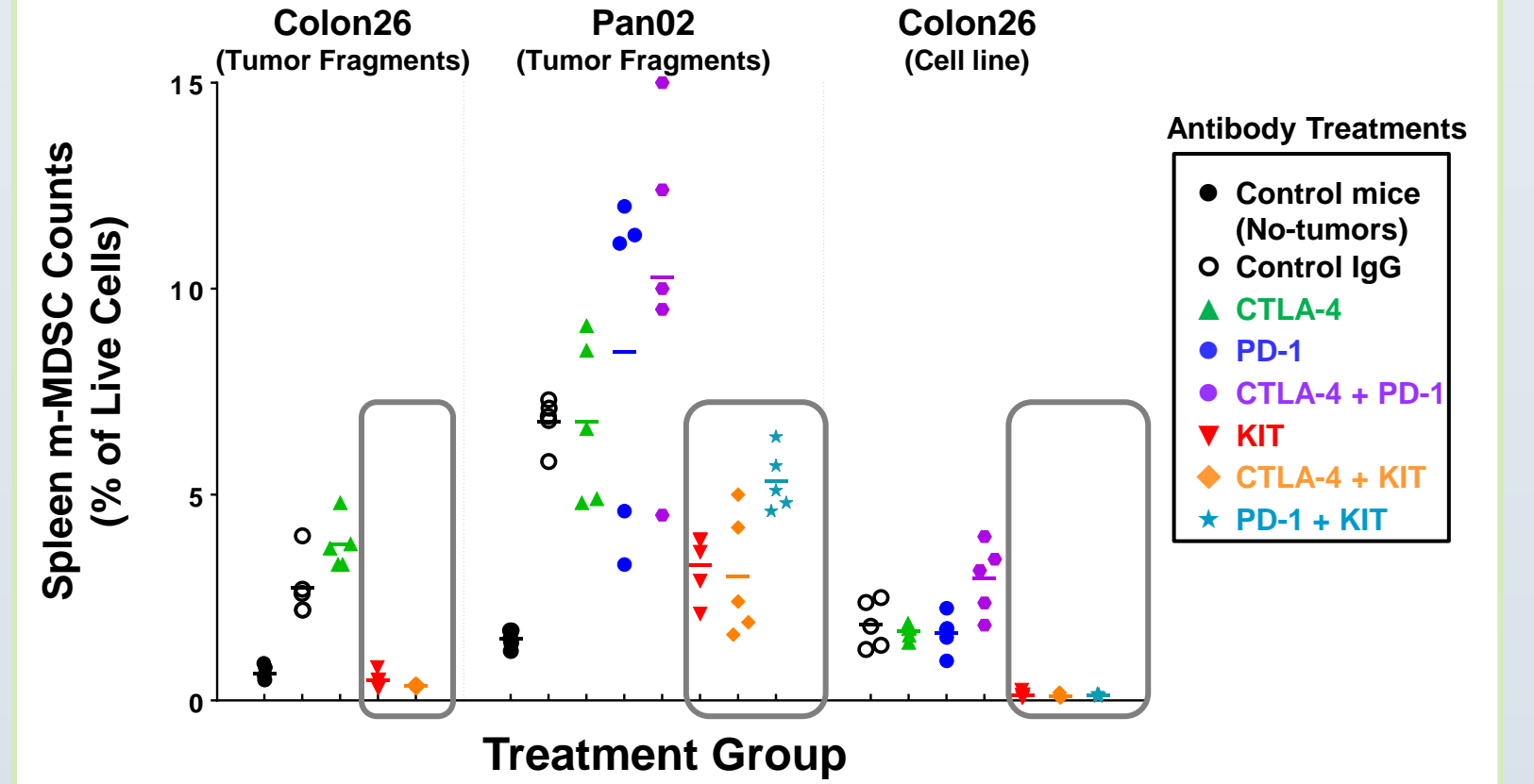
- Combination of anti-KIT and anti-PD-1, or anti-CTLA-4 and anti-PD-1, yielded additional anti-tumor activity in the CloudmanS91 melanoma model compared to single agent treatments.

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.