

Effects of Crenolanib (CP-868596), a Highly Selective Platelet-derived Growth Factor Receptor (Pdgfr) Tyrosine Kinase Inhibitor, on the Proliferation of Interstitial Cell of Cajal (ICC) Precursors and *Kit*-mutant Gastrointestinal Stromal Tumor (GIST) Cells

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Background 1

➤ **Kit** and **Pdgfra** are closely related type 3 receptor tyrosine kinases found in distinct interstitial cell types of the GI *tunica muscularis*:

➤ **Kit** is expressed in **ICC** and **Pdgfra** is expressed in **'fibroblast-like cells' (FLC)**

Sanders *et al.*, Neurogastroenterol Motil 1999; 11: 311-38
Iino & Nojyo, Arch Histol Cytol 2009; 72: 107-15

➤ Most **GISTs** harbor oncogenic, mutually exclusive **Kit** or **Pdgfra mutations**, which are in most cases heterozygous

Heinrich *et al.*, Science 2003; 299: 708-10
Negri *et al.*, J Pathol 2009; 217: 103-112

Background 2

- However, both fetal and adult ICC stem cells (**ICC-SC**), as well as most **GISTs co-express wild-type Kit and Pdgfra**

Kurahashi *et al.*, Neurogastroenterol Motil 2008; 20: 521-31

Bardsley *et al.*, Gastroenterology 2010;139:942-52

Negri *et al.*, J Pathol 2009; 217: 103-12

- Signaling mediated by wild-type **Kit/Pdgfra heterodimers contributes to GIST growth** even after blocking oncogenic signaling by Kit/Pdgfra inhibitors such as **imatinib**

Negri *et al.*, J Pathol 2009; 217: 103-112

- Thus, Kit and Pdgfra signaling **may not be as compartmentalized** as previously suggested and **Pdgfra inhibition may further inhibit Kit⁺ GIST growth**

Aims

To investigate

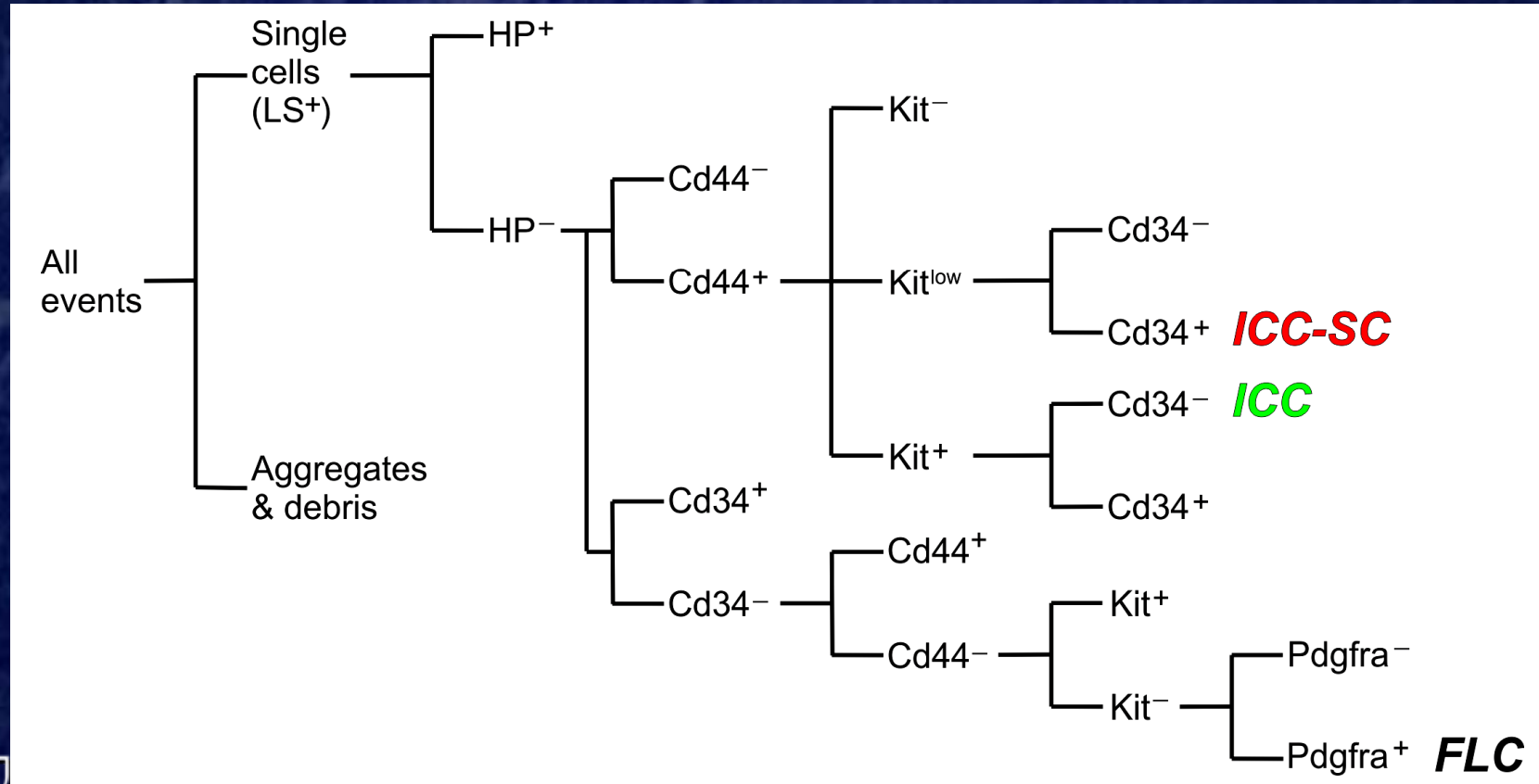
- the **co-expression of Kit, Pdgfra and Pdgfrb** in cells of the ICC lineage and GIST;
- and
- the effect of **Pdgfr inhibition on cell proliferation** in Kit⁺ and Kit⁻, Pdgfr-expressing cells

Detection of ICC, ICC-SC and FLC by Flow Cytometry

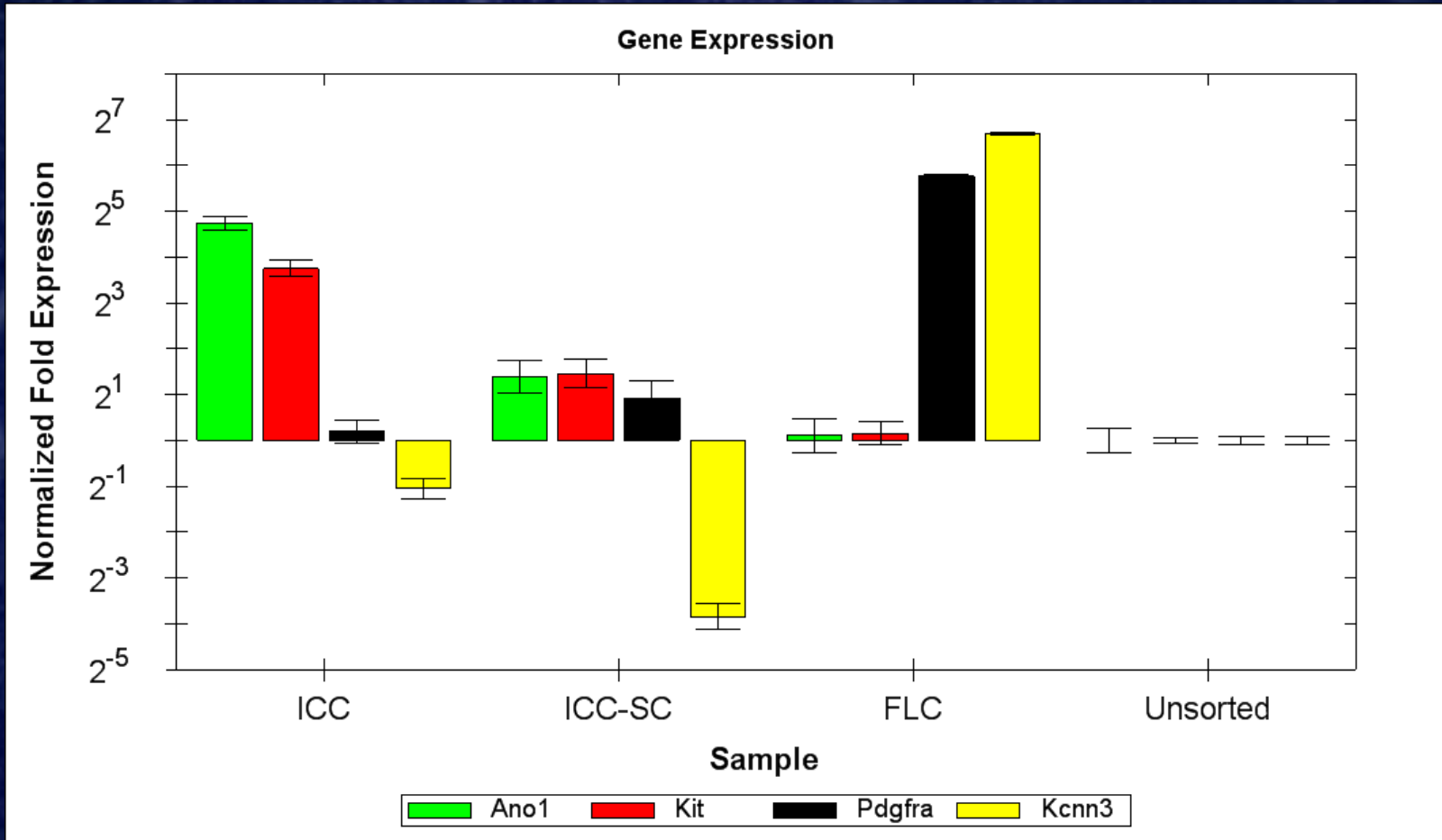
ICC: Kit⁺Cd44⁺Cd34⁻

ICC-SC: Kit^{low}Cd44⁺Cd34⁺ (e.g., Bardsley et al., Gastroenterology 2010)

FLC: Pdgfra⁺Kit⁻Cd44⁻Cd34⁻ (or Cd34^{low}; Iino&Nojyo, Arch Histol Cytol 2009)

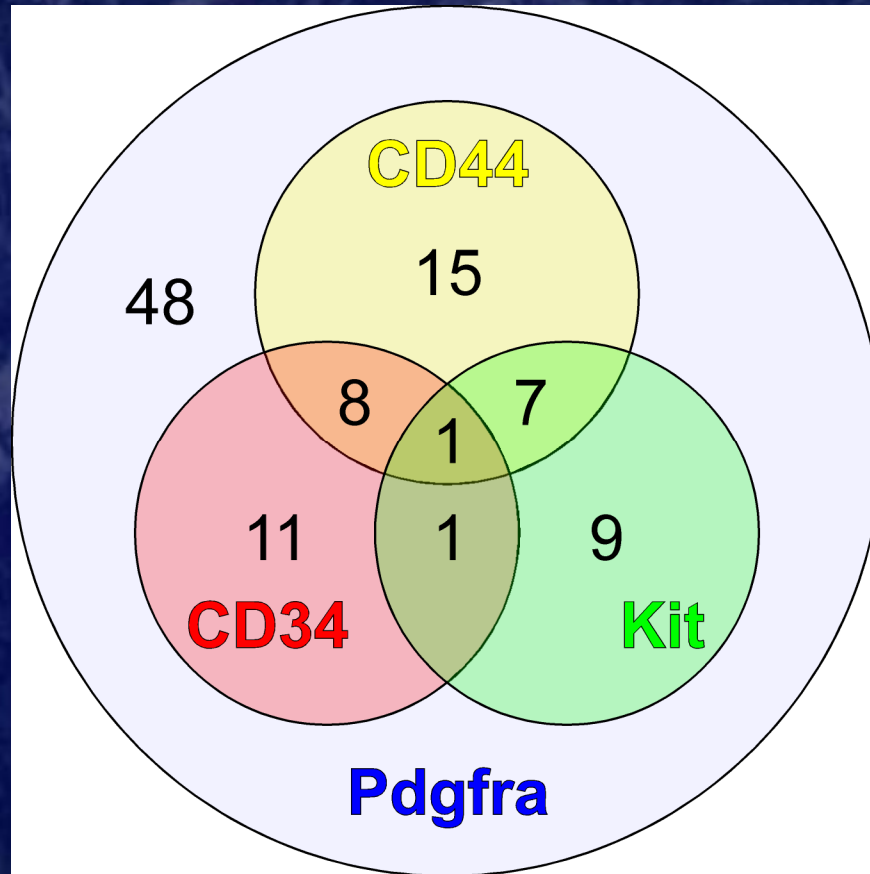


Validation of Flow Cytometry/FACS by qRT-PCR



Day 20 BALB/c corpus+antrum

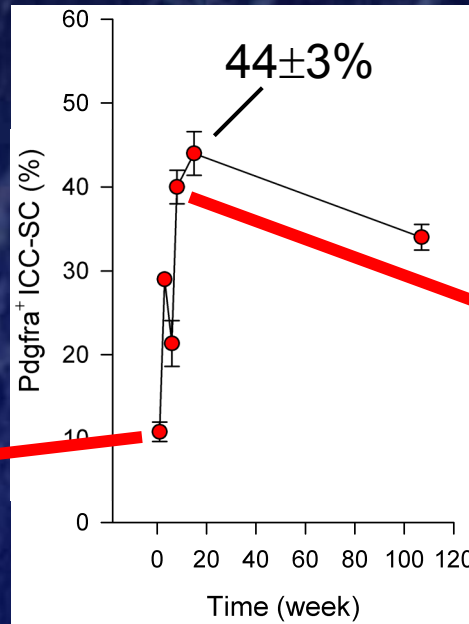
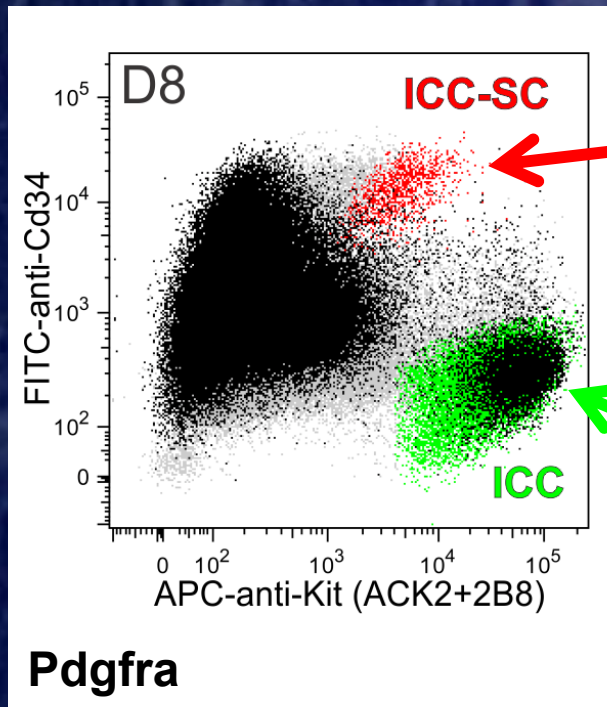
Distribution of Pdgfra Immunoreactivity



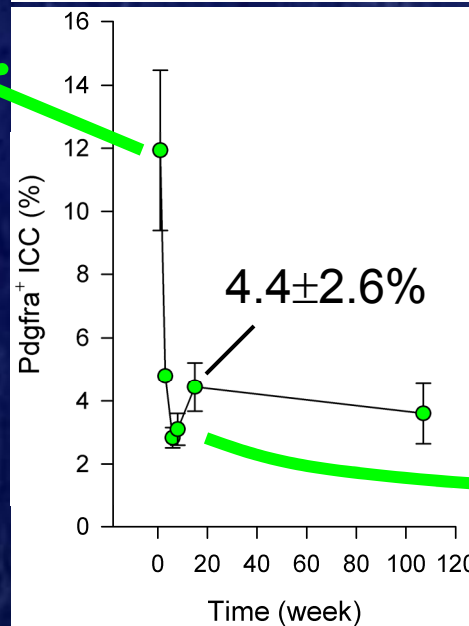
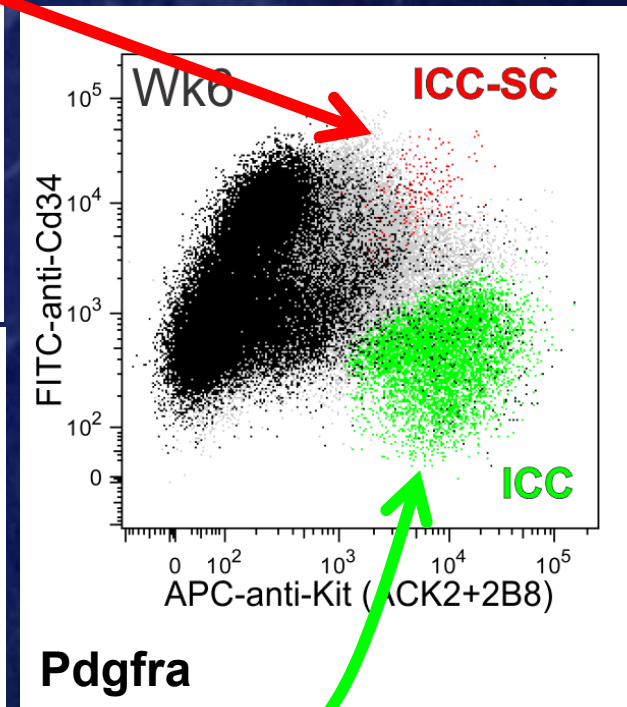
Day 21 BALB/c corpus+antrum

Pdgfra is Expressed by Subsets of ICC & ICC-SC

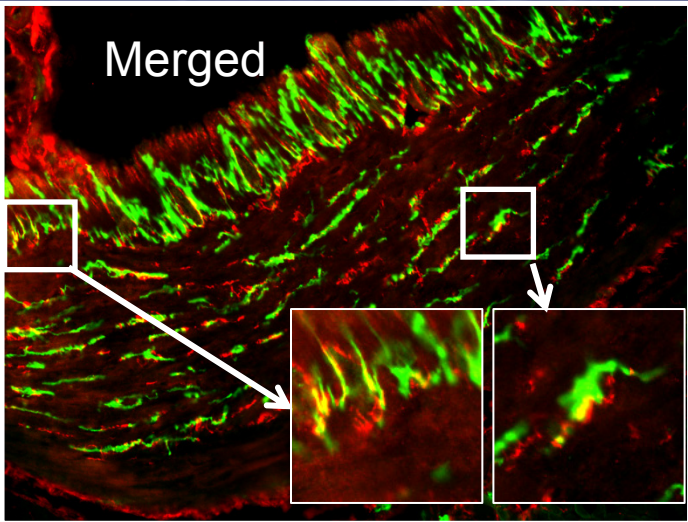
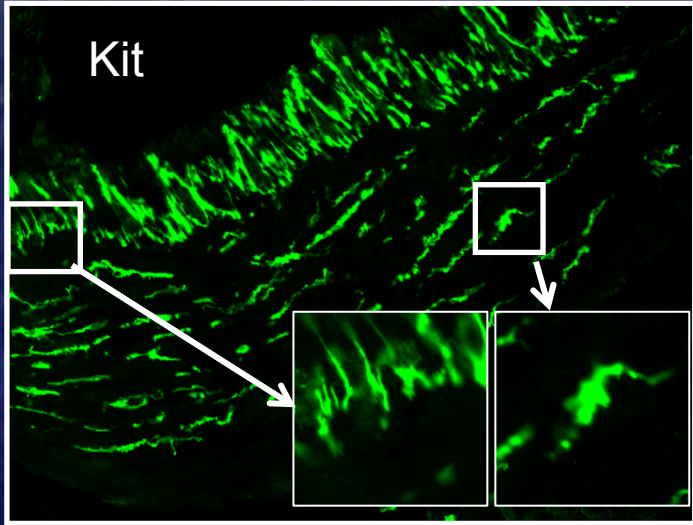
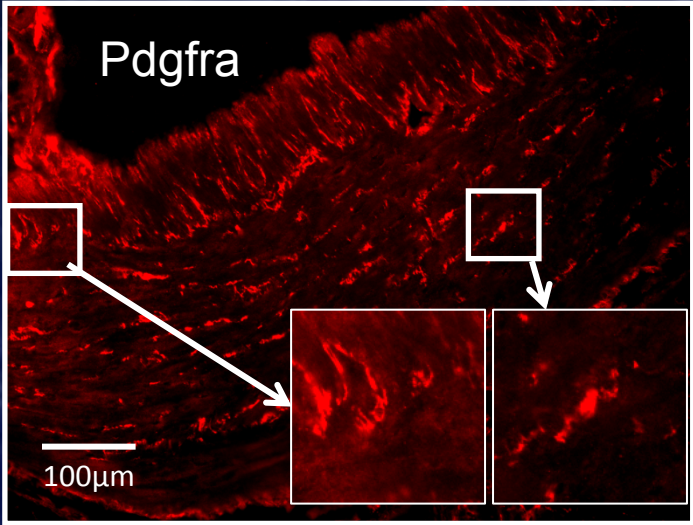
Age: 8 days



Age: 6 weeks



Co-expression of Pdgfra and Kit



Colocalized:
4.6% of ICC

Adjacent/overlapping

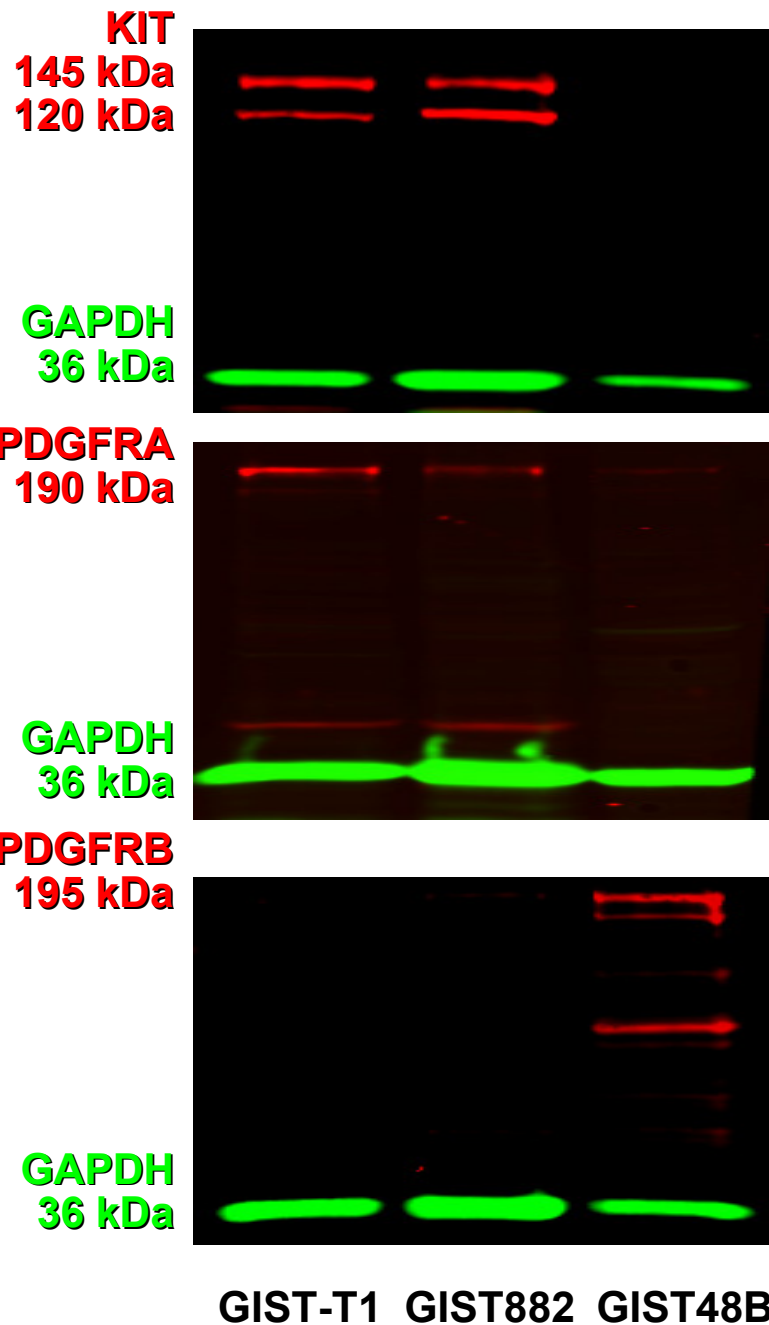
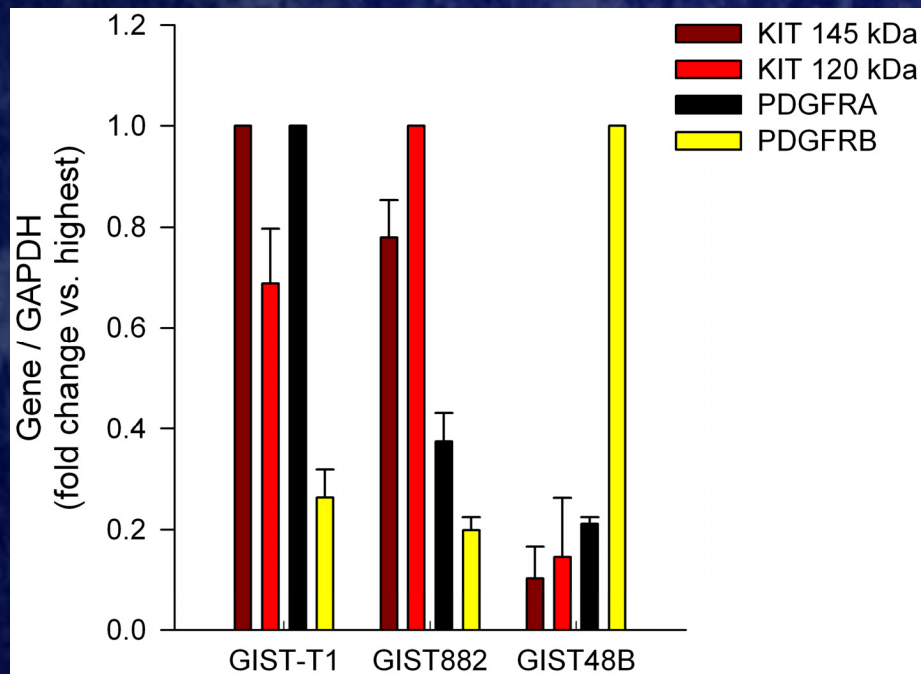
Summary 1

- **Kit and Pdgfra are co-expressed** in a subset of murine ICC
- The proportion of **Kit⁺Pdgfra⁺ ICC** and **ICC-SC** changes with age

Human GIST Cell Lines

- **GIST-T1**: Heterozygous *KIT* mutant, **KIT⁺**, imatinib-sensitive
- **GIST882**: Homozygous *KIT* mutant, **KIT⁺**, imatinib-sensitive
- **GIST48B**: Homozygous & heterozygous double *KIT* mutant, **KIT⁻**, imatinib-resistant

Both KIT⁺ and KIT⁻ GIST Cells Express PDGFR Isoforms



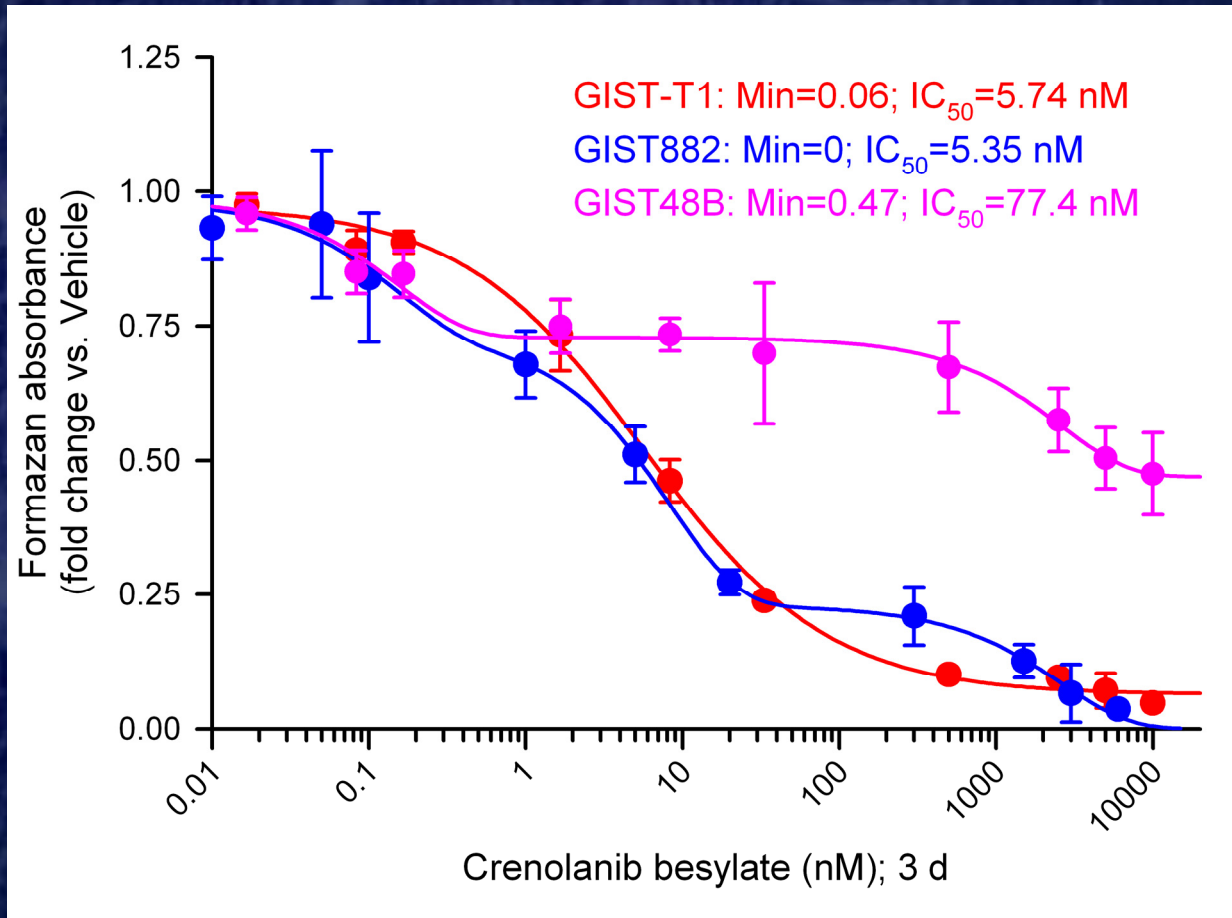
Crenolanib besylate (CP-868,596)

- **Crenolanib** (AROG pharmaceuticals) is an orally bioavailable **Pdgfra/b tyrosine kinase inhibitor**

Lewis *et al.*, J Clin Oncol 2009; 27: 5262-9

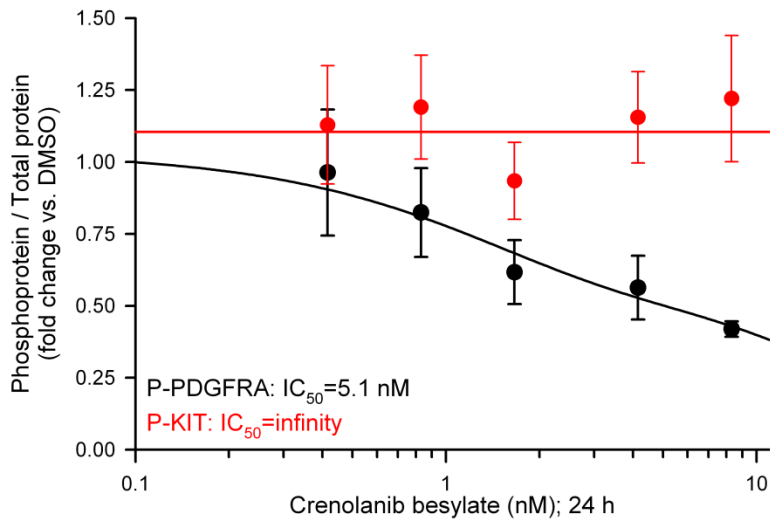
- **Highly selective** for Pdgfra/b over other tyrosine kinases including Kit: >100x
- **Highly potent**: IC₅₀: Pdgfra: 1.7 nM; Pdgfrb: 0.67nM

Crenolanib Effectively Inhibits the Proliferation of KIT⁺ But Not KIT⁻ GIST Cells (MTS Assay)

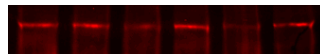


Mechanisms of Crenolanib's Effect on GIST-T1 Cells

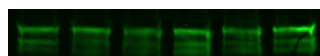
Crenolanib inhibits PDGFRA phosphorylation but not KIT phosphorylation



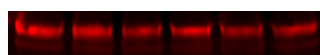
P-Y742 PDGFRA 190 kDa



PDGFRA 190 kDa



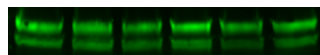
P-Y719 KIT 145 kDa



P-Y719 KIT 120 kDa



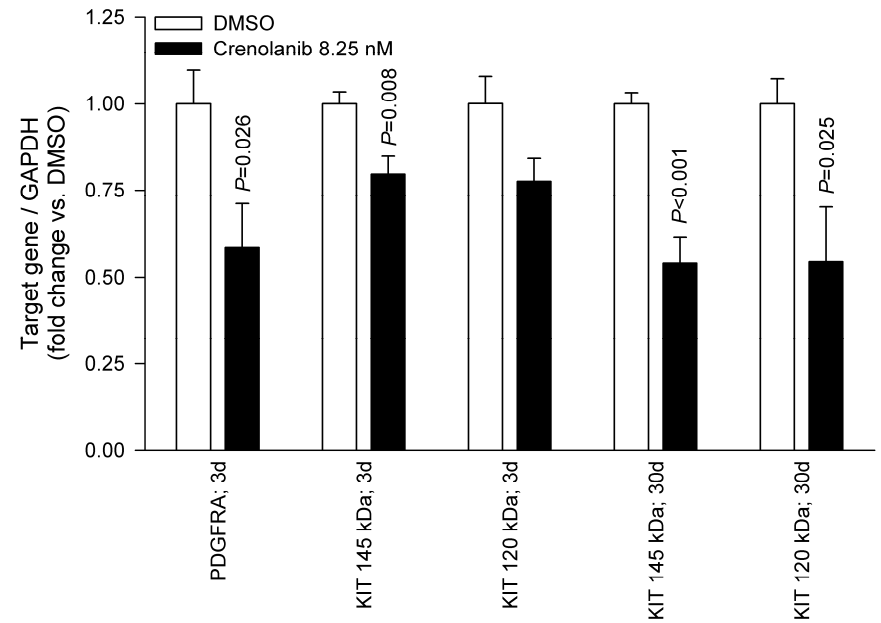
KIT 145 kDa



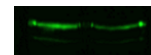
KIT 120 kDa



Crenolanib inhibits PDGFRA & KIT expression



PDGFRA 190 kDa



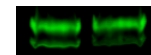
36 kDa GAPDH



KIT 145 kDa 120 kDa



36 kDa GAPDH



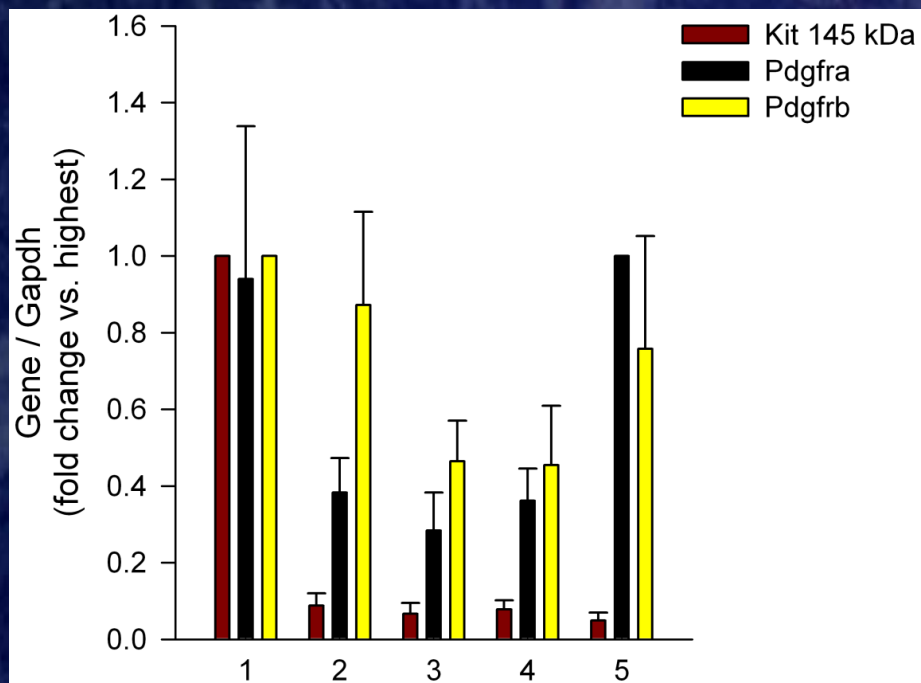
Summary 2

- Crenolanib inhibited the proliferation of **KIT⁺ human GIST** cells with an IC_{50} comparable to that of imatinib
- Crenolanib's **anti-proliferative effect** in these cells paralleled the inhibition of Pdgfr phosphorylation but appeared to require **expression of KIT protein**

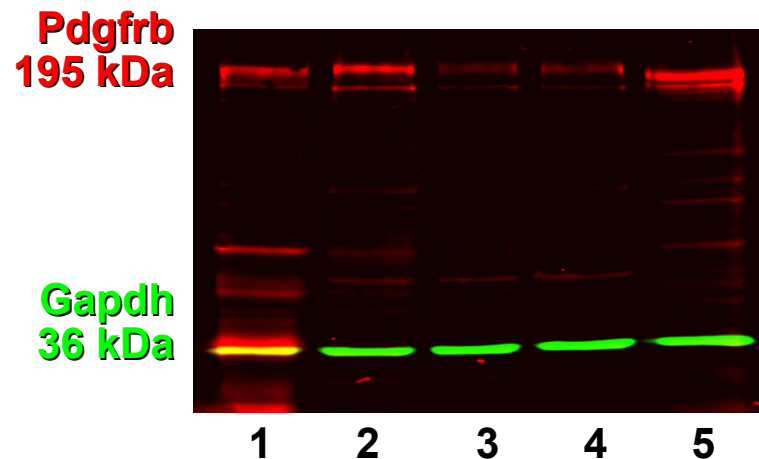
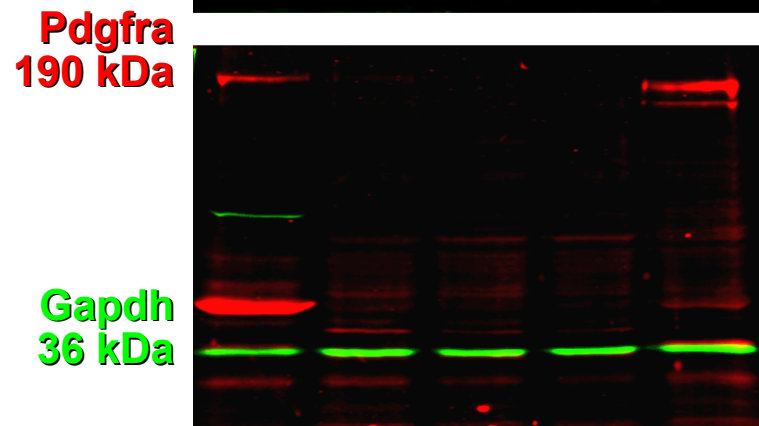
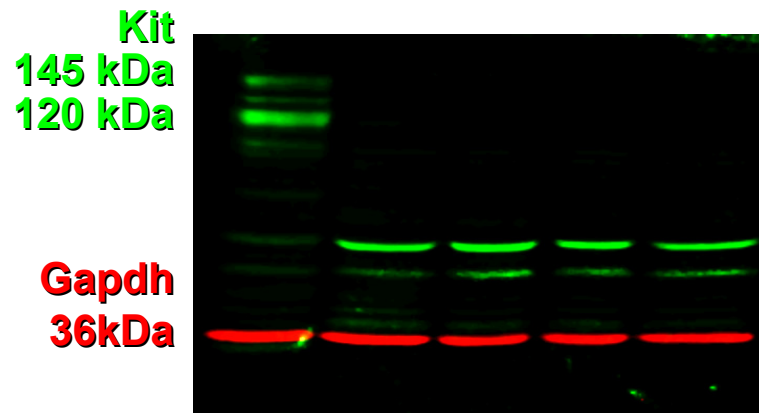
Cell Lines Derived From Murine ICC Lineage

- **ICL2A**: Conditionally immortalized ICC
- **D2211B, 2xSCS70**: Spontaneously transformed, $\text{Kit}^{\text{low/-}}$ ICC-SC lines
- **2xSCS2F10**: Wild-type $\text{Kit}^{\text{low/-}}$ ICC-SC line

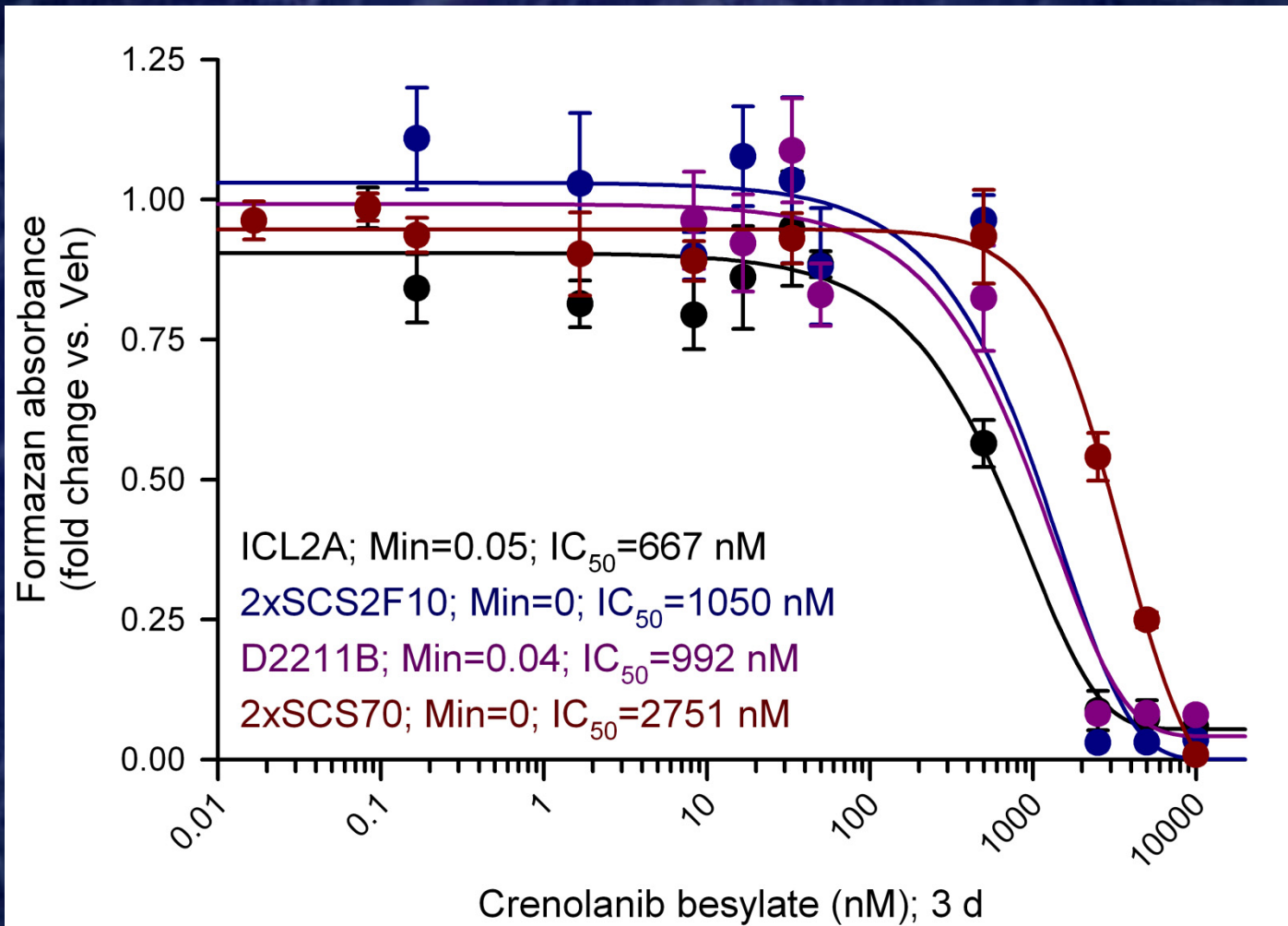
ICC-Related Cells Lacking Full-Length Kit Express PDGFR Isoforms



- 1: BALB/c corpus+antrum
- 2: ICL2A
- 3: D2211B
- 4: 2xSCS70
- 5: 2xSCS2F10

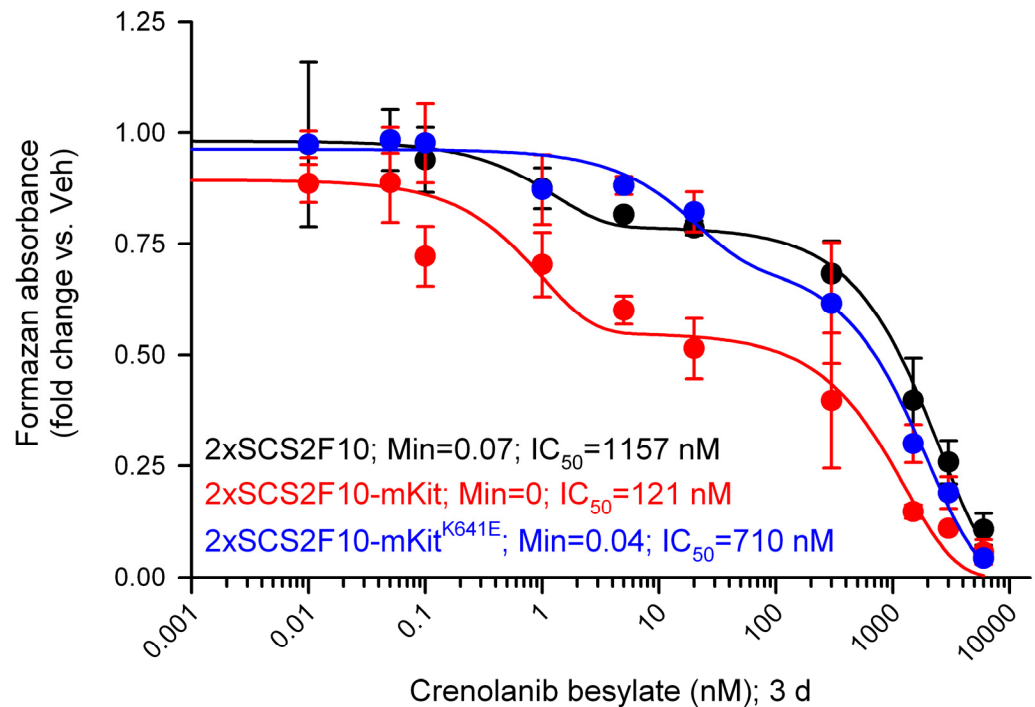
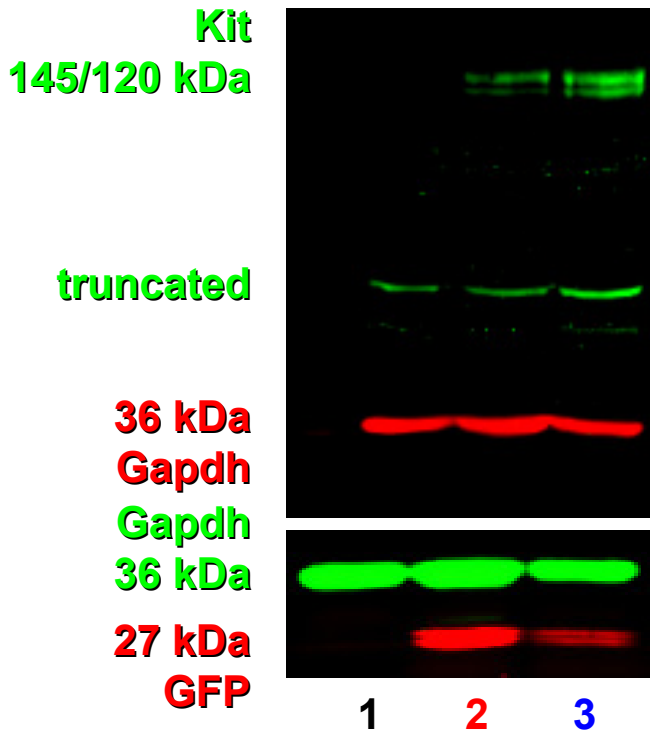


Crenolanib Poorly Inhibits the Proliferation of KIT⁻ ICC-Related Cells



Expression of Wild-type but not Constitutively Active Kit in Pdgfr⁺ ICC-SC Increases Their Sensitivity to Crenolanib

2xSCS2F10 cells (1) were retrovirally transduced with full-length wild-type murine Kit (**2xSCS2F10-mKit**; 2) or murine Kit bearing the activating mutation K641E (**2xSCS2F10-mKit^{K641E}**; 3)



Summary 3

- Crenolanib did not effectively inhibit the proliferation of **Kit⁻ cells expressing wild-type Pdgfra and/or Pdgfrb**
- Retroviral transduction with wild-type murine **Kit** increased the cells' **sensitivity to crenolanib**

Conclusion

- Inhibition of wild-type PDGFRA expressed in some **KIT mutant, KIT-addicted GIST** can potently inhibit cell proliferation
- **The inhibitory effect of crenolanib** in cells expressing wild-type PDGFR isoforms appears to **require the presence of KIT protein**
- **Co-expression of Pdgfr with wild-type Kit may enhance the effects of crenolanib**

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