CRISPR-Sublines	PDGFRa mutation
T1α^{D842V}	D842V
T1α$^{D842V/QGPins}$	D842V + P653_H654insQGP
T1α$^{D842V/G652E}$	D842V + G652E
T1α$^{D842V/V658A}$	D842V + V658A
T1α$^{D842V/V658A/G652E}$	D842V + V658A + G652E
T1α$^{D842V/T670I}$	D842V + T670I
T1α$^{D842V/G680R}$	D842V + G680R

Gene editing by CRISPR-Cas9

Further cell lines with endogenous PDGFRA mutations (T1α^{D842V}, T1α$^{D842V/QGPins}$, T1α$^{D842V/G652E}$, T1α$^{D842V/V658A}$, T1α$^{D842V/V658A/G652E}$, T1α$^{D842V/T670I}$, T1α$^{D842V/G680R}$) were generated by CRISPR/Cas9 mediated gene editing, as described previously [1], with the following deviations: We used recombinant Cas9 from IDT Labs (60µM) in a 1:2 ratio with specific single guide RNA (sgRNA). First, PDGFRA^{D842V} was introduced into parental GIST-T1 and cells were selected with IM 200nM until outgrowth of a resistant population, which carried heterozygous PDGFRA^{D842V}. In a second round of editing, one sgRNA specific for *PDGFRA* resistance mutations (exons 14 and 15) and an ssODN template to introduce the desired mutation (P653_H654insQGP, G652E, V658A, G652E/V658A, T674I, and G680R, respectively) was combined with a guide targeting exon 1 (for G652E, G652E/V658A, T674I, and G680R) or mutant exon 11 (for D842V-only, QGPins, V658A) of KIT. Cells were then treated with avapritinib 100nM and after outgrowth of a resistant population, single cell clones were derived. Heterozygous D842V and in-cis secondary mutations as well as frameshift mutations in mutant exon 1 and 11 of KIT were confirmed by sanger sequencing and/or next generation panel sequencing. Strong reduction of KIT expression and phosphorylation were confirmed by western blotting. For a complete list of sequences used in CRISPR experiments see supplemental material [2].

1. Muhlenberg, T., et al., *KIT-Dependent and KIT-Independent Genomic Heterogeneity of Resistance in Gastrointestinal Stromal Tumors - TORC1/2 Inhibition as Salvage Strategy.* Mol Cancer Ther, 2019. **18**(11): p. 1985-1996.
2. Grunewald, S., et al., *Resistance to Avapritinib in PDGFRA-Driven GIST Is Caused by Secondary Mutations in the PDGFRA Kinase Domain.* Cancer Discov, 2021. **11**(1): p. 108-125.

If you are interested, please contact contact@husarc.org for future procedure (MTA, shipping costs, collaboration options etc.)