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Effect of secondary KIT mutations on growth of GIST cells in the absence of selective pressure by imatinib in isogenic models of imatinib resistance

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Background
Secondary mutations of KIT are commonly found in progressing lesions of imatinib (IM)-resistant GIST and represent a major factor of drug resistance. Development of salvage treatments is complicated by the genomic heterogeneity of secondary mutations. We hypothesize that resistant clones require selective pressure to overgrow sensitive GIST and that mixed sensitive/resistant populations may still display drug sensitivity.

Methods:
Based on parental IM-sensitive GIST-T1 (KIT Ex11 57bp del) we have developed IM-resistant isogenic sublines harbouring critical secondary mutations (T670I, D816E, D820A, A829P). Each subline was transduced with specific marker proteins allowing detection of resistant subclones by FACS or IHC. Proliferation and cell viability for individual cell lines and mixed cell populations (+/- IM) were evaluated in vitro and in vivo.

Results:
IM-resistant cell-lines grew faster in the presence of IM compared to no treatment. Resistant sublines were xenogenic in nude mice but were overgrown by parental GIST-T1 in mixed populations in the absence of IM. Tumors derived from mixed GIST populations contained high levels of resistant clones in IM-treated mice. To estimate the proportion of resistant cells within a population necessary to display resistance, we mixed resistant sublines with sensitive cells at known percentages. Sensitivity to IM was lost when the fraction of resistant cells was >50% compared to parental cells (3- and 6 day time point).

Conclusion:
GIST with acquired resistance likely harbor mixed populations of drug-sensitive and resistant cells which exhibit differential growth rates. Our studies show that tumor populations with low levels of resistant clones may still respond to treatment. Secondary KIT mutations confer a growth disadvantage in the absence of IM. Selective pressure by IM results in partial KIT inhibition which may reduce the oncogenic stress of kinase hyperactivation in GIST harbouring secondary mutations. Altering the selective pressure could therefore delay the emergence of resistant clones and our models could be applied toward optimization of drug selection and dosing strategies.

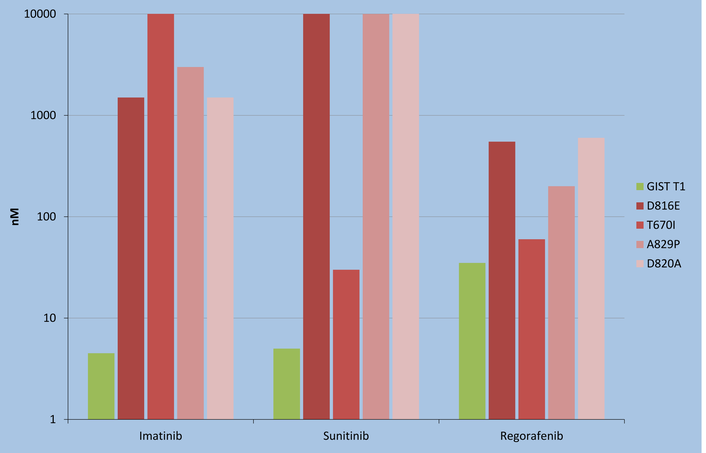


Figure 1

Secondary KIT mutations in exon 14 and 17 confer resistance to most clinically relevant KIT inhibitors in vitro as measured by SRB-assay (3 day treatment).

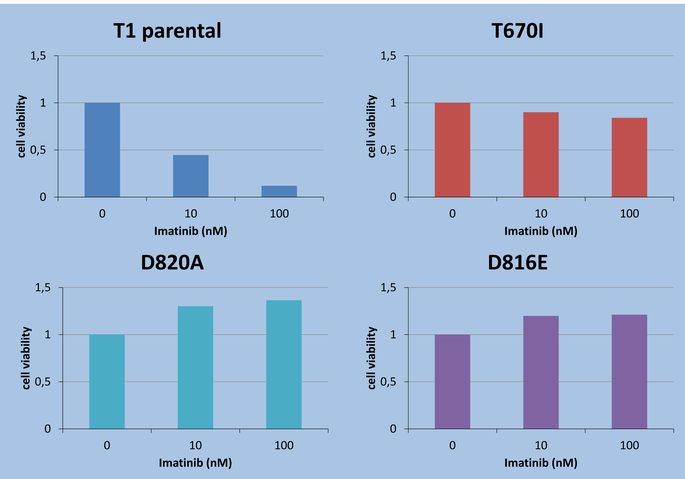


Figure 2

Imatinib may promote cell growth in the presence of secondary resistance mutations in vitro (SRB-assay, 3-day treatment).

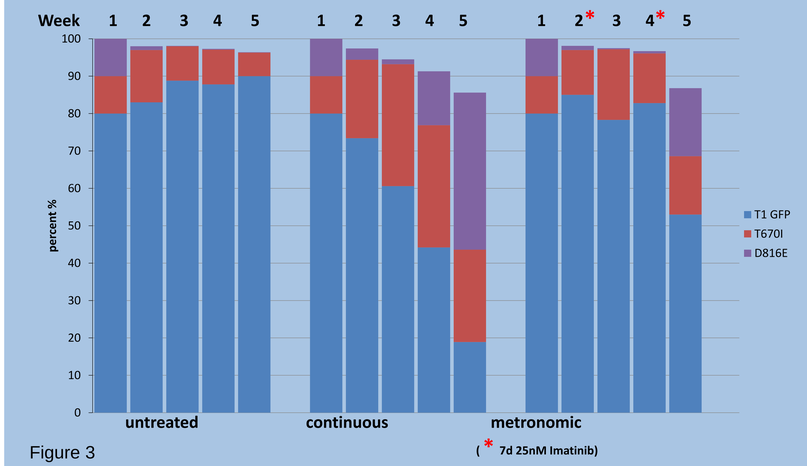


Figure 3

Metronomic imatinib treatment delays outgrowth of mixed populations of imatinib-sensitive and resistant clones in vitro as measured by FACS.

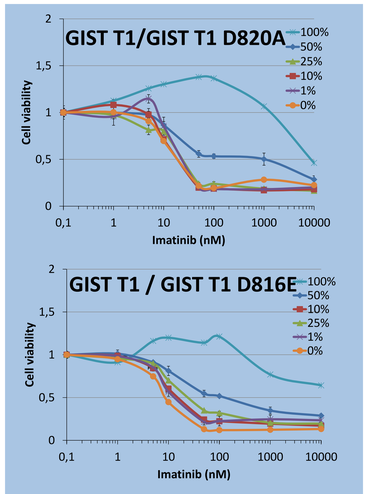


Figure 4

Determination of resistant phenoytype of a mixed population of imatinib-sensitive and resistant GIST subclones.

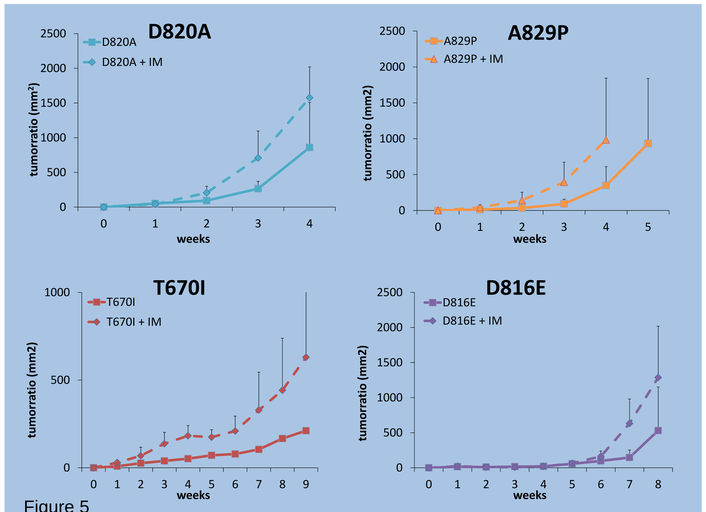
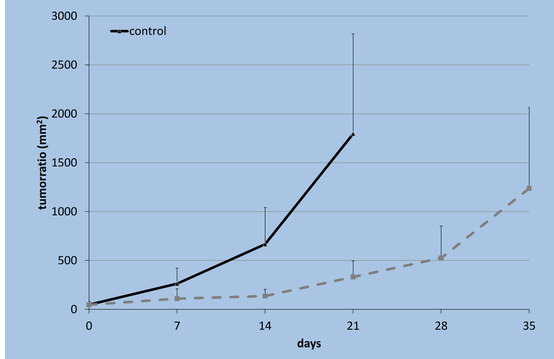


Figure 5

Population dynamics in imatinib-resistant GIST xenografts: Imatinib promotes tumor growth in the presence of secondary resistance mutations.

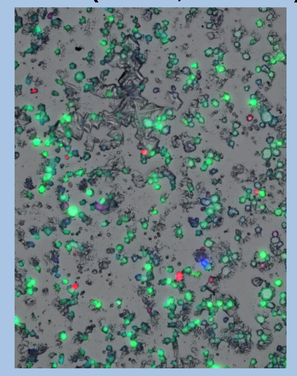


tumor composition: 70% T1 parental + 10% D816E + 10 % T670I + 10% A829P

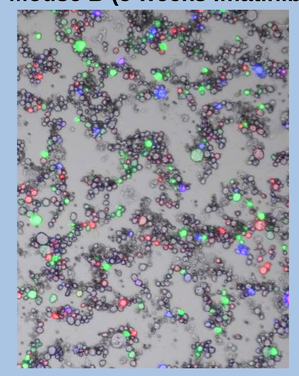
Figure 6

Population dynamics in xenografts: Tumors derived from mixed GIST populations grow slower with Imatinb

Mouse A (control, untreated)



Mouse B (5 weeks Imatinib)



tumor composition: 70% T1 parental + 10% D816E + 10 % T670I + 10% A829P

Figure 7

Population dynamics of mixed populations of imatinib-sensitive and resistant GIST xenografts following oral treatment with imatinib for 5 weeks. Each subline carries a specific fluorophore.Tumors were enzymatically disaggregated and measured by FACS.