Exome-scale liquid biopsy characterization of emerging immune resistance mechanisms in treatment-resistant GIST

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Background

Metastatic gastrointestinal stromal tumors (GIST) are lethal tumors of the GI tract characterized by gain of function mutations in KIT or PDGFRα. Transient first-line control is achieved through the inhibition of tyrosine kinase signaling using the KIT inhibitor imatinib, though most patients progress after 2-3 years. Progression through successive lines of therapy results in a molecularly heterogeneous disease with diverse subtypes, driven by distinct collections of exon-specific KIT mutations which directly inform therapy decisions. To address the unmet need of comprehensive understanding of GIST, we used tumor-informed whole exome liquid biopsy to identify and track the evolution of multiple concurrent heterogeneous resistance mechanisms in individual patients receiving tyrosine kinase inhibitors (TKIs).

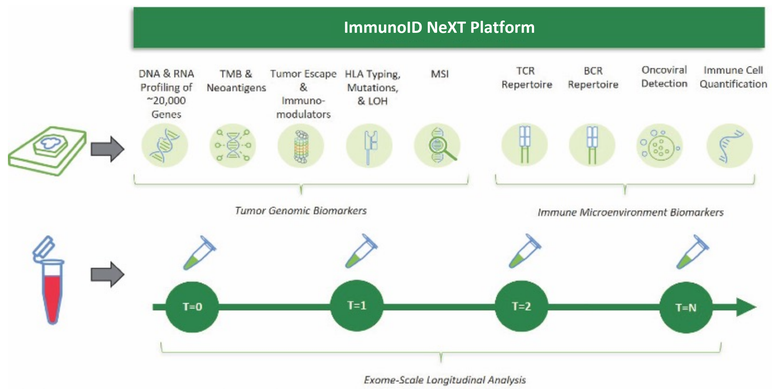
Methods

Cohort

Baseline matched tumor, normal and longitudinal plasma samples were obtained from 15 metastatic, heavily pretreated GIST patients. Following baseline sample collection, all patients received systemic TKI therapy, and were monitored until disease progression.

Solid tumor

The ImmunoID NeXT Platform®, an augmented exome/transcriptome platform and analysis pipeline was used to profile paired tumor and normal samples. Gene expression profiling, comprehensive tumor mutation information, neoantigen characterization including our composite neoantigen burden score NEOPS™, HLA typing and allele-specific LOH, TCR repertoire profiling, and tumor microenvironment profiling were generated as outlined in the plot below.



Whole exome sequencing from plasma

Exome-scale cfDNA profiling of matched serial plasma samples was performed using the NeXT Liquid Biopsy™ platform. Sensitive, exome-scale variant detection was achieved using an enhanced exome assay and chemistry that augments difficult to sequence genomic regions yielding more uniform, high average depth coverage (2000X) across the exome, with boosted coverage (5000X) for 247 clinically relevant genes. To analyze the data we used tools which incorporate an error suppression model estimated from a panel of normal individual plasma samples, and ad hoc filters including a dedicated blacklist.

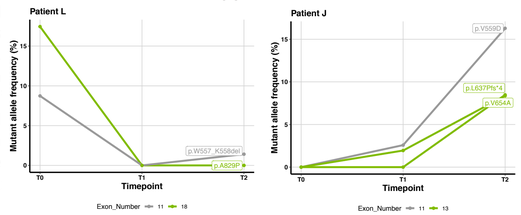
Results

Sensitizing KIT and PDGFRA mutation detection in baseline tumor and longitudinal plasma

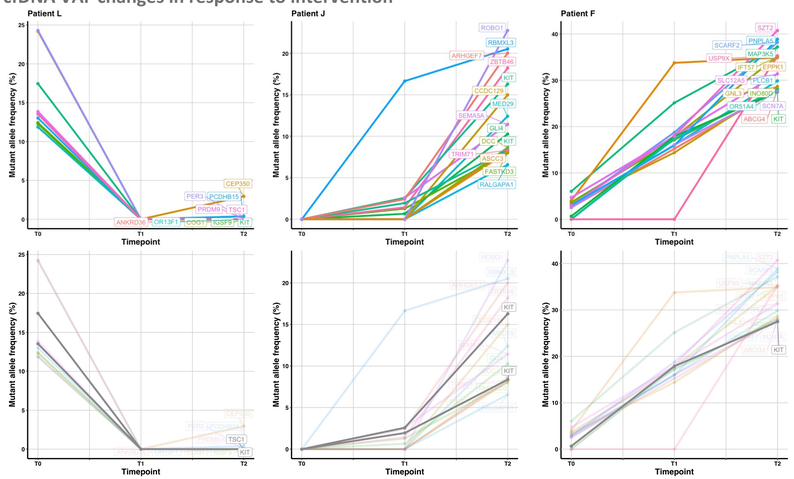
Baseline solid tumor WES identified primary sensitizing KIT/PDGFRA mutations in all 15 (100%) patients, and secondary KIT mutations in 7/15 patients (47%). WES of cfDNA from plasma confirmed KIT/PDGFRA mutations in 14/15 patients (93%), demonstrating feasibility of cfDNA-based disease monitoring.

Evolution of secondary KIT mutations across course of therapy

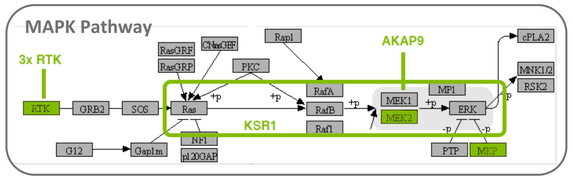
Serial plasma WES identified evolution and expansion of clones harboring newly formed, druggable, exon-specific KIT mutations which evolved prior to identification of tumor progression using standard imaging techniques. Patient L (shown at left) presented with stable disease in response to systemic therapy at T1, and maintained SD at T2. The changes in tumor were paralleled by reduced plasma VAF of exon 11 and 18 KIT mutations detected in this patient. Patient J (right) had initial SD at T1. Evaluation at T2 uncovered a new lesion, which was preceded by an increase in VAF of 3 separate plasma-detected KIT mutations.



cfDNA VAF changes in response to intervention

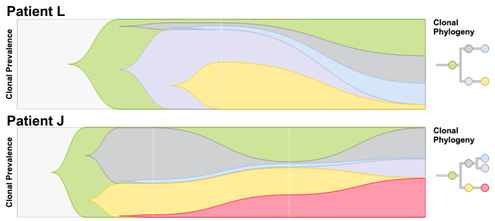


Despite the low mutational burden in this cohort (median TMB = 0.8), we were able to sensitively and repeatably characterize a broad set of variants in serial plasma samples. VAF of the most dynamic variants shift consistently with changes in tumor volume (top row). Interestingly, we found that the majority of variants detected fell outside of those captured by typical panel-based assays (shown in grey, second row), highlighting the strength of an exome-scale approach. Pathway-level investigation of plasma variants, revealed consistent involvement of the PI3K-AKT pathway which was enriched for mutations in patients with shorter overall survival (OS). Additionally, we detected multiple deleterious variants in the classical MAPK pathway with an enrichment of variants detected in patients with reduced OS. Shown at right is a focused view of the mutated elements detected in patients with shorter OS.

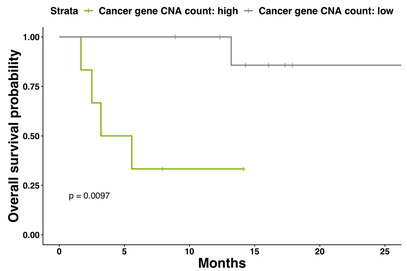


Clonal architecture in GIST

All cfDNA-detected variants (SNVs and INDELs) were clustered by their CCF values to determine the clonal architecture, as well as evolution of resistance in this cohort. Contracting populations in Patient L included clones harboring KIT and CD36 mutations, potentially contributing to the longer OS observed in this patient. Characterization of clonal temporal evolution during the treatment of Patient J revealed a structurally complex phylogeny, and an increase in the abundance of two subclonal populations, one which involves KIT pathway signaling and another enriched for apical junction activity, which increases prior to disease progression. Additionally, a new HRAS clone emerged in ctDNA at T2, potentially contributing to reduced OS by inducing cellular proliferation in the tumor.



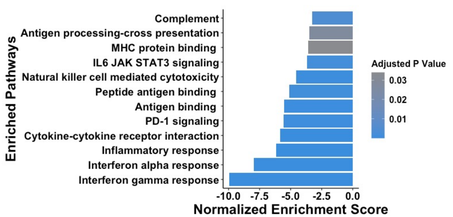
Copy number variants in the GIST genome



Contrary to the low TMB observed in this cohort, copy number alteration (CNA) burden was relatively high (median 232 regions with CNAs), and was correlated with degree of microsatellite instability (Spearman R=.64, P=.01). Next, we considered the copy number status of regions overlapping with cancer-related genes, and the association with OS, as CNAs can often involve oncogenes and tumor suppressors which directly impact cancer development and disease progression. Here, we detected significant association between gene copy-number profiles and duration of survival (P=.0097).

Characterization of baseline immune infiltration status

Investigation of immune signatures using univariate cox modeling revealed a significant association between TCRβ diversity and OS (HR = 2.55, log rank P = .04). Pathway analysis revealed significant associations between patient survival and downregulation of general inflammatory response, interferon gamma response and antigen presentation, among others.



Conclusions

Comprehensive genomic profiling (WES and RNA-Seq) of paired tumor tissue and WES of serially collected ctDNA identified evolving druggable KIT mutations and other molecular alterations which preceded clinical disease progression. These findings suggest liquid biopsy-based monitoring of late-stage GIST malignancies may be useful for early identification of treatment resistance, providing treatment guidance prior to traditional approaches.

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