Exome-scale longitudinal tracking of emerging therapeutic resistance in GIST via analysis of circulating tumor DNA

#5161

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Background

Gastrointestinal stromal tumors (GIST) are lethal tumors characterized by constitutively activating mutations to KIT or PDGFRA. Transient disease control in the first-line setting is achieved via inhibition of tyrosine kinase signaling using the KIT inhibitor imatinib. As patients progress through subsequent lines of therapy a molecularly heterogeneous disease evolves, characterized by distinct subtypes and shifting repertoires of exon-specific KIT variants which directly impact treatment outcomes. Here, we use tumor-informed exome-scale liquid biopsy to identify and track the evolution of multiple resistance mechanisms in patients receiving tyrosine kinase inhibitors (TKIs) to address the unmet need of comprehensive understanding of GIST evolution in response to therapy.

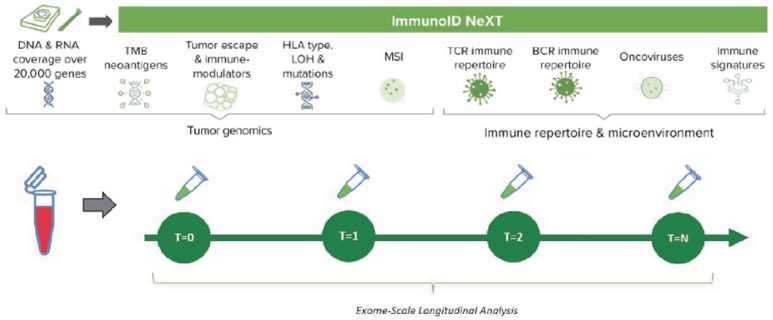
Methods

Cohort

Matched tumor, normal and serial plasma samples were obtained from 15 heavily pretreated metastatic 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 and solid tumor

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.

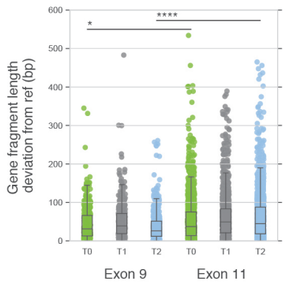
Results

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

Primary sensitizing KIT/PDGFRA mutations were detected in tumors of 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 the feasibility of cfDNA-based disease monitoring.

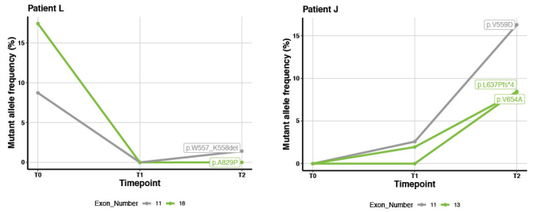
cfDNA fragmentation patterns vary with KIT mutational profile

Exome-wide cfDNA fragmentation patterns were characterized at three timepoints (T0, T1, and T2). Here, we subtracted the mean length of variant supporting reads from the mean length of reference reads, yielding gene-level fragment size distributions. The absolute value of this difference represents gene-specific cancer-derived variation from normal read length (y-axis). ctDNA-derived fragments from KIT exon 11 mutated tumors varied on average 10 bases more (*MWU, p=0.02) than those with exon 9 mutations when compared to reference fragments, a difference that increased to 22 bases by the end of the study (****MWU, p=10-14). We next focused on fragments originating from the KIT gene, and found that patients with larger mutant KIT fragments compared to reference were at a significantly reduced risk of death in a cox proportional hazard model (HR: -7.75; p=0.02).

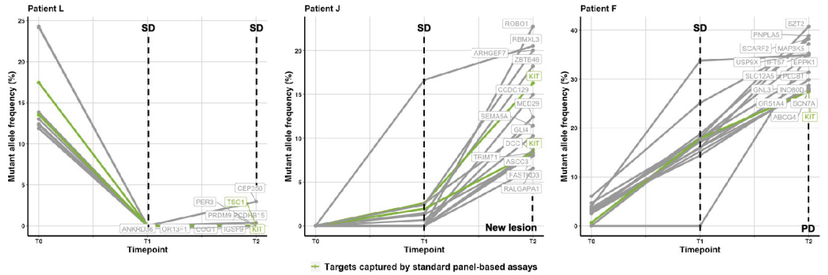


Evolution of secondary KIT mutations detected in plasma ctDNA

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 (SD) in response to systemic therapy at T1, and maintained SD at T2. The changes in tumor were paralleled by reduced plasma variant allele frequency (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 variants.



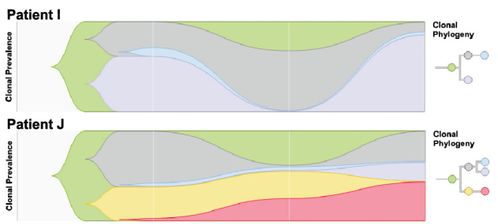
ctDNA VAF changes over the course of intervention



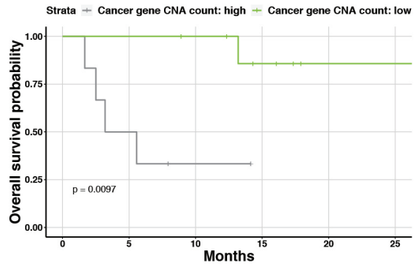
Despite the low mutational burden in this cohort (median TMB = 0.8), we were able to sensitively and repeatedly characterize a broad set of variants in serial plasma samples. VAF of the most dynamic variants shifts consistently with changes in tumor volume (stable disease, SD, and progressive disease, PD, indicated by dashed line) and appearance of new lesions. In patient F, increasing VAF preceded radiographic detection of PD. Interestingly, we found that the majority of variants detected fell outside of those captured by typical panel-based assays (shown in grey), 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.

Clonal architecture in GIST

ctDNA-derived variants (SNVs and INDELs) were clustered by their CCF values to characterize clonal architecture, and evolution of resistance in this cohort. Expanding populations in Patient I included clones harboring KIT mutations, likely contributing to the short OS observed in this patient. Characterization of clonal temporal evolution during the treatment of patient J revealed a structurally complex phylogeny, and increasing abundance of two distinct subclonal populations which increase prior to disease progression. The first was enriched for apical junction activity, and a second involving KIT pathway signaling. Emergence of a novel HRAS clone was detected in ctDNA at timepoint two in this patient, potentially contributing to reduced OS by inducing cellular proliferation in the tumor.



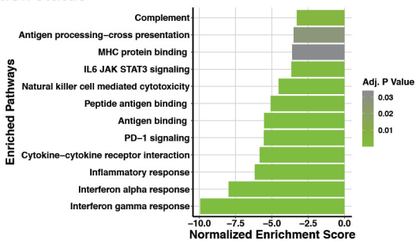
Solid tumor copy number variants in GIST



Copy number alteration (CNA) burden was relatively high (median 232 regions with CNAs) despite low observed TMB in this cohort, and was correlated with degree of microsatellite instability (Spearman R=.64, P=.01). As CNAs can often involve oncogenes and tumor suppressors that drive tumor development and disease progression, we next evaluated the association between OS and copy number status of regions overlapping with cancer-related genes. These analyses revealed significant association between gene copy-number profiles and survival duration (P=.0097).

Characterization of baseline immune infiltration status

Immune signatures associated with survival were characterized using univariate cox modeling, revealing 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 profiling of paired tumor tissue (WES and RNA-Seq) and WES of serially collected ctDNA sensitively and repeatedly identified evolving KIT mutations and other molecular alterations prior to radiologically confirmed disease progression. These findings suggest plasma-based monitoring of late-stage GIST malignancies may be useful for non-invasive disease tracking, providing treatment guidance prior to traditional approaches.

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Main points for ePoster 5161:
Exome-scale longitudinal tracking of emerging therapeutic resistance in GIST via analysis of circulating tumor DNA

1. Work is in collaboration with MD Anderson in gastrointestinal stromal tumors.
2. Larger mutant KIT fragments compared to reference were at a significantly reduced risk of death in a cox proportional hazard ratio (HR: -7.75, p=02)
3. These signals represent an early signal of disease recurrence that is more sensitive than traditional RECIST measurements.
4. Evaluating variants in serial plasma samples post-treatment show that ctDNA VAF shifts consistently with changes in tumor volume, demonstrating an association with response to therapy.