Antonescu Research lab synopsis – Proposal for 2024-2025 Funding:

Progress Report:

As a result of the generous GCRF funding, we were able in the past year to complete an ambitious project on defining genomic biomarkers to better assess risk of malignancy in GIST and guide the patient's selection for adjuvant therapy (*Dermawan J et al, Clinical Cancer Research, 29:3974-85, 2023*). This study was triggered by the many existing limitations of the current risk stratification schemes in GIST. To mention a few, the conventional guidelines for risk assessment were defined in the pre-imatinib era and rely solely on clinicopathologic metrics: tumor site, tumor size, and mitotic activity. These risk models have performed inconsistently in differentiating potentially aggressive from indolent clinical behavior, offering limited guidance on adjuvant therapy. Additional significant limitations of the current prognostication include the lack of integrated molecular biomarkers and not accounting for the imatinib sensitivity of the primary GIST genotype.

In our recent study, we specifically investigate means of extracting additional information from large-scale genomic landscape signatures, focusing on selecting and validating prognostic biomarkers of tumor progression. By applying a targeted hybrid capture-based next-generation sequencing assay to determine the mutational and copy-number landscape in a large GIST cohort with comprehensive and detailed clinicopathologic annotation from a single tertiary center, we were able to identify molecular determinants of aggressive clinical behaviors that can be used as the next-generation, genomic based risk prediction in GIST in the present imatinib era.

The following is a brief summary of our recent study main findings:

- *Genomic landscape comparison between gastric vs small bowel GIST*. Gastric GISTs more frequently harbored alterations in PDGFRA, SDHA, SDHB, whereas small bowel GISTs were enriched in inactivating alterations in MAX/MGA and RB1, deletions in chr1p and chr22q deletion, and amplifications in chr1q, chr5p, and chr5q. The frequencies of CDKN2A and chr14q deletion were relatively similar in gastric and small bowel GISTs.
- *Genomic risk stratification in gastric GIST*. Among gastric GIST, our genomic risk stratification model revealed that SDHB status correlated with high risk. This SDHB-deficient group has few co-occurring alterations.
- *Genomic risk stratification in SDH-deficient GIST*. Within SDH deficient cases, TP53 mutations or chr1p deletions portends poor outcome even within this high-risk group. This is significant as we have shown previously that conventional pathologic

criteria (mitotic count) do not correlate well with outcome (metastatic potential) in the molecular subset of SDH-deficient GIST.

- *Genomic risk stratification in small bowel GIST.* In the small bowel cohort, alterations in MYC/MAX/MGA axis were associated with a significantly worse recurrence free survival (RFS, log-rank P = 0.003) by multivariate Cox regression analysis. In addition, presence of CDKN2A deletions or RB1 loss-of-function alterations also conferred high-risk status in small bowel GISTs.
- *Longitudinal sequencing* uncovered that unlike acquired KIT second site mutations, most chromosomal arm level CNA and cooccurring “secondary” gene-level alterations occurred at baseline and remained stable during disease progression.
- *Comparison between genomic and conventional risk stratification schemes*. The results showed that the genomic risk stratification not only upgrades but also downgrades. This suggests that conventional risk stratification can miss some high-risk patients (underestimates), but also tends to overestimate risks, implying that patients who belong to the low genomic risk groups may potentially be over treated.
- *Wide application of the proposed genomic risk stratification*. Most KIT-independent genomic risk factors found to be prognostically significant occur at the gene level (MYC/MGA/MAX, CDKN2A, RB1, etc.). Thus, it is likely that these alterations will be consistently detected by various platforms.

Proposed Aims for the next funding year (2024-2045).

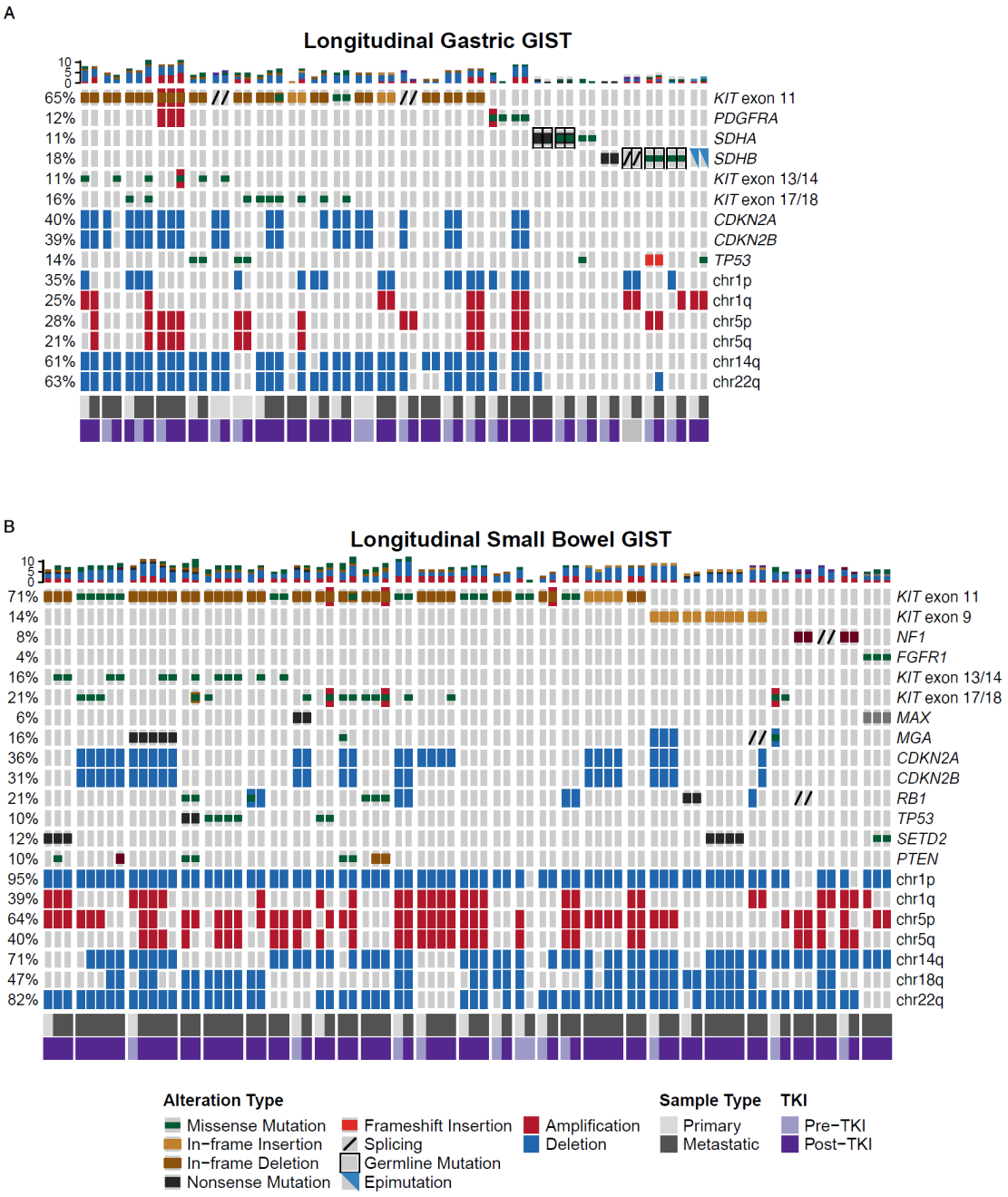
(1) identifying molecular biomarkers of primary and secondary resistance to imatinib and other tyrosine kinase inhibitors (TKI) beyond secondary KIT mutations.

(2) investigating alternative mechanisms of imatinib failure in GIST such as the presence of *KIT* amplifications.

Aim 1. Genomic determinants of Imatinib and other TKI resistance using molecular predictors and machine learning (ML) approaches.

SubAim 1A*. Longitudinal sequencing analysis of paired primary/non-treated and metastatic/resistant GIST to define novel mechanisms of resistance.* In a preliminary analysis we investigated a cohort of 56 GIST patients (26 gastric, 30 small bowel) with multiple samples available for genomic analysis. We reviewed each of these cases to determine whether the sample was pre- or post-imatinib treatment at the time of sequencing. Our results showed that unlike acquired *KIT* second site mutations (*KIT* exon 13/14, exon 17/18), most chromosomal arm level CNA (deletion in chr1p, 14q, 22q; amplification in chr1q, 5p, 5q) and co-occurring “secondary” gene-level alterations

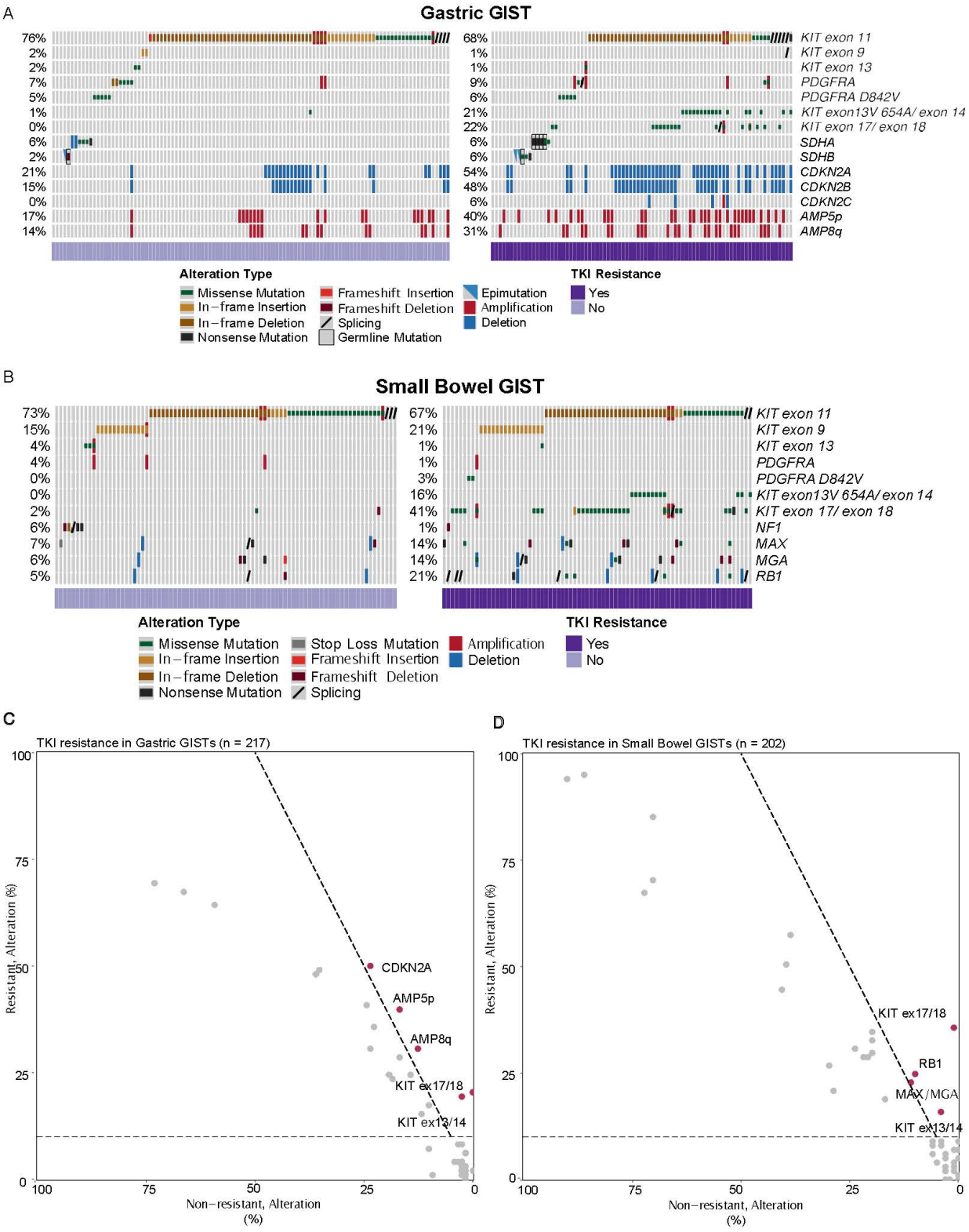
occurred at baseline and remained stable during disease progression. Among gastric GISTs, *CDKN2A* deletions and *TP53* mutations were mutually exclusive, while *RB1* alterations were rare (only 1 case with *RB1* inactivating mutation). Among small bowel GISTs, *CDKN2A* deletions*, RB1* deletion/inactivating mutations, *TP53* mutations, and *SETD2* mutations were mutually exclusive except for one case; *TP53* and *PTEN* mutations were exclusively seen in imatinib-resistant GISTs harboring 2nd site *KIT* mutations; chr1p deletions were highly prevalent, all of which were detected at baseline (**Figure 1**). This preliminary data is encouraging to further investigate a larger cohort of 100 GIST patients during the proposed application to determine patterns of tumor progression, independent from the main primary or secondary *KIT/PDGFRA* driver mutations.



SubAim 1B: KIT-independent Genomic Risk Factors in TKI Resistance.

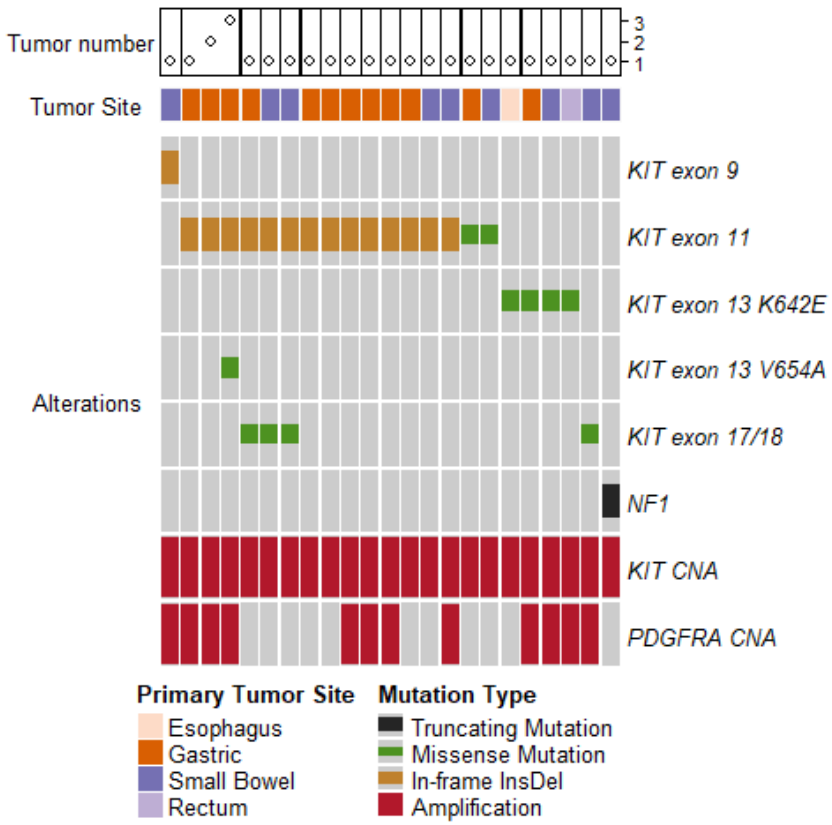
In a different pilot analysis, we reviewed a cohort of treated GIST cases (neoadjuvant, adjuvant, palliative) that had available targeted DNA sequencing. The genetic alterations were analyzed by site and by the association of *KIT* second site mutations and *KIT*-independent alterations with imatinib resistance. Cases were reviewed for evidence of TKI resistance, i.e., progression of disease (recurrence, increase in size,

metastasis) despite compliance with imatinib treatment. Patients who progressed due to intolerance or toxicity to imatinib or other TKIs were excluded. On multivariate logistic regression analysis, we included only alterations with at least 10% allele frequency in resistant cases and 2X frequency in resistant versus sensitive cases. In addition to *KIT* exon 13 V654A/14/17/18 second-site mutations, among treated gastric GISTs, the presence of *CDKN2A* deletions or chr5q / chr8q amplifications were significantly associated with imatinib resistance (**Figure 2A**). Among treated small bowel GISTs, the presence of *MAX/MGA* or *RB1* inactivating mutations or deletions were significantly associated with imatinib resistance (**Figure 2B**). Moreover, the distribution of these non-*KIT* alterations was not significantly different between TKI-resistant GIST cases with or without *KIT* second site mutations. We will further perform genomic analyses on an extended number of resistant GIST patients with various TKIs to define various mechanisms of drug failure not related to KIT mutations. We will also use Machine Learning (OncoCast) for integrating large number of genomic alterations and clinicopathologic data to predict duration of response, as well as biomarkers for TKI resistance. OncoCast is a computational tool developed at MSK, to incorporate genomic data from broad panel sequencing platforms such as MSK-IMPACT to predict individual patient prognosis and compare risk characteristics. OncoCast uses an ensemble learning strategy by repeatedly splitting the cohort into training and test sets that generate classifiers with varying genes and gene combinations. We hypothesize that these molecular predictive biomarkers may complement the optimal conventional risk stratification model for improved prediction of GIST recurrence and resistance.


A
Gastric GIST
76%
2%
2%
7%
5%
1%
0%
6%
2%
21%
15%
0%
17%
14%
68%
1%
1%
9%
6%
21%
22%
6%
6%
54%
48%
6%
40%
31%
KIT exon 11
KIT exon 9
KIT exon 13
PDGFRA
PDGFRA D842V
KIT exon13V 654A/ exon 14
KIT exon 17/ exon 18
SDHA
SDHB
CDKN2A
CDKN2B
CDKN2C
AMP5p
AMP8q
Alteration Type
Missense Mutation
In−frame Insertion
In−frame Deletion
Nonsense Mutation
Frameshift Insertion
Frameshift Deletion
Splicing
Germline Mutation
Epimutation
Amplification
Deletion
TKI Resistance
Yes
No
B
Small Bowel GIST
73%
15%
4%
4%
0%
0%
2%
6%
7%
6%
5%
67%
21%
1%
1%
3%
16%
41%
1%
14%
14%
21%
KIT exon 11
KIT exon 9
KIT exon 13
PDGFRA
PDGFRA D842V
KIT exon13V 654A/ exon 14
KIT exon 17/ exon 18
NF1
MAX
MGA
RB1
Alteration Type
Missense Mutation
In−frame Insertion
In−frame Deletion
Nonsense Mutation
Stop Loss Mutation
Frameshift Insertion
Frameshift Deletion
Splicing
Amplification
Deletion
TKI Resistance
Yes
No
C
TKI resistance in Gastric GISTs (n = 217)
Resistant, Alteration (%)
Non−resistant, Alteration (%)
CDKN2A
AMP5p
AMP8q
KIT ex17/18
KIT ex13/14
D
TKI resistance in Small Bowel GISTs (n = 202)
Resistant, Alteration (%)
Non−resistant, Alteration (%)
KIT ex17/18
RB1
MAX/MGA
KIT ex13/14

Aim 2. Investigating the role of KIT amplification in tumor progression and resistance using DNA targeted sequencing (MSK-IMPACT).

Using MSK IMPACT we have identified a preliminary cohort of 20 GIST patients harboring KIT amplification. 75% of patients had primary/non-treated disease, while remaining 25% of patients showed co-existing secondary KIT mutations in keeping with imatinib/TKI resistant disease (**Figure 3**). We will specifically investigate the heterogeneity of resistance mechanisms taking advantage of the large number of patients with available serial next generation sequencing (NGS) from different time points of their clinical course.



RECENT REFERENCES:

1. Dermawan JK, Kelly C, Gao Z, Smith S, Jadeja B, Singer S, Tap WD, Chi P, **Antonescu CR**. Novel Genomic Risk Stratification Model for Primary Gastrointestinal Stromal Tumors (GIST) in the Imatinib and Adjuvant Therapy Era. *Clinical Cancer Research*; **29**:3974-85, 2023.

2. Dermawan JK, Vanderbilt CM, Chang JC, Untch BR, Singer S, Chi P, Tap WD, **Antonescu CR**. FGFR2::TACC2 fusion as a novel KIT-independent mechanism of targeted therapy failure in a multidrug-resistant gastrointestinal stromal tumor. *Genes Chromosomes Cancer* **61**:412-419, 2022.

3. Torrence D, Xie Z, Zhang L, Chi P, **Antonescu, CR**. Gastrointestinal stromal tumors with BRAF gene fusions. A report of two cases showing low or absent KIT expression resulting in diagnostic pitfalls. *Genes Chromosomes Cancer* **60**, 789-795, 2021.

Proposed Budget:

Salary Support for:

- Sr Research technician
- Sr Bioinformatician

Core Facilities – for sequencing

Other Lab Reagents