GIST Cancer Research at Fox Chase Cancer Center

Prepared for Tania and Robert Stutman

September 2024

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The GIST Cancer Research Fund at Fox Chase Cancer Center

The GIST Cancer Research Fund graciously supports the research efforts of Margaret von Mehren, MD and Lori Rink, PhD, who manage a lab devoted to GIST research. With your support, Dr. von Mehren, Dr. Rink and their team continue to focus on advancing our understanding of GIST in pursuit of novel therapies.

The GIST Translational Laboratory

Lori Rink, PhD

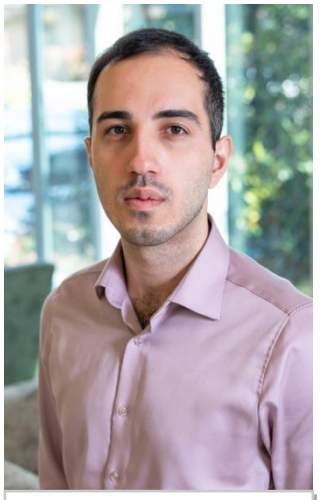
Associate Professor

Dr. Rink leads the GIST laboratory, which includes two graduate students, Delia Zumpano and Adam Chatoff, with the assistance of Jane Koshy, a Scientific Technician. The lab said goodbye to Ashot Nazaretian at the end of August 2024. Throughout the year, several students have rotated in the lab including Sneha Anmalsetty (Medical Student, Lewis Katz School of Medicine), Gulnaz Alekbaeva (PhD candidate, Drexel University) and Joseph Koski (Undergraduate Fellow, University of Delaware). We are anticipating Uliana Novoyatlova joining sometime in September of 2024.

Lori Rink, PhD

Your continued support has helped advance research in five distinct areas:

1. Characterization of Persistent GIST Cells Receiving Targeted Therapy.



Ashot Nazaretian, Graduate Student

RESEARCHERS: Mr. Nazeretian, Dr. Rink, Dr. Greco, and Dr. von Mehren

The tyrosine kinase inhibitors (TKIs) imatinib mesylate (IM) and avapritinib (AVA) are first-line therapies in advanced *KIT* and *PDGFRA* exon 18-mutated GIST, respectively, with initial tumor response observed in greater than 80 percent of patients. Despite this, most patients progress within two years. Responses to post-IM lines of TKIs (sunitinib, regorafenib, and ripretinib) are brief (3-9 months), and no approved second-line therapy exists for patients with acquired resistance to AVA. Once all approved TKI therapies are ineffective, patients with advanced GIST are left without further treatment options.

Despite the success of IM and AVA, complete response to therapy is rare (about 5%). Most GIST patients with advanced disease demonstrate either partial response (40-68%) or stable disease (14-32%) following TKI treatment. Therapeutic benefit is observed by a decrease in tumor density and volume on CT imaging, with the maximum tumor shrinkage typically occurring after $\geq$ six months of treatment. Similarly, *in vivo* studies

performed in our laboratory using TKI-sensitive patient-derived GIST xenograft (PDX) models have recapitulated the clinical experience. TKI treatment in these sensitive PDX models leads to significant tumor shrinkage as early as 1-2 weeks into treatment, yet compete response is never achieved, and ultimately these tumors will exhibit resistance following ~5-6 weeks of therapy. Histologic examination of these responding "remnant" tumors (PDX and patient samples) revealed the presence of viable, KIT-positive GIST cells.

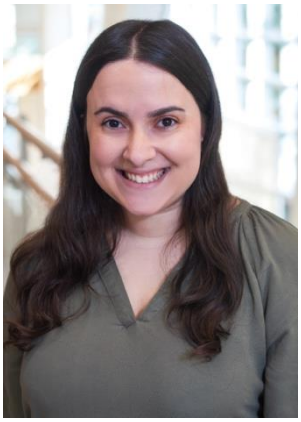
We hypothesize that these non-cycling or "senescence-like" residual tumor cells that remain after the bulk of the tumor has been eliminated by therapy may represent a form of tumor dormancy that may play an important early role in TKI resistance. In collaboration with Dr. Stephanie Greco, (Department of Surgical Oncology at Fox Chase Cancer Center) who has studied senescence in colon cancer, we are currently using innovative single cell-RNA sequencing of treated tumors to characterize the surviving cells and non-tumoral components (immune cells, cancer-associated fibroblasts). These studies have identified markers of interest that we are interrogating to determine if they may be involved in contributing to this dormant phenotype. During this last year, Dr. Greco and Dr. Rink have received approval for an IRB protocol to investigate the feasibility of isolating dormant disseminated tumor cells from GIST patient specimens to interrogate these potential biomarkers. We believe these studies will allow us to develop future studies to investigate the biology of cancer dormancy and recurrence in GIST and to potentially identify novel vulnerabilities to target these surviving cells before they can reemerge, potentially with polyclonal acquired drug-resistant mutations.

2. Identification of Wee1 as a Target in GIST by Kinome Profiling.

RESEARCHERS: Mr. Zumpano, Dr. Duncan, Dr. Rink, and Dr. von Mehren

GIST has three major genotypic subtypes: *KIT* mutant, *PDGFRA* mutant and SDH deficient GIST. Mutational subsets confer different natural histories and primary response to Gleevec. Limited options exist for those patients whose disease is refractory to primary treatment. Therefore, there is an urgent need to identify novel targets to provide additional therapeutic options and combinations for refractory GIST. Inhibition of cancer-promoting cell signaling pathways by targeting additional protein kinases active in GIST may provide these options.

In collaboration with Dr. James Duncan (Cancer Biology Program, FCCC), we have obtained kinome profiles for these subsets using an innovative mass spectrometry-based technique that allows us to analyze the majority of the human kinome simultaneously. We have successfully profiled the kinome



Delia Zumpano, BS
Graduate Student

using primary tumors from each of the three major GIST subtypes and have demonstrated that the basal activation state of the kinome in these distinct GIST subtypes cluster separately from each other and from normal gastric tissue. We have identified uniquely activated kinases in each group, as well as kinases that are overexpressed in both *KIT* and *PDGFRA*-mutant GIST. We showed that GIST cells are exquisitely dependent on the cell cycle-related kinase, WEE1, identified in our kinome profiling study. WEE1 is the main controller of the G2/M checkpoint in the cell cycle. Further studies have suggested a role for WEE1 in other non-canonical functions, such as maintaining replication fork stability and safeguarding against replication stress. Furthermore, we demonstrated that targeting WEE1 in combination with avapritinib, both in cell lines and in mouse models revealed significant efficacy. This work was published in JCI Insight (https://insight.jci.org/articles/view/143474). We have been awarded a five-year NCI R01 grant to elucidate the critical role of WEE1 in GIST with a specific focus on the protein's non-canonical roles. Recent studies have identified that GIST cell lines treated with WEE1 inhibitors in combination with TKIs show decreased replication fork speed and delays the cell cycle. Pausing the cell cycle could lead to errors in DNA replication and DNA damage, leading to cell death.

While GIST are generally refractory to genotoxic chemotherapeutic agents, we hypothesized that WEE1 inhibitors could potentially sensitize GIST cells to DNA damaging agents, agnostic of mutation status of the tumor. We have shown in preliminary studies in the TKI-refractory setting that doxorubicin, a topoisomerase II inhibitor, in combination with WEE1 inhibitors, increases apoptosis and reduces cell viability. This is because the doxorubicin causes DNA damage to the cells, and the WEE1i causes the unrepaired DNA damage to go through the G2/M checkpoint, causing replication fork stalling and mitotic catastrophe, leading to cell death. In addition to the cell line results, animal studies using doxorubicin + WEE1 inhibitor in both xenograft and PDX model mice led to decreased tumor volume and overall survival compared to the vehicle and single agent treatments. This work is currently ongoing.

3. Development of Novel Patient-Derived Xenograft (PDX) Models of GIST.

RESEARCHERS: Mr. Nazaretian, Dr. Rink, and Dr. von Mehren

A limitation of the study of GIST is the lack of available models to study in the laboratory. There are limited cell lines that have been developed, and attempts at developing them have been very challenging. One method to overcome this difficulty has been to implant tumor tissue removed at the time of surgery and directly implant the tissue into mice. This approach, called PDX models, is one that we have been taking in collaboration with our surgical oncology colleagues. To date, we have successfully developed fourteen models with a variety of *KIT*/*PDGFRA* mutations and TKI sensitivities, including a novel avapritinib-resistant PDX model. Once developed, this approach allows for the testing of novel drugs and drug combinations, including those described in section 2, in animal models representing

different genotypes to expand the available models for further preclinical testing of novel agents and or combinations of agents. We have recently drafted a manuscript describing our established PDX models and comprehensive genomic characterizations of these models, entitled "Establishment and Genomic Validation of Novel Patient-Derived Xenograft Models for Drug Discovery in Gastrointestinal Stromal Tumor".

4. Metabolic profiling of SDH-Deficient GIST

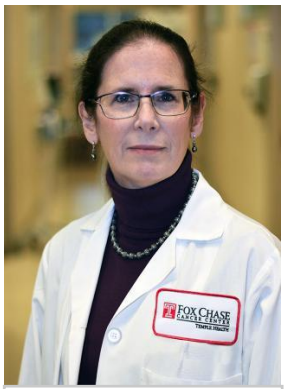
Adam Chatoff, BS
Graduate Student

RESEARCHERS: Mr. Chatoff, Dr. Snyder, Dr. Rink, and Dr. von Mehren

There is no current first-line therapeutic option for SDH-deficient GIST. This is in stark contrast to therapeutic targeting with the front-line TKIs, IM, and AVA, which have transformed therapy for advanced KIT/PDGFRA-mutant GIST. In collaboration with Dr. Nathaniel Snyder (Center for Metabolic Disease Research, Lewis Katz School of Medicine at Temple University), we are profiling the metabolome in SDH-deficient GIST using SDH-deficient GIST cell lines, clinical samples, and genetic models. We hypothesize that SDH-deficiency in GIST may reveal a wider network of metabolic rewiring and changes in enzyme activity to deal with this rewiring with the potential to identify novel therapeutic vulnerabilities in this GIST subtype.

5. Testing and evaluating new therapies in GIST

Margaret von Mehren, MD
Chief, Division of Sarcoma Medical Oncology
Physician Director, Clinical Trials Office
Associate Director, Clinical Research
Professor, Department of Hematology/Oncology



Margaret von Mehren, MD

The primary goal of Dr. von Mehren's work is the testing and evaluation of new therapies for soft tissue sarcomas. There are approximately 7,000 new cases of soft tissue sarcomas diagnosed annually with only 50 percent of patients surviving their disease, and with the limitations of current therapies, a far lower rate of survival if the sarcoma recurs. Dr. von Mehren has a particular interest in novel therapies for Gastrointestinal Stromal Tumors (GIST), the most common sarcoma of the intestinal tract. GIST is characterized by the presence of a growth factor, named KIT, found on the surface of the tumor cells. The gene for KIT, or in rare cases PDGFRA, BRAF, or SDH, undergoes mutations that activate the protein, causing tumor cells with these mutated genes to continuously grow and divide.

Dr. von Mehren and the GIST laboratory have long focused on understanding tumors that *do not* respond well to standard targeted directed therapy. These include the subset of tumors that

don’t carry activating mutations (SDH deficient, NF-1 mutated or quadruple negative GIST) and those GISTs that have become resistant to the standard therapies. Fox Chase Cancer Center has been a clinical trial site for testing many types of targeted therapies as well as novel therapeutic agents. We completed accrual to “An Open-Label, Phase 2 Efficacy Study of Temozolomide (TMZ) In Advanced Succinate Dehydrogenase (SDH)-Mutant/Deficient Gastrointestinal Stromal Tumor (GIST)" and the Peak Trial that explored the benefit of adding a second agent, CGT9486, to suntinib in the second line setting. The phase one-half study of THE-630 in patients with advanced GIST closed due to side effects, with Dr von Mehren spearheading the writeup of the study. We are actively enrolling patients on phase I trials evaluating IDRX-42, a pan-KIT inhibitor, and on a study combining ripretinib with a novel agent DCC-3116 that affects the autophagy pathway. We are collaborating with SARC on SARC044, a study further exploring the benefits of sunitinib and bezuclastinib advanced GIST. Lastly our team is developing a trial for management of rectal GIST and a study to assess the impact of the gut microbiome in patients with GIST.

Dr. von Mehren, Dr. Rink and their laboratory members are grateful for the generous support of the GIST Cancer Research Fund. You have contributed $1.5 million to the Fox Chase mission over more than 20 years!