MENIN INHIBITOR ZIFTOMENIB SYNERGIZES WITH IMATINIB IN TYROSINE KINASE INHIBITOR (TKI)-RESISTANT GASTROINTESTINAL STROMAL TUMOR MODELS

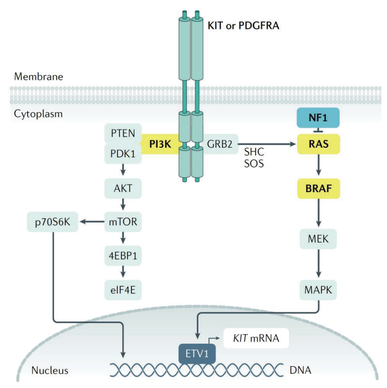
Abstract #227 Asako McCloskey[1], Quinn Reilly[1], Judy Wu[1], Shivani Malik[1] and Francis Burrows[1]

[1] Kura Oncology, Inc., San Diego, CA, USA

BACKGROUND

- GIST is the most common mesenchymal neoplasm of the digestive tract. Most cases are driven by gain-of-function oncogenic mutations in the receptor tyrosine kinase KIT.
- Despite the successful disease control achieved with imatinib in advanced GIST patients, most patients eventually progress due to acquired secondary *KIT* mutations. TKIs such as sunitinib can target imatinib-resistant genotypes and are approved in later lines, but response rates and long-term outcomes are modest, so new therapeutic options are needed.
- Epigenetic transcriptional upregulation mediated by the menin-KMT2A complex was recently suggested to underpin oncogenic overexpression of mutant *KIT* in GIST cells[1].
- **Ziftomenib is a potent, selective oral menin inhibitor currently in clinical testing for acute leukemias. Here we report the first results of ziftomenib-imatinib combination treatment in imatinib-sensitive (1L) and -resistant (2L/3L) GIST patient-derived xenograft (PDX) models.**

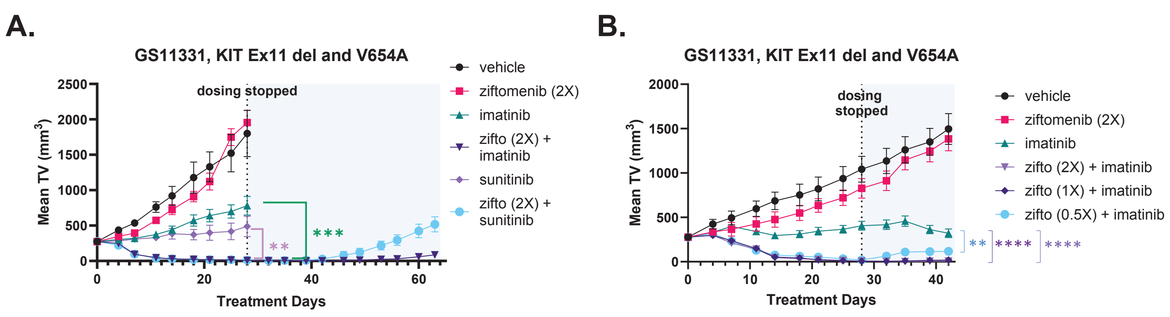
Signaling pathways of KIT-mutated GIST[2]



1. Hemming M (2022) *Cancer Discov.*; 2. Blay JY (2021) *Primer*, modified

RESULTS

Combination treatments of ziftomenib and TKIs manifested synergistic antitumor activity in TKI-resistant GIST PDX models

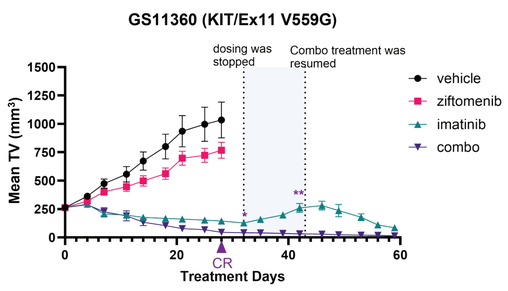


Bauer S (2021) *Front. Oncol.*, modified

Data were presented as Mean ± SEM; Two-way ANOVA followed by Dunnett test; *P<0.05,**P<0.01,***P<0.001,****P<0.0001, not significant (ns)

Figure 1. Ziftomenib-TKI combination treatments showed robust and durable antitumor activity in GIST PDX models harboring various TKI-resistant mutations. A- E. Growth of GIST PDX models harboring indicated *KIT* status, treated with vehicle, ziftomenib (0.5X, 1X or 2X, QD), imatinib (100 mg/kg QD), sunitinib (10 mg/kg, 5-days ON/2-days OFF) or combination. **A and B.** Dosing was stopped on Day 28 and tumor regrowth was monitored (pale blue shading). **B.** Dose titration of ziftomenib was performed. **C.** Body weight in **B.** was monitored. **D & E.** Combination showed synthetic lethal activity in GIST PDX models carrying *KIT* Exon 17 mutations. **F.** The biochemical sensitivities of KIT mutations to different clinical TKIs.

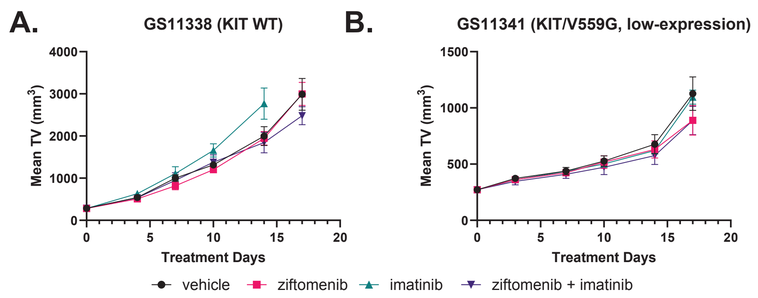
The ziftomenib-imatinib combination displayed durable antitumor activity in a first line GIST PDX model



Data were presented as Mean ± SEM; t-test; *P<0.05,**P<0.01,***P<0.001,****P<0.0001, not significant (ns)

Figure 2. The combination treatment showed superior antitumor activity to imatinib monotherapy. GIST PDX model carrying *KIT* V599G mutation was treated with ziftomenib (1X, QD) and imatinib (100 mpk, QD). The treatment was stopped on Day 33 and tumor regrowth was monitored for 10 days. The imatinib group and combination group were each treated with the combination from Day 43 to Day 60.

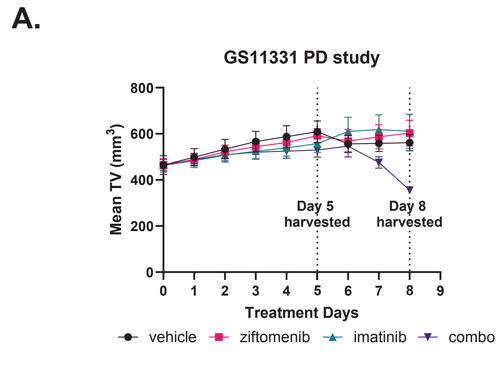
KIT-independent GIST PDX models did not respond to the combination, suggesting a KIT-dependent mechanism



Data were presented as Mean ± SEM

Figure 3. Oncogenic KIT expression is required for the synergistic combination activity. A and B. Growth of GIST PDX models harboring indicated *KIT* status, treated with vehicle (QD), ziftomenib (1X, QD), imatinib (100 mg/kg QD), or the combination.

Combination treatment eliminated KIT protein expression and shut down downstream oncogenic signaling in an imatinib-resistant GIST PDX model



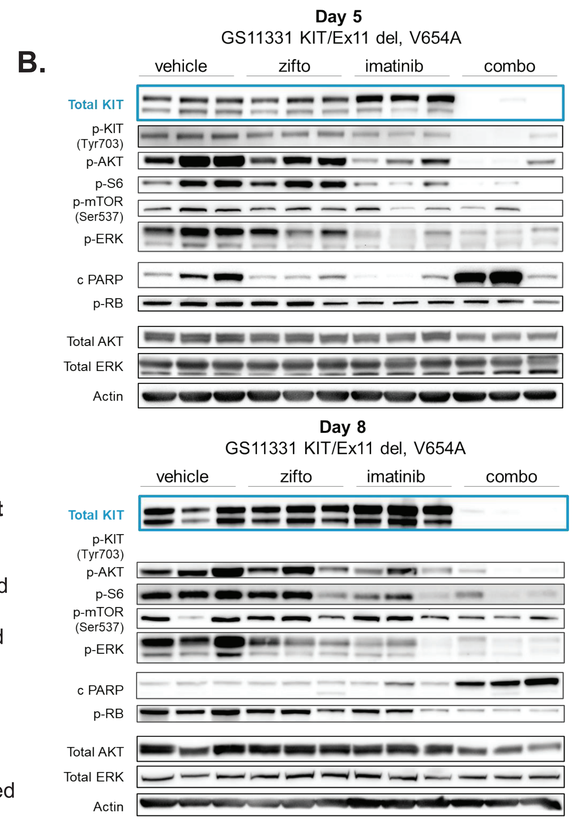
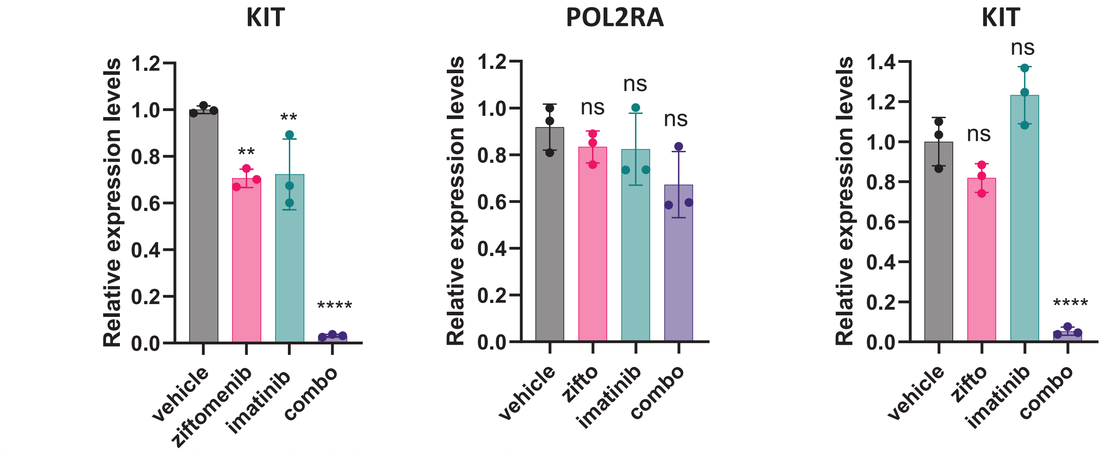


Figure 4. Combined ziftomenib and imatinib treatment abolished KIT protein expression, shut down AKT/mTOR and ERK pathways, and strongly induced cell cycle arrest and apoptosis. A. Tumor growth curve of pharmacodynamic (PD) study in the GS11331 model. Tumors were treated with vehicle (QD), ziftomenib (1X, QD), imatinib (100 mpk, QD) and the combination. Tumors were harvested on Days 5 and 8. **B.** Immunoblots of total or phosphorylated KIT proteins, indicated MAPK/PI3K pathway components and apoptotic markers. Combination treatment resulted in the disappearance of total KIT protein, strong inhibition of AKT activity/target phosphorylation (phosphorylated mTOR and AKT), cell cycle arrest (RB phosphorylation), and cell death (cleaved PARP). The signaling response to the combination treatment deepened on Day 8 compared to Day 5.

Combination treatment synergistically downregulated *KIT* transcription in an imatinib-resistant GIST PDX model



Two-way ANOVA followed by Dunnett test; *P<0.05,**P<0.01,***P<0.001,****P<0.0001, not significant (ns)
Data presented as Mean ± SD.

Figure 5. Combination treatment selectively reduced KIT mRNA levels. A and B. Total RNAs were extracted from GS11331 PD tumor samples (Fig. 4A). *KIT* and house-keeping gene *POL2RA* mRNA levels were quantified by qPCR, normalized by *IPO8*. The combination treatment decreased KIT mRNA levels, but did not affect POLR2A mRNA levels. The ziftomenib and imatinib monotherapies showed limited effects on KIT mRNA levels.

Ziftomenib and imatinib combine to silence KIT in a mutation-independent manner

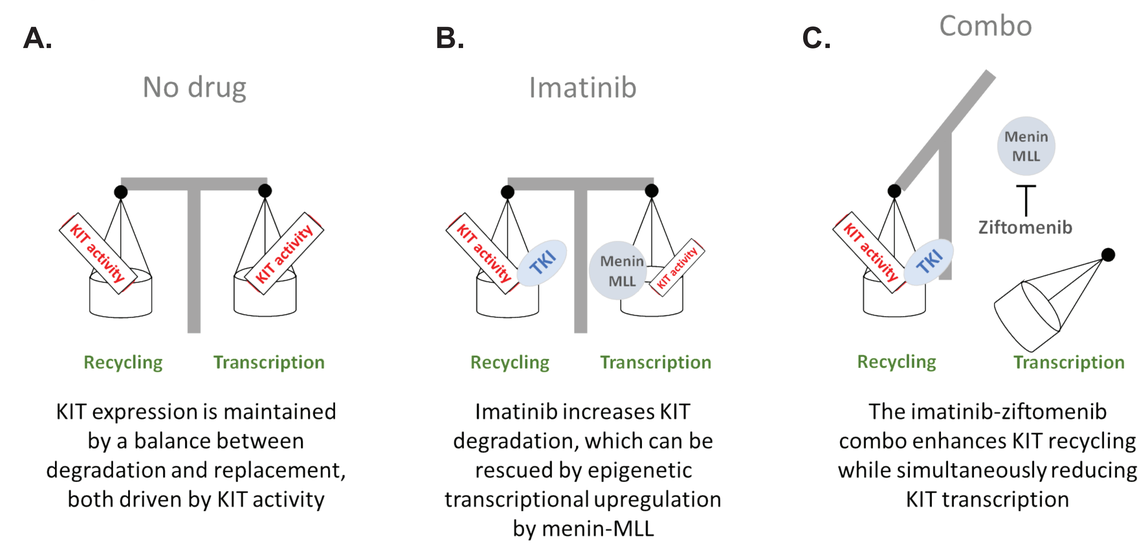


Figure 6. Possible mechanism of action. Imatinib causes vulnerability that can be targeted by ziftomenib at the transcriptional and/or protein stability levels, creating synthetic lethality.

CONCLUSIONS

- Ziftomenib-imatinib combination treatment unexpectedly showed robust antitumor activity not only in imatinib-sensitive but also in imatinib-resistant KIT-dependent GIST models representing the full GIST treatment continuum.
- The combination exerts antitumor activity by a synthetic lethal mechanism through which ziftomenib epigenetically targets a vulnerability of GIST tumors actively induced by even ineffective TKI treatments.
- A proof-of-concept study of ziftomenib + imatinib in patients with advanced GIST after imatinib failure is expected to start in 1H 2025.

Abbreviations: AV, avapritinib; CR, complete response; IM, imatinib; RE, regorafenib; RI, ripretinib; SU, sunitinib; TV, total volume; WT, wild type

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