Uncovering Mechanisms of Action and Resistance for KRAS G12D Inhibitor MRTX1133 Using *Drosophila melanogaster* Models

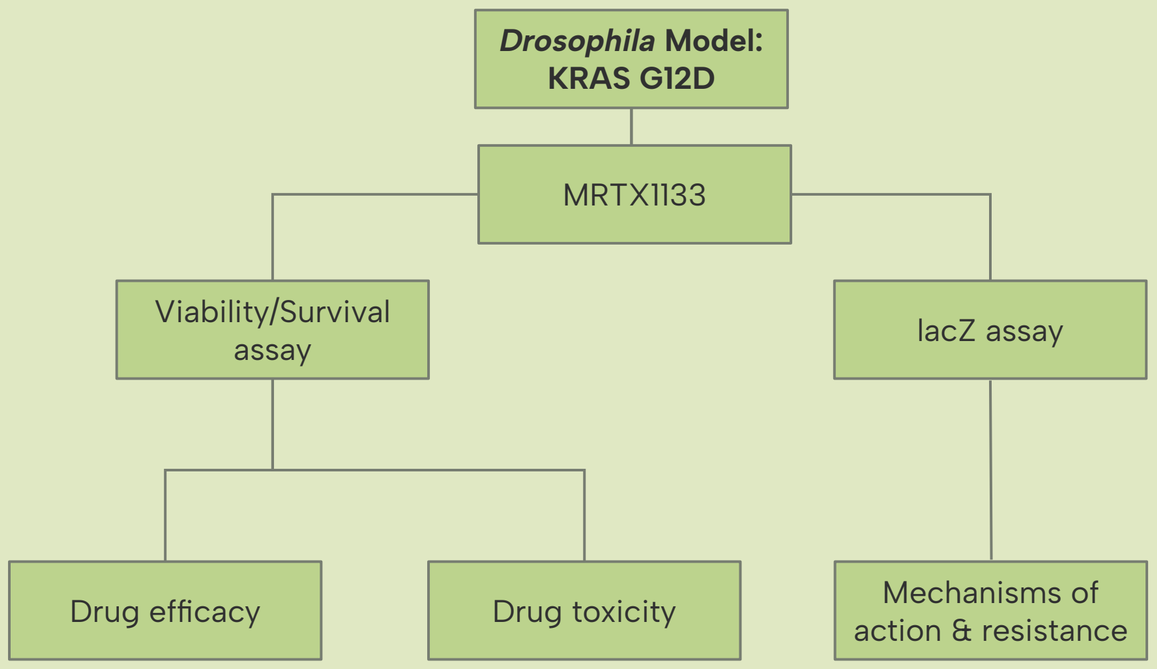
Introduction

The **KRAS protein**, thought to be "undruggable" because of its unique structure, is mutated in 30% of cancers worldwide (Nat'l Cancer Institute, 2022). A common mutation is **KRAS G12D**, present in 4.2% of cancers in a nationwide database.

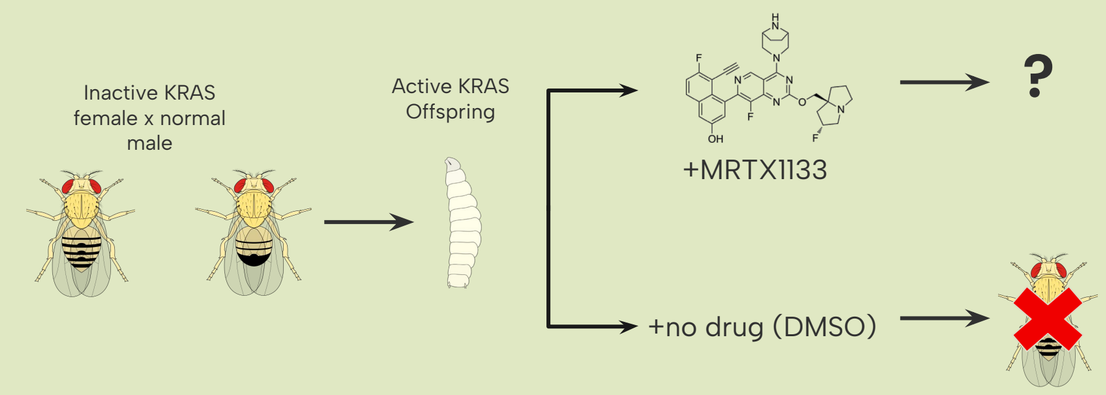
In 2021, Mirati Therapeutics developed the drug **MRTX1133** to inhibit KRAS G12D. MRTX1133 is effective in human cell and mouse models, and is in early phases of clinical testing in humans. However, it is unclear **how MRTX1133 behaves in the body** (Wang, 2021). **Notably, preemptive identification of mechanisms of resistance to promising cancer drugs like MRTX1133 remains an unmet public health need.**

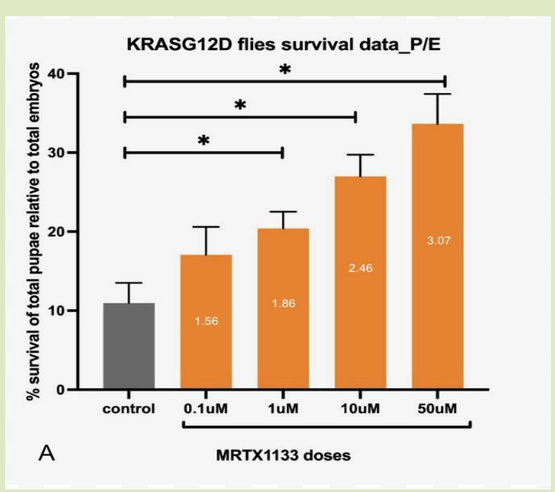
To explore this behavior, I utilize ***Drosophila melanogaster***, the fruit fly. *Drosophila* share approximately 60–70% of disease-causing genes with humans, and the RAS pathway is highly conserved between the two.

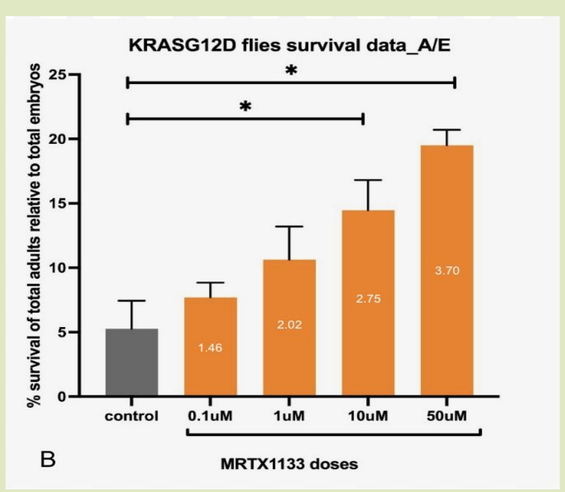
Experimental Design



Drug Efficacy: Can MRTX1133 improve survival of KRAS G12D-expressing flies?

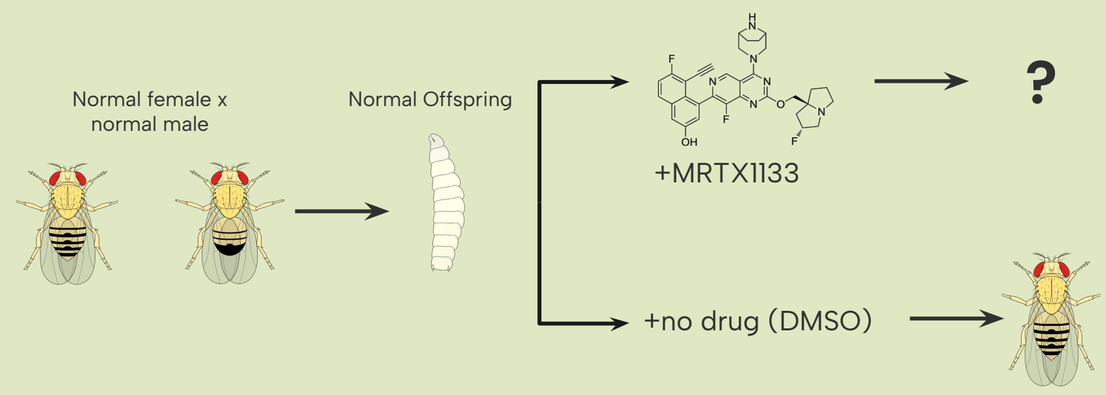


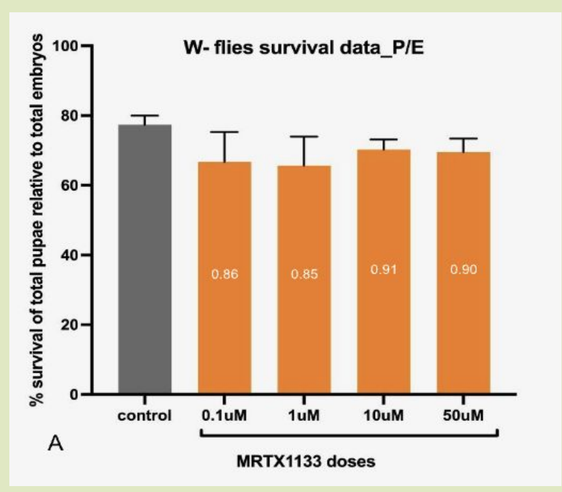


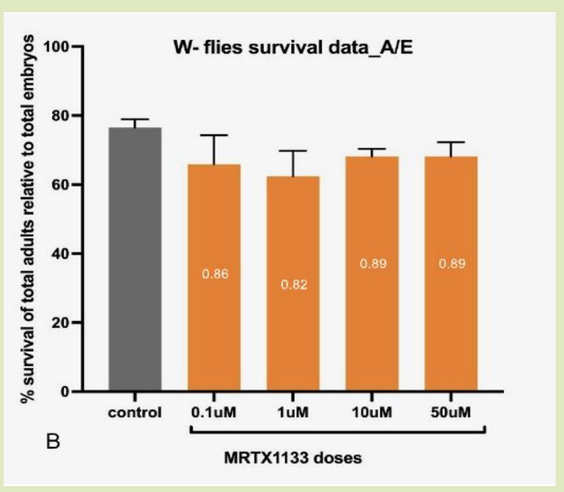


Assay indicates **strong rescue** in MRTX1133-treated flies, and a **correlation between higher rescue and higher dosage**.

Drug Toxicity: What are potential side effects of MRTX1133 in healthy fly tissue?



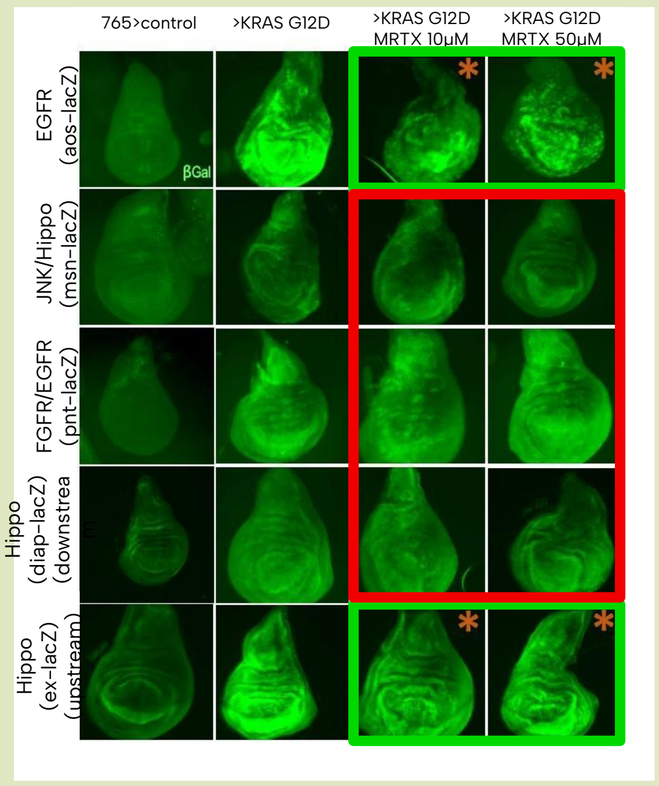




Assay indicates that MRTX1133 **does not significantly negatively impact survival, even at high dosages,** indicating that MRTX1133 is **relatively non-toxic**.

Mechanisms: Which specific pathways does MRTX1133 inhibit? What are potential avenues for resistance?

When wing disks from flies with the reporter gene are stained with antibodies, the intensity of their fluorescence indicates activation of the corresponding pathway. Each pathway is implicated in KRAS G12D cancers.



Assay indicates that **EGFR and upstream Hippo** pathways show **partial rescue** when treated, while **JNK, FGFR, & downstream Hippo do not**. The latter set may be an avenue for cell proliferation and ultimately resistance to MRTX1133. None of the pathways I examined showcased complete rescue, indicating that MRTX1133 may exhibit **single-pathway action**.

Data and graphics generated by student researcher

Conclusions & Discussion

- *Drosophila* are **established as a viable model** for modeling the KRAS G12D genotype and the effects of KRAS inhibitors for the first time.
- MRTX1133 is **highly effective** in terms of full-organism rescue, especially at higher doses.
- MRTX1133 is **well tolerated by the model**, even at high doses, supporting its classification as a low-toxicity drug (Issahaku *et. al.*, 2022).
- FGFR, JNK, and downstream Hippo pathways are **potential mechanisms of resistance**, and might serve as potential avenues for **combination therapies**.

- MRTX1133 may be used in the **treatment of other RAS pathway diseases** (RASopathies); this KRAS G12D model has already been established as a RASopathy model (Das *et. al.*, 2021).
- *Drosophila* may be used for **further analysis** for mechanisms of action and resistance for KRAS inhibitors in early stages of development/testing. Given their short lifespan, they may best act as a **preliminary test** capable of **efficiently screening high volumes of drugs** to select promising therapies for testing in more humanlike models.