# DHX9 Inhibition as a Novel Therapeutic for Cancers With Microsatellite Instability or Loss-of-Function Mutations in the DNA Damage Repair Genes BRCA1 or BRCA2

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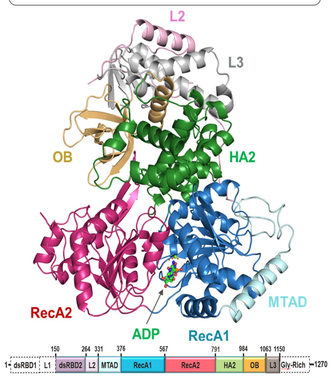
## RNA Helicase DHX9 Plays an Important Role in Maintaining Genome Stability

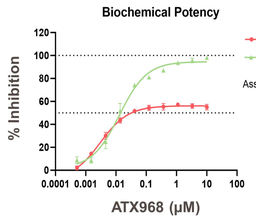
DHX9 is an NTP-dependent DExH-box helicase enzyme which binds and resolves numerous secondary nucleic acid structures, including DNA-RNA hybrids (R-loops), DNA and RNA G-quadruplexes, and circular RNAs. Through these functions, DHX9 plays a role in replication, transcription, translation, RNA splicing and RNA processing[1-3], highlighting its importance in maintaining genome stability. In addition, DHX9 can interact with key proteins in DNA damage repair pathways such as BRCA1, ATR, Ku86, and WRN. DHX9 is overexpressed in many cancer types, including colorectal cancer (CRC), breast and ovarian cancer. DHX9 is a compelling first-in-class oncology target in MSI-H tumors defective in mismatch repair (dMMR)[4]. Here we show that loss-of-function alterations in BRCA1 and/or BRCA2 enriches for sensitivity to DHX9 inhibitor in breast cancer models both *in vitro* and *in vivo,* expanding the opportunity for DHX9 inhibition to provide therapeutic benefit in solid tumors for patients beyond MSI-H CRC.

Crystal Structure of Human DHX9 (2.62 Å)[5]

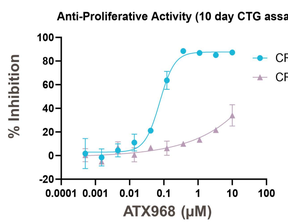



## Accent Has Developed Novel First-in-Class DHX9 Inhibitors

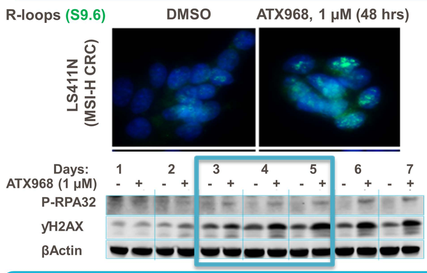
### ATX968 Inhibits DHX9 ATPase and Helicase Activity

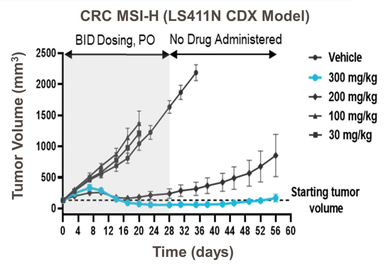



### ATX968 Selectively Inhibits Proliferation of MSI-H vs MSS CRC Cells

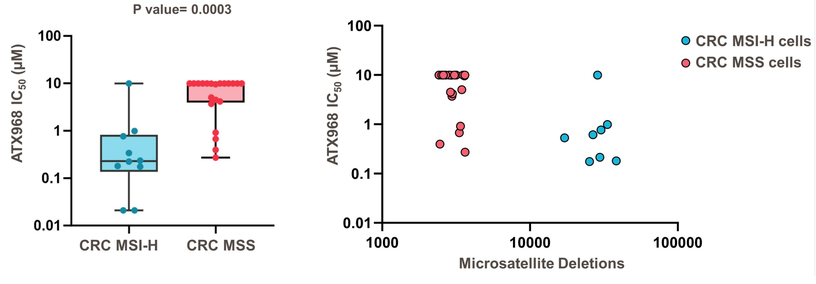



## DHX9 Inhibition Selectively Leads to Increased R-loops Causing Unresolved Replication Stress/DNA Damage Resulting in Robust Tumor Regression in MSI-H CRC Models

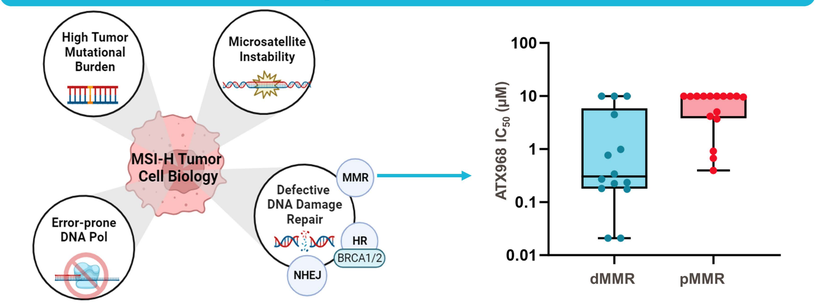



## DHX9 Inhibitor Sensitivity in CRC does not Correlate to Microsatellite Deletion Number[7]

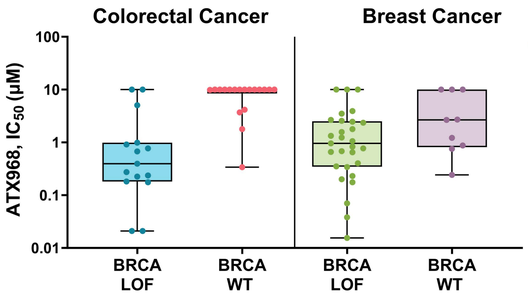



## MSI-H Tumors are Associated with dMMR; DHX9 Inhibitor Sensitivity is Enriched in CRC with dMMR Status




- **Are there additional genetic changes that predict DHX9i sensitivity beyond MSI-H and dMMR?**
- MSI tumors have frequent mutations in additional DNA repair pathways beyond MMR, such as Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ)
- **DHX9 has been shown to interact with BRCA1 and exhibits a role in HR**

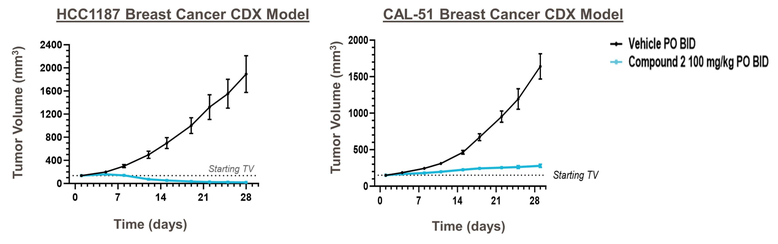
## DHX9 Inhibitor Sensitivity is Enriched in BRCA LOF Cancers



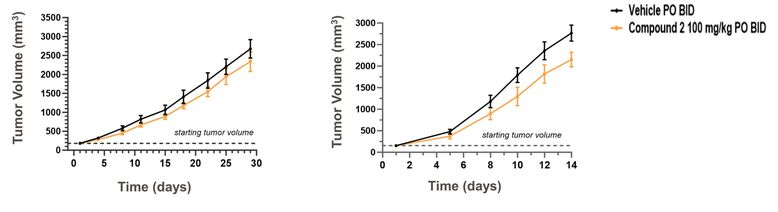

- BRCA LOF = BRCA1 and/or BRCA2 damaging or possibly damaging mutations and copy number loss as identified in Sanger Cell Model Passports

## Treatment with a DHX9 Inhibitor Leads to Selective Tumor Growth Inhibition in BRCA LOF Breast Cancer Models

### DHX9 Inhibitor Efficacy in BRCA LOF Xenograft Models

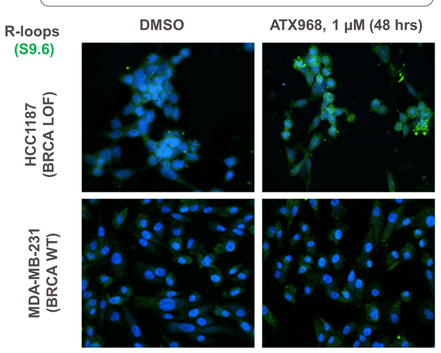



### DHX9 Inhibitors are not Efficacious in BRCA WT Xenograft Models

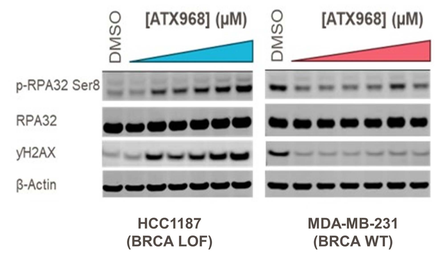


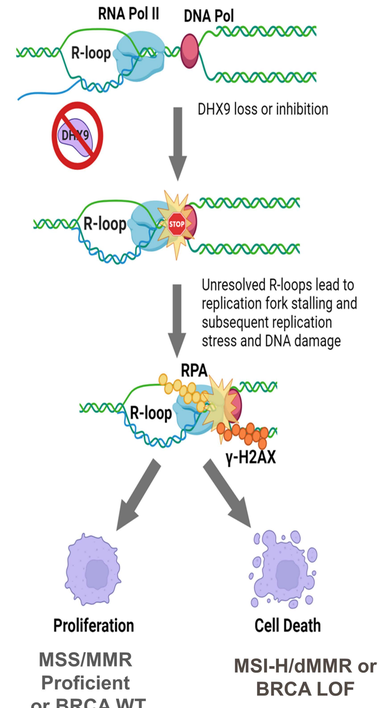

## DHX9 Inhibition Selectively Increases R-loops Causing Unresolved Replication Stress and DNA Damage in BRCA LOF Breast Cancer

### Increase in R-loops Selective to BRCA LOF Cells




### Replication Stress and DNA Damage Selective to BRCA LOF Cells




- Replication stress and DNA damage observed in BRCA LOF breast cancer is similar to CRC MSI-H/dMMR

## Conclusions

- Novel DHX9 small molecule inhibitors have been previously disclosed that demonstrate efficacy in CRC MSI-H cancer cells
- DHX9 inhibitor sensitivity in CRC MSI-H does not correlate to the number of microsatellite deletions, sensitivity is enriched in models with defective DNA repair pathways such as MMR and HR through BRCA1 and/or BRCA2 LOF alterations
- Beyond CRC and MSI-H, DHX9 inhibition leads to anti-proliferative activity in multiple BRCA LOF breast cancer models *in vitro* and *in vivo*
- DHX9 inhibitor mechanism of action in BRCA LOF breast cancer is similar to what has been observed previously in CRC MSI-H, including increased replication stress and DNA damage
- Studies are in progress to further interrogate DHX9 inhibitor mechanism and efficacy in HRD and other defective DNA repair pathways, as well as other tumor types

## References

[1]Lee and Pelletier, Oncotarget (2016)
[2]Chakraborty et al, Nature (2018)
[3]Gulliver et al, Future Science (2020)
**[4]Castro J, Daniels MH, Lu C, et al. AACR; 2023. Abstract #1136**
**[5]Lee et al, Acta Crystallogr D Struct Biol. (2023)**
**[6]Gotur et al, SLAS Discovery (2023)**
[7] Chan et al, Nature (2019)

## Acknowledgements

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