DHX9 Inhibition as a Novel Therapeutic Modality in Microsatellite Instable Colorectal Cancer Exhibiting Defective Mismatch Repair

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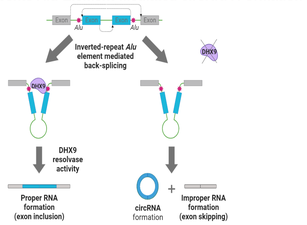
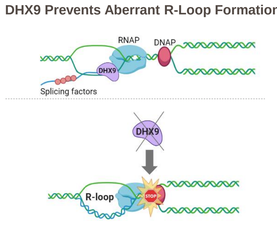
RNA Helicase DHX9 Plays an Important Role in Maintaining Genome Stability

DHX9 is an ATP-independent DEAH-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[1-4], highlighting its importance in maintaining genome stability. DHX9 is overexpressed in many cancer types, including colorectal cancer (CRC) and lung cancer. In particular, microsatellite instable (MSI) tumors exhibiting defective mismatch repair (dMMR) show a strong dependence on DHX9, making this helicase an attractive target for oncology drug discovery.

DHX9 Prevents *Alu* Element-Mediated circRNA Formation



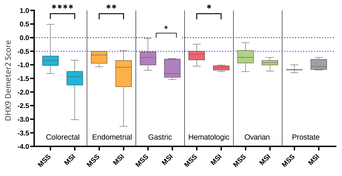
DHX9 Prevents Aberrant R-Loop Formation



DHX9 is a Novel Oncology Target with a Selective Dependency Profile in Microsatellite Instable Tumors

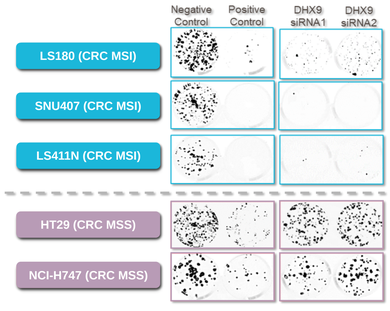
- Publicly available pan-cancer RNAi screens in the Broad Institute DepMap portal reveal a DHX9-dependency (as measured by Demeter2 score) in MSI cell line models, especially in CRC
- Follow-up colony formation and proliferation assays in multiple CRC cell lines show that depletion of DHX9 by siRNA knockdown results in cell growth inhibition in CRC MSI but not CRC MSS models

DHX9 Dependency in Relationship to MSI Status



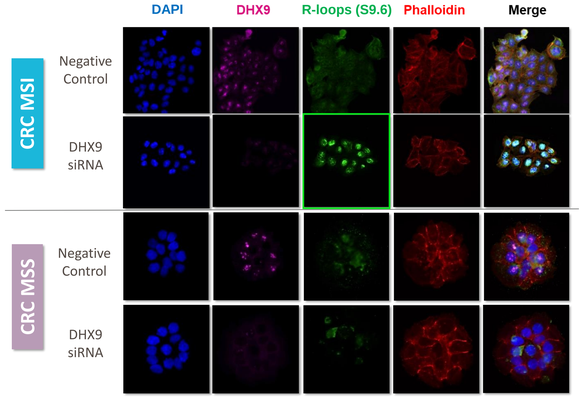
*DepMap Portal

DHX9 Knockdown Leads to CRC MSI Selective Antiproliferative Effect in Colony Formation Assay



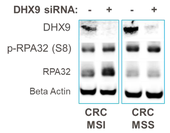
DHX9 Knockdown Selectively Increases R-loops in CRC MSI Cells

- Depletion of DHX9 by siRNA knockdown for 3 days results in elevated DNA/RNA hybrids (R-loops) in CRC MSI but not CRC MSS cell lines
- Aberrant R-loops can lead to DNA damage and replication stress

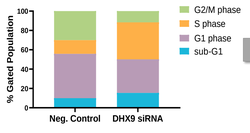


Induction of Replication Stress, Cell Cycle Arrest and Apoptosis in CRC MSI upon DHX9 Knockdown

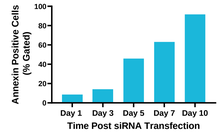
RPA Accumulation in CRC MSI 3 Days Post DHX9 KD



S-phase Cell Cycle Arrest in CRC MSI 5 Day Post DHX9 KD



Onset of Apoptosis in CRC MSI after DHX9 KD Over Time

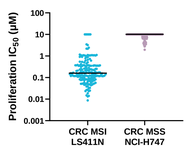


- DHX9 siRNA knockdown leads to accumulation of RPA in CRC MSI cells, but not CRC MSS cells, indicating increased replication stress
- Cell cycle arrest in S-phase is observed at 5 days post-transfection in CRC MSI
- Apoptotic cells (as measured by Annexin staining) increase over time upon DHX9 knockdown, consistent with timing of cell cycle arrest

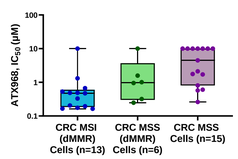
Small Molecule DHX9 Inhibitors Exhibit Preferential Dependency in CRC Cancer Cells with dMMR

- Lead series small molecule DHX9 inhibitors recapitulate CRC MSI anti-proliferative selectivity observed by DHX9 siRNA KD during target validation
- DHX9 tool compound ATX968 tested in a panel of 34 CRC cell lines results in strong and selective antiproliferative effect in CRC cells exhibiting dMMR

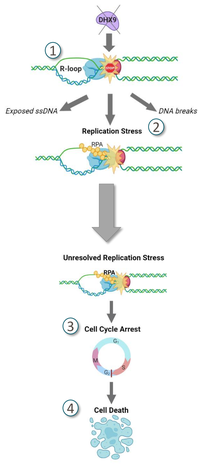
DHX9 Lead Series Compounds Exhibit CRC-MSI Selective Anti-Proliferative Activity



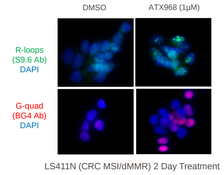
CRC Focused Cell Panel Proliferation Screen with Tool Compound Confirms Dependency in cells with dMMR



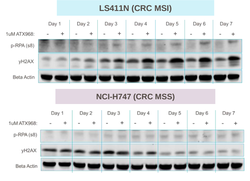
DHX9 Inhibitors Increase DNA/RNA Secondary Structures and Replication Stress Markers Prior to Cell Cycle Arrest and Ultimately Cell Death



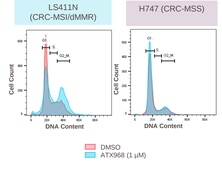
1 R-Loops and G-Quadruplexes Increase Upon DHX9 Inhibition



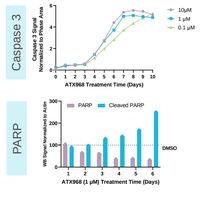
2 DNA Damage and Replication Stress Markers Increase upon DHX9 Inhibition only in CRC MSI



3 DHX9 Compound Inhibition Leads to Cell Cycle Arrest in G2/M Phase in dMMR CRC (Day 4-5)



4 DHX9 Inhibition Leads to a Later Onset Apoptosis in CRC MSI (Day 6-10)

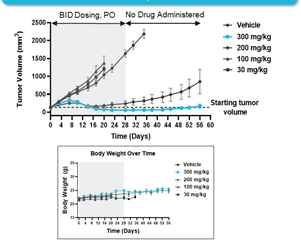


1. Increase of R-loops and G-quadruplexes observed in CRC MSI/dMMR cells as early as 1-2 days post ATX968 treatment
2. Increased γH2AX (DNA damage) after 2 days of treatment and increased p-RPA (replication stress) after 4-5 days only in CRC MSI/dMMR
3. Increase in G2/M arrest in CRC MSI/dMMR but not MSS 4-5 days post-ATX968 treatment
4. Cell death by apoptosis consistent with onset timing of cell cycle arrest

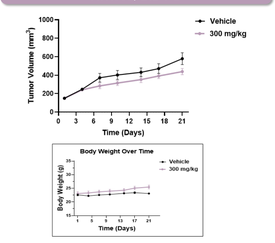
DHX9 Inhibitor ATX968 is Well Tolerated *in vivo* and Exhibits Durable Tumor Regression Selective to CRC MSI/dMMR

- Tool compound ATX968 demonstrates robust tumor growth inhibition in CRC MSI/dMMR xenograft model LS411N, achieving durable tumor regression at a well-tolerated dose of 300 mg/kg
- CRC MSS xenograft model SW480 did not exhibit significant tumor growth inhibition at high dose of compound

CRC MSI/dMMR LS411N Xenograft Efficacy Study ATX968, BID PO

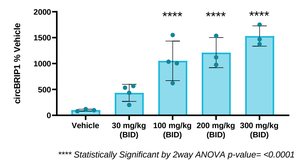


CRC MSS SW480 Xenograft Efficacy Study ATX968, BID PO

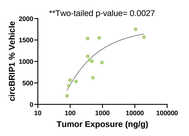


ATX968 Achieved Dose Dependent Intra-tumoral circBRIP1 PD with a Well-Correlated PK/PD/Efficacy Relationship

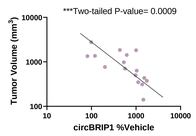
circBRIP1 Induction in LS411N Tumors



Tumor Exposure Correlates to Intra-tumoral circBRIP1 PD



Tumor Volume Correlates to Intra-tumoral circBRIP1 PD



**** Statistically Significant by 2way ANOVA p-value= <0.0001

*Data shown are from tumor samples collected at day 21, 12 hours post last dose

- Inhibition of DHX9 results in increased *Alu* element mediated circular RNAs such as circBRIP1[2], which can be used as a PD marker both *in vitro* and *in vivo*
- Intra-tumoral circBRIP1 induction correlates with both tumor exposure and tumor volume
- DHX9 inhibition leads to dose-dependent circBRIP1 induction in all cells tested, including human PBMCs (data not shown)

Conclusions

- DHX9 is an RNA helicase with important roles in maintaining genome stability, including prevention of R-loops and other secondary structures
- Novel inhibitors of DHX9 demonstrate selective anti-proliferative activity tied to unresolved replication stress in CRC cancer cells with dMMR
- Oral dosing of mice bearing human CRC MSI/dMMR tumors with ATX968 results in robust and durable tumor regression with correlated intra-tumoral induction of the PD biomarker circBRIP1
- PD biomarker circBRIP1 can also be measured in human PBMC, making circBRIP1 a potential non-invasive PD biomarker for clinical applications
- Sensitivity of other tumor types to DHX9 inhibition is currently under investigation

References

[1]Lee and Pelletier, Oncotarget (2016)
[2]Aktas et al, Nature (2017)
[3]Chakraborty et al, Nature (2018)
[4]Gulliver et al, Future Science (2020)

Acknowledgements

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