circBRIP1 RNA as a Non Invasive Target Engagement Pharmacodynamic Biomarker for DHX9 Inhibition

David Brennan*, Jennifer Castro, Matthew H. Daniels, Monique Laidlaw, Sunaina Nayak, Stephen J. Blakemore, Serena J. Silver, P. Ann Boriack-Sjodin, Kenneth W. Duncan, Jason A. Sager, & Robert A. Copeland

Accent Therapeutics, Lexington, MA / *Presenting Authors

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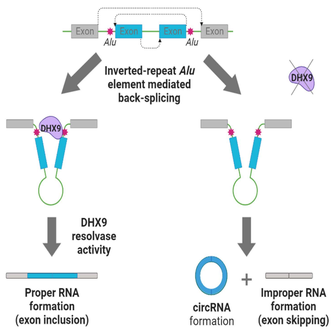
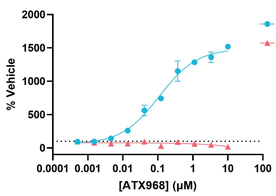
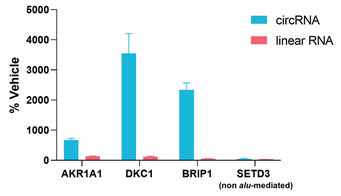
RNA Helicase DHX9 Plays an Important Role in Replication and Transcription

DHX9 is a multifunctional DExH-box NTP-dependent RNA helicase which has been reported to play important roles in replication, transcription, translation, RNA splicing and RNA processing[1-4] which contribute to DHX9's role in maintenance of genomic stability. DHX9 can bind specifically to inverted repeat *Alu* elements, preventing back-splicing events that lead to circular RNA and exon skipping of the linear transcript[2]. Upon DHX9 inhibition, a robust induction of *Alu*-mediated circular RNA is observed. For example, circBRIP1 is an *Alu*-mediated circular RNA that is robustly elevated upon DHX9 loss, with no observed change to levels of linear BRIP1. Here we describe data demonstrating circBRIP1 as a proximal DHX9 specific target engagement pharmacodynamic (PD) biomarker and its potential utility as a non-invasive clinical PD biomarker.



DHX9 Inhibition Increases *Alu* Element-Mediated circRNA Formation

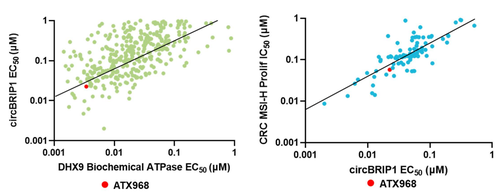




- DHX9 inhibition for 72 hours increases circRNA formation as compared to the linear form of the respective gene in a concentration-dependent manner
- ATX968 treatment leads to an increase in *Alu*-mediated specific circRNAs but not for the non-Alu-mediated circSETD3

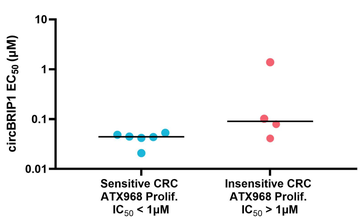
Potency of circBRIP1 Induction has Strong Correlation with ATPase Activity and Antiproliferative Activity in MSI-H CRC Cells

- Multiple compounds of the same chemotype series as ATX968 exhibit a strong positive correlation between circBRIP1 EC_{50} and biochemical ATPase activity
- circBRIP1 EC_{50} also has a strong correlation with antiproliferative IC_{50} in MSI-H CRC cells



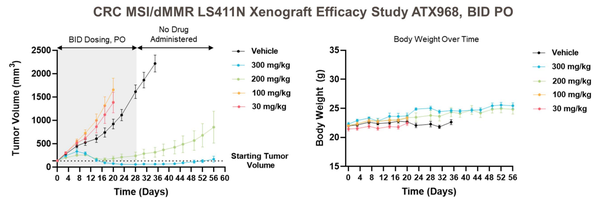
circBRIP1 Induction Mediated by DHX9 Inhibition is not an Indicator of Sensitivity

- Induction of circBRIP1 was observed in both DHX9-dependent and independent CRC cell lines
- Similar potency of circBRIP1 induction across sensitive and insensitive cells lines makes it a robust DHX9-specific target engagement marker

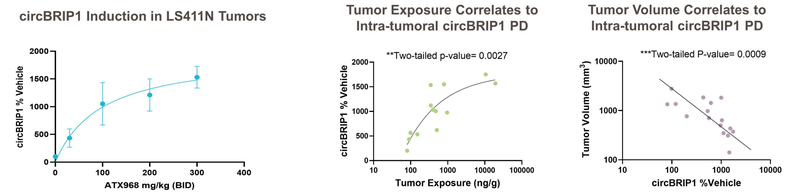


DHX9 Inhibitor ATX968 is Well Tolerated *in vivo* and Exhibits Durable Tumor Regression in CRC MSI/dMMR

Tool compound ATX968 demonstrates robust tumor growth inhibition in CRC MSI/dMMR xenograft model LS411N, achieving durable tumor regression at a well-tolerated oral dose of 300 mg/kg BID



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

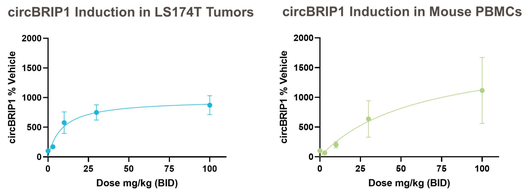


*Data shown are from tumor samples collected at day 21, 12 hours post last dose

- Inhibition of DHX9 *in vivo* results in increased intra-tumoral *Alu* element mediated circBRIP1, highlighting utility as an *in vivo* PD marker
- Intra-tumoral circBRIP1 induction correlates with both tumor exposure and tumor volume

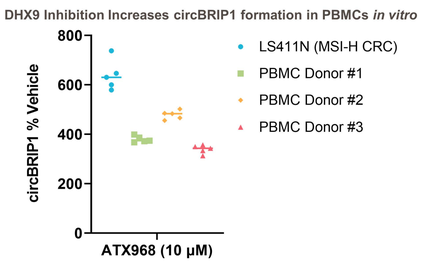
DHX9 Inhibitor Induces circBRIP1 Formation in CRC MSI/dMMR Xenograft Tumors and Mouse PBMCs

Treatment of CRC MSI/dMMR xenograft model LS174T with DHX9 compound 2 induces circBRIP1 formation in tumors and circulating mouse PBMCs after 28 days of treatment.

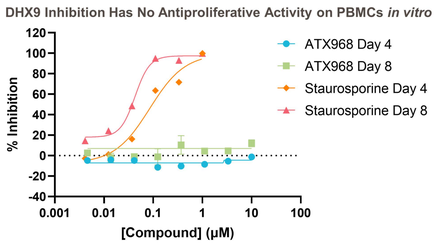


DHX9 Inhibition in Human PBMCs Elevates circBRIP1 Formation with No Effect on Proliferation *in vitro*

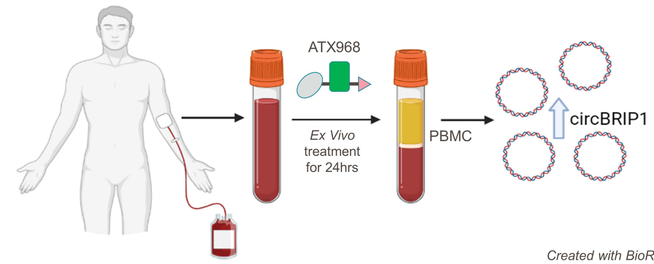
- 24 hours of ATX968 treatment *in vitro* resulted in substantial increase in circBRIP1 formation in human PBMCs
- Using pre-isolated frozen PBMCs as a proof-of-concept for examining circBRIP1 induction gave evidence for expansion into *ex vivo* experiments



- PBMCs were stimulated with IL-15, IL-21, CD40L for proliferation study[5,6]
- No antiproliferative activity observed through 10 days of ATX968 treatment compared to staurosporine control



Ex vivo Treatment of Human Whole Blood with DHX9 Inhibitor Induces circBRIP1 Formation



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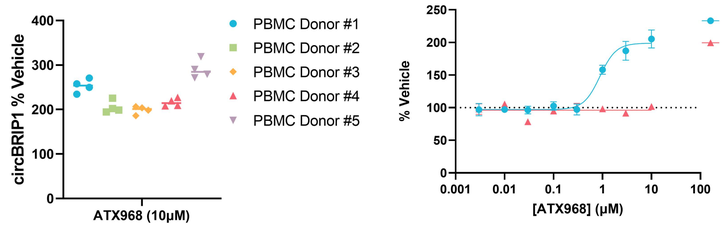
Human Whole Blood Treatment with DHX9 Inhibitor Induces circBRIP1 Formation in PBMCs



- *Ex vivo* treatment of human whole blood for 24 hours induced circBRIP1 formation in PBMCs
- PBMCs were isolated from human whole blood post 24 hour DHX9 treatment
- Minimal linear BRIP1 changes were observed across donors tested

Conclusions

- DHX9 is an RNA helicase with important roles in replication and transcription, including a role in prevention of inverted-repeat *Alu* element mediated back-splicing
- Treatment with novel inhibitors of DHX9 lead to robust increases of *Alu* element mediated circRNAs including circBRIP1 as compared to the linear form
- circBRIP1 accumulation following DHX9 inhibition is observed in both DHX9-dependent and independent cell lines, and can be used as a measure of target engagement
- ATX968 treatment results in robust tumor growth inhibition in CRC MSI xenograft models and intra-tumoral circBRIP1 levels correlates with both tumor exposure and tumor volume
- DHX9 inhibitor treatment results in circBRIP1 formation in mouse PBMCs *in vivo* and human PBMCs *in vitro* without affects on proliferation
- *Ex vivo* treatment of human whole blood with ATX968 induces circBRIP1 formation highlighting its potential as a non-invasive target engagement biomarker for DHX9 inhibitors in the clinic

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)
[5]Marsman et al, Frontiers in Immunology (2022)
[6]Dunne et al, Journal of Immunology (2001)

Acknowledgements

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