The PKC-β Inhibitor MS-553 Displays Preclinical Efficacy in BTK inhibitor Resistant Chronic Lymphocytic Leukemia

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Background

- The treatment of chronic lymphocytic leukemia (CLL) has been revolutionized through usage of targeted therapies against the B-cell receptor (BCR) signaling cascade, mainly through Bruton's tyrosine kinase (BTK) inhibitors.
- Resistance to BTK inhibition has emerged in patients through the acquisition of mutations in BTK[1,2]. Despite these mutations, BCR signaling remains intact, suggesting targeting molecules downstream of BTK may be an effective therapeutic strategy.
- Protein Kinase C-β (PKCβ) is a downstream component of the BCR pathway and has been demonstrated as an effective therapeutic target in CLL.[3]
- MS-553 is a potent, ATP competitive, reversible inhibitor of multiple PKC isoforms including PKCβ.
- Develop therapies for CLL patients who are resistant to either covalent BTKi (cBTKi) or non-covalent BTKi (ncBTKi).
- We hypothesize the inhibition of PKCβ through MS-553 can be a therapeutic strategy for the treatment of treatment-naïve or cBTKi or ncBTKi resistant CLL.

PKC isoform inhibition by MS-553

Human PKC isoform	IC_{50} (nM)
Alpha (α)	2.3
Beta I (βI)	8.1
Beta II (βII)	7.6
Theta (θ)	25.6
Gamma (γ)	57.5
Mu (μ)	314
Epsilon (ε)	808
Delta (δ), eta (η), iota (ι), zeta (ζ)	>1000

Figure 1. Inhibition of human PKC isoforms by MS-553. *In vitro* biochemical assays were used to determine the IC_{50} of MS-553 on multiple PKC isoforms.

References and Acknowledgments

References: [1] Woyach, JA et al. (2014). *NEJM* 370, 2286-2294
[2] Wang, E et. Al (2022). *NEJM* 386, 735-746
[3] El-Gamal, D et al. (2014). *Blood* 124, 1481-1491

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MS-553 inhibits BCR dependent signaling

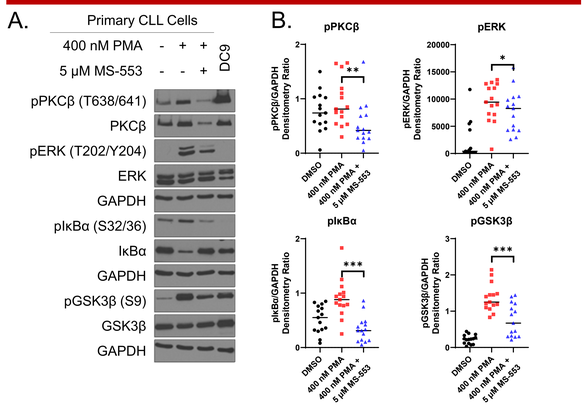

Figure 2. Primary CLL cells were treated with 5 μM MS-553 for 24 hours and stimulated with 400 nM PMA for 90 minutes. **A)** Representative immunoblot showing decrease phosphorylation of PKCβ and its downstream targets. **B)** Quantification of immunoblots (n=15), results are reported as fold change in expression compared to vehicle control.

MS-553 inhibits β-Catenin signaling

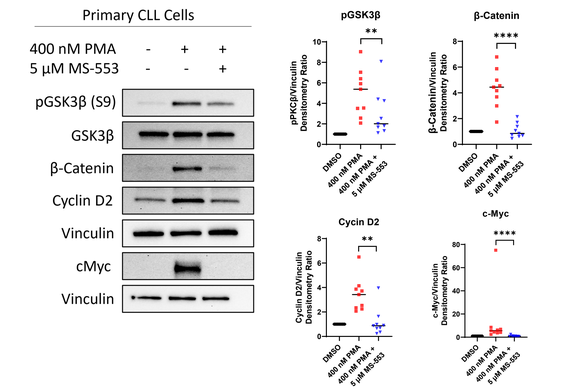

Figure 3. Primary CLL cells were treated with with 5 μM MS-553 for 24 hours and stimulated with 400 nM PMA for 90 minutes. **A)** Representative immunoblot showing decrease in β-Catenin expression and its downstream targets. **B)** Quantification of immunoblots (n=9), results are reported as fold change in expression compared to vehicle control.

MS-553 inhibits β-Catenin signaling

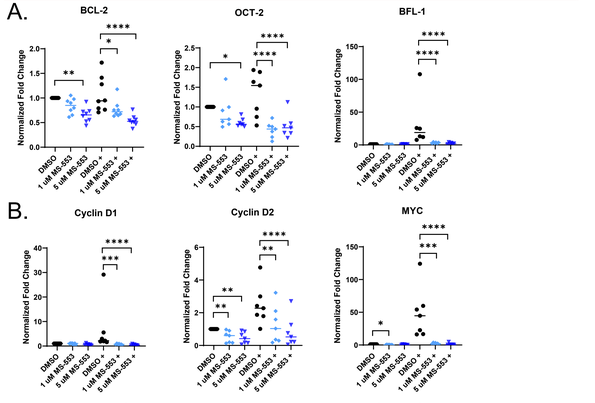

Figure 4. Primary CLL cells were treated with 1 or 5 μM MS-553 for 48 hours and stimulated with 400 nM PMA for 90 minutes. **A)** Graphs showing decreased mRNA expression of downstream NFκB genes. (n=7) **B)** Graphs showing decreased mRNA expression of downstream WNT pathway targets (n=7).

MS-553 retains efficacy in Primary cBTKi resistant samples

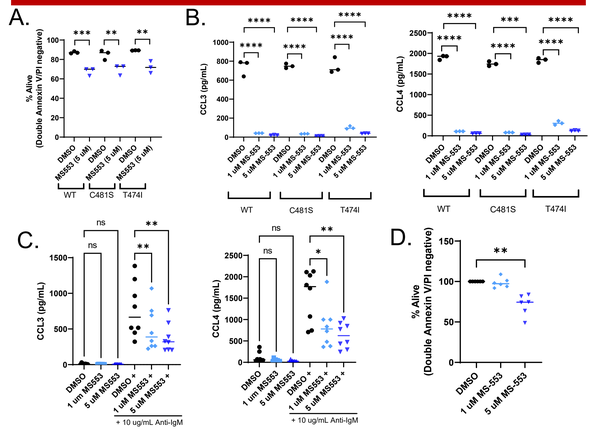

Figure 5. Primary cBTKi resistant CLL cells and TMD8 cells harboring either WT, C481S, or T474I BTK mutations were treated with MS-553 and assessed for viability and downstream cytokine expression. **A)** WT, C481S, and T474I TMD8 cells were treated with 5 μM MS-553 for 48 hours and assessed for viability via Annexin V/PI. **B)** TMD8 cells WT, C481S, or T474I cells were treated with either 1 μM or 5 μM MS-553 for 24 hours and assessed for CCL3 and CCL4 expression (n=3). **C)** CLL cells harboring C481S BTK were treated with either 1 μM or 5 μM MS-553 for 24 hours and assessed for CCL3 and CCL4 expression (n=8). **D)** CLL cells harboring C481S BTK were treated with either 1 μM or 5 μM MS-553 for 72 hours and assessed for viability via Annexin V/PI.

MS-553 improves survival in the Eμ-MTCP1 mouse model

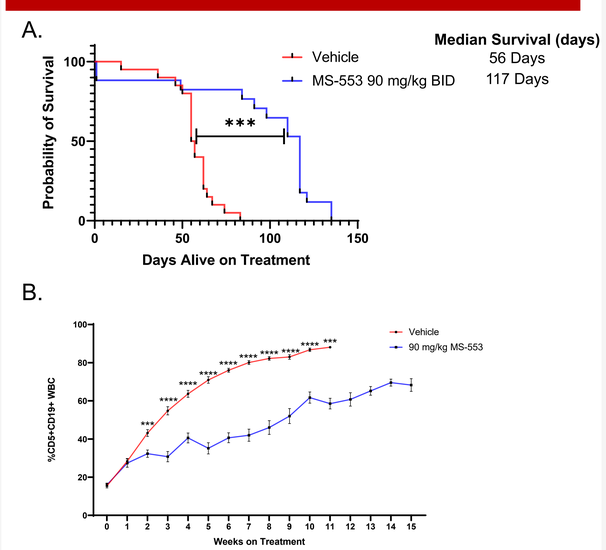

Figure 6. C57BL/6J mice were engrafted with 10^6 splenocytes from leukemic Eμ-MTCP1 mice and monitored for survival and peripheral disease progression. **A)** Measurement of overall mice survival of mice treated with vehicle or 90 mg/kg MS-553 BID (vehicle n=20, 90 mg/kg MS-553 n=17). **B)** Measurement of peripheral disease characterized by CD45+/CD5+/CD19+ % in peripheral blood.

Conclusions

- MS-553 is a potent inhibitor of PKCβ and displays potency in primary CLL cells.
- MS-553 inhibits BCR depend signaling shown by reduced phosphorylation of PKCβ and its downstream targets.
- In treatment-naïve samples, MS-553 displays modest cytotoxicity and the ability to overcome stromal protection while also inhibiting downstream pro-inflammatory cytokine expression.
- MS-553 inhibits downstream β-Catenin signaling shown by reduced levels of β-Catenin and its downstream targets.
- In both covalent and non-covalent resistant TMD8 cells, MS-553 maintained its ability to induce modest cytotoxicity and inhibition of downstream pro-inflammatory cytokine expression.
- Treatment of Eμ-MTCP1 mice with MS-553 leads to significantly longer OS and decreased peripheral disease.
- Together our results establish MS-553 as a potential therapeutic to treat CLL patients regardless of BTK status.