MCL1 inhibitor PRT1419 Demonstrates Antitumor Activity in PBRM1-altered Clear Cell Renal Cancer and Synergizes with Standard of Care Agents

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6147

Background

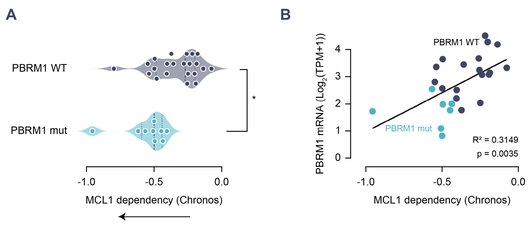
- Induced myeloid leukemia cell differentiation protein (MCL1) is a member of the B-cell lymphoma-2 (BCL2) family of apoptosis regulators and plays a critical role in promoting cancer cell survival[1].
- We previously described PRT1419, a novel, potent, and orally bioavailable MCL1 inhibitor that demonstrates anti-tumor efficacy in various preclinical models and is currently under evaluation in phase 1 clinical trials in hematologic and solid malignancies.[2]
- Gene dependency analyses to identify biomarkers of MCL1 inhibitor sensitivity revealed that clear cell Renal Cancer (ccRCC) cell lines with deleterious alterations in PBRM1/BAF180 (Polybromo 1) displayed a strong dependency on MCL1. We had previously described alterations in other mammalian SWI/SNF factors as biomarkers of MCL1 inhibitor sensitivity.[3]
- PBRM1 is a chromatin-targeting subunit of mammalian pBAF (SWI/SNF-B) complexes, which is frequently altered in various human cancers with a particularly high alteration rate in ccRCC (~40% of tumors harbor damaging PBRM1 alterations).[4]
- Current standard of care (SoC) agents in ccRCC include multi-targeted Receptor Tyrosine Kinase inhibitors, mammalian Target of Rapamycin (mTORc) inhibitors, HIF2α inhibitors and immune checkpoint blockade.[5]

Key Findings

- Here we show that PBRM1-loss is associated with sensitivity to MCL1 inhibition in ccRCC and provide rationale for the evaluation of PRT1419 in combination with SoC agents for the treatment of PBRM1-deficient ccRCC

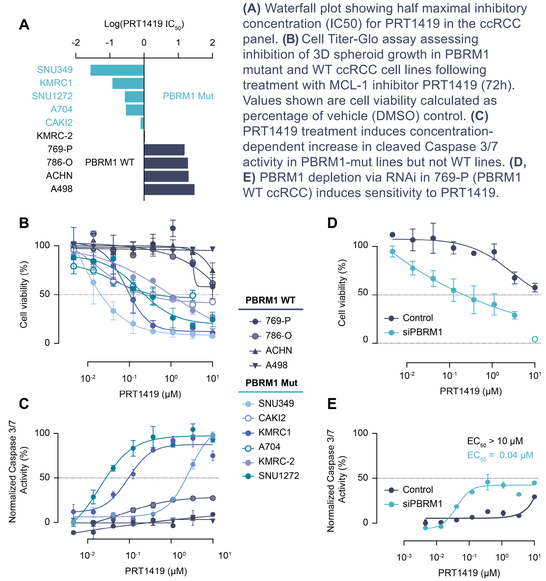
Results

Figure 1. Damaging Mutations in *PBRM1* are associated with MCL1 dependency in ccRCC



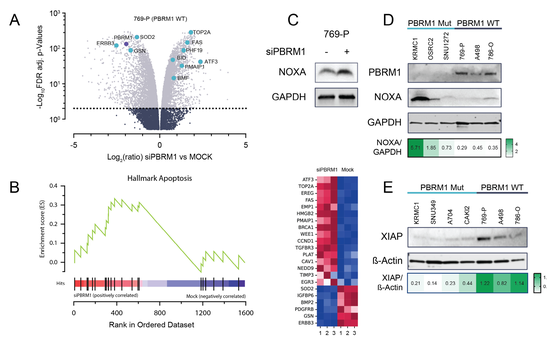
(A) MCL-1 gene dependency analysis in ccRCC cell lines with damaging PBRM1 mutations compared to WT. Dependency scores (Chronos, 22Q2) were retrieved from DepMap (https://depmap.org) **(B)**. PBRM1 mRNA levels in ccRCC cell lines inversely correlate with MCL1 dependency. *P<0.05, versus WT by t-test.

Figure 2. ccRCC cell lines with PBRM1 mutations are sensitive to PRT1419 *in vitro*



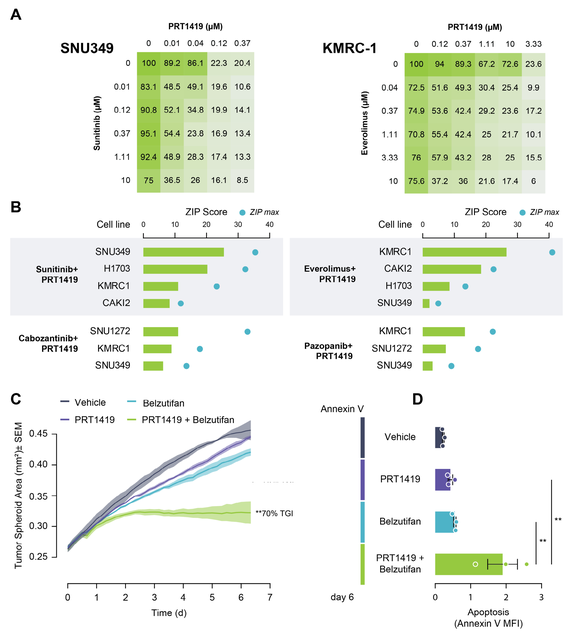
(A) Waterfall plot showing half maximal inhibitory concentration (IC50) for PRT1419 in the ccRCC panel. **(B)** Cell Titer-Glo assay assessing inhibition of 3D spheroid growth in PBRM1 mutant and WT ccRCC cell lines following treatment with MCL-1 inhibitor PRT1419 (72h). Values shown are cell viability calculated as percentage of vehicle (DMSO) control. **(C)** PRT1419 treatment induces concentration-dependent increase in cleaved Caspase 3/7 activity in PBRM1-mut lines but not WT lines. **(D, E)** PBRM1 depletion via RNAi in 769-P (PBRM1 WT ccRCC) induces sensitivity to PRT1419.

Figure 3. PBRM1 loss in ccRCC is associated with pro-apoptotic signaling, priming cells for cell death following treatment with PRT1419



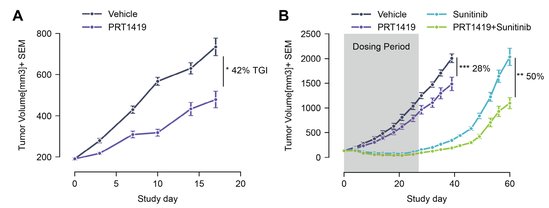
(A) Volcano plots of global RNA expression data in 769-P (PBRM1 WT ccRCC) cells following RNAi-mediated PBRM1 depletion and 24h spheroid culture; genes mediating apoptosis are highlighted in blue. **(B)** Unbiased Gene Set Enrichment Analysis (GSEA) analysis (MSigDB Hallmark geneset)[6] demonstrates that PBRM1 depletion increases expression of a pro-apoptosis gene signature in ccRCC. **(C)** Western blot showing increased expression of pro-apoptotic MCL1 inhibitor, NOXA (*PMAIP1*), following PBRM1 depletion. **(D, E)** Western blot showing increased endogenous expression of NOXA and reduced expression of XIAP, a caspase inhibitor, in PBRM1 mutant ccRCC cell lines cultured as spheroids, suggesting apoptosis "priming."

Figure 4. PRT1419 synergizes with Standard of Care (SoC) agents in PBRM1-mutant ccRCC



A) CellTiter-Glo assay demonstrating synergistic inhibition of spheroid growth in PBRM1-mutant cell lines following treatment with PRT1419 and SoC agents: Sunitinib, Everolimus, Cabozantinib and Pazopanib. Values shown are cell viability calculated as percentage of vehicle control. **(B)** ZIP synergy scores from synergy proliferation assays were computed using SynergyFinder 2.0 (https://synergyfinder.fimm.fi/). **(C, D)** Incucyte-based measurement of spheroid growth and apoptosis (Annexin V) in KMRC2 (VHL protein loss, PBRM1 mutant) following treatment with PRT1419 (0.5 μM) and/or Belzutifan (HIF2α inhibitor, 1 μM) for 6 days. Data represented as mean + SEM. N=3, ** P<0.01 (A) one-way ANOVA

Figure 5. Combination of PRT1419 and S.o.C agents leads to improved and durable response in vivo



Intravenously administered PRT1419 is efficacious in monotherapy and in combination with Sunitinib, administered orally, to potently repress tumor growth in CDX models of PBRM1-loss ccRCC and NSCLC. **(A)** Female BALB/c nude mice were injected subcutaneously with OSRC-2 (ccRCC, PBRM1 mutant) in the right front flank region (1 x 10^6 cells) **(B)** Female NOD/SCID mice were injected subcutaneously in the right front flank region with PBRM1-protein loss NSCLC cell line NCI-H1703 (1 x 10^7 cells). Animals were dosed with PRT1419 once a week and/or Sunitinib(10 mg/kg) daily. Data represented as mean + SEM. N=8, * P<0.05, **P<0.001, ***P<0.001 by (A) Mann-Whitney U test or (B) unpaired t test

Figure 6. PRT2527, a potent and selective CDK9 inhibitor, similarly demonstrates antitumor activity in PBRM1-loss ccRCC

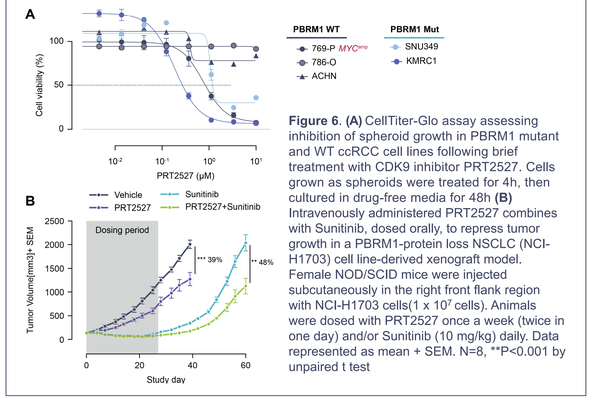
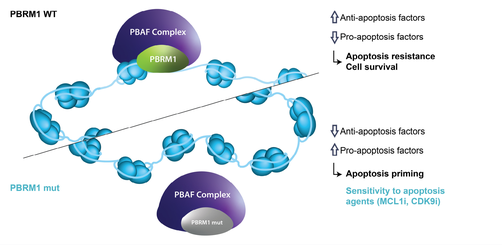


Figure 6. **(A)** CellTiter-Glo assay assessing inhibition of spheroid growth in PBRM1 mutant and WT ccRCC cell lines following brief treatment with CDK9 inhibitor PRT2527. Cells grown as spheroids were treated for 4h, then cultured in drug-free media for 48h **(B)** Intravenously administered PRT2527 combines with Sunitinib, dosed orally, to repress tumor growth in a PBRM1-protein loss NSCLC (NCI-H1703) cell line-derived xenograft model. Female NOD/SCID mice were injected subcutaneously in the right front flank region with NCI-H1703 cells(1 x 10^7 cells). Animals were dosed with PRT2527 once a week (twice in one day) and/or Sunitinib (10 mg/kg) daily. Data represented as mean + SEM. N=8, **P<0.001 by unpaired t test

Conclusions

- PBRM1 loss is associated with sensitivity to MCL1 inhibition in preclinical models of ccRCC
- PBRM1 loss is associated with pro-apoptotic signaling, priming cells for apoptosis following MCL1 inhibition.
- PRT1419 synergizes with SoC (multitargeted TKIs, mTOR inhibitors and HIF2α inhibitor) in inhibiting tumor growth *in vitro* and *in vivo*.
- Indirect inhibitors of MCL1 expression, such as selective CDK9 inhibitor PRT2527, are similarly efficacious in PBRM1-deficient ccRCC.
- PRT1419 and PRT2527 are currently being evaluated in Phase I clinical trials in patients with relapsed/refractory hematologic and solid malignancies. [2,7]



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Disclosures

Authors are or were employees of Prelude Therapeutics, Inc at the time of research, and may own equity in the Company.