Preclinical Characterization of PRT1419, a Potent, Selective and Orally Bioavailable Inhibitor of MCL1

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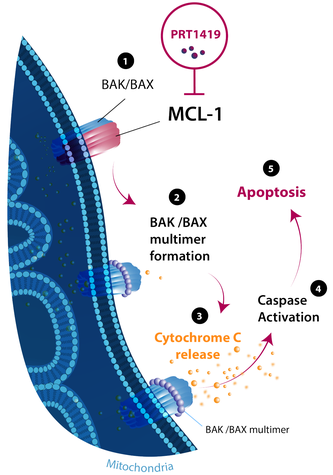
INTRODUCTION

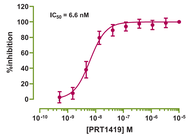
- MCL1 is a member of the anti-apoptotic BCL2 family of proteins and plays a critical role in maintaining cellular homeostasis and promoting cell survival.
- It is frequently found to be amplified or overexpressed in both solid tumors and hematologic malignancies.
- Increased expression of MCL1 is associated with a higher grade and poor prognosis in multiple cancer types.
- MCL1 has been implicated in mediating resistance to chemotherapeutic agents as well as targeted therapies.



PRT1419 is designed to a potent, selective and orally bioavailable MCL1 inhibitor currently under evaluation in a Phase I clinical trial in patients with relapsed/refractory hematologic malignancies (NCT04543305).

RESULTS

PRT1419 IS A POTENT AND SELECTIVE MCL1 INHIBITOR WITH ROBUST PRO-APOPTOTIC ACTIVITY AND ORAL BIOAVAILABILITY



> 200-fold selectivity over BCL2 and BCL-xL

Summary of in vitro properties of MCL1 inhibitors in Ph 1 studies

Assay	AMG176	AZD5991	MIK665	PRT1419
Proliferation IC_{50} (nM)	150	31	4.5	80
C-Caspase 3 EC_{50} (nM)[a]	1800	320	430	210
Caco-2 (x10^{-6}cm/s)	6	<0.1	0.2	11
Human Hepatic CI (%HBF[b])	42	78	35	71
Solubility at pH 7.4 (µg/mL)	13	31	14	>1000
Route of administration in Phase 1 study	IV	IV	IV	Oral

[a] *Cleaved Caspase 3 activity evaluated in OPM2 cells spiked in human whole blood*
[b] *Hepatic Blood Flow*

Summary of PRT1419 PK parameters

	Rat (3 mg/kg)	Dog (3 mg/kg)
t½ (h)	3.68	9.22
F(%)	53	86.3

Figure 1: A. Concentration-dependent inhibition of human MCL1-BIM binding by PRT1419 in a fluorescence polarization binding assay. Data represent mean ± SD. n = 15. **B.** Concentration-dependent activation of cleaved Caspase 3/7 in the OPM2 cell line following 4 hours of treatment with PRT1419, correlates with anti-proliferative activity in a 24-hour CellTiter-Glo® assay

PRT1419 SHOWS POTENT ANTI-PROLIFERATIVE ACTIVITY IN HEMATOLOGIC CANCER CELL LINES

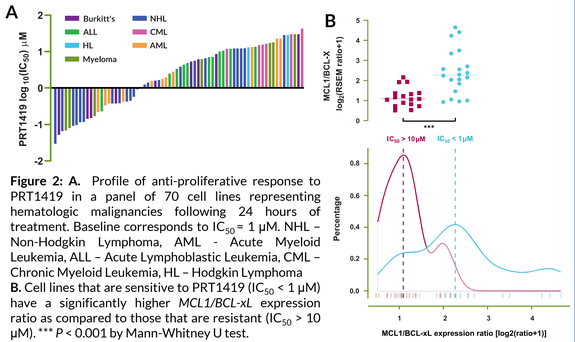


Figure 2: A. Profile of anti-proliferative response to PRT1419 in a panel of 70 cell lines representing hematologic malignancies following 24 hours of treatment. Baseline corresponds to IC_{50} = 1 µM. NHL – Non-Hodgkin Lymphoma, AML - Acute Myeloid Leukemia, ALL – Acute Lymphoblastic Leukemia, CML – Chronic Myeloid Leukemia, HL – Hodgkin Lymphoma **B.** Cell lines that are sensitive to PRT1419 (IC_{50} < 1 µM) have a significantly higher *MCL1/BCL-xL* expression ratio as compared to those that are resistant (IC_{50} > 10 µM). *** *P* < 0.001 by Mann-Whitney U test.

PRT1419 TREATMENT SHOWS RAPID INDUCTION OF APOPTOSIS AND POTENT ANTI-TUMOR ACTIVITY IN MULTIPLE CELL LINE-DERIVED XENOGRAFT MODELS

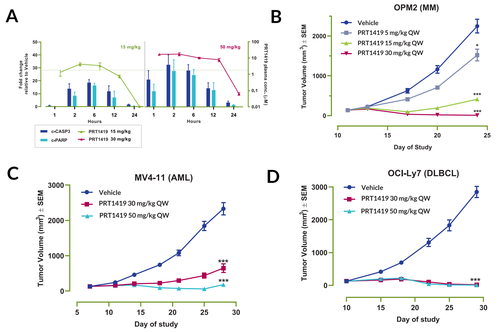


Figure 3: A. Dose-dependent increase in PRT1419 plasma concentration and levels of cleaved caspase-3 and cleaved PARP in OPM2 tumor tissue following oral administration of PRT1419. Dotted line refers to c-caspase3 EC_{50} from whole blood assay. Oral administration of PRT1419 leads to tumor regressions in **B**. OPM2 MM, **C**. MV4-11 AML, and **D**. OCI-Ly7 DLBCL cancer xenograft models. Data represent mean ± SEM. * *P* < 0.05, *** *P* < 0.001 vs. vehicle by Mann-Whitney U test.

PRT1419 DEMONSTRATES SYNERGY IN COMBINATION WITH TARGETED AGENTS IN VITRO AND IN VIVO

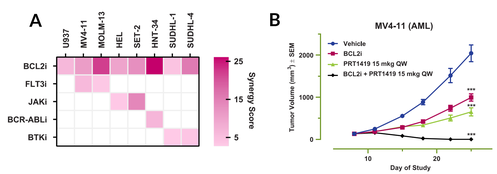


Figure 4: A. ZIP synergy scores in various cell lines treated with PRT1419 in combination with targeted agents including a BCL2 inhibitor or tyrosine kinase inhibitors **B**. Oral administration of a combination of PRT1419 and a BCL2 inhibitor results in complete tumor regressions in the MV4-11 CDX model. Data represent mean ± SEM. ** *P* < 0.01 vs. Vehicle by Mann-Whitney U test

PRT1419 TREATMENT DEMONSTRATES SIGNIFICANT ANTI-TUMOR ACTIVITY AND IMPROVED SURVIVAL IN PDX MODELS OF B-CELL LYMPHOMA

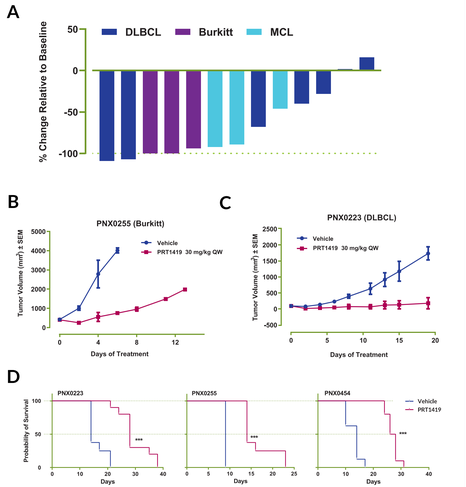


Figure 5: A. Once weekly oral administration of PRT1419 shows potent anti-tumor activity in PDX models of human lymphoma. DLBCL – Diffuse Large B-Cell Lymphoma, MCL – Mantle Cell Lymphoma. **B, C.** Tumor growth curves in a model of Burkitt lymphoma and DLBCL. **D.** PRT1419 treatment leads to a significant improvement in survival in several PDX models of lymphoma. *** *P* < 0.001 by log-rank (Mantel-Cox) test

PRT1419 SHOWS POTENT SINGLE AGENT ACTIVITY AND CAN OVERCOME RESISATNCE TO TARGETED THERAPIES IN PRIMARY AML CELLS

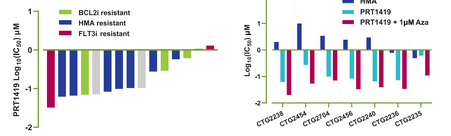


Figure 6: A. Ex vivo PRT1419 treatment demonstrates concentration-dependent inhibition of proliferation in a 2-day 2-D assay of primary AML blasts including FLT3 mutant samples that were resistant to a FLT3 inhibitor (purple bars) and samples that were resistant to a BCL2 inhibitor (blue bars) or a hypomethylating agent (green bars). Baseline corresponds to IC_{50} = 1 µM. **B.** Combination of PRT1419 with an HMA shows greater activity than either agent alone in primary AML models ex vivo.

PRT1419 DEMONSTRATES ANTI-TUMOR ACTIVITY IN SOLID TUMOR PDX AND CELL LINE MODELS

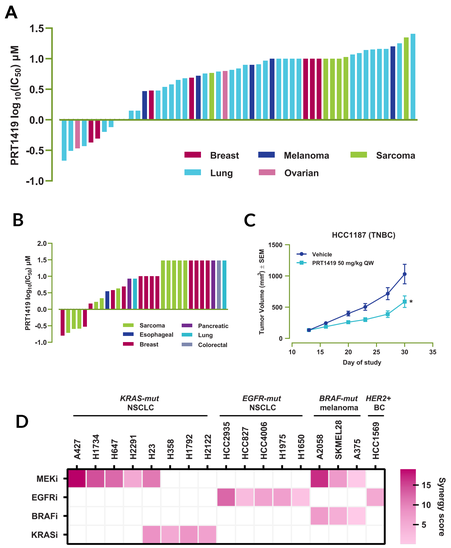


Figure 7: A. Profile of anti-proliferative response to PRT1419 in a panel of solid tumor **A.** cell lines and **B.** PDX models following 48 hours of treatment. Baseline corresponds to IC_{50} = 1 µM. **C.** Oral administration of PRT1419 shows significant anti-tumor activity in the HCC1187 CDX model of triple negative breast cancer. Data represent mean ± SEM. * *P* < 0.05 vs. vehicle by Mann-Whitney U test. **D.** ZIP synergy scores in various cell lines treated with PRT1419 in combination with targeted agents.

CONCLUSIONS

- **PRT1419 is designed to be a potent, selective and orally active MCL1 inhibitor.**
- **PRT1419 demonstrates potent oral activity as monotherapy and in combination with standard of care therapies in various tumor types.**
- **PRT1419 can overcome resistance to multiple targeted therapies, particularly in myeloid malignancies.**
- **PRT1419 is currently under evaluation as an oral agent in a Phase I clinical trial in patients with relapsed/refractory hematologic malignancies (NCT04543305).**