Development of Pharmacodynamic Assays for Quantifying SMARCA2 Protein Degradation and Target Gene Expression in Response to a SMARCA2 Degrader (PRT3789)

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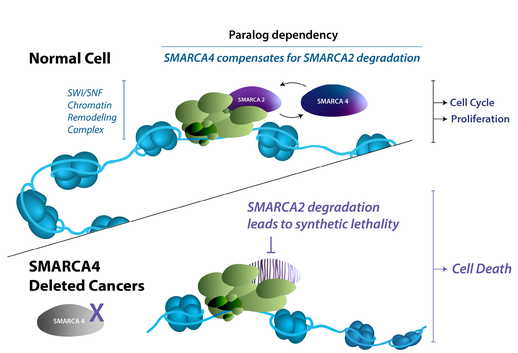
2792

Background

- The SWI/SNF complex plays an important role in controlling gene expression via chromatin remodeling.
- Certain cancers are heavily dependent on one of its catalytic subunits, SMARCA2, in the absence of another subunit, SMARCA4.
- Targeting SMARCA2 with selective protein degraders has therapeutic potential in these SMARCA4 deficient human cancers.
- PRT3789 is a highly potent and selective SMARCA2 degrader[1] that is currently being evaluated in a Phase 1 clinical study in patients with advanced solid tumors with loss of SMARCA4.

Figure 1. SMARCA2 Degradation Leads to Synthetic Lethality in SMARCA4 Deleted Cancers



Objectives

- Develop a plate-based MSD® assay to quantify SMARCA2 protein degradation in human peripheral blood mononuclear cells (PBMCs) in response to PRT3789 treatment.
- Develop a secondary quantitative PCR assay to assess changes in SMARCA2 transcriptional targets in human PBMCs in response to degradation by PRT3789.

Development of MSD® Assay for SMARCA2 Protein Quantification

Figure 2. MSD® Assay Development Scheme

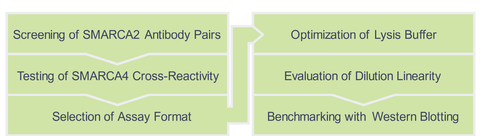
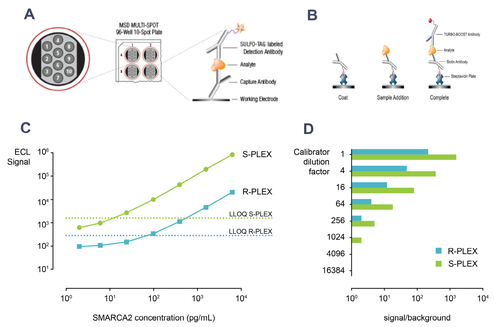
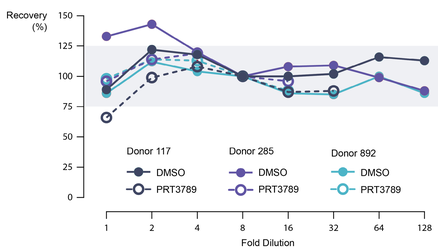


Figure 3. MSD S-PLEX® Format Demonstrates High Sensitivity and Signal-to-Background



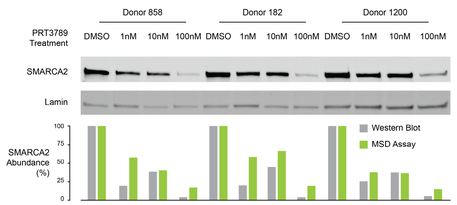
(A) Schematic of MSD R-PLEX®, the standard MSD® assay format.[2]
(B) Schematic of MSD S-PLEX®. S-PLEX® format utilizes a TUBRO-BOOST® label that results in improved sensitivity.[3]
(C) The S-PLEX® format resulted in a higher dynamic range of quantification and sensitivity compared to the R-PLEX®. Lower Limit of Quantification (LLOQ) = 3X Blank RLU
(D) The S-PLEX® format resulted in a higher signal-to-background ratio compared to the R-PLEX® format.

Figure 4. MSD® Assay Exhibits Broad Dilution Linearity



PBMCs from healthy donors were treated with DMSO and PRT3789, lysed and serially diluted.

Figure 5. SMARCA2 Degradation Measured by MSD® Assay is Similar to Western Blotting



Significant SMARCA2 degradation was achieved across cultured PBMCs from three healthy donors treated with increasing doses of PRT3789. Consistent results were observed by both methods.

Development of qPCR Assay

Figure 6. qPCR Assay Development Scheme

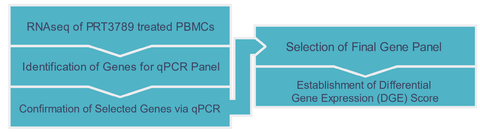
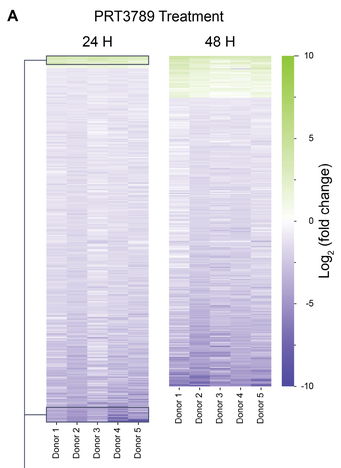


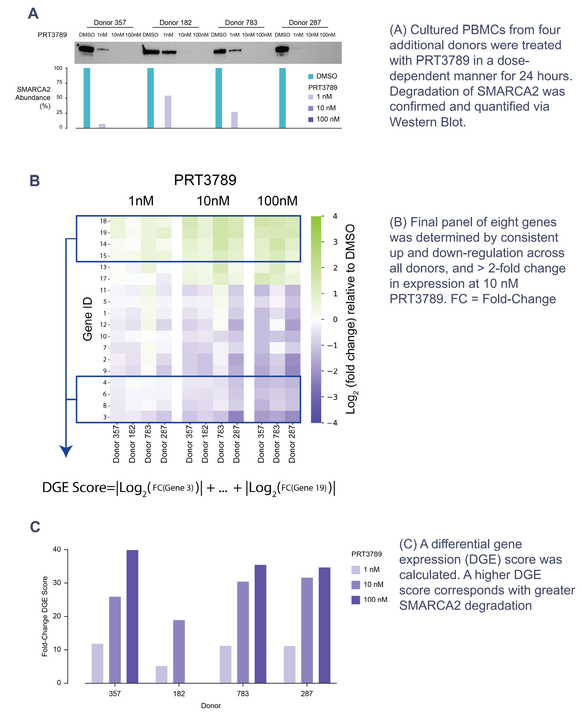
Figure 7. Identification of SMARCA2 Target Genes in PBMCs



(A) PBMCs from 5 healthy donors were treated ex vivo with PRT3789 for 24 and 48 hours. RNA sequencing was performed to identify differentially expressed genes in response to SMARCA2 degradation. A significant number of genes were both up- and down-regulated in response to treatment.

(B) Nineteen genes were chosen for confirmation.
(C) Expression was verified by TaqMan qPCR in the same samples used for RNA-Seq.

Figure 8. Selection of Final Panel and Calculation of DGE Score



(A) Cultured PBMCs from four additional donors were treated with PRT3789 in a dose-dependent manner for 24 hours. Degradation of SMARCA2 was confirmed and quantified via Western Blot.

(B) Final panel of eight genes was determined by consistent up and down-regulation across all donors, and > 2-fold change in expression at 10 nM PRT3789. FC = Fold-Change

(C) A differential gene expression (DGE) score was calculated. A higher DGE score corresponds with greater SMARCA2 degradation

Conclusions

- We developed an MSD® based assay to quantify SMARCA2 degradation in human PBMCs in response to treatment with PRT3789.
- The assay is quantitative in the pg/mL range, has no cross-reactivity with SMARCA4, and exhibits a broad range of dilution linearity in cultured PBMCs and whole blood.
- An additional TaqMan qPCR assay was developed to assess differential gene expression in eight target genes in response to SMARCA2 degradation in PBMCs.

References
1. Hulse, et al. Cancer Res 2022;82(12_Suppl):Abstract nr 3263
2, 3. Figures used with permission from Meso Scale Discovery

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Disclosures
1.Authors are or were employees of Prelude Therapeutics, Inc. at the time of research and may own equity in the Company.
2.Authors are employees of Meso Scale Discovery

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