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ORAL ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

RSK1 Is an Exploitable Dependency in Myeloid Malignancies

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Myeloid malignancies harbor distinct molecular drivers but share convergence of oncogenic signaling pathways and propagation by ripe pro-inflammatory niches. To delineate these hallmarks across the spectrum of myeloid disease states, we established the largest comprehensive atlas of myeloproliferative neoplasms (MPN) and secondary acute myeloid leukemia (sAML) through RNA-sequencing and mass cytometry (CyTOF) of 370 primary samples encompassing CD34+ hematopoietic stem/progenitor cells and CD14+ monocytes, revealing aberrant of PI3K/AKT/mTOR signalling and NFκB-mediated hyper-inflammation. As a central mediator of these hyperactive signaling pathways, ribosomal S6 kinases (RSKs) represent potentially attractive novel therapeutic targets warranting further evaluation.

Using both genetic approaches and a first-in-class oral RSK inhibitor, PMD-026, that is currently being evaluated in phase 1/1b clinical trials (NCT04115306) in metastatic and triple negative breast cancer, we found that RSK1 (*RPS6KA1*) inhibition potently induced apoptosis and G2/M arrest in leukemia models in conjunction with suppression of PI3K/AKT/mTOR and NFκB signaling. Further NFκB cascade profiling following RSK1 perturbation revealed reduced phosphorylation of IKKα/β and p65/RELA and prevention of p65/RELA nuclear translocation. Using RNA-seq, ATAC-seq and H3K27ac CUT&Tag, we found that PMD-026 treatment altered chromatin accessibility and inhibited NFKB1, TNF, and cell cycle regulators CCNA1/2 and CDK1/2. In cytokine CyTOF, PMD-026 nearly abrogated all induction of inflammatory cytokines including TNF, IL-6, IL-8, CCL3, and CCL4 in primary MPN monocytes to levels substantially below basal conditions.

We further extended our findings to *de novo* AML harboring *FLT3* internal tandem duplication (*FLT3*-ITD), where *FLT3*-ITD patients exhibited elevated *RPS6KA1* expression and those who developed resistance to gilteritinib demonstrated progressively increased *RPS6KA1* expression. *FLT3-ITD* cells exhibited preferentially sensitivity to RSK1 inhibition, where we uncovered bi-directional regulation. Through cycloheximide, MG-132, and ubiquitination assays, we found that RSK1 regulates FLT3-ITD activity and protein stability through deubiqutinase USP1, and where either *RPS6KA1* or *USP1* inhibition resulted in concomitant downregulation of FLT3 protein and cell lethality. Multivariate analysis of the TCGA and BeatAML cohorts (n= 568 patients) revealed high median *USP1* or *RPS6KA1* expression to be significantly associated with poor survival, thus defining a RSK1-USP1-FLT3 axis with prognostic relevance.

Across *in vivo* models, PMD-026 ameliorated MPN disease features driven by *MPL* W515L including leukocytosis, splenomegaly, and bone marrow fibrosis, while prolonging survival, in conjunction with suppression of inflammatory cytokines including TNF, IL-6 and IL-1b. We then evaluated PMD-026 across five patient-derived xenograft (PDX) models spanning the spectrum of myeloid malignancies including myelofibrosis (MF), sAML, and chronic myelomonocytic leukemia (CMML) encompassing various driver and multiple high-risk mutations. Treatment in MF PDX mice decelerated disease progression, extended survival, and reduced splenomegaly, while in CMML/sAML PDXs, PMD-026 led to a 67 to 96 percent reduction in human CD45+ cells engraftment. Finally, PMD-026 treatment in both mice xenografted with MV4-11 cells and syngeneic *Flt3*^{ITD} *Tet2*^{KO} mice significantly reduced leukemic cell engraftment and prolonged survival. These data indicate potent efficacy of RSK inhibition across diverse models of chronic and acute myeloid malignancies.

Lastly, we investigated the impact of PMD-026 on hematological parameters from 41 breast cancer patients enrolled in the phase 1/1b clinical trial. Overall, there was no sustained suppressive effect on hematological parameters and there were no

hematological PMD-026-related toxicities meeting criteria for dose modification during the trial period. These clinical data demonstrate that PMD-026 did not cause myelosuppression.
Our findings uncover a novel therapeutic avenue for a conserved RSK1 dependency across chronic and acute myeloid neoplasms. The potent and consistent disease-ameliorating effects across numerous leukemia mouse models demonstrates promise for repurposing PMD-026 for the treatment of myeloid malignancies.

Disclosures Huynh: *Phoenix Molecular Designs:* Current Employment. **Los:** *Phoenix Molecular Designs:* Current Employment. **Dunn:** *Phoenix Molecular Designs:* Current Employment. **Challen:** *Incyte, Ajax Therapeutics, ReNAgade Therapeutics Management:* Consultancy, Research Funding. **Oh:** *CTI BioPharma, Bristol Myers Squibb, Disc Medicine, Blueprint Medicines, PharmaEssentia, Constellation/MorphoSys, Geron, AbbVie, Sierra Oncology/GSK, Cogent, Incyte, Morphic, Protagonist:* Consultancy.

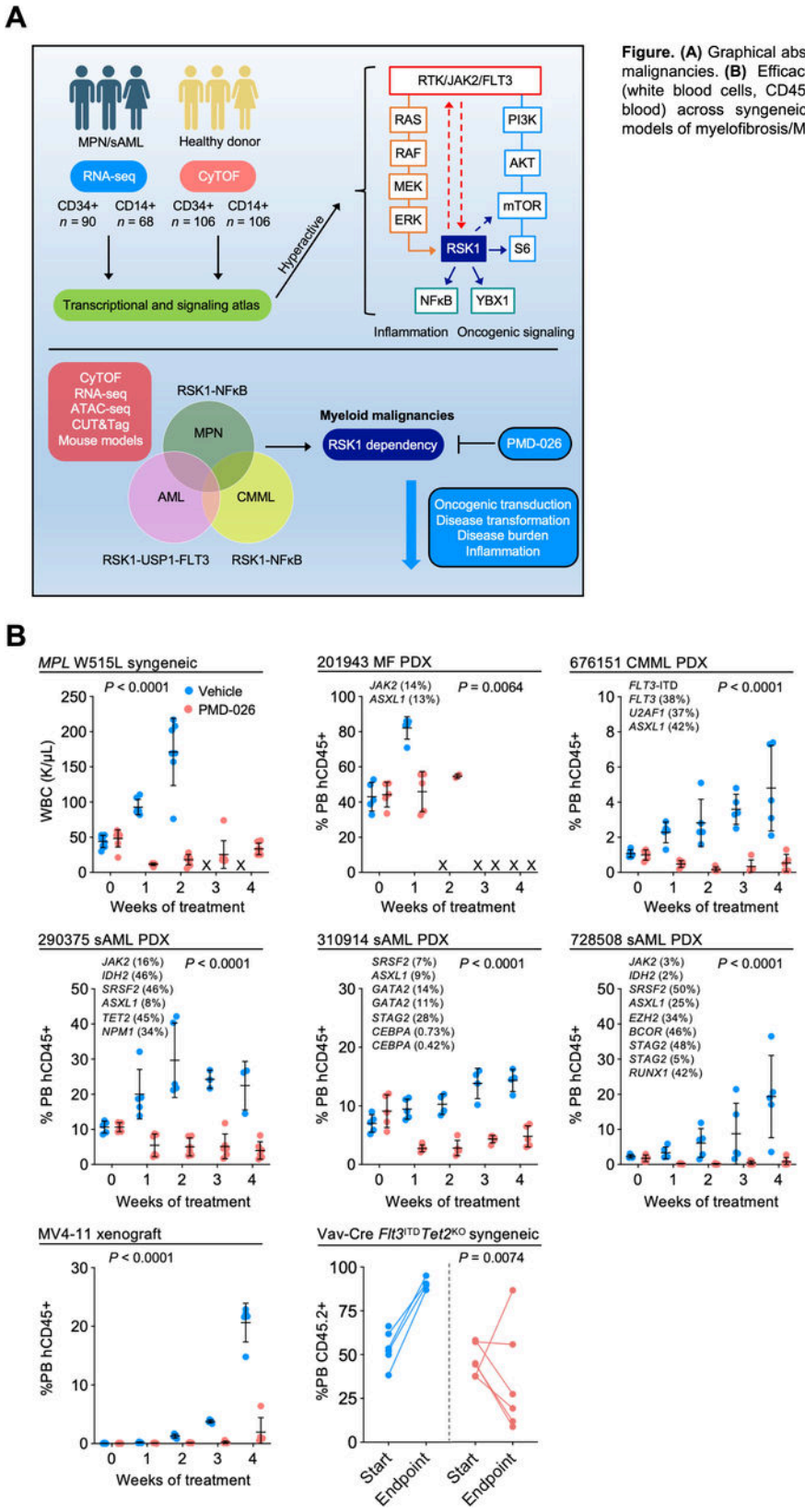

Figure. (A) Graphical abstract summarizing RSK1 dependency across myeloid malignancies. **(B)** Efficacy of PMD-026 treatment in reducing leukemic burden (white blood cells, CD45.2+ cells, or human CD45+ cells in the peripheral blood) across syngeneic, xenograft, and patient-derived xenograft mouse models of myelofibrosis/MPN, CMML, sAML, and *FLT3*-ITD mutant AML.

Figure 1

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