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Blood 142 (2023) 3225–3227

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Novel Clinically Useful Inhibitor of Mediator Complex, RVU120, Relives Differentiation Block in MDS/AML

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Background:
Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukemias (AML) are characterized by a block in hematopoietic differentiation that can often be traced to aberrant stage-specific transcription. The kinase module of the mediator complex, consisting of CDK8, its paralog CDK19, Cyclin C, MED12, and MED13, have been identified as a molecular switch that regulates gene expression. A variety of cancers have exploited this mechanism to maintain stemness and an undifferentiated state. RVU120 is a first-in-class, specific, and selective inhibitor of CDK8/19, currently in Phase Ib clinical trial in patients with AML or High-Risk MDS (HR-MDS) (NCT04021368). RVU120 has been shown to stimulate erythroid differentiation in transformed CD34+ cord blood (CB) cells and in CD34+ cells from Diamond-Blackfan Anemia (DBA). To evaluate the further therapeutic potential of CDK8/19 inhibition, we confirmed aberrant expression of mediator complex components and erythroid-stimulating activity in a collection of CD34+ primary samples of AML and MDS.

Results:
We investigated the expression of mediator complex components in a large database of MDS CD34+ marrow samples and age-matched controls. Our findings revealed that MED12, a critical component of the mediator complex, was significantly overexpressed in MDS samples from refractory anemia with excess blasts (RAEB), a higher risk subset of MDS(p=0.018) (Fig A). Furthermore, we observed that this overexpression of MED12 was associated with a higher rate of transformation to AML. To delve deeper, we examined the expression of MED12 in sorted leukemic stem cells from primary AML samples and found significantly elevated levels in Long Term Hematopoietic Stem Cells from AML samples with both normal karyotype and complex karyotypes compared to healthy controls.
Next, we conducted an evaluation of the efficacy of the mediator kinase module inhibition in MDS-derived cell line (MDS-L). Inhibition of CDK8/19 with RVU120 led to increased erythroid differentiation in this model which confirmed our observations in transformed CD34+ CB cells in CB cells, these phenotypic changes were correlated with the inhibition of CDK8-dependent phosphorylation marker S726 on STAT5 and a profound increase in the expression of genes involved in erythroid commitment and hemoglobin metabolism. In order to confirm therapeutic potential of CDK8/CDK19 inhibition in MDS and AML, we performed colony-forming assay on primary samples of MDS and AML (n= 15). The treatment with RVU120 resulted in increased erythroid differentiation in a majority of samples, evident from an augmented number of colonies. Detailed analysis indicated

changes in the expression of erythroid differentiation markers, including increased CD71 and Glycophorin A expression on colonies, as assessed by FACS analysis (Figure B, shows representative sample).

Conclusion

Our data highlight that inhibition of the overexpressed mediator complex proteins can alleviate the differentiation blocks seen in MDS/AML. These results provide a compelling rationale for further developing the CDK8/19 inhibitor RVU120 for transfusion- dependent MDS/AML patients.

Disclosures Pakulska: *Ryvu Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Obacz:** *Ryvu Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Adamczyk:** *Ryvu Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Zaucha:** *Medical University of Gdańsk:* Current Employment; *BMS:* Research Funding; *Pierre Fabre, Takeda, BMS, Gilead, Novartis, Pfizer, Amgen, F. Hoffmann-La Roche Ltd, Astra Zeneca, Abbvie:* Honoraria; *MSD:* Research Funding. **Steidl:** *Aileron Therapeutics:* Consultancy, Research Funding; *Novartis:* Consultancy; *Roche:* Consultancy; *Stelexis Therapeutics:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Pfizer:* Consultancy; *Bayer Healthcare:* Research Funding; *GlaxoSmithKline:* Research Funding. **Shastri:** *Kymera Therapeutics:* Honoraria, Research Funding; *Gilead Sciences:* Honoraria; *Rigel Pharmaceuticals:* Honoraria; *Janssen Pharmaceuticals, Inc.:* Consultancy, Honoraria. **Zhao:** *Albert Einstein COM:* Current Employment. **Mazan:** *Ryvu Therapeutics:* Current Employment, Current equity holder in publicly-traded company. **Verma:** *Bristol Myers Squibb:* Research Funding; *Stelexis:* Consultancy, Current equity holder in private company, Honoraria; *Bakx:* Consultancy, Current equity holder in private company; *Janssen:* Honoraria; *Curis:* Research Funding; *GSK:* Research Funding; *Incyte:* Research Funding; *Medpacto:* Research Funding; *Eli Lilly:* Research Funding; *Novartis:* Consultancy; *Acceleron:* Consultancy; *Throws Exception:* Current equity holder in private company; *Celgene:* Consultancy; *Prelude:* Research Funding.

https://doi.org/10.1182/blood-2023-186227

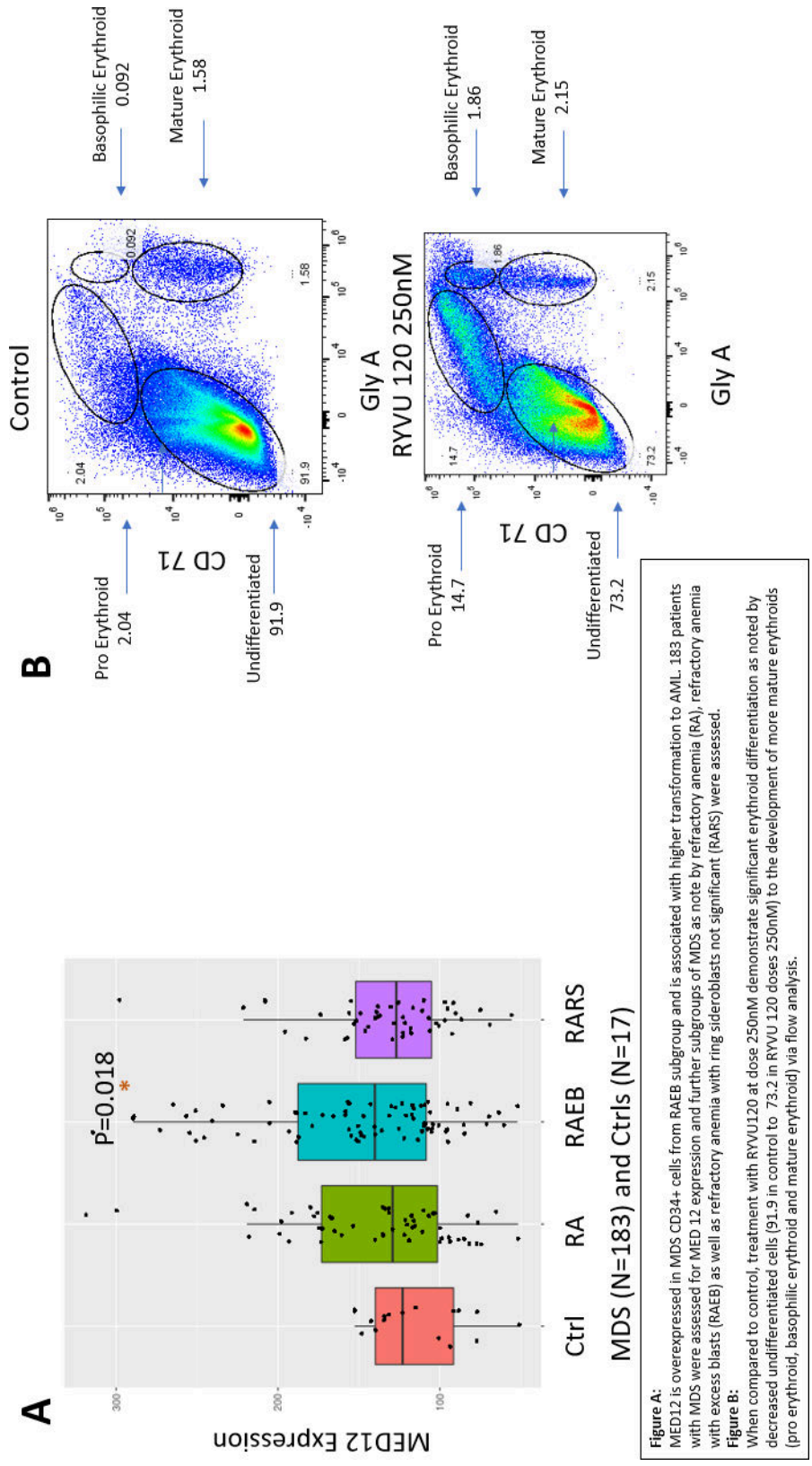

Figure A:
MED12 is overexpressed in MDS CD34+ cells from RAEB subgroup and is associated with higher transformation to AML. 183 patients with MDS were assessed for MED 12 expression and further subgroups of MDS as note by refractory anemia (RA), refractory anemia with excess blasts (RAEB) as well as refractory anemia with ring sideroblasts not significant (RARS) were assessed.
Figure B:
When compared to control, treatment with RYVU120 at dose 250nM demonstrate significant erythroid differentiation as noted by decreased undifferentiated cells (91.9 in control to 73.2 in RYVU 120 doses 250nM) to the development of more mature erythroids (pro erythroid, basophilic erythroid and mature erythroid) via flow analysis.

Figure 1