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RVU120 – a CDK8/19 Inhibitor for the Treatment of AML and HR-MDS Can Induce Erythroid Differentiation

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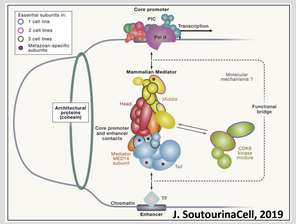
SUMMARY

Myelodysplastic syndrome (MDS) is a disorder of hematopoietic cells that, as a result of genetic and epigenetic changes, do not produce mature blood cells and can often evolve to acute myeloid leukemia (AML). RVU120 (formerly known as SEL120) is a specific, selective inhibitor of CDK8 and its paralog CDK19. A first-in-human Phase Ib clinical trial with RVU120 in patients with AML or HR-MDS is currently ongoing. Preclinical studies indicated strong antileukemic potential of RVU120 that was often associated with multilineage commitment of CD34+ AML cells. Moreover, efficacy of RVU120 was also confirmed in PDX samples. The major aim of this work was to evaluate erythroid differentiation potential of RVU120 in primary MDS and transformed CD34+ cord blood cells characterized with an early block in erythroid differentiation. RVU120 treatment leads to transcriptional reprogramming of transformed CD34+ cord blood (CB) cells. The most profound changes included decreased CDK8 occupancy followed by increased STAT5 and RNA Pol II loading at transcription start site and gene bodies. RVU120 could repress many genes associated with stem cells and importantly induce the expression of genes involved in erythroid commitment. Detailed analysis by flow cytometry at early and late time points reflected sequential changes in the expression of lineage-specific surface markers, leading to erythroid differentiation. Observed differentiation phenotype strikingly resembles previously reported effects of RVU120 in Diamond-Blackfan Anemia (DBA) cells caused by disruption of genes encoding ribosomal proteins. Further studies are warranted to investigate efficacy of RVU120 in AML and MDS and in anemias associated with bone marrow failures in these patients.

INTRODUCTION



MDS is a group of hematologic disorders characterized by the lack of mature blood cells or development of dysfunctional cells. MDS can often evolve to AML. Primary clinical goals in MDS are to achieve remissions, alleviate symptoms associated with cytopenias and to minimize the transfusion burden. While supportive red blood cell transfusions, erythropoiesis-stimulating agents and novel targeted agents may lead to clinical improvement, an allogeneic bone marrow transplant (BMT) is the only potential cure for patients with MDS.

RVU120 (SEL120) is a specific, selective inhibitor of CDK8 and its paralog CDK19. A first-in-human Phase Ib clinical trial with RVU120 in patients with AML or HR-MDS is ongoing. Preclinical studies indicated strong antileukemic potential of RVU120 that was often associated with multilineage commitment of CD34+ AML cells. Moreover, RVU120 could improve proliferation and induce erythroid differentiation of CD34+ cells derived from Diamond-Blackfan anemia patients.
Primary aim was to evaluate erythroid differentiation potential of RVU120 in primary MDS and transformed cord blood CD34+ blood cells characterized with a block in erythroid differentiation.

RVU120 INDUCES ERYTHROID COMMITMENT IN TEX MODEL WITH STEM CELL CHARACTERISTICS

Primary cellular model was based on cord blood cells transduced with TLS-ERG - a fusion gene generated from t(16;21)(p11;q22) translocation associated with primary AML and secondary AML associated MDS. Transformed cells displayed increased capacity for self-renewal, proliferation and altered erythroid differentiation (Warner et al. 2005). **This system establishes a foundation to explore responsiveness to a broad array of stimulators, providing a valuable model to study lineage commitment pathways in human cells.**

Morphology of RVU120 treated TEX cells

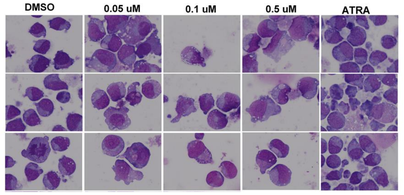


Fig. 2. Changes in cell morphology induced by RVU120.
TEX cell morphology was assessed by May-Grunwald Giemsa staining and analyzed under a light microscope. AML TEX cells were treated with RVU120 or ATRA for 14 days. Representative plots are shown, N=2.

Differentiation of TEX cells after RVU120 treatment

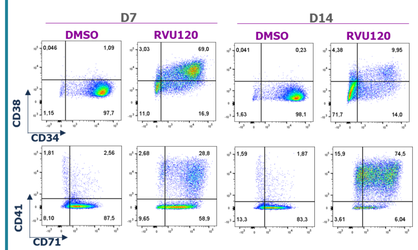


Fig. 3. RVU120 induces differentiation into erythroid lineage.
The expression of surface markers was analyzed by flow cytometry after 7 and 14 days of RVU120 treatment in conditions with or without EPO. Representative plots are shown, N=2.

GIEMSA assay indicated that RVU120 treated cells possess brightened cytoplasm, granular formation and decreased ratio of nucleus to cytoplasm compared to control cells. **Altered morphology of RVU120 treated AML strongly suggests differentiation potential of the compound.** RVU120 differentiation potential was also assessed by flow cytometry. **RVU120 induces commitment into erythroid (and possibly megakaryocytic) lineage which is shown by appearance of the CD71+CD41+ progenitors in liquid culture**. This early induction of erythroid differentiation is EPO independent. **These results clearly indicate differentiation potential of RVU120.**

TRANSCRIPTOME PROFILING REVEALS ENRICHMENT IN GENES REGULATING ERYTHROID COMMITMENT AND HEMOGLOBIN METABOLISM

GESA RNAseq of RVU120 treated TEX cells

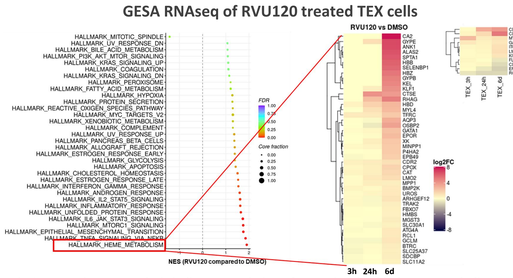
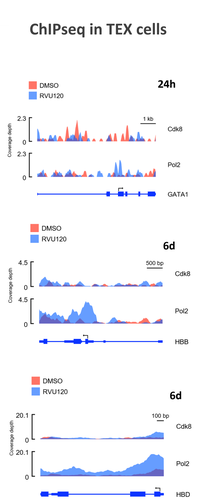


Fig.4. Transcriptome analysis indicates regulation of genes involved in heme metabolism and erythroid differentiation. (A) GSEA summary plot for top 33 gene sets sorted according to |NES| (normalized enrichment score) and with FDR < 0.5; Shown is 6 day time point (B) Heat map showing RNA-seq data. RNA seq was performed 3h, 24h and 6 days after RVU120 treatment. Shown is heatmap with expression (rlog-transformed values).

ChIPseq in TEX cells



Transcriptome analysis of TEX cells treated with RVU120 showed significant deregulation of transcription. **The most significant enrichment observed after prolonged 6 days treatment revealed genes involved in erythroid commitment and hemoglobin metabolism**. In addition, ChIPseq analysis confirmed changes in chromatin loading for master regulators of erythropoiesis known from normal cells, such as GATA1. Induction of GATA1 is associated with displacement of CDK8 from the promoter and recruitment of Polymerase II. At later time points very significant increase in Pol II occupancy at hemoglobin genes is observed.

Fig.5. ChIPseq data revealed induction of GATA1, a master regulator of the erythroid differentiation. Large scale view of the occupancy of CDK8 and Pol II around GATA1 loci at 24h of RVU120 treatment (A) and around HBB loci (B) and HBD loci (C) in TEX cell after 6 days of treatment.

DIFFERENTIATION IS DEPENDENT ON TRANSACTIVATION OF STAT5 BY RVU120

ChIPseq profiling of TEX cells further revealed significant deregulation of transcription upon RVU120 treatment. Decreased CDK8 occupancy and increased Pol II, STAT5 and EZH2 occupancy at promoter regions was observed. Moreover, ATACseq analysis confirmed increased accessibility of chromatin for STAT5-dependent genes in RVU120 treated cells. Mechanistically RVU120 treatment in TEX cells results in induction of STAT5- dependent genes (many of which are involved in erythroid differentiation) as a result of CDK8 displacement from TSS, inhibition of pSTAT5 S727 followed by STAT5 transactivation (pSTAT5 Y694) and Pol II engagement at TSS and gene body. **Overall, these results indicate that erythroid differentiation is at least partially driven by transactivation of STAT5 by RVU120.**

ChIPseq and ATACseq of RVU120 treated TEX cells

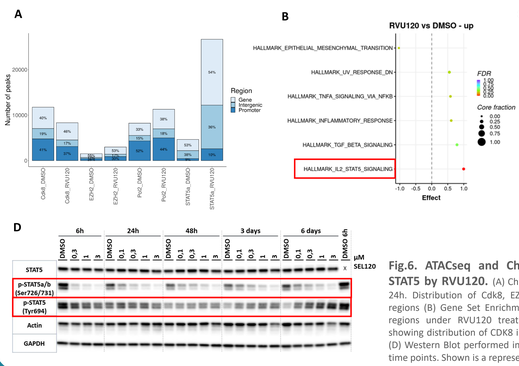
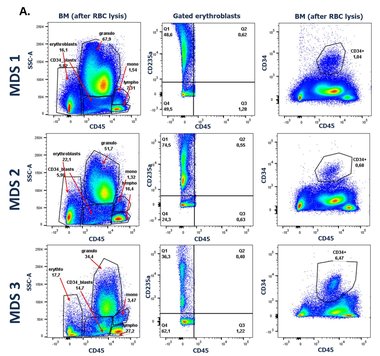


Fig.6. ATACseq and ChIPseq profiling revealed activation of STAT5 by RVU120. (A) ChIPseq analysis of TEX cells treated with RVU120 for 24h. Distribution of Cdk8, EZH2, Pol2, STAT5a ChIPseq peaks across genomic regions (B) Gene Set Enrichment of ATACseq peaks located in the nearest TSS regions under RVU120 treatment compared to DMSO (C) ChIPseq analysis showing distribution of CDK8 in gene body after treatment with RVU120 for 24h. (D) Western Blot performed in TEX cell after treatment with RVU120 at different time points. Shown is a representative plot, n=3.

RVU120 IMPROVES ERYTHROID DIFFERENTIATION IN MDS PATIENT DERIVED CELLS

Analysis of BM from MDS patients



B.

Molecular alterations

GENE	EXON	DNA/cDNA	PROTEIN	VAF	MATERIAL
SF3B1	15	c.2096A>G	p.Lys700Glu	42	DNA/BM
TET2	3	c.1743_1758dup	p.Pro587*	45	DNA/BM
TET2	10	c.4380del	p.Val1461Serfs*9	26	DNA/BM
ZRSR2	7	c.524A>G	p.Tyr175Cys	11	DNA/BM

GENE	EXON	DNA/cDNA	PROTEIN	VAF	MATERIAL
SF3B1	14	c.1866G>C	p.Glu622Asp	41	DNA/BM
TET2	3	c.3409_1G>A	p.splice site mutation	20	DNA/BM
TET2	3	c.832del	p.Gln278Argfs*15	8	DNA/BM
TET3	9	c.4118C>A	p.Ala1373Glu	2	DNA/BM

MDS: 5q-
Karyotype: 46,XX,del(5)(q14q34)

Fig. 7. Analysis of BM samples of MDS patients. (A) Flow cytometry analysis of fresh BM samples after red cell lysis performed within 14h from BM aspiration. (B) Molecular alteration in BM samples.

Differentiation potential of RVU120 was studied in MDS patients. Analysis of BM showed abnormalities in the erythroid lineage (high amount of erythroblasts and/or low levels of CD235a positive cells). Two patients had molecular alteration in common genes such us SF3B1 and TET2. Third patient belonged to deletion 5q subtype of MDS. RVU120 treatment increased the fraction of CD34+CD38+ early progenitors in all patients at early time points (similarly to Senexin B, another CDK8 inhibitor). Importantly, RVU120 improved erythroid differentiation in these patients which is shown by the appearance of higher numbers of CD71+CD235a+ cells at later time points. The effect was mostly pronounced in case of del 5q patient. **These results clearly show that RVU120 can improve erythroid differentiation not only of cell line models but also of MDS patient derived cells.** RVU120 did not exert a significant effect on cell number at these conditions.

Erythroid differentiation of BM CD34+ cells derived from MDS patients

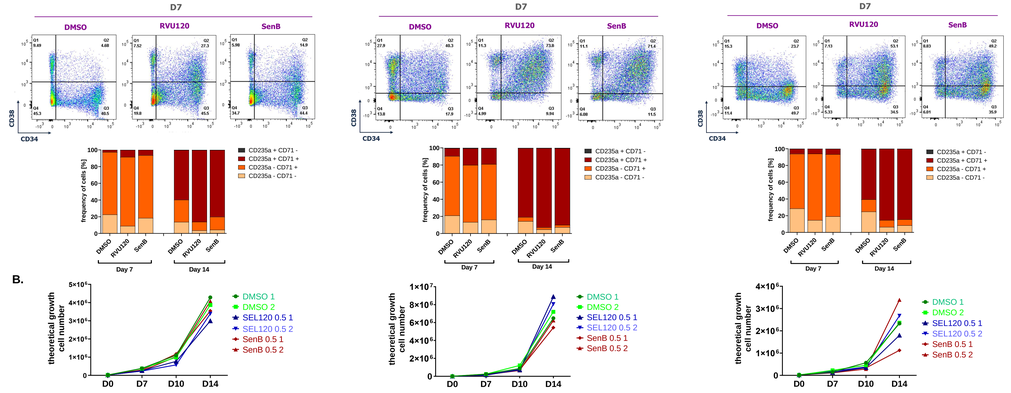


Fig. 8. RVU120 improves erythroid differentiation of CD34+ BM cells derived from MDS patients.
(A) Fresh CD34+ isolated from BM of MDS patients were cultured in conditions promoting erythroid differentiation. Stages of erythroid differentiation were followed by the analysis of surface marker expression using flow cytometry. Representative plots a day 7 and 14 are shown. (B) Accumulative growth curve of cells from the experiment shown in A.

CONCLUSIONS

- Presented results indicate strong erythroid differentiation potential of RVU120 in (Lin-) CD34+ cells that acquired genetic abnormalities resulting in arrested erythroid commitment – a characteristic of many MDS and AML subtypes.
- Detailed transcriptomic, ChIPseq and RNAseq profiling strongly associated differentiation with enrichment of genes representing regulators of erythroid commitment/hemoglobin metabolism. It also indicates at least partial dependence of differentiation on STAT5 activation.
- Further studies are warranted to investigate the efficacy of RVU120 in chronic anemias associated with bone marrow failures in AML and MDS patients.

LEUKEMIA & LYMPHOMA SOCIETY

The concept of RVU120 has been validated through the support of The Leukemia & Lymphoma Society, the largest blood cancer foundation in the world which has supported multiple breakthrough therapies through its highly-competitive venture philanthropy initiative, the Therapy Acceleration Program.

Therapy Acceleration Program (TAP)

European Funds Smart Growth

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