Modulation of the immune landscape in lower-risk MDS with imetelstat-induced transfusion independency

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Conflict of interest

• Research funding from Geron Corp.
Introduction

• Around 50% of lower-risk MDS patients are resistant to ESA
• Innate immune signaling and inflammation underlies MDS pathogenesis
• Targeted therapies for MDS-5q or MDS-SF3B1 are available
• Imetelstat, a telomerase inhibitor, is in clinical development

Results from IMerge (MDS3001, NCT02598661):

- **Phase II (open-label, single-arm study)**
  - 23% of 57 highly transfused LR-MDS achieved 24-wk transfusion independency (TI) with a median duration of 65 wk
  - 65% obtained hematological improvement (HI) - erythroid
  - ≥1 year sustained, continuous TI in 29% of patients with transfusion dependent, non-del(5q) LR-MDS relapsed/refractory to ESAs and lenalidomide/HMA naive

- **Phase III (Double blind, randomized, imetelstat vs placebo)**
  Imetelstat demonstrated highly statistically significant and clinically meaningful efficacy compared with placebo:
  - 8-week TI = 40% vs 15%,
  - 24-week TI = 28% vs 3.3%
  - 1-year TI = 18% vs. 1.7%

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Objective: Identify biological pathways associated with the clinical response in a subset of patients enrolled in the imetelstat MDS3001 phase II clinical trial.

Patient samples:
- 10 LR-MDS patients: 6 Transfusion Independence Responder (TIR), 4 Transfusion Independence Non-Responder (TINR) with a median follow-up of 140.7 weeks
- Samples at baseline and 4-7 months post-imetelstat treatment (total 24 samples)

Methods
- BMMNC transcriptomes were analyzed by RNA-sequencing
- PBMC immune profiles were assessed by mass cytometry (CYToF) using the Maxpar® Direct Immune Profiling assay
- Cytokine profiling was performed using quantitative multiplex panel of 47 cytokines in PB plasma samples collected from 21 patients (15 TIR, 6 TINR) at 2 to 5 time points.
## Patients’ Baseline Characteristics and Clinical Response

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Gender</th>
<th>WHO</th>
<th>Karyotype</th>
<th>Mutations</th>
<th>IPSS</th>
<th>Treatment duration in cycles</th>
<th>Follow-up (wks)</th>
<th>Longest transfusion free interval (wks)</th>
<th>≥1-year Transfusion independency</th>
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<tbody>
<tr>
<td>1</td>
<td>78</td>
<td>M</td>
<td>MDS-RS</td>
<td>46,XY</td>
<td>SF3B1_E622D</td>
<td>Intermediate-1</td>
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<td>3</td>
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<td>del(5q)</td>
<td>46,XX,del(5)(q13;q33)</td>
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<td>Low</td>
<td>6</td>
<td>141.1</td>
<td>3.7</td>
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</table>
Low innate immune features and T cell activation at baseline in ≥1 year TI Responders (TIR) Compared to Non-Responders (TINR)

**Bone marrow MNC transcriptomes in TIR (n=6) vs TINR (n=4):**
1185 differentially expressed genes ($P = 0.05; \log_2(FC) = 1\)I)
1150 down (blue) & 35 up-regulated (red)

**Gene set enrichment**
- Innate immune response
- Inflammation
- DNA replication
- Nucleoside biosynthesis
- Telomerase RNA localization
- Adaptive immune response
- Ribosome biogenesis
- mRNA modification
- positive regulation of interferon gamma production
- MYD88 dependent toll like receptor signaling pathway
- pyroptosis
- T cell mediated cytotoxicity
- positive regulation of interferon beta production
- inflammasome complex assembly
- negative regulation of I kappaB kinase NF-kappaB signaling
- positive regulation of macrophage cytokine production
High heterogeneity of immune cells repartition at baseline

- High heterogeneity between samples in both TIR and TINR
- No differences of B, T and NK cell proportion
- Difference in monocytes repartition

Blood samples at baseline (n=9)
Maxpar® Direct Immune Profiling assay (CyTOF)

Unsupervised analysis in TIR (n=6) vs TINR (n=3)
Low inflammatory features and immune suppression in TIR at baseline

- High heterogeneity between samples in both TIR and TINR

- No differences of B, T and NK cell proportion

- Difference in monocytes repartition

Maxpar® Direct Immune Profiling assay (CyTOF)

Blood samples at baseline (n=9)

Unsupervised analysis in TIR (n=6) vs TINR (n=3)
Low inflammatory features in TIR at baseline demonstrated by relative low levels of CXCL9 and IL-18

Cytokine profiling (Milliplex® kit) in PB plasma samples collected at baseline

Analysis in TIR (n=15) vs TINR (n=6)

- No difference at baseline for plasmatic amounts of IFNγ, IL-4, IL-5, IL-6

- Some monocyte-derived chemokine or interleukin are less expressed in TIR
Immune cell activation associates with the response to Imetelstat

Bone marrow MNC transcriptomes in TIR (n=6) post treatment vs baseline:

127 differentially expressed genes ($P = 0.05$; $\log_2(FC) = I1I$)

37 down (blue) & 90 up-regulated (red)
Imetelstat induces modification of immune cells repartition in TIR

Blood samples at baseline and after treatment (n=9)

Maxpar® Direct Immune Profiling assay (CyTOF)

Unsupervised analysis in baseline vs post treatment for TIR (n=6) and TINR (n=3)
Imetelstat induces modification of immune cells repartition in TIR

**In TIR (n=6):**

- Modification of repartition of monocytes subpopulations with decreased of HLA-DR-/low monocytes
- Increased of CD8+ terminal effector T cells
- Increased of B cells
Early modulation of immunosuppressive S100A8/A9 and pro-inflammatory cytokines after 2 cycles of imetelstat treatment

Pro-Inflammatory

S100A8/A9
Calprotectin

Immune suppression

**Log2 (ratio C2D1/screening)**

- **TNFα**
  - TIR: ns
  - TINR: p=0.053

- **IL-17F**
  - TIR: p=0.067

- **IL-6**
  - TIR: ns

**Log2 (ratio C2D1/screening)**

- **CXCL9**
  - TIR: p=0.053

- **IL-5**
  - TIR: *

B cells Stimulation

**Log2 (ratio C2D1/screening)**
Conclusion

- **At baseline** TIR are characterized by **immune suppression, low innate immune features and T cell activation** attested in blood by:
  - Increase proportion of immunosuppressive monocytes (HLA-DR-/low)
  - Lower level of some pro-inflammatory chemokines (IL-18 and CXCL9)
  - Lower level of pro-inflammatory monocytes (CD16+)

- **Imetelstat treatment induces in TIR an immune cell activation** attested in blood by remodeling of:
  - immune cell repartition (monocytes, T CD8+ cells and B cells)
  - pro-inflammatory and immunosuppressive cytokines

**Future studies will be necessary to**
- Determine the contribution of immunomodulation to the erythroid response
- Validate theranostic and predictive biomarkers of response to imetelstat treatment
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