The association between Gal9 and TIM-3 as prognostic marker for Selinexor+HiDAC Mito regimen.

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The increase of TIM-3 expression was higher in TF compare to CR patients

Median Fluorescence Intensity (MFI) was calculated by FlowJo-10 software and relative normalized to comparing TIM-3 expressing T cells (CD4+ and CD8+) and PDL-1+ CD34+ cells in CR and TF patients at time of diagnosis and end of induction (A-B). The Mann-Whitney test was used to compare the 2 sub-groups. Bars represent medians. P < 0.05 was considered statistically significant. The colored overlay dot plots shows the co-expression of TIM-3 and PD-1 on CD4+ and CD8+ cells comparing expression levels of these receptors in representative patients CR (blue dot) and TF (red dot) at diagnosis vs end of induction.
Strategy of the previous study and samples collection.
26 patients divided in two group enrolled to a phase I dose escalation trial that combined increasing doses of Selinexor (SINE) with age-adjusted HiDAC/Mito (NCT02573363) at time of diagnosis. Patients experienced induction failure was taken off of protocol therapy due to death or documented induction failure. HiDAC (3 g/m2, or 2 g/m2 if > 70 years, intravenously over 4 h) followed immediately by Mito (30 mg/m2, or 20 mg/m2 if > 70 years, intravenously over 1 h) were administered on days 1 and 5. Selinexor was given orally on days 2, 4, 9, and 11. Initial Selinexor dose was 60 mg (~35 mg/m2 for an average adult) followed by dose escalation to a target level of 80 mg (~50 mg/m2). Bone Marrow (BM) and blood samples were collected at the time of diagnosis and at the end of induction/treatment (days range 19-56).

Supplementary Fig.1-B

Kaplan-Meier Plot curves depicting CR (black) and TF (red) patients survival (percent) since the time of the diagnosis. The median of the days elapsed since the diagnosis was 346 and 176 days for CR and TF respectively and Hazard Ratio (Mantel-Haenszel) TF/CR was ~1.7, Mantel-Cox test was used to compare the 2 groups. The shadow in the chart indicates the timewise of samples collection and analysis.
Supplementary Fig. 2
Sequential gating to identify PD-1. Multi-parameter flow-cytometry was performed on blood and bone marrow (BM) aspirates. PD-1 was stained with Pe (BioLegend Clone EH12.2H7). A Fluorescence Minus One (FMO) controls were used to determine the median fluorescence intensity (MFI) and frequency among the parent population of each costimulatory and coinhibitory molecule. Here we show the frequency of PD-1 gated on CD4 and CD8 population.
Supplementary Fig. 3
Statistical trend toward higher frequencies of CD34+ cells in TF patients
CD34+ cells were stained with FITC (BioLegend Clone 541). Here is shown the frequency (Log scale) in comparison between TF and CR cohorts at the time of diagnosis. The Mann-Whitney U test was used to compare the 2 groups. Bars represent medians. P < 0.05 was considered statistically significant.
Supplementary Fig.4
PD-1/PDL-1 axes at the time of diagnosis as a prognostic factor.

In (A) is shown the frequency (Log scale) of CD8+ (FITC-BioLegend Clone HIT8a)–PD-1+ (Pe-Biolegend Clone EH12.2H7) cells in comparison of TF and CR cohorts at the time of diagnosis. The Mann-Whitney U test was used to compare the 2 groups. Bars represent medians. P < 0.05 was considered statistically significant. (B) Spearman correlation coefficients in TF (above) and CR (bottom) populations between CD8+ PD-1+ cells and CD34+ (FITC-BioLegend Clone 541)–PDL-1+ (BV-421-BioLegend Clone 29E.2A3) cells. According to the Deming procedure linear regression equation is shown. P < 0.05 is considered statistically significant. In (D) Frequency (Log scale) CD34+ PDL-1+ cells in comparison of TF and CR cohorts at the time of diagnosis. The Mann-Whitney U test was used to compare the 2 groups. Bars represent medians. P < 0.05 was considered statistically significant.
Supplementary Fig. 5
TIM-3 expression in TF and CR patients at diagnosis and at the end of induction.

In (A) is shown the frequency (Log scale) of CD4+ (PerCPcy-5.5-Biolegend Clone SK3) TIM-3+ (APC-Cy7-BioLegend clone F38-2E2) cells at the time of diagnosis and at the end of remission in CR (left) and TF (right) patients. In (B) is shown the frequency (Log scale) of CD8+ (FITC-BioLegend Clone HIT8a) TIM-3+ cells. The Mann-Whitney U test was used to compare the two groups. Bars represent medians. P<0.05 was considered statistically significant.
**Supplementary Fig. 6**

TIM-3 expression in BM compartment is significant higher in the both subsets of CD4 and CD8 populations

In (A) is shown the frequency (Log scale) of CD4⁺ (PerCP Cy5.5 BioLegend Clone SK3) TIM-3⁺ (APC-Cy7 BioLegend clone F38-2E2) cells (right) and CD8⁺ (FITC BioLegend Clone HIT8a) (left) at the time of diagnosis in comparison between peripheral blood and bone marrow compartments. p<0.05 was considered statistically significant.

In (B) is shown the same comparison in the frequency (Log scale) of CD8⁺ (FITC BioLegend Clone HIT8a) PD-1⁺ (PerCPCy5.5 BioLegend Clone EH12.2H7) cells. The Mann-Whitney U test was used to compare the 2 groups. Bars represent medians. P<0.05 was considered statistically significant.
Gal9 EXPRESSION

Freq. Parent Log
Gated on CD34 [-]

Freq. Parent Log
Gated on CD34 [+]

CR De Novo AML
CR Secondary
TF De Novo AML
TF Secondary

NS
NS
*

CR De Novo AML
CR Secondary
TF De Novo AML
TF Secondary
CD34+ EXPRESSION

* NS
  NS
### Table 1. Characteristic of the patients

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Complete Remission</th>
<th>Treatment Failure</th>
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<tbody>
<tr>
<td><strong>Patient Characteristics</strong></td>
<td>Number (%)</td>
<td>Number (%)</td>
</tr>
<tr>
<td>Total patients enrolled</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Female</td>
<td>6 (37%)</td>
<td>8 (80%)</td>
</tr>
<tr>
<td>Median Age (years, range)</td>
<td>61 (35-75)</td>
<td>62 (38-74)</td>
</tr>
<tr>
<td>Disease State on enrollment*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated AML</td>
<td>12 (80%)</td>
<td>2 (22%)</td>
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<tr>
<td>Relapse or refractory AML</td>
<td>3 (20%)</td>
<td>7 (78%)</td>
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<tr>
<td><strong>Initial AML diagnosis</strong></td>
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<tr>
<td>De Novo AML</td>
<td>8 (53%)</td>
<td>5 (55%)</td>
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<tr>
<td>Secondary AML after MDS</td>
<td>7 (47%)</td>
<td>4 (45%)</td>
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<tr>
<td><strong>Acquired Mutation Status</strong></td>
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</tr>
<tr>
<td>FLT3</td>
<td>3 (20%) NMP1 mutated</td>
<td>2 (22%) NMP1 mutated</td>
</tr>
<tr>
<td>CEPBA</td>
<td>1 (6%)</td>
<td>2 (22%)</td>
</tr>
<tr>
<td>NMP1</td>
<td>5 (34%)</td>
<td>1 (11%)</td>
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<tr>
<td><strong>Blast (CD34+)</strong></td>
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<tr>
<td>Median</td>
<td>9.90%</td>
<td>48.90%</td>
</tr>
<tr>
<td>Range</td>
<td>4.5-89.7</td>
<td>7.9-76.6</td>
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</tbody>
</table>

* CR pts=15 TF* pts=9
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CD34-Gal9+ cells and TIM-3 expressing T cells correlated to worse outcome

Selinexor + HiDAC/Mito Regimen
High-dose Cytarabine Mitoxantrone