TP53 abnormalities correlate with immune infiltration and are associated with response to flotetuzumab, an investigational immunotherapy, in acute myeloid leukemia

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Background

• Chemotherapy remains the standard of care for most patients with AML, despite recent approvals of novel drugs

• We have identified immune subgroups of AML (‘immune-infiltrated’ and ‘immune-depleted’) that predict chemotherapy resistance but also response to flotetuzumab immunotherapy (Vadakekolathu J, et al. Under revision)
  
  • The genetic drivers of immune infiltration in AML are presently unknown

• TP53 mutations occur in 8-10% of de novo AML cases and are associated with chemotherapy resistance, high risk of relapse and dismal prognosis even after hematopoietic stem cell transplantation

• The functional consequences of TP53 mutation/inactivation on host immune regulation have been largely overlooked in AML
  
  • The TP53 mutants studied thus far in AML do not show any evidence of gain-of-function mechanisms (Boettcher S, et al. Science 2019)
Objectives

• To determine whether TP53 abnormalities correlate with the composition and functional orientation of the tumor immunological microenvironment (TME) in AML

• To determine whether TP53 abnormalities identify a subgroup of patients with AML that may benefit from immunotherapy with flotetuzumab, a CD123×CD3 bispecific DART® molecule for redirecting host T cells to AML (Chichili GR, et al. Science Translational Medicine 2015) in the CP-MGD006-01 clinical trial (NCT#02152956)
**Graphical ‘cohorts and methods’**

**A - In silico analyses (TCGA-AML)**
- 147 non-promyelocytic AMLs (14 with TP53 mutations)
- Immune cell type and biological activity gene signatures (computed as in Danaher P, et al. JITC 2017 and 2018)
- Correlation with prognostic molecular lesions (TP53 mutational status, NPM1 mutational status, FLT3-ITD status, CHIP-defining mutations) and clinical outcomes (Cox PH)

**B - Primary AML blasts**
- TP53-mutated (n=42)  
  - RNA extraction
  - Targeted Immune GEP (PanCancer IO 360™ Panel) - RUO
  - DE genes
  - GO ontologies (METASCAPE)
  - Network analysis
- TP53-wt (n=22)

**C - Flotetuzumab cohort (n=35)**
- TP53-mutated and/or 17p abnormalities with genomic loss of TP53 (n=11)
- Predictors of response
TP53 mutations associate with an immune-infiltrated TME in TCGA-AML

N=118 cases with available information on prognostic molecular lesions, including TP53 mutations (n=14)
**TP53–related immune profiles in primary BMs**

### A

- 84% TP53 missense
- 12% TP53 no missense
- 4% N.A.

### B

**TP53 status**
- Mutated
- WT/NA/ND

### C

**TP53 status**
- Mutated
- WT/NA/ND

### D

**mRNA**
- PD-L1
- LAG3
- FoxP3
- FN1
- CD8A
- PD-L1

**P-values**
- *P*<0.0001
- *P*<0.0001
- *P*=0.0042
- *P*=0.0013
- *P*<0.0001
- *P*<0.0001
A unique immune *TP53* classifier
**TP53–related immune genes stratify survival**

In *silico* prognostic power in TCGA-AML cases (18 upregulated genes in *TP53* mutated AML)

<table>
<thead>
<tr>
<th>KEGG Pathway</th>
<th>Description</th>
<th>Count in gene set</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa04060</td>
<td>Cytokine-cytokine receptor interaction</td>
<td>9 of 263</td>
<td>5.56×10⁻⁹</td>
</tr>
<tr>
<td>hsa05323</td>
<td>Rheumatoid arthritis</td>
<td>6 of 84</td>
<td>6.37×10⁻⁸</td>
</tr>
<tr>
<td>hsa04657</td>
<td>IL-17 signaling pathway</td>
<td>6 of 92</td>
<td>8.02×10⁻⁸</td>
</tr>
<tr>
<td>hsa04621</td>
<td>NOD-like receptor signaling pathway</td>
<td>6 of 166</td>
<td>1.56×10⁻⁶</td>
</tr>
<tr>
<td>hsa04668</td>
<td>TNF signaling pathway</td>
<td>5 of 108</td>
<td>4.16×10⁻⁶</td>
</tr>
</tbody>
</table>

“Altered”: mRNA up-regulation amplification deep deletion mis-sense mutations

- **Relapse-free survival time (months)**
  - Altered (median RFS=11.4 mo.)
  - Not altered (median RFS=24.1 mo.)

- **Overall survival time (months)**
  - Altered (median OS=11.4 mo.)
  - Not altered (median OS=27.0 mo.)

- Log rank \(\chi^2=7.32; HR=1.86 \text{ (95%CI 1.12-3.1)}\)
- Log rank \(\chi^2=10.94; HR=1.88 \text{ (95%CI 1.24-2.85)}\)
- \(P=0.0068\)
- \(P=0.0009\)
# Flotetuzumab immunotherapy cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NCT#02152956</th>
<th>Patients (n=35)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (median and range)</strong></td>
<td></td>
<td>54 years (27-74)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>16 (46%)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>19 (54%)</td>
</tr>
<tr>
<td><strong>Disease status at study entry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late relapse (CR with initial duration &gt;6 months)</td>
<td></td>
<td>7 (20%)</td>
</tr>
<tr>
<td>Refractory to HMA</td>
<td></td>
<td>2 (5.7%)</td>
</tr>
<tr>
<td><strong>Refractory</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary induction failure (PIF; ≥2 induction attempts)</td>
<td></td>
<td>20 (57.1%)</td>
</tr>
<tr>
<td>Early relapse (CR with initial duration &lt;6 months)</td>
<td></td>
<td>6 (17.2%)</td>
</tr>
<tr>
<td><strong>2017 ELN risk stratification</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td></td>
<td>3 (8.6%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
<td>8 (22.9%)</td>
</tr>
<tr>
<td>Adverse</td>
<td></td>
<td>24 (68.6%)</td>
</tr>
<tr>
<td><strong>Secondary AML</strong></td>
<td></td>
<td>11 (31.4%)</td>
</tr>
<tr>
<td><strong>Number of prior lines of therapy (median and range)</strong></td>
<td></td>
<td>3 (1-9)</td>
</tr>
</tbody>
</table>

*Subgroup of 35/50 patients treated at the RP2D for whom BM samples were available

Response assessment criteria employed in analysis:

- **Anti-leukemic activity (ALA):** CR/CRh, PR, “other benefit” (>30% decrease in BM blasts compared to baseline)
- **Non-responders (NR):** treatment failure, stable disease, progressive disease
Flotetuzumab cohort – **TP53** mutations associate with an immune-infiltrated TME

Immune infiltration int.-to-high in 7/9 patients

ALA in 45.5% (5/11) evaluable patients with **TP53** mutations and/or 17p abnormalities (2 CR, 1 CRh, 1 morphologic leukemia-free state [MLFS], and 1 OB)
Response to flotetuzumab in TP53 mutated patients

A. BM blasts (TP53 mut./17p abn.)

- Best change from baseline (%)
- N=11 with TP53 mut./17p abn.
- Median=4.0 months (n=11)

- 42% blast reduction on average

B. TIS score

- P=0.016

- Inflammatory chemokine score

- P=0.016

- Treg score

- P=0.032

C. HOVON (TP53 mutated)

- PIF (n=6)
- CR (n=7)

- Log-rank $\chi^2=6.77$; $P=0.0093$
- HR=7.1 (95%CI 1.62-30.96)

D. HOVON cohort

- TP53 mutated (n=13; median OS=3.58 mo.)
- PIF (n=125; median OS=3.78 mo.)
Conclusions

• Immune transcriptomic analyses of *in silico* and wet-lab cohorts of *TP53* mutated AML suggest the presence of high T-cell infiltration and high expression of immune checkpoints and IFN-γ signaling molecules compared with AML subgroups with other risk-defining molecular lesions.

• Immunotherapy with flotetuzumab may be efficacious in individuals with altered *TP53* status, with an overall reduction of BM blasts averaging 42% and with evidence of ALA in 45.5% (5/11) of the patients.

• The overall response rate observed in *TP53*-mutated patients treated with flotetuzumab encourages further study of this immunotherapeutic approach.
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