Adaptive Immune Gene Signatures Correlate with Response to Flotetuzumab, a CD123 × CD3 Bispecific DART® Molecule, in Patients with Relapsed/Refractory Acute Myeloid Leukemia

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Background

- Cytotoxic chemotherapy remains the standard-of-care for most patients with acute myeloid leukemia (AML), in spite of the recent approval of novel agents
  - The investigation of new molecularly-targeted and immuno-modulating agents remains a high priority
- Immunotherapies such as monoclonal antibodies, bispecific molecules, immune checkpoint blockade (ICB) and CD123-CAR T cells are currently under investigation in AML
- There is an urgent need for predictive biomarkers to help identify patients who are more likely to respond to cancer immunotherapy
  - Tumor Mutational Burden (TMB) identifies responders to pembrolizumab in KEYNOTE clinical trials across 22 solid tumor types (Cristescu R, et al. Science 2018; 362:6411)
- Flotetuzumab, a CD123 × CD3 bispecific DART® molecule, is being tested in a phase 1 clinical trial of relapsed/refractory AML (NCT#02152956)
- See also presentation #764. Monday, December 3, 2018: 3:00PM
  - Dr. John DiPersio, Session #616. Acute Myeloid Leukemia: Novel Therapy Seaport Ballroom F (Manchester Grand Hyatt San Diego)
Diversity of immune landscapes in AML

**Immune-inflamed TME is associated with resistance to cytotoxic chemotherapy**

Immune profiles in the tumor microenvironment (TME)
1. Innate (PMN, macrophages)
2. Adaptive (T, B, NK, CTL)
3. Mast cells, exhausted CD8+ T cells

**Discovery cohort (n=62)**
34 non-promyelocytic de novo childhood AML
(Dr. Sarah K. Tasian, Children’s Hospital of Philadelphia, USA)
28 non-promyelocytic de novo adult AML
(Professor Martin Bornhäuser, Dresden, Germany)

Expression of IFN-stimulated genes in BM associates with poor prognosis in AML

A Altered in 18 (11%) of 162 sequenced patients in TCGA-AML

<table>
<thead>
<tr>
<th>Genetic Alteration</th>
<th>CD274</th>
<th>MX1</th>
<th>IFIT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplification</td>
<td>4%</td>
<td>9%</td>
<td>4%</td>
</tr>
<tr>
<td>mRNA Upregulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No alterations</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

416 genes up in patients with abnormalities in query genes
174 genes down in patients with abnormalities in query genes

B

-10 >-8 >-6 >-4 >-2 0 2 4 6 Log Ratio

<0.005 P-value

Significance

Under-expressed
Over-expressed

Intermediate (n=5)
- 7+3 (n=9)
- HMA (n=6)
- Lenalidomide (n=1)
- None (n=2)

Adverse (n=13)
- Yes (n=2 MUD; n=2 MRD)
- No (n=14)

CR (n=3)
- No CR (n=14)
- Persistent disease (n=1)

C

TP53

P=0.000537

D

Alterations in query genes (median=10.3 mo)
No alterations in query genes (median=20.8 mo)

HR=2.67 (95% CI: 0.87-8.17)

Relapse-Free Survival Time (Months)

Number at risk
Query genes not altered
- 142
- 18
Query genes altered
- 69
- 3

Overall Survival Time (Months)

Number at risk
Query genes not altered
- 144
- 18
Query genes altered
- 86
- 8
Research questions

IFN-\(\gamma\)-related signatures reflecting an “inflamed” TME are associated with adverse prognosis in patients with AML receiving conventional chemotherapy

Are immune-infiltrated/inflamed TMEs, and IFN-\(\gamma\) gene signatures, associated with sensitivity to flotetuzumab?
Patients and methods

• Immune gene expression was analyzed in 65 bone marrow (BM) samples from patients with relapsed/refractory AML treated with flotetuzumab in NCT#02152956 (Vey, et al. ESMO 2017; Uy, et al. ASH 2017; Uy, et al. ASH 2018)
  • 38 samples collected at baseline (35 with clinical outcome data)
    • 4 patients, 300 ng/kg/day
    • 28 patients, 500 ng/kg/day (RP2D)
    • 6 patients, 700 ng/kg/day
  • 27 samples collected “on treatment” (post-cycle 1)

• The NanoString PanCancer IO360™ assay interrogates the expression of 770 genes, including the abundance of 14 immune cell types and 32 immuno-oncology signatures
  • Signature scores were calculated as pre-defined linear combinations (weighted averages) of biologically relevant gene sets
# Patients’ characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median and range)</td>
<td>64 years (29-82)</td>
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<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (42.1%)</td>
</tr>
<tr>
<td>Female</td>
<td>22 (57.9%)</td>
</tr>
<tr>
<td>Disease status at time of enrolment</td>
<td></td>
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<tr>
<td>Relapse</td>
<td>8 (21.1%)</td>
</tr>
<tr>
<td>Primary refractory (73.7%)§</td>
<td></td>
</tr>
<tr>
<td>Hypomethylating agents (HMA)</td>
<td>12 (31.6%)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>16 (42.1%)</td>
</tr>
<tr>
<td>Not classifiable (Failed ≤ 2 cycles of HMA)</td>
<td>2 (5.2%)</td>
</tr>
<tr>
<td>2017 ELN risk stratification</td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>7 (18.4%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>12 (31.6%)</td>
</tr>
<tr>
<td>Adverse</td>
<td>13 (34.2%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (15.8%)</td>
</tr>
<tr>
<td>Number of prior lines of therapy (median and range)</td>
<td>3 (1-11)</td>
</tr>
</tbody>
</table>

§Primary refractory: Chemotherapy-refractory (≥2 induction attempts or 1st CR with initial CR duration <6 months) HMA-refractory (failure of ≥4 cycles of HMAs)

Response assessment criteria: Anti-leukemic activity (CR/CRi, PR, “other benefit”*)
Non-responders (treatment failure, stable disease, progressive disease)

*Other benefit defined as >30% decrease in BM blasts
Immune gene signatures at baseline (I)

Immune-infiltrated (Innate\textsuperscript{pos}Adaptive\textsuperscript{pos})

N=21

Anti-leukemic activity
31.6% (6/19)
3 CR, 2 OB, 1 PR

No response
13/19

N.A.*
2/21

ELN cytogenetic risk at time of initial diagnosis (all patients)
Favorable (n=5)
Intermediate (n=9)
Adverse (n=5)
N.A. (n=2)

Favorable (n=2)
Intermediate (n=3)
Adverse (n=8)
N.A. (n=4)

Immune-depleted (Innate\textsuperscript{neg}Adaptive\textsuperscript{neg})

N=17

Anti-leukemic activity
12.5% (2/16)
1 CRi, 1 OB

No response
14/16

N.A.*
1/17

ELN cytogenetic risk at time of initial diagnosis (patients with evidence of anti-leukemic activity)
Favorable (n=1)
Intermediate (n=3)
Adverse (n=1)
N.A. (n=1)

Favorable (n=0)
Intermediate (n=0)
Adverse (n=2)
N.A. (n=0)

*Response data available in 35/38 patients

AA = Anti-leukemic activity
NR = No response
NA = Not available
immune gene signatures at baseline (ii)

AA = Anti-leukemic activity
NR = No response
NA = Not available

Exhausted

Dysfunctional T cells?
Immune gene signatures at baseline (III)

AA = Anti-leukemic activity
NR = No response
NA = Not available

Immune-inflamed

Immune-exhausted

Immune-infiltrated (Innate^{pos}Adaptive^{pos})

"IFN-γ-dominant" TME?
# Immune signatures and flotetuzumab response

## Table

<table>
<thead>
<tr>
<th></th>
<th>Immune-inflamed (n=5)</th>
<th>Immune-exhausted (n=16)</th>
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<tbody>
<tr>
<td><strong>Anti-leukemic activity</strong></td>
<td>40% (2/5)</td>
<td>29% (4/14)</td>
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<tr>
<td></td>
<td>1 CR, 1 OB</td>
<td>2 CR, 1 OB, 1 PR</td>
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<tr>
<td><strong>No response</strong></td>
<td>3/5</td>
<td>10/14</td>
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<tr>
<td>*<em>N.A.</em></td>
<td>0/5</td>
<td>2/16</td>
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<tr>
<td><strong>Previous HMA treatment</strong></td>
<td>40% (2/5)</td>
<td>62.5% (10/16)</td>
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<td><strong>ELN cytogenetic risk at time</strong></td>
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<td>Favorable (n=1)</td>
<td>Favorable (n=4)</td>
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<td>Intermediate (n=0)</td>
<td>Intermediate (n=9)</td>
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<td>Adverse (n=4)</td>
<td>Adverse (n=1)</td>
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<td>N.A. (n=0)</td>
<td>N.A. (n=2)</td>
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<td>Favorable (n=1)</td>
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<td>Intermediate (n=3)</td>
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<td></td>
<td>Adverse (n=1)</td>
<td>Adverse (n=0)</td>
</tr>
<tr>
<td></td>
<td>N.A. (n=0)</td>
<td>N.A. (n=1)</td>
</tr>
</tbody>
</table>

*Response data available in 35/38 patients

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*PLEASE DO NOT POST*
Increased immune exhaustion signatures in HMA-refractory vs. chemotherapy-refractory patients

**A**

- Down-regulated in HMA-refractory versus chemotherapy-refractory
- Up-regulated in HMA-refractory versus chemotherapy-refractory

- Proliferation
- JAK-STAT loss
- Endothelial cells
- BT-H3
- APM loss
- Glycolysis
- Mast cells
- Cytotoxicity
- Cytotoxic cells
- CD8 T cells
- Lymphoid
- T-cells
- Treg
- CTLA4
- TIGIT
- NK cells
- NK CD56dim cells
- NK cells
- Apoptosis
- Hypoxia
- ARG1
- IL10
- IFN gamma
- Macrophages
- Myeloid
- Neutrophils
- PD-L2
- Stromal
- DC
- MAOEs
- IDO1
- B-cells
- PD-1
- NOS2
- Inflam chemokines
- PD-L1
- CD45
- Exhausted CD8
- Immunoproteasome
- APM
- IFN downstream
- Myeloid inflam
- MHC2
- TGFB-beta
- MMR loss

**B**

- **TIGIT expression**
  - P=0.006

- **PD-L1 expression**
  - P=0.0096

- **Exhausted CD8 T cells**
  - P=NS

- **Treg cell abundance**
  - P=0.009

*Evaluated in a subset of 22 patients (8 HMA-refractory, 14 chemotherapy-refractory)

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Flotetuzumab treatment enhances tumor inflammation, antigen presentation and IFN-γ signaling signatures.

**A**

Down-regulated post-cycle 1 versus baseline

Up-regulated post-cycle 1 versus baseline

- Proliferation
- JAK-STAT loss
- Endothelial cells
- BT-H3
- APM loss
- Glycolysis
- Mast cells
- Cytotoxicity
- Cytotoxic cells
- CD8 T cells
- Lymphoid
- T-cells
- Treg
- CTLA4
- TIS
- T helper cells
- TIGIT
- NK CD56dim cells
- NK cells
- Apoptosis
- Hypoxia
- ARID1
- IL10
- IFN gamma
- Macrophages
- Myeloid
- Neutrophils
- PD-L2
- Stroma
- DC
- MAGEs
- IDO1
- B-cells
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- Exhausted CD8
- Immunoproteasome
- APM
- IFN downstream
- Myeloid inflam
- MHC2
- TGF-beta
- MMR loss

**B**

**B**

Tumor inflammation signature (TIS) score

- Log2 fold-change

**B**

IFN-γ signaling score

- P=0.015

**B**

Immunoproteasome score

- P=0.0002

**B**

Antigen processing machinery (APM) score

- P=0.0015

- P=0.06

Non-responders

Anti-leukemic activity

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IFN-γ signaling scores are associated with response to flotetuzumab

Down-regulated in anti-leukemic activity versus non-responders
- Proliferation
- JAK-STAT loss
- Endothelial cells
- B7-H3
- APM loss
- Glycolysis
- Mast cells
- Cytotoxicity
- Cytotoxic cells
- CD8 T cells
- Lymphoid
- T-cells
- Treg
- CTLA4
- Tim3
- Th1 cells
- TIGIT
- NK CD56dim cells
- NK cells
- Apoptosis
- Hypoxia
- ARG1
- IL10

Up-regulated in anti-leukemic activity versus non-responders
- Macrophages
- Myeloid
- Neutrophils
- PD-L2
- Stromal DC
- MAGEs
- IDO1
- B-cells
- PD-1
- NOS2
- Inflamm chemokines
- PD-L1
- CD45
- Exhausted CD8
- Immunoresponse
- APM
- IFN downstream
- Myeloid inflam
- MHC2
- TGF-beta
- MMR loss

Receiver operating characteristic (ROC) curve analysis
- AUC = 0.815
- 97.5% CI = 0.805 (SE = 0.15)
- Z-score = 3.188
- P = 0.0014

*PLEASE DO NOT POST*
Predictors of ICB response in solid tumors

Ayers M, et al.

Cristescu R, et al.
Science 2018; 362 (6411): eaar3593

18-gene score (Tumor Inflammation Signature) for a cohort of 96 patients with HNSCC from KEYNOTE-012

IFN-γ signaling signature for flotetuzumab
Conclusions

• Evidence for a **range of immune profiles** in the AML TME was previously presented and confirmed here.

• As opposed to prior experience with chemotherapy, most patients showing evidence of anti-leukemic activity with flotetuzumab [6/8 (75%)] in this initial data set had a gene signature consistent with higher immune infiltration in the bone marrow.

• More specifically, **IFN-γ-related gene profiles** at baseline may associate with clinical response to flotetuzumab.

• Patients previously treated with HMAs showed an immune-exhausted TME:
  • We hypothesize that flotetuzumab could invigorate an immune-exhausted TME (increased tumor inflammation, antigen processing/presentation and IFN-γ signaling scores).

• Patients with an immune-infiltrated TME had increased immune checkpoint expression, suggesting potential enhanced benefit from flotetuzumab in combination with immune checkpoint blockade.
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