Immune Landscapes Predict Chemotherapy Resistance and Anti-Leukemic Activity of Flotetuzumab, an Investigational CD123 × CD3 Bispecific DART® Molecule, in Patients with Relapsed/Refractory Acute Myeloid Leukemia

Sergio Rutella¹,², Jayakumar Vadakekolathu¹, Mark D. Minden³, Tressa Hood⁴, Sarah E. Church⁴, Stephen Reeder¹, Heidi Altmann⁵, Amy H. Sullivan⁴, Elena J. Viboche⁴, Tasleema Patel⁶, Narmin Ibraghimova³, Sarah E. Warren⁴, Andrea Arruda³, Yan Liang⁴, Marc Schmitz⁷, Alessandra Cesano⁴, Peter J.M. Valk⁸, Bob Löwenberg⁸, A. Graham Pockley¹, Martin Bornhäuser⁵, Sarah K. Tasion⁶, Michael P. Rettig⁹, Jan Davidson-Moncada¹⁰, John F. DiPersio⁹

¹John van Geest Cancer Research Centre and ²Centre for Health, Ageing and Understanding Disease (CHAUD), School of Science and Technology, Nottingham Trent University, Nottingham, UK; ³Princess Margaret Cancer Centre, Toronto, Canada; ⁴NanoString Technologies, Inc., Seattle, WA; ⁵Department of Internal Medicine I, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany; ⁶Division of Oncology and Centre for Childhood Cancer Research, Children's Hospital of Philadelphia and University of Pennsylvania School of Medicine, PA; ⁷Institute of Immunology, Medical Faculty, Technische Universität Dresden, Dresden, Germany; ⁸Department of Hematology, Erasmus University Medical Centre, Rotterdam, The Netherlands; ⁹Division of Oncology, Department of Medicine, Washington University School of Medicine, St Louis, MO; ¹⁰MacroGenics, Inc., Rockville, MD

sergio.rutella@ntu.ac.uk
Background

- Cytotoxic chemotherapy remains the standard-of-care for most patients with acute myeloid leukemia (AML), despite the recent approval of novel agents.
- 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 to identify predictive biomarkers in the tumor immunological microenvironment (TME).
- Flotetuzumab, a CD123 × CD3 bispecific DART® molecule, is being tested in a phase 1 clinical trial of relapsed/refractory (R/R) AML (NCT#02152956).
- See also presentation #733. Monday, December 9, 2019: 2:45PM
  - Dr. Geoffrey Uy, Session #613. Acute Myeloid Leukemia: Clinical Studies: Treatment of Relapsed/Refractory Disease. Tangerine 3 (Orange County Convention Center).
Diversity of immune landscapes in AML

The AML tumour immunological 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
(Sarah K. Tasian, Children’s Hospital of Philadelphia, USA)
28 non-promyelocytic de novo adult AML
(Martin Bornhäuser, Dresden, Germany)

Patient series and methods

**Wet-lab cohorts**

<table>
<thead>
<tr>
<th></th>
<th>PMCC*</th>
<th>CHOP^</th>
<th>SAL^^</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr of patients</td>
<td>290</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>Age (y)</td>
<td>52 (18-81)</td>
<td>10 (0.1-20)</td>
<td>52.5 (23-75)</td>
</tr>
<tr>
<td>Disease status</td>
<td>Onset</td>
<td>Onset</td>
<td>Onset/CR/Relapse</td>
</tr>
</tbody>
</table>

**In silico cohorts**

<table>
<thead>
<tr>
<th></th>
<th>HOVON</th>
<th>Beat AML Master Trial</th>
<th>TCGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr of patients</td>
<td>618</td>
<td>267</td>
<td>147</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Adult</td>
<td>Adult</td>
<td>Adult</td>
</tr>
<tr>
<td>Disease status</td>
<td>Onset</td>
<td>Onset</td>
<td>Onset</td>
</tr>
</tbody>
</table>

- The PanCancer Immune Profiling Panel (NanoString Technologies, Seattle, WA) was used to measure mRNA expression in bulk BM samples (n=770 immune-related genes).
- Immune signature scores and biological activity scores were calculated as pre-defined linear combinations (weighted averages) of biologically relevant gene sets.
- Gene expression data have been deposited in NCBI's Gene Expression Omnibus and will be accessible through GEO Series accession number GSE134589.

*PMCC = Princess Margaret Cancer Centre, Toronto, Canada – **Discovery cohort**

^CHOP = Children’s Hospital of Philadelphia, Philadelphia, PA

^^SAL = Studienallianz Leukämie, Dresden, Germany
Immune landscapes assist stratification

**Discovery cohort**
(n=290 patients)

- **“IFN module” gene score**
- Myeloid inflammation
- Inflammatory chemokines
- Downstream IFN signaling
- IFN-γ
- PDL1
- PDL2
- MAGEs
- IL10

**Immunoproteasome**
- Infiltrated (n=136)
- Depleted (n=154)

(C) IFN-stimulated genes
- IFN-γ mRNA
- IRF1 mRNA
- IRF7 mRNA
- MX1 mRNA
- PRF1 mRNA
- CXCL10 mRNA

(T-cell and cytotoxicity markers)
- CD8A mRNA
- CD274 mRNA
- HAVCR2 (Tim-3) mRNA
- GZMB mRNA
- PRF1 mRNA

(A) Immune checkpoints and immunotherapy targets
- HAVCR2 (Tim-3) mRNA

**Antigen processing and presentation**
- HLA-A mRNA
- HLA-B mRNA
- HLA-C mRNA
- HLA-A-DQ mRNA

Prediction of chemotherapy response

Therapy resistance (‘3+7’ backbone) was defined as failure to achieve CR in patients who survive at least 28 days (primary refractory AML) or as early relapse (less than 3 months after achieving CR).

A) PMCC discovery series (n=290)

- **Variable**: Immune scores, ELN risk
- **AUROC**: 0.815, 0.702
- **SE**: 0.031, 0.038
- **95% CI**: 0.755–0.876, 0.628–0.776

B) Beat-AML Master Trial validation series (n=196)

- **Variable**: IFN module score, ELN risk
- **AUROC**: 0.921, 0.709
- **SE**: 0.04, 0.021
- **95% CI**: 0.88–0.961, 0.629–0.788

Translational research question

IFN-γ-related gene signatures reflecting an immune-infiltrated TME are associated with adverse prognosis in patients with AML receiving conventional chemotherapy.

Are immune-infiltrated TMEs, and IFN-γ gene signatures in particular, associated with sensitivity to targeted immunotherapy with flotetuzumab, a CD123 × CD3 DART bispecific molecule?
Flotetuzumab immunotherapy

- Immune gene expression was analyzed in a subgroup of patients (n=30/50) with relapsed/refractory AML treated with flotetuzumab (NCT#02152956) at RP2D (500 ng/kg/day; Uy, et al. ASH 2017; Uy, et al. ASH 2018; Rutella, et al. ASH 2018)
  - 30 BM samples analyzed at baseline
  - 19 BM samples analyzed “on treatment” (post-cycle 1)
- The NanoString PanCancer IO360™ assay was used to interrogate 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 and Methods

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=30)*</th>
</tr>
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<tbody>
<tr>
<td>Age (median and range)</td>
<td>57 years (27-74)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16 (53%)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (47%)</td>
</tr>
<tr>
<td>Disease status at study entry</td>
<td></td>
</tr>
<tr>
<td>Relapse (CR with initial duration &gt;6 months)</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>Refractory</td>
<td></td>
</tr>
<tr>
<td>Primary induction failure (PIF; ≥2 induction attempts)</td>
<td>17 (57%)</td>
</tr>
<tr>
<td>Early relapse (CR with initial duration &lt;6 months)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>2017 ELN risk stratification</td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>7 (23%)</td>
</tr>
<tr>
<td>Adverse</td>
<td>17 (57%)</td>
</tr>
<tr>
<td>Secondary AML</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Number of prior lines of therapy (median and range)</td>
<td>3 (1-9)</td>
</tr>
</tbody>
</table>

*Subgroup of 30/50 patients treated at the RP2D for whom BM samples were available

Response assessment criteria employed in analysis:

- **Anti-leukemic activity (ALA):** CR/CRi, PR, “other benefit” (>30% decrease in BM blasts)
- **Non-responders (NR):** treatment failure, stable disease, progressive disease
‘Hot’ TME in chemorefractory AML

Refractory = Primary induction failure (PIF) + early relapse (ER)

Mann Whitney U test for unpaired determinations

Ref. = Refractory
Rel. = Relapse
IFN-related profiles and response to flotetuzumab

Flotetuzumab modulates the TME

Matched baseline-post-C1 BMs available for 19 patients treated with flotetuzumab

A

B

C

GeoMx Digital Spatial Profiling of BM FFPEs (50+ IO proteins)

1 patient

P=0.0006

P=0.002

P=0.004

P=0.0062

Anti-leukemic activity  No response  Wilcoxon matched-pairs signed rank test
Flotetuzumab modulates the TME

GeoMx Digital Spatial Profiling of 2 BM FFPEs (50+ IO proteins)

2 patients achieving CR

Region of interest (ROI) with no 'T-cell clustering'

ROI with 'T-cell clustering'

High in ROIs with 'no T-cell clustering'

High in ROIs with 'T-cell clustering'

Conclusions

• Transcriptional programs that reflect high immune infiltration and IFN-γ signaling enrich in a subset of patients with AML and predict chemotherapy resistance

• IFN-γ-related mRNA profiles at baseline correlate with anti-leukemic activity of flotetuzumab at the RP2D

• A subgroup of patients with an immune-infiltrated TME show high expression of immune checkpoints, including PD-L1, suggesting potential enhanced benefit from flotetuzumab in combination with ICB

• A phase I study of flotetuzumab combined with MGA012, an anti-PD1 antibody, is ongoing in patients with R/R AML (Wei AH, et al. Poster #2662; ASH 2019)
Acknowledgements

Co-authors and Collaborators

JVGCRC, NTU
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PhD Students
Jenny Ashforth
Melissa Courtney

Mark D. Minden
Toronto, Canada

The Princess Margaret Hospital Foundation

Tasleema Patel
Sarah K. Tasian
Philadelphia, PA

University of Regensburg
Carl Gustav Carus University Hospital
Heidi Altmann
Martin Luther University Halle – Wittenberg, Germany

Universität Regensburg
Martin Bornhäuser
Jörn Meinel
Marc Schmitz
SAL Studienallianz Leukämie

Dresden, Germany

University of Regensburg
Katja Dettmer-Wilde
Peter Oefner
Institute of Functional Genomics
Regensburg, Germany

University of Regensburg
Franziska Müller

Regensburg, Germany

University of Regensburg
Ernst Holler
Peter Siska
Poliklinik für Innere Medizin III
Regensburg, Germany

nanoString
Leonido Luznik
Sidney Kimmel Comprehensive Cancer Centre
Baltimore, MD

nanoString
Joseph M. Beechem
Alessandra Cesano

Thomas Smith
James Gowen-MacDonald
Michael Bailey

Sarah E. Church
Tressa Hood
Sarah E. Warren
Seattle, WA

International Union Against Cancer

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Toronto, Canada

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Toronto, Canada

John Muth
Rockville, MD

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Regensburg, Germany

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