Microenvironmental Immune Senescence and Exhaustion in Acute Myeloid Leukemia Associate with Response to Flotetuzumab, an Investigational CD123×CD3 Bispecific DART® Molecule


1John van Geest Cancer Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK; 2NanoString Technologies, Inc., Seattle, WA; 3Division of Oncology, Washington University in St. Louis, St. Louis, MO; 4Gehr Family Center for Leukemia Research, City of Hope, Duarte, CA; 5Marseille Immunopole, Marseille, France; 6University of Maryland Greenebaum Comprehensive Cancer Center; 7University of California San Francisco, San Francisco, CA; 8Clinical Research Division, Fred Hutch, Seattle, WA; 9UNC Lineberger Comprehensive Cancer Centre, Chapel Hill, NC; 10Winship Cancer Institute of Emory University, Atlanta, GA; 11Erleie A. Chiles Research Institute, Providence Cancer Center, Portland, OR; 12Moore’s Medical Center, University of California San Diego, La Jolla, CA; 13Medical College of Wisconsin, Milwaukee, WI; 14Loyola University Medical Center, Maywood, IL; 15San Raffaele Scientific Institute, Milan, Italy; 16INSERM U802, Nantes, France; 17Department of Hematology and Cell Therapy, Tours University Hospital, Tours, France; 18Institut Universitaire du Cancer de Toulouse Oncopole, Toulouse, France; 19Leukemia Program, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH; 20University Hospital Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany; 21Duke University School of Medicine, Durham, NC; 22University of Groningen, The Netherlands; 23Erasmus Medical Center, Rotterdam, The Netherlands; 24Universitätsklinikum Würzburg, Würzburg, Germany; 25MD Anderson Cancer Centre, Houston, TX; 26Institute L. & A. Seragnoli, University of Bologna, Bologna, Italy; 27MacroGenics, Inc., Rockville, MD; 28Department of Oncology, Johns Hopkins University, Baltimore, MD; 29Centre for Health, Ageing and Understanding Disease (CHAUD), School of Science and Technology, Nottingham Trent University, Nottingham, UK
• Research funding, NanoString Technologies, Seattle, WA
• Research funding, MacroGenics, Rockville, MD
• Research funding, Kura Oncology, San Diego, CA
Chemotherapy refractoriness and disease relapse continue to be significant obstacles to therapeutic success in AML. We have recently shown that bone marrow (BM) RNA profiles stratify patients with AML into immune-infiltrated and immune-depleted subtypes, and that type I/II interferon (IFN)-related gene signatures associate with complete response to flotetuzumab (Vadakekolathu J, et al. Sci. Transl. Med. 2020; 12: eaaz0463). Within the AML tumor microenvironment (TME), CD8+ T cells exhibit features of immune exhaustion and senescence (IES; Knaus HA, et al. JCI Insight 2018; 3: e120974). IES are dysfunctional states driven by metabolic alterations in the TME, and are emerging targets for cancer immunotherapy.
To determine whether gene sets reflective of IES in the BM TME predict response of relapsed-refractory (R/R) AML to flotetuzumab in the CP-MGD006-01 clinical trial (NCT#02152956)
Patients and Methods

<table>
<thead>
<tr>
<th>All patients (n=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years, median and range)</strong></td>
</tr>
<tr>
<td>61 (27-82)</td>
</tr>
<tr>
<td><strong>Males/Females, n/n</strong></td>
</tr>
<tr>
<td>46/25</td>
</tr>
<tr>
<td><strong>AML risk stratification (2017 ELN; n, %)</strong></td>
</tr>
<tr>
<td>Favorable: 6 (8.4%)</td>
</tr>
<tr>
<td>Intermediate: 18 (25.4%)</td>
</tr>
<tr>
<td>Adverse: 47 (66.2%)</td>
</tr>
<tr>
<td><strong>Secondary AML (n, %)</strong></td>
</tr>
<tr>
<td>29 (40.8%)</td>
</tr>
<tr>
<td><strong>Disease status at study entry (n, %)</strong></td>
</tr>
<tr>
<td>Primary induction failure*: 30 (42.2)</td>
</tr>
<tr>
<td>Early relapse (CR1 duration &lt; 6 months; ER6): 10 (14.1)</td>
</tr>
<tr>
<td>Late relapse (CR1 duration ≥ 6 months; LR): 31 (43.7)</td>
</tr>
<tr>
<td><strong>Number of prior lines of therapy (median and range)</strong></td>
</tr>
<tr>
<td>2 (1-4)</td>
</tr>
</tbody>
</table>

- 139 BM samples from 71 patients with R/R AML treated with FLZ at the RP2D of 500 ng/kg/day (Uy J, et al. Blood 2020); BMs collected at time of study entry (n=71; n=66 with response data) and longitudinally post-cycle (PC) 1 (n=40), PC2 (n=18), PC3 and PC4 (n=4) and end of treatment (n=6);
- Microenvironmental RNAs were profiled using the PanCancer IO 360™ gene expression panel on the nCounter® platform;
- Disease status was assessed by modified International Working Group (IWG) criteria (Uy J, et al. Blood 2020).

*Lack of response to at least two cycles of induction chemotherapy
Derivation of an IES Gene Expression Score

TCGA and Beat AML Master Trial Cohorts

“Immunologic signature” gene sets (C7; n=4,872) (MSigDB)

Gene Set Enrichment Analysis (GSEA)

68-gene IES signature score

An Immune Senescence and Exhaustion-Related RNA Profile Predicts Clinical Outcomes in Acute Myeloid Leukemia (Vadakekolathu J, et al. ASH Oral Presentation #33; December 5th, 2020)
IES Stratify BM Samples and Associate with Response to Flotetuzumab

Baseline BM samples (n=66 patients evaluable for response)

<table>
<thead>
<tr>
<th>Group</th>
<th>CR+PR</th>
<th>Response Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>IES\text{high}</td>
<td>11/19</td>
<td>58%</td>
</tr>
<tr>
<td>IES\text{int}</td>
<td>10/32</td>
<td>31.2%</td>
</tr>
<tr>
<td>IES\text{low}</td>
<td>2/15</td>
<td>13.3%</td>
</tr>
</tbody>
</table>

**Graphs:**
- **A:** Heatmap showing baseline BM samples stratified by IES.
- **B:** Box plots comparing IES scores between responders and non-responders post-treatment.
- **C:** Box plots comparing IES scores between responders and non-responders post-treatment.
- **D:** Box plots showing IES scores at baseline and post-treatment for responders and non-responders.
Flotetuzumab Modulates the Immunological TME

BM aspirates of 22 subjects, prior to and post flotetuzumab treatment (21-marker flow cytometry panel)
GeoMx Digital Spatial Profiling of the TME Identifies Protein Changes after Flotetuzumab

CD3
CD123
DNA

Up in responders

Log2 fold change

Up in non-responders

8 FFPE BM biopsies

183 Regions of interest/Areas of illumination
Conclusions

- Immune exhaustion and senescence gene sets are over-expressed in BM samples from patients with evidence of chemotherapy resistance (PIF/ER6) compared with LR
- Flotetuzumab modulates the immunological TME by enhancing T cell activation and the expression of immune checkpoints
- Transcriptomic features of immune and exhaustion and senescence are associated with response (CR+PR) to flotetuzumab
- T cell functional rejuvenation by flotetuzumab could benefit patients with R/R AML by counteracting pre-existing immune dysfunction
Acknowledgements

Co-authors and Collaborators

Tasleema Patel
Sarah K. Tasian
Philadelphia, PA

Heidi Altmann
Martin Bornhäuser
Marc Schmitz
SAL Studienallianz Leukämie
Dresden, Germany

Stephen Reeder
Payton Tau
Jayakumar Vadakekolathu

PhD Students
Jenny Ashforth
Melissa Courtney

Ivana Gojo
Leo Luznik
Sidney Kimmel Comprehensive Cancer Centre
Baltimore, MD

Mark D. Minden
Toronto, Canada

Nottingham Trent University

NANOSTRING
Joseph M. Beechem
Alessandra Cesano
Michael Bailey
James Gowen-MacDonald
Thomas Smith
Sarah E. Church
Tressa Hood
Sarah E. Warren
Seattle, WA

MACROGENICS
Patrick Kaminker
Jan K. Davidson-Moncada
John Muth
Rockville, MD

Funding Sources

NANOSTRING
Joseph M. Beechem
Alessandra Cesano
Michael Bailey
James Gowen-MacDonald
Thomas Smith
Sarah E. Church
Tressa Hood
Sarah E. Warren
Seattle, WA

MACROGENICS
Patrick Kaminker
Jan K. Davidson-Moncada
John Muth
Rockville, MD

Please email your questions to sergio.rutella@ntu.ac.uk