ELSEVIER

Blood 142 (2023) 2806–2807

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

AML Drug Tolerant Persister (DTP) Cells Survive Chemotherapy By Transiently Altering Cellular Lipidomics to Increase Plasma Membrane Rigidity, but Also Increases Sensitivity to Immune Cell Killing

Yael Morgenstern[1], JongBok Lee[2], Yoosu Na[2], William Gwynne[3], Rose Hurren[1], Li Ma[1], Marcela Gronda[4], Chaitra Sarathy, PhD[1], Arvind Mer[1], Andrea Arruda[5], Avraham Frisch, MD[6], Tsila Zuckerman[7], Yishai Ofran, MD[8,9], Mark D. Minden[10], Li Zhang[2], Catherine O'Brien[1], J. Rafael Montenegro-Burke[3], Aaron D. Schimmer, MD PhD[1]

[1] University Health Network, Princess Margaret Cancer Centre, Toronto, Canada
[2] Department of immunology, University of Toronto, Toronto, Canada
[3] Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Canada
[4] UHN, Princess Margaret Cancer Centre, Toronto, Canada
[5] Princess Margaret Cancer Centre, University Health Network, Toronto, Canada
[6] Department of Hematology and Bone Marrow Transplantation, Rambam Health Care Campus, Rappaport Faculty of Medicine - Technion, Haifa, Israel
[7] Department of Hematology and BMT, Rambam Health Care Campus, Haifa, Israel
[8] Department of hematology and Bone Marrow Transplant, Sharee Zedek Medical Center, Jerusalem, Israel
[9] Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel
[10] Princess Margaret Cancer Centre, University Health Network, Toronto, Canada

Emerging evidence indicates that cancer persister cells, including AML, stochastically and transiently upregulate stress pathways to survive acute exposure to chemotherapy. Here, we explore the biology of these persister cells and identify a T cell-based immunotherapy that targets this surviving fraction.

To investigate the drug-tolerant persister (DTP) state, we established a model of DTP AML cells. AML cell lines (MOLM13, OCI-AML2, OCI-AML3, MV4-11, THP1 and NB4) were treated with a combination of Daunorubicin and Ara-C, at their IC90 - a concentration at which 90% of the cells were dead by day 7. Surviving cells displayed a lag phase in their proliferation for 7 to 15 days post-chemotherapy. As determined by EdU (5-ethynyl-2'-deoxyuridine) labeling, persisting cells actively proliferated during this time, albeit slower than parental cells. Upon cell regrowth, rates of proliferation returned to normal and cells regained chemosensitivity similar to parental cells, demonstrating the DTP phenotype.

To understand mechanisms of the DTP state, we used liquid chromatography-mass spectrometry (LC-MS) to analyze the metabolome and lipidome of MOLM13 and OCI-AML2 cells before Daunorubicin and Ara-C treatment, 7 days posttreatment as well as 12 days after treatment removal (i.e., recovery period). Among the most prominent changes in the DTP cells was an alteration in the global lipidome, with an increase in the levels of unsaturated and elongated fatty acids across the main lipid classes (e.g., triglycerides, phosphatidylcholines, phosphatidylethanolamines, etc.).

Increased unsaturated and elongated fatty acids have been associated with increased plasma membrane rigidity and we previously demonstrated that increased plasma membrane rigidity impairs the uptake of chemotherapeutic agents such as Daunorubicin. Therefore, we measured membrane rigidity in AML persisters by staining cells with a lipophilic pyrene dye. DTP AML cells (MOLM13, OCI-AML2, OCI-AML3, MV4-11, THP1 and NB4) acutely increased membrane rigidity upon exposure to chemotherapy and membrane rigidity returned to baseline after recovery from chemotherapy when proliferation and chemosensitivity returned to baseline. We also demonstrated that primary AML cells isolated from a patient on d5 of "7+3" induction chemotherapy had increased membrane rigidity compared to their cells prior to chemotherapy.

As a non-pharmacologic approach to mimic the DTP state and increase membrane rigidity, we cultured AML cells at lower temperature (31 °C). Culturing cells at 31 °C mimicked the DTP phenotype as the cells that survived culture at reduced temperature displayed slower proliferation and were resistant to Daunorubicin and Ara-C. Upon returning cells to 37 °C, proliferation and chemosensitivity returned to baseline, similar to DTP cells. Interestingly, the lipidome alterations of AML cells at lower temperatures matched the DTP state with increased unsaturation and elongated fatty acids across the main lipid classes.

Increasing membrane rigidity can increase sensitivity to immune-mediated killing. Therefore, we explored the sensitivity of persisting AML cells to immune-mediated killing using Double Negative T (DNT) cells, a subset of CD3+ CD4- CD8- T cells that possess anti-cancer properties. We established a mouse model of AML persisters by engrafting primary AML cells into immune-deficient mice and treating mice with Ara-C or vehicle control. Eight days after treatment, primary AML cells were isolated from Ara-C treated or control mice. Isolated primary AML cells were treated with DNTs for 2 hours. AML DTPs from mice treated with Ara-C were more susceptible to DNT cell-mediated killing compared to AML cells from vehicle-treated mice. Likewise, AML cells that persisted at 31 °C displayed increased sensitivity to DNT-mediated killing compared to cells cultured at 37 °C.
In conclusion, AML persister cells adapt and survive chemotherapy by transiently increasing fatty acid unsaturation and elongation resulting in increasing plasma membrane rigidity. Although these cells survive chemotherapy, they have increased susceptibility to T cell-mediated killing, thus highlighting a potential therapeutic strategy for this disease.

Disclosures Zhang: *WYZE Biotech Co. Ltd::* Consultancy, Honoraria. **Schimmer:** *BMS:* Research Funding; *Medivir AB:* Research Funding; *Novartis:* Consultancy, Honoraria; *Jazz:* Consultancy, Honoraria; *Otsuka Pharmaceuticals:* Consultancy, Honoraria; *UHN:* Patents & Royalties: the use of DNT cells to treat AML; *Medical and Scientific Advisory Board of the Leukemia and Lymphoma Society of Canada:* Membership on an entity's Board of Directors or advisory committees; *Takeda Pharmaceuticals:* Consultancy, Honoraria, Research Funding.

https://doi.org/10.1182/blood-2023-178995