

The Selective Targeting of Unique Metabolic Properties of Leukemic Stem Cells in Acute Myeloid Leukemia

Benjamin A. Brakel^{1,2}

1. McMaster University, Biomedical Discovery and Commercialization Program, Class of 2022

2. McMaster University, Stem Cell and Cancer Research Institute

Received | 19 January 2021

Accepted | 5 February 2021

Published | 12 April 2021

SUMMARY

Acute myeloid leukemia is currently treated with chemotherapy and radiotherapy, effectively eradicating most cancerous cells. However, shortly after the depletion of these cells, the re-emergence of cancer often occurs. This has been attributed to the presence of leukemic stem cells, a small population of cells capable of resisting chemotherapy and radiotherapy. Further, the stem-like properties of such cells allow them to regenerate the cancerous cells, which the disease primarily consists of, limiting our treatments and greatly increasing death and suffering. Attempts have been made to target leukemic stem cells; however, no standard therapies have been approved for this purpose. One method of targeting these cells may be through their metabolic differences from healthy cells. Recently, several unique metabolic properties of leukemic stem cells have been discovered and targeted, successfully reducing the ability of leukemia to return after treatment.

ABSTRACT

Current therapeutic options in the treatment of acute myeloid leukemia often succumb to high instances of relapse and subsequent mortality. Chemotherapy and radiotherapy have long been used as the standard treatment for this disease, remaining stagnant over the past few decades. Recently, a small self-renewing population of leukemic stem cells have been identified as drivers of cancer relapse and progression due to their increased resistance to anticancer therapeutics. This enables these cells to maintain a minimal residual disease and results in downstream differentiation, leading to relapse. Targeting these cells may lead to effective therapies that reduce relapse and mortality. Recently, the metabolic properties of leukemic stem cells have begun to be elucidated. Here, we discuss recent discoveries regarding the metabolism of leukemic stem cells and approaches to targeting their unique metabolic properties.

Keywords: Acute myeloid leukemia, leukemic stem cells, cancer stem cells, stem cell metabolism, cancer relapse

INTRODUCTION

Acute leukemias are a rare set of cancers with disproportionately low rates of survival. Although these illnesses make up less than three percent of all cancers, they result in more deaths than any other cancer in those under 39 years of age.¹ Acute myeloid leukemia (AML) is the most common leukemia diagnosed in adults and carries with it the highest mortality rate among all leukemias.² AML occurs when immature leukocytes, called blast cells, begin to occupy the bone marrow and prevent normal blood formation. Blast cells originate from myeloid progenitor stem cells, which differentiate from hematopoietic stem cells and further differentiate into leukocytes under normal

circumstances.

Standard treatment of AML consists of a combination of chemotherapy and radiotherapy. Treatment standards have remained stagnant over the past few decades and are often followed by damage to healthy tissue and a high incidence of relapse. Despite achieving complete remission in a majority of AML patients, over 70 percent of adults and 30 percent of children will relapse and succumb to the disease within five years of their initial diagnosis.³ A unique subset of cancerous cells called cancer stem cells (CSC) have been implicated in relapse. These cells have been shown to resist typical anticancer therapies used in AML treatment and can remain in a quiescent state and maintain a

minimal residual disease.⁴ While chemotherapy is effective against the bulk of cancer cells, the persistence of CSCs after chemotherapy is thought to be responsible for the emergence of relapse, where CSCs differentiate into various cancer cells and the disease regenerates. This regenerating ability makes the eradication of CSCs necessary to effectively cure AML.⁵ The specific CSCs involved in AML have been identified as leukemic stem cells (LSCs). Several treatments have been developed which target leukemic stem cells; however, no drug has been able to eradicate LSCs.^{6,7} One method of doing so is through metabolic differences. LSC metabolism has been somewhat understudied until now, with recent breakthroughs showing key metabolic differences capable of being targeted in an LSC-specific manner.

TARGETING LSC AMINO ACID DEPENDENCY

Several drugs have been developed to address the need for a more effective, well-tolerated therapy of AML. One therapeutic option is B-cell lymphoma 2 (BCL-2) inhibitors. The BCL-2 protein inhibits cancer cell apoptosis and is highly expressed in LSCs. BCL-2 inhibitors such as Venetoclax reduce the expression of BCL-2 in AML patients, inducing apoptosis and reducing oxidative phosphorylation.^{8,9} Venetoclax has shown promise in clinical trials, but once again is not sufficient to cure AML.¹⁰ Hypomethylating agents, such as azacitidine, have also been developed as an alternative to chemotherapy for elderly AML patients. Gene hypermutation is extensive in AML and contributes to its progression; hypomethylating agents reverse this state and slow the disease.¹¹ Preclinical models have shown BCL-2 inhibitors to work synergistically with azacitidine, leading to a clinical study of the venetoclax-azacitidine (ven/aza) combination therapy.^{12,13} The combination was able to elicit high rates of remission and durable responses in patients with AML, suggesting that these effects were due to the selective targeting of LSCs.

In a recent study by Pollyea et al. in 2018, the metabolic state of LSCs was compared to blast cells, which make up the bulk of cancerous cells.¹⁴ The study found decreased oxygen consumption after ven/aza treatment in LSCs and metabolite levels consistent with decreased oxidative phosphorylation. Following this, decreased energy production was found in the ven/aza-treated LSCs, aligning with previous findings that LSCs rely selectively on oxidative phosphorylation for energy production, and alluding to the mechanism behind ven/aza treatment.⁸ Importantly, these effects were specific to LSCs and were not seen in blast cells or normal hematopoietic stem cells isolated from these same patients, showing selectivity of ven/aza towards

LSCs.

In a later study by Jones et al. in 2018, amino acid metabolism was found to be a large contributor to LSC energy production.¹⁵ Global metabolic profiling revealed increased amino acid content and uptake in LSCs compared to blast cells, suggesting that amino acid metabolism plays a key role in LSC survival. This was supported by decreased LSC viability and colony-forming potential when cultured in amino acid-depleted media, while blast cells saw minimal changes. LSCs grown in amino acid-depleted media further showed decreased oxidative phosphorylation, revealing that these amino acids are needed for oxidative phosphorylation and suggesting that LSCs lack metabolic flexibility, being unable to compensate using other fuel sources. This dependency was confirmed when these amino acid-depleted LSCs and blast cells were supplemented with fatty acids; while blast cells increased fatty acid uptake and metabolism, LSCs did not.¹⁵

Ven/aza treatment was then investigated and found to lower amino acid levels in LSCs, indicating that the drug targeted this dependence.¹⁵ Supporting this finding, gene expression of common amino acid transporters was significantly reduced in LSCs after ven/aza treatment, indicating the involvement of amino acid uptake inhibition in the ven/aza-induced reduction of oxidative phosphorylation. A causal relationship was established by flooding these LSCs with high concentrations of amino acids prior to ven/aza treatment. This pretreatment elevated amino acid levels remained post-ven/aza and rescued LSC viability. The amino acid reduction was further shown to cause a reduction in LSC oxidative phosphorylation, which the amino acid pretreatment was also able to rescue.¹⁵ This confirmed that the reduction of amino acids caused by ven/aza treatment reduced oxidative phosphorylation in LSCs.

METABOLIC FLEXIBILITY OF RELAPSED LSCs

Recently, reduced responses to ven/aza treatment in relapsed or refractory (R/R) AML patients after previous chemotherapy have been found, suggesting resistance among these LSCs.¹⁶ With ven/aza inhibiting oxidative phosphorylation of amino acids, Jones et al. in 2018 suggested that altered metabolic properties and dependencies may exist among R/R LSCs.¹⁵ To test this, the metabolic properties of pre-chemotherapy (de novo) and R/R LSCs from AML patients were compared.¹⁵ The study found that while oxygen consumption was decreased by ven/aza in de novo LSCs, R/R LSCs were not affected and were resistant to the ven/aza-induced depletion of oxidative

phosphorylation. R/R LSCs cultured in amino acid-depleted media further showed higher cell viability and oxygen consumption rates than de novo LSCs, revealing a loss of dependency on amino acid metabolism and suggesting a gained metabolic flexibility and shift to other sources of energy. Indeed, amino acid-depleted R/R LSCs showed increased fatty acid levels followed by elevated citrate, indicating that R/R LSCs could switch to fatty acid metabolism and explaining their resistance to ven/aza. By treating R/R LSCs with a fatty acid uptake inhibitor, the cells were re-sensitized to the treatment. These findings pose the combination of amino acid and fatty acid metabolism as a potential therapeutic target in reducing relapse in AML.¹⁵

NICOTINAMIDE MEDIATES VEN/AZA RESISTANCE

Another study published by Jones et al. in late 2020 observed global metabolite levels of de novo and R/R LSCs, identifying increased nicotinamide levels after relapse.¹⁷ Nicotinamide is a substrate for NAD⁺ production, which was also elevated in R/R LSCs. Stemming from the role of NAD⁺ as an essential coenzyme in various energy production pathways, R/R LSCs generated higher levels of ATP compared to de novo LSCs. This NAD⁺-mediated increase in energy production was shown to occur through both increased amino acid metabolism and fatty acid oxidation, explaining how R/R LSCs gain metabolic flexibility and compensate for amino acid depletion to resist ven/aza treatment. To confirm the causal relationship between increased nicotinamide and resistance to ven/aza treatment, de novo LSCs were pre-treated with concentrated nicotinamide and subsequently treated with ven/aza. Compared to those without the nicotinamide pre-treatment, pre-treated LSCs showed rescued viability and supported the finding that increased nicotinamide levels mediate ven/aza resistance.¹⁷

The researchers then inhibited NAMPT, the enzyme responsible for synthesizing NAD⁺ from nicotinamide, which selectively decreased R/R LSC viability and revealed a reliance on nicotinamide.¹⁷ In paired de novo and R/R LSCs, standard chemotherapy had little effect on either sample, while ven/aza treatment caused a decrease in de novo LSC viability only. NAMPT inhibitors, APO866 and KPT-9274, both selectively decreased R/R LSC viability, showing efficacy and selectivity of NAMPT inhibition for targeting R/R LSCs. A reduction in oxygen consumption was found after NAMPT inhibition, suggesting it targets oxidative phosphorylation.¹⁷ Supporting this, a decrease in NAD⁺-dependent TCA cycle enzyme activity was seen in R/R LSCs, while not in NAD⁺-independent enzymes, suggesting NAMPT inhibition targets R/R

LSCs through reducing nicotinamide levels and NAD⁺ production. NAMPT inhibition in R/R LSCs further showed decreased amino acid levels, confirming that the nicotinamide increase in R/R LSCs increases amino acid metabolism. Fatty acid metabolism was also reduced, indicating that increased nicotinamide allows R/R LSCs to become more metabolically flexible and use fatty acids for energy production.¹⁷

CONCLUSION

The high rate of relapse accompanying chemotherapy and radiotherapy has led to the development of many drugs; however, no effective therapies have been able to replace the standard treatment or overcome relapse. Ven/aza therapy has effectively targeted LSCs, but remission is still not achieved in many de novo AML patients and relapsed LSCs resist the treatment through shifting their metabolic dependencies. NAMPT and other factors related to nicotinamide metabolism have shown potential as therapeutic targets for reducing AML relapse; however, more studies are needed to fully characterize the metabolic properties of LSCs before and after relapse. Future efforts targeting metabolic processes of both de novo and relapsed LSCs would benefit from considering the possibility of nicotinamide-mediated therapy resistance and the involved metabolic pathways. The processes behind relapse in AML have important implications in the design of therapeutics targeting LSCs. For future drug development, therapeutics targeting de novo LSCs could benefit from avoiding selective pressures which may induce metabolic flexibility and lead to resistance. Additionally, drug development targeting relapsed LSCs should consider the process by which these cells have become resistant and their resulting differences, which could lead to more selective and effective targeting.

ACKNOWLEDGEMENTS

I would like to thank Dr. Sheila Singh and Dr. Michelle MacDonald for their mentorship and guidance in the areas of cancer stem cell biology and metabolism. This work did not receive funding. There are no conflicts of interest.

REFERENCES

- (1) Jemal A, Siegel R, Ward E, Murray T, Xu J, Smigal C, et al. Cancer statistics, 2006. *CA-cancer J Clin.* 2006 Apr;56(2), 106–130.
- (2) Hernández JA, Land KJ, McKenna RW. Leukemias, myeloma, and other lymphoreticular neoplasms. *Cancer.* 1995 Jan;75, 381–394.
- (3) Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017 Jan;129(4), 424–447.
- (4) Shlush LI, Mitchell A, Heisler L, Abelson S, Ng S, Trotman-Grant A, et al. Tracing the origins of relapse in acute myeloid leukaemia to stem cells. *Nature.* 2017 Jul;547(7661), 104–108.

- (5) Dick JE. Stem cell concepts renew cancer research. *Blood*. 2008 Dec;112(13), 4793–4807.
- (6) Tallman M. S. New strategies for the treatment of acute myeloid leukemia including antibodies and other novel agents. *Hematology Am Soc Hematol Educ Program*. 2005 Jan;2005(1),143–150.
- (7) Tallman MS, Gilliland DG, Rowe JM. Drug therapy for acute myeloid leukemia. *Blood*. 2005 Aug;106(4), 1154–1163.
- (8) Lagadinou ED, Sach A, Callahan K, Rossi RM, Neering SJ, Minhajuddin M, et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. *Cell Stem Cell*. 2013 Mar;12(3), 329–341.
- (9) Pan R, Hogdal LJ, Benito JM, Bucci D, Han L, Borthakur G, et al. Selective BCL-2 inhibition by ABT-199 causes on-target cell death in acute myeloid leukemia. *Cancer Discov*. 2014 Mar;4(3), 362–375.
- (10) Konopleva M, Pollyea DA, Potluri J, Chyla B, Hogdal L, Busman T, et al. Efficacy and Biological Correlates of Response in a Phase II Study of Venetoclax Monotherapy in Patients with Acute Myelogenous Leukemia. *Cancer Discov*. 2016 Oct;6(10), 1106–1117.
- (11) Kroeger H, Jelinek J, Estécio MR, He R, Kondo K, Chung W, et al. Aberrant CpG island methylation in acute myeloid leukemia is accentuated at relapse. *Blood*. 2008 Aug;112(4), 1366–73.
- (12) Tsao T, Shi Y, Kornblau S, Lu H, Konoplev S, Antony A, et al. Concomitant inhibition of DNA methyltransferase and BCL-2 protein function synergistically induce mitochondrial apoptosis in acute myelogenous leukemia cells. *Ann Hematol*. 2012 Dec;91(12), 1861–1870.
- (13) DiNardo CD, Pratz KW, Letai A, Jonas BA, Wei AH, Thirman M, et al. Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. *Lancet Oncol*. 2018 Feb;19(2), 216–228.
- (14) Pollyea DA, Stevens BM, Jones CL, Winters A, Pei S, Minhajuddin M, et al. Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. *Nat. Med*. 2018 Dec;24(12), 1859–1866.
- (15) Jones CL, Stevens BM, D'Alessandro A, Reisz JA, Culp-Hill R, Nemkov T, et al. Inhibition of Amino Acid Metabolism Selectively Targets Human Leukemia Stem Cells. *Cancer Cell*. 2018 Nov;34(5), 724–740.
- (16) DiNardo CD, Rausch CR, Benton C, Kadia T, Jain N, Pemmaraju N, et al. Clinical experience with the BCL2-inhibitor venetoclax in combination therapy for relapsed and refractory acute myeloid leukemia and related myeloid malignancies. *Am J Hematol*. 2018 Mar;93(3), 401–407.
- (17) Jones CL, Stevens BM, Pollyea DA, Culp-Hill R, Reisz JA, Nemkov T, et al. Nicotinamide Metabolism Mediates Resistance to Venetoclax in Relapsed Acute Myeloid Leukemia Stem Cells. *Cell Stem Cell*. 2020 Nov;27(5), 748–764.

ARTICLE INFORMATION

Senior Editor

Reza Khorvash

Reviewers and Section Editors

Lavanya Sinha, Kasia Tywonek

Formatting and Illustrations

Angelina Lam