Lowering mTORC1 Drives CAR T-Cells Home in Acute Myeloid Leukemia

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SUMMARY

Cellular therapies have demonstrated limited efficacy thus far in acute myeloid leukemia (AML). A recent study shows that mTOR complex 1 activation downregulated CXCR4 reducing marrow infiltration of EpCAM-targeting chimeric antigen receptor (CAR) T-cells in AML. Abrogating mTOR signaling by cotreatment with mTOR inhibitors during IL2-mediated *ex vivo* expansion upregulated CXCR4 and bolstered bone marrow migration and AML elimination by CAR T-cells.

See related article by Nian et al., p. 6026

In this issue of *Clinical Cancer Research*, Nian and colleagues investigated mechanisms leading to reduced bone marrow infiltration and consequently hampered leukemia killing, as well as therapeutic interventions to augment such infiltration by chimeric antigen receptor (CAR) T-cells in acute myeloid leukemia (AML; ref. 1). They demonstrated that aberrant activation and downstream signaling from mTOR complex 1 (mTORC1) downregulated expression of the chemokine receptor CXCR4 on EpCAM targeting CAR T-cells, consequently decreasing CXCR4-mediated CAR T-cell migration to the bone marrow (**Fig. 1**). The mTOR signaling was noted during *ex vivo* expansion with IL2 as well as during tonic CAR signaling and eventually reduced the capacity of CAR T-cells to eliminate AML blasts *in vivo*. Subsequently, they show that the abrogation of mTORC1 activity through pretreatment with rapamycin during IL2-mediated *ex vivo* expansion of EpCAM-targeting CAR T-cells increased CXCR4 expression and bolstered CAR T-cell migration to the bone marrow and elimination of marrow-resident AML cells in cell line and primary sample xenografts. Common rapalogs including everolimus and temsirolimus also exerted similar effects on CXCR4 expression on CAR T-cells tested *in vitro*. The authors used CAR T-cells employing CD8 transmembrane domain, 4-1BB costimulatory domain, and CD3ζ signaling domain, xenograft models using AML cell lines and patient samples, and replicated their findings using CD33 targeting CAR T-cells.

These novel findings have important implications for the field of cellular therapies in leukemia. While CAR-based cellular therapies have shown groundbreaking responses in lymphoid malignancies, responses are lower in patients with high burden disease and relapses are common in up to 50% of patients. Relapsed or refractory AML continues to have dismal prognosis with CR/CRi rates of 10% to 30% and dismal median survival of 4 to 10 months, in spite of the recent advent and approval of FLT3- and IDH-targeted therapies, making this an area of high unmet need. A number of efforts are ongoing to develop various immunotherapy approaches including leveraging the adaptive immune system via T-cell checkpoint inhibitors (PD-1, PD-L1), bispecific antibodies, and more recently to harness the innate immune system via CD47-SIRPα inhibition or activated non-CAR and CAR-NK cellular therapies (2). On the basis of promising preclinical data, a number of CAR T-cell therapy–targeting antigens, such as CD33, CD123, entered clinic for AML over the last 3–5 years, but have either been stalled by slow enrollment, low efficacy, or high toxicity, especially prolonged myelosuppression. Recent approaches targeting potentially more AML-restricted antigens such as CLL1 have shown early encouraging responses and tolerability in relapsed pediatric AML (3). Approaches to improve the quality and fitness of modified or unmodified cellular therapies in AML are urgently needed.

Previous work has shown that the *ex vivo* expansion of CAR T-cells with IL15 improved proliferative capacity of CAR T-cells, conferred higher antiapoptotic properties, and exhibited lower exhaustion markers (4). This resulted in preservation of stem cell memory T phenotype and translated into better *in vivo* antitumor activity. The benefit of IL15 was thought to be partially mediated through decreased mTORC1 activity, and consequent reduction of glycolytic enzymes and improvement of mitochondrial fitness. These findings were recapitulated by adding rapamycin when IL2 was used for *ex vivo* expansion, congruent with the findings reported by the authors (1). In addition, inhibition of mTORC1 by mTOR or AKT inhibitors may also shift immune cell metabolism from glycolysis to fatty acid oxidation conferring superior metabolic fitness and improved T-cell longevity. Furthermore, mTOR activation may decrease T-cell exhaustion via reducing FOXO, but this effect may be context dependent and consequently the overall effect of priming CAR T-cells with mTOR inhibitors needs further evaluation (4).

The CXCR4 chemotactic receptor is of singular importance for bone marrow homing of hematopoietic and immune cells. Gain-of-function (GOF) mutation in *CXCR4* noted in WHIM syndrome led to superior bone marrow homing of NK cells (5). Transfecting NK cells to overexpress CXCR4 or with GOF-mutant variant of CXCR4 could be leveraged for adoptive cellular therapies for leukemia (5). Other similar approaches have been explored to improve CAR T-cell trafficking into the tumor microenvironment including forced expression of other chemokines including CCR4, CCR2b, CSF-1R, to local infusion of CAR T-cells into solid tumors (6). In contrast to these interventions, the authors' work provides a target independent and simple strategy without needing to resort to forced expression of specific receptors.

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Clin Cancer Res 2021;27:5739–41

doi: 10.1158/1078-0432.CCR-21-2574

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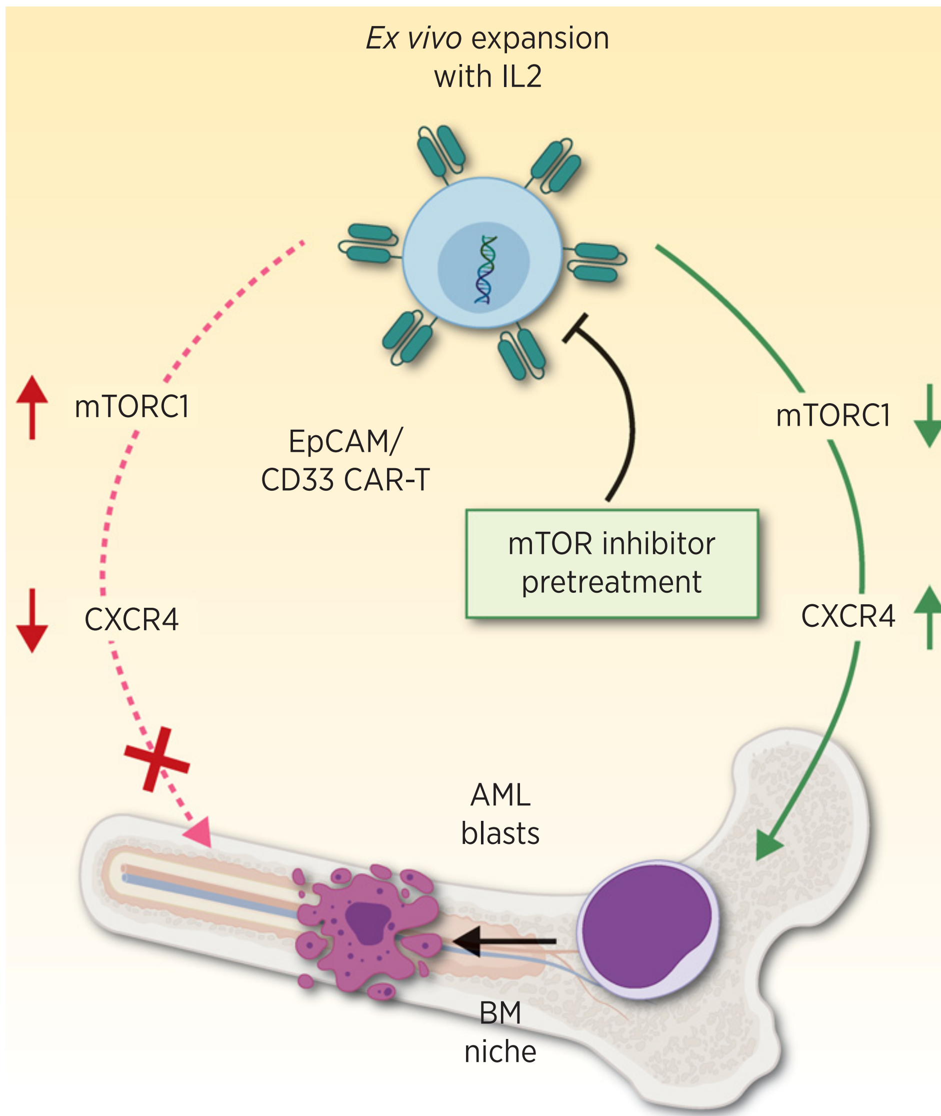

Figure 1.
mTORC1-mediated CXCR4 expression, resultant bone marrow (BM) homing, and mTOR inhibitor effect on EpCAM- or CD33-targeting CAR T cells in AML. Adapted from an image created with BioRender.com.

Other functional and clinical consequences of such *ex vivo* treatment including long-term durability of CXCR4 upregulation after one-time treatment, resultant CAR T-cell capacity for *in vivo* expansion, differentiation, persistence, exhaustion, and toxicity remain to be explored. These findings lead to other questions including optimal dose, duration, and relative superiority of such *ex vivo* treatment approaches with rapalogs, AKT inhibitors, glycolysis inhibitors, BET inhibitors, versus usage of IL15 for *ex vivo* expansion instead of IL2 to augment CAR T-cell fitness versus combinatorial approaches to further enhance the CAR T-cell fitness (4). For example, could combining CAR T-cell pretreatment with rapalogs or using IL15 for expansion, and appropriately timed patient pretreatment with leukemia stem cell–mobilizing agents or growth factors offer a simple approach to augment efficacy? Could mTOR inhibitors or IL15 be a solution for similar phenomenon observed with other unmodified adoptive cellular therapies for leukemias where *ex vivo* expansion downregulates CXCR4? (5) Would *in vivo* long-term treatment with mTOR inhibitors have a similar effect? Optimization of *ex vivo* expansion techniques, for example, with mTOR inhibitor cotreatment or with IL15, implementation of alternative costimulatory domains, or pharmacologic modulation of tonic signaling, for example, with dasatinib, or modular CAR approaches minimizing tonic signaling may help further reduce such chemokine downregulation and improve CAR T-cell homing.

In summary, Nian and colleagues elegantly demonstrate the adverse impact of *ex vivo* expansion with IL2 and tonic signaling on mTORC1 upregulation and consequent CXCR4 downregulation impairing bone marrow migration and antitumor activity of EpCAM1 and CD33-targeting CAR T-cells in AML. Subsequently, they demonstrate the capacity to potentially reverse such phenotype with simple *ex vivo* pretreatment with mTOR inhibitors. These findings may provide an important piece of the puzzle toward improving CAR T-cell fitness in the field of cellular therapies in AML, an area of unmet need and growing interest.

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Authors' Disclosures

A. Maiti reports grants and other support from Celgene outside the submitted work. N.G. Daver reports grants from Abbvie, Genentech, Astellas, Daiichi-Sankyo, Pfizer, BMS, Immunogen, Novimmune, and Forty-seven, and personal fees from Abbvie, Genentech, Astellas, Daiichi-Sankyo, Pfizer, BMS, Immunogen, Jazz Pharmaceuticals, Trillum, Forty-seven, Gilead, Kite, and Novartis outside the submitted work.

Acknowledgment

This work was supported in part by the MD Anderson Cancer Center Support Grant CA016672 from the NCI. Figure created with BioRender.com.

Received August 13, 2021; revised August 20, 2021; accepted August 24, 2021; published first September 1, 2021.

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