

from Roche, Celgene, Janssen, AbbVie, and Gilead Sciences. M.N. declares no competing financial interests. ■

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## IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Daher et al, page 624

# Releasing the brake in CAR natural killer cells

Sophia Chen | Memorial Sloan Kettering Cancer Center

**In this issue of *Blood*, Daher et al<sup>1</sup> present a strategy to improve natural killer (NK) cell effector function that combines chimeric antigen receptor (CAR) engineering and gene editing of a cytokine-related immune checkpoint. Their study provides preclinical data on the mechanistic synergy of these 2 approaches and might have an impact on the future development of NK cell-based cancer immunotherapy.**

Recent advances in cancer immunotherapy have mainly focused on engineering T cells to express a CAR against specific tumor antigens. Although significant clinical efficacy has been achieved in B-cell malignancies, the application of the existing CAR T-cell products approved by the US Food and Drug Administration still faces logistic and clinical challenges, such as timely collection and expansion of sufficient numbers of autologous gene-modified

T cells in heavily pretreated patients. NK cells are attractive alternative candidates for novel approaches in cancer immunotherapy because they mediate potent cytotoxicity against tumor cells and can potentially be used as readily available off-the-shelf products.<sup>2</sup>

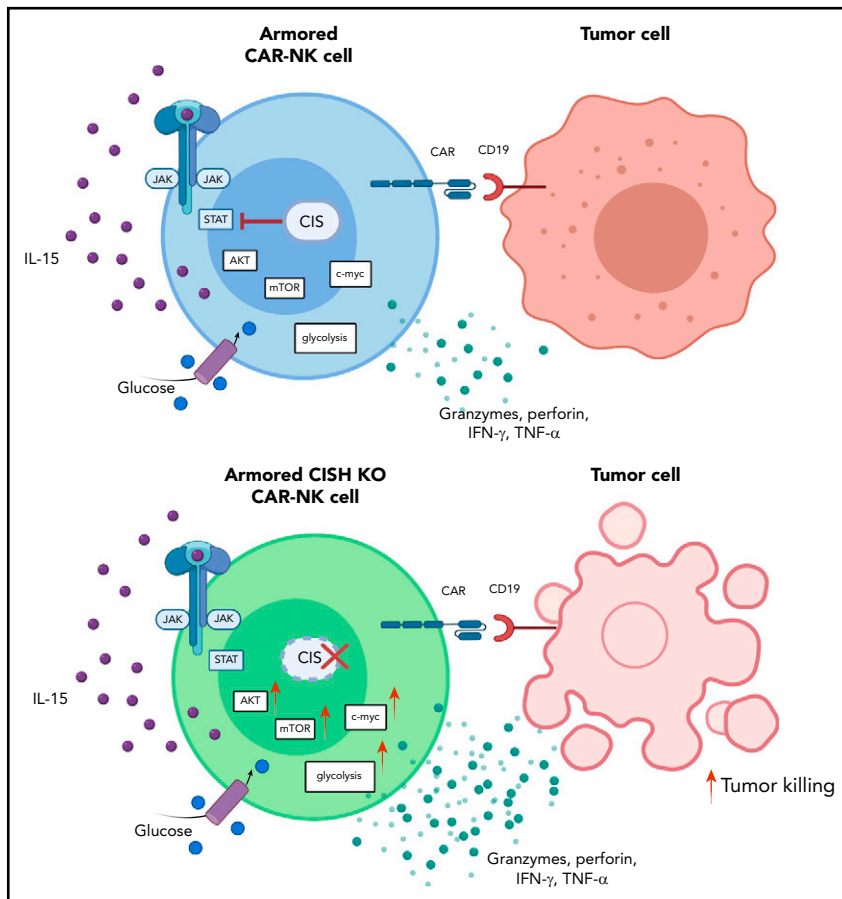
Liu et al have previously demonstrated that cord blood-derived NK cells engineered to secrete interleukin-15 (IL-15) and

to express a CD19-targeted CAR exhibit potent antitumor activity and long-term persistence.<sup>3</sup> In the Daher et al study, the authors investigate the question of whether the effector function and persistence of these IL-15-secreting, so-called “armored” CD19 CAR-NK cells can be further improved by deleting cytokine-inducible Src homology 2-containing protein (CIS), a key negative regulator of IL-15 signaling.

CIS, which is encoded by the *CISH* gene, is a member of the suppressor of cytokine signaling (SOCS) family of proteins. CIS is induced by cytokines such as IL-15 and IL-2 and acts as an important intracellular immune checkpoint in NK cells.<sup>4</sup> Specifically, it uses a negative feedback loop to suppress signaling of the pleiotropic cytokine IL-15, which is known to drive NK-cell activation, expansion, and persistence.<sup>4</sup>

By using a series of in vitro and in vivo experiments, the authors provide a detailed characterization of CD19 CAR-NK cells undergoing CRISPR/Cas9-mediated *CISH* knockout (KO). On a phenotypic level, *CISH* deletion was shown to be associated with prominent features of activation, proliferation, and cytotoxicity. Interestingly, despite the activation phenotype caused by *CISH* KO, no evidence of activation-induced cell death was observed after stimulation with tumor targets in vitro. The effects of *CISH* deletion on the transcriptome included upregulation of genes related to inflammatory and immune responses as well as cytokine signaling. Notably, pathways involved in signaling of interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , IL-2/STAT5 and IL-6/JAK/STAT3 were enhanced.

Taken together, these results support the hypothesis that targeting CIS in CAR-NK cells removes a critical immune checkpoint. When tested against CD19<sup>+</sup> Raji lymphoma cells in vitro, *CISH* KO CAR-NK cells displayed greater cytotoxicity against their targets. From a metabolic standpoint, the study provides evidence that this gain of effector function can be attributed to enhanced IL-15 signaling secondary to removal of the CIS checkpoint, which leads to activation of the AKT/mTOR pathway and c-Myc pathway (see figure). The authors suggest that endogenously secreted IL-15 by CAR-NK cells has to overcome a lower threshold of activity to trigger the AKT/mTOR pathway, which is known to be involved



Deletion of CIS in CAR-NK cells enhances IL-15 signaling by removing an immune checkpoint, which leads to increased activation of the AKT/mTOR pathway and, in the presence of tumor cells, enhanced c-Myc activation. These signaling pathways result in greater glycolytic capacity of CAR-NK cells and subsequently increased cytotoxicity against tumor targets. IFN- $\gamma$ , interferon- $\gamma$ ; JAK, Janus kinase; mTOR, mammalian target of rapamycin; STAT, signal transducer and activator of transcription; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ . See the visual abstract in the article by Daher et al that begins on page 624.

in proliferation and cytotoxicity.<sup>5</sup> In contrast, c-Myc, a mediator of glycolysis,<sup>6</sup> was upregulated only in the presence of Raji lymphoma cells, thereby increasing NK-cell metabolic fitness in response to target cells. These results on improved metabolic function contrast with previous findings which indicated that continuous treatment of human NK cells with IL-15 results in functional changes consistent with exhaustion and thus warrants further in-depth analyses.<sup>7</sup> When Daher et al used an in vivo Raji lymphoma model in their study, CIS checkpoint disruption combined with CAR engineering improved antitumor activity and the persistence of CAR-NK cells.

Although greater tumor control is desirable, unleashing the effector function of CAR-NK cells raises possible concerns regarding the safety of the proposed strategy. In the given setting, the cells not

only ectopically express IL-15, thereby driving inflammatory responses and cell expansion, but in addition, the physiologic negative feedback control of IL-15 signaling is disrupted. With regard to cytokine release syndrome, apparent toxicities such as rapid weight loss or early death were not observed in the present mouse model. However, the NSG mouse model used in the Daher et al study has limitations for assessing cytokine release syndrome, and more detailed investigations will be needed.<sup>8</sup> Furthermore, aberrant IL-15 is known to potentially cause NK lymphoma and to initiate malignant transformation in large granular lymphocytes through induction of Myc.<sup>9</sup> In the Daher et al study, CISH KO did not lead to uncontrolled growth patterns of NK cells or increased expression of genes related to chromosomal instability or DNA damage. Nevertheless, further investigations on prolonged exposure to

IL-15 will be necessary to evaluate potential oncogenic effects of IL-15. With these possible challenges in mind, it is noteworthy that the armored CAR-NK cells used by Daher et al were equipped with an inducible caspase-9 suicide gene as a safety mechanism, which was effective at eliminating CAR-NK cells in vitro and in vivo upon application of a small molecule dimerizer.

In summary, Daher et al propose a strategy for improving NK-cell effector function in the context of IL-15-secreting CD19 CAR-NK cells. Early results of a recent phase 1/2 trial have shown that these CAR-NK cells can induce responses in patients with high-risk CD19<sup>+</sup> cancers without major toxic effects.<sup>10</sup> The Daher et al study suggests that additional deletion of the immune checkpoint CIS releases the brake on IL-15 signaling, which leads to improved tumor control and CAR-NK-cell persistence, and which is an interesting novel aspect to consider in the development of NK cells for cancer immunotherapy.

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## LYMPHOID NEOPLASIA

Comment on Armand et al, page 637

# Checkpoint blockade in FL: eluding the king

Carla Casulo | University of Rochester

**In this issue of *Blood*, Armand and colleagues present results of the phase 2 CheckMate 140 study of programmed death-1 antibody nivolumab in recurrent follicular lymphoma. Although efficacy was limited, the authors did reveal important mechanistic insights into the immune response to nivolumab.<sup>1</sup> Targeted inhibition of cellular immune checkpoints has proven to be a transformative therapeutic advance in both solid tumors and Hodgkin lymphoma. As a heterogeneous disease whose clinical behavior is heavily influenced by properties of the nonmalignant tumor microenvironment, follicular lymphoma is a logical setting in which to investigate immune modulation as treatment. The programmed cell death (PD) pathway, comprising the PD receptors (PD-1 and PD-2) and their ligands (PD-L1/L2), is activated by interactions between PD ligands and receptors, resulting in attenuation of downstream T-cell receptor signaling.<sup>2</sup> This immune checkpoint is among several that play a critical role in modulating the innate antitumor immune response. Multiple tumors exploit this interaction to escape natural antitumor immunity.**

Although follicular lymphoma rarely expresses PD-L1/L2, nonmalignant tumor infiltrating T cells and macrophages in the tumor microenvironment do, and they also express PD-1. Pivotal studies have shown that, in general, elevated numbers of macrophages in the tumor microenvironment are associated with unfavorable outcomes, increased risk of disease progression, and death (immune response 2), whereas increased numbers of T cells correlate with a favorable prognosis (immune response 1).<sup>3</sup> More recently, reduced intratumoral T cells have also been found to portend worse outcomes and increase the risk of early disease progression.<sup>4</sup>

It stands to reason that augmenting the antitumor immune response through checkpoint inhibition would be a promising approach in the treatment of follicular lymphoma. Moreover, elucidating the mechanisms involved in antitumor

immunity would be important for the development of novel therapeutics. In a phase 1 study of 14 patients with follicular lymphoma, the anti-CTLA-4 antibody ipilimumab was well tolerated. The overall response rate was modest at 11% (2 in 18 patients), yet the responses were durable, lasting 1 to 2 years.<sup>5</sup> In combination with rituximab, PD-1 inhibition appears more efficacious, with overall response rates of ~65% (50% to 54% complete response).<sup>6,7</sup> In a phase 1 study of nivolumab, responses were observed in 4 of 10 patients with follicular lymphoma and lasted >1 year.<sup>8</sup> These data led to the CheckMate 140 study, a prospective, open-label, multicenter phase 2 study of adult patients with relapsed or refractory follicular lymphoma following 2 or more therapies. Each therapy must have contained an anti-CD20 antibody or alkylating agent, and at least 1 therapy must have included rituximab. Ninety-two patients

were enrolled and received nivolumab every 2 weeks until disease progression or toxicity. At the discretion of the treating physician, patients were permitted to remain on therapy if clinical benefit beyond progression was perceived. The primary endpoint was objective response rate by independent review committee.

After 12 months of follow-up, objective responses were minimal: 4% by independent review committee and 11% by investigator assessment. Median progression-free survival was 2.2 months, and duration of response was 11 months. Treatment-related adverse events occurred in more than half of patients. Three patients stopped therapy due to immune-mediated adverse events, and there were 3 treatment-related deaths due to respiratory failure, toxic epidermal necrolysis, and erythema multiforme.

Previous studies with more robust responses had combined anti-PD-1 antibodies with rituximab in less heavily pretreated populations, suggesting either synergistic activation of the immune response or a heterogeneous patient population. However, if follicular lymphoma pathogenesis is dependent on complex interactions between the nonmalignant tumor microenvironment and the neoplastic B cell, then interrupting immune tolerance should amplify the antitumor response. Therefore, why does PD-1 blockade monotherapy result in such limited responses? Why do a few patients have durable responses? In an attempt to answer these questions, the authors conducted several exploratory post hoc analyses to look for any biomarkers of response.

Multispectral immunofluorescence was performed on pretreatment samples from subsets of responding and nonresponding patients to identify expression of CD3, CD68, PD-1, and PD-L1. Follicular lymphoma specimens from 4 responding patients and 6 nonresponders revealed that responding patients had significantly higher infiltrating CD3<sup>+</sup> T cells, of which most also expressed PD-1 ( $P = .016$ ). Gene expression profiling was performed in 56 patients, and RNA sequencing was performed in 11 patients, to validate known immune response signatures. As demonstrated in the original pivotal study, increased expression of genes from the immune response 2 signature was strongly associated with poor progression-free survival. Patients with higher than average baseline levels