

whereupon the enzyme glutamate dehydrogenase converts it to α -ketoglutarate. The enzyme α -ketoglutarate dehydrogenase consists of 3 enzymatic subunits; the E1 subunit is responsible for decarboxylating α -ketoglutarate and converting it to the 4-carbon succinate molecule that enzymatically binds to CoA. The enzyme aminolevulinic acid synthase condenses succinyl-CoA with glycine in the mitochondrial matrix, forming the first heme precursor, aminolevulinic acid (ALA). ALA is exported to the cytosol, where several enzymes gradually build the porphyrin ring, which reenters the mitochondria to undergo the 2 final heme biosynthetic steps, which include insertion of iron at the center of the porphyrin ring. Because the pathway from exogenous glutamine to succinate CoA needs large amounts of the enzyme α -ketoglutarate dehydrogenase to direct glutamate into succinyl-CoA, the authors checked levels of the E1 subunit of α -ketoglutarate, and they found that levels increased about fourfold during differentiation, indicating that the cell was able to regulate and remodel important metabolic steps to maintain metabolic functions of the full citric acid cycle despite diversion of large amounts of succinyl-CoA into heme synthesis.

Similar to other studies that alter a widely accepted paradigm, the article by Burch et al raises several questions that merit further study. How does the erythroid cell remodel expression of α -ketodehydrogenase to more efficiently funnel succinyl-CoA into heme biosynthesis? Are levels of the plasma membrane glutamine importer, the mitochondrial glutamate importer, or the plasma membrane and mitochondrial glycine importers also increased in heme-synthesizing erythroid cells? The authors conclude that glutamine plays another undefined role in promoting early erythropoiesis that extends beyond its role in serving as a precursor to heme and nucleotide synthesis. Notably, enzymatic activities of aconitase and isocitrate dehydrogenase were high in erythroid cells, which is interesting because these enzymes are important in a process known as reductive carboxylation, in which α -ketoglutarate is carboxylated by isocitrate dehydrogenase and converted to citrate by aconitase.⁴ A mitochondrial citrate exporter could export a precursor of fatty acid synthesis to the cytosol, which could alter metabolism in other yet unrecognized ways.

The heme biosynthetic pathway has been an unending source of wonder for decades.

Here, the article by Burch et al reminds us that much remains to be learned, and many assumptions may be overturned by experimental interrogation in the future. Newer methodologies such as metabolomics have opened the way to fresh insights into the process of heme biosynthesis.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Svoboda et al, page 1022

CART19 in Hodgkin lymphoma: are we driving the right model?

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In this issue of *Blood*, Svoboda and colleagues describe, for the first time, the safety, feasibility, and activity of a nonviral RNA chimeric antigen receptor (CAR) modified T-cell (CART) construct targeting CD19 in relapsed Hodgkin lymphoma (HL).¹

CART therapy has demonstrated significant activity in relapsed diffuse large B-cell lymphoma and acute lymphocytic leukemia (ALL), resulting in approval by the US Food and Drug Administration in both of these diseases. As CARs move from bench to bedside in other malignancies, there are several key questions that remain. In relapsed HL, there are important benchmarks that an investigational new agent must meet. Is the spectrum of toxicity comparable to that of existing therapies? Can it be safely delivered or are there significant or unusual toxicities? If it is more toxic, does this therapy have significantly greater efficacy than existing therapies? How does response rate and durability compare with those of existing therapies? Does this therapy possess a novel mechanism that expands on therapeutic options for patients with relapsed HL?

HL has a unique biology, in which the Hodgkin and Reed-Sternberg (HRS) tumor cells make up a small fraction (0.1%) of the cells in the HL microenvironment and reside in a milieu of reactive inflammatory cells.

These nonmalignant inflammatory cells produce soluble and membrane-bound molecules that promote tumor cell growth, evasion of self-immunity, and survival.² The T cells in the HL microenvironment demonstrate anergy to recall antigen when stimulated, and an association between high numbers of CD68⁺ tumor-associated macrophages and shortened survival has been described.³

This biology suggests that CART approaches could be uniquely potent and toxic. A review of the current literature reveals 2 studies that report CD30 CARs in HL using viral vectors, which do not report any unusual or excessive toxicity,^{4,5} suggesting that in small numbers of patients with CD30-targeted CARs, the unique HL tumor microenvironment (TME) does not lead to unusual or excessive toxicity. In this context, with this data, it is unclear why the investigators chose their approach of transfecting T cells with messenger RNA using electroporation in contrast to a viral vector. This approach leads to a transient expression of the CAR with the intention

of attenuating adverse effects such as cytokine release syndrome or neurotoxicity, which is justified by the authors by their desire to minimize toxicity. There is a clear safety signal with this approach: primary grade 1 headache and confusion are confirmatory; however, these CARs were extremely transient and had disappeared in all patients by day 21.

The choice of CD19 as a target is similarly curious for HL. The expression of CD30 on HRS cells is ubiquitous, and the activity of brentuximab vedotin (BV), the antibody drug conjugate (ADC) against CD30, clearly shows the viability of CD30 as a therapeutic target.⁶ In contrast, activity was modest in both CD30 CART studies,^{4,5} suggesting that although CD30 is an appropriate target for an ADC, it may be less than optimal for immunotherapy. The high and durable response rate of the checkpoint inhibitors pembrolizumab and nivolumab in relapsed HL clearly demonstrate the activity of therapies that activate the TME rather than targeting the HRS cells directly.^{7,8} However, the role of CD19⁺ B cells in the TME is controversial, and although the rich cytokine cross talk in the TME contributes to HRS growth and survival, it is far from clear that CD19⁺ B cells are the prime driver.

The clinical activity described for the anti-CD19 CART (CART19) in the Svoboda et al study was modest. One patient was taken off study. For 4 patients (3 of whom received bridging therapy and all of whom received conditioning chemotherapy), the response rate was 50% (1 complete response and 1 partial response), with progression at 3 months in the patient who had a complete response. The short duration of progression-free survival, even in responders, shows that this therapy is more a proof of concept than a potential novel therapy. Current approved therapies such as BV or the checkpoint inhibitors have high response rates and long response durations in responding patients. How then, can this therapy be improved upon, and what place does it have as a potential new therapy?

Rational design of CARs for relapsed HL should begin from the ground up. Because toxicity has been mild to date, design of future therapies using lentiviral vectors should be considered, potentially adding a molecular suicide option which could provide a safety valve. To date, neither CD19 nor CD30 has proved to be

a clear winner as a therapeutic target for HL CARs. But as the data for the combination of checkpoint inhibitors and BV clearly show, dual CARs targeting both the HRS cells and immune activation of the TME might overcome this. Perhaps CD19 is the ideal target for cotargeting with CD30. However, macrophage or natural killer cell targets should also be considered. The question of the optional target(s) in HL remains to be answered. For CARTs to find a place among therapies for relapsed HL, they will need to do this.

In summary, the article by Svoboda et al provides a proof of concept for the safety and feasibility of a nonviral CART19 in relapsed HL. Toxicity is mild, but with a viral vector and greater CAR persistence, will it increase? Activity of the CART19 in relapsed HL is modest and does not appear to be durable. Although the data for checkpoint blockade plus BV or chemotherapy will set a high bar for therapies in relapsed HL,^{9,10} there remains a population of patients who are relapsed or refractory to standard therapies, to BV, and to checkpoint inhibitors. For these young patients who have no viable therapeutic options, these new CARs cannot come down the road soon enough.

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LYMPHOID NEOPLASIA

Comment on Morelli et al, page 1050

A miRaculous new therapy in myeloma?

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In this issue of *Blood*, Morelli et al describe a novel method for targeting cancers with dysregulated c-MYC (MYC), such as multiple myeloma, by inhibiting the micro-RNA 17-92 (miR-17-92) cluster through degradation of its precursor RNA.¹

Early work by Evan and colleagues initially established that dysregulation of MYC could sensitize cells to apoptosis,

providing evidence that overcoming apoptosis is required for cells to survive the inappropriate proliferation induced