# Epigenetic Inhibition Puts Target Antigen in the Crosshairs of CAR T Cells

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The successes of chimeric antigen receptor (CAR) T cell therapies targeting hematologic malignancies have not yet translated to solid tumors due to challenges with antigen selection, tumor trafficking, T cell persistence, and the tumor microenvironment (TME). In this issue of *Molecular Therapy*, Kailayangiri et al.[1] report that inhibiting Enhancer of Zeste Homolog 2 (EZH2) to upregulate tumor antigen GD2 synthase (GD2) expression in Ewing sarcoma cells circumvents its variable expression. The combination of EZH2 inhibition therapy with CAR T cell therapies may therefore improve outcomes in Ewing sarcoma and potentially other malignancies.

CAR T cell therapies combine the signaling receptor complex of a T cell with the specificity of a monoclonal antibody, typically via a single chain variable fragment. Studies using a CD19-targeted CAR T cell for the treatment of B cell leukemia and lymphoma have produced impressive response rates, and two of these products received US Food and Drug Administration (FDA) approval.[2,3] Despite the impressive results of CAR T cell therapy for CD19+ hematologic malignancies, there are formidable obstacles that limit its success against the vast majority of solid tumors.[4] These challenges include the limited *in vivo* expansion and persistence of adoptively transferred T cells and tumor-mediated inhibition of T cell-induced responses as well as difficulties identifying target antigens expressed selectively at high densities on tumor tissue but not normal cells.

GD2 is a target that is consistently expressed on neuroblastoma cells, and several trials of T cells genetically modified with a GD2-CAR have reported clinical activity with no off-target toxicity.[5,6] Extending the use of GD2-CAR T cells beyond neuroblastoma to the other tumors that express this antigen is challenging, as their expression of the GD2 antigen is lower and more heterogeneously expressed. Kailayangiri and colleagues[1] focus on Ewing sarcoma, an aggressive solid tumor in bone and soft tissues with a limited number of targetable antigens. GD2 is present in Ewing sarcoma, but only a small number of tumor cells highly express GD2, limiting its value as a target. Here, the authors report an epigenetics-based solution to enable GD2-CAR T cells to target this antigen-sparse tumor.

Epigenetic modification has been evaluated in other cancers where inhibition of histone deacetylases (HDACs), a group of enzymes responsible for regulating gene expression, has improved immunogenicity and recognition by the immune system of some solid cancers. For example, HDAC inhibition in a melanoma tumor model enhanced the anti-tumor efficacy of an anti-programmed cell death 1 (PD-1) antibody.[7] Through an elegant sequence of experiments, Kailayangiri et al.[1] established that EZH2 is a potent histone methyltransferase and its upregulation in Ewing sarcoma silences genes involved in cell differentiation and potentiates tumorigenicity. Based on these data, the authors hypothesized that EZH2 may be involved in the regulation of synthesis of GD2 in Ewing sarcoma and, thus, that EZH2 inhibitors may promote GD2 expression.

The authors found that high doses (10–12 μM) of a small molecule EZH2 inhibitor induced GD2 expression in six of nine previously GD2-negative cell lines, while low doses (4 uM) increased expression in five of nine negative lines for up to 28 days in a multilayered three-dimensional (3D) tumor model. Their studies further demonstrated that this effect was selective to Ewing sarcoma by showing that inhibiting EZH2 did not lead to upregulation of GD2 in Jurkat cells, rhabdoid tumor cells, or mesenchymal stromal cells, thereby reducing the risk of on-target, off-tissue toxicity. Importantly, manipulation of GD2 expression did not change *in vitro* proliferation, tumorigenicity, or chemosensitivity of the tumor cells. Lastly, the authors found that GD2-CAR T cells showed increased activation and cytolysis *in vitro* in coculture with GD2+ Ewing sarcoma cells previously treated with EZH2 inhibitor compared to the same cell lines without prior forced expression of GD2 by EZH2 inhibition. The strategy proposed herein is therefore a clinically relevant approach to enable recognition of tumor cells with low or negative target expression.

As GD2-CAR T cells have shown only modest antitumor activity even against Ewing sarcoma cells with significant GD2 expression in previous mouse studies, Kailayangiri et al.[1] tested their pharmacological approach against multiple Ewing sarcoma cell lines in a 3D *in vitro* model. These prior mouse studies reflect the additional difficulties of targeting solid tumors with CAR T cells.[8] For instance, the immunosuppressive TME of solid tumors blocks T cell activation through molecules such as programmed cell death ligand 1 (PDL-1) and transforming growth factor β (TGF-β), which inhibit the immunostimulatory cytokines necessary for preserving optimal T cell activity. Several strategies are being explored to improve the activity of CAR T cells in solid tumors like Ewing sarcoma, including a combination with checkpoint inhibition and genetic engineering of the T cells to overexpress cytokine-signaling

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systems (interleukin 7 [IL-7], IL-2, IL-15).[9,10] Thus, the epigenetic approach described here to increase target antigen expression would likely need to be combined with other strategies to overcome additional tumor evasion mechanisms.

In summary, the work performed by Kailayangiri et al.[1] identifies a novel epigenetic mechanism to overcome one major obstacle facing CAR T cell therapy for Ewing sarcoma: heterogeneous and low target antigen expression. As there remain major limitations to the efficacy of CAR T cells for the treatment of solid tumors, this strategy to amplify tumor-associated antigens will likely need to be combined with other approaches to enhance the function and persistence of adoptively transferred CAR T cells.

## AUTHOR CONTRIBUTIONS

D.H.M.S. and H.E.H. wrote the article.

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# AAV Engineering Identifies a Species Barrier That Highlights a Portal to the Brain

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In this issue of *Molecular Therapy*, Hordeaux et al.[1] present a new chapter in the fascinating story on gene transfer across the blood-brain barrier (BBB). Few in our field—and beyond—would have predicted the twists and turns in the story of this unexpected biology with transformative therapeutic implications.

The tale starts in 2008 with a series of observations in which a 25 nm proteinaceous adeno-associated virus (AAV) particle at a high dose could transduce targets in the peripheral and CNS via systemic routes of administration in mice[2,3] and larger animals.[3,4] Moreover, the efficiencies of gene transfer supported remarkable levels of correction of disease models, most notably in spinal muscular atrophy (SMA).[3,5] A decade later, these findings culminated in a successful Ph1/2 study for SMA type 1 and the likelihood of a drug approval for this otherwise fatal disorder.[6] This initial academic success was translated commercially by Avexis (now acquired by Novartis), and this SMA program is currently being reviewed for drug approval by the US Food and Drug Administration (FDA).

These findings and developments have energized a field of vector discovery to further improve on this unique ability that allows gene transfer to the CNS and peripheral nervous system via a non-invasive injection route. That potential was reached in 2016 when Deverman et al.[7] identified an AAV variant that leapfrogged AAV9 in its ability to traverse the BBB.

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