

of the dimer are not identical in conformation. A shift of the  $\alpha 2$  helix keeps the active site “open” in one subunit but “closed” in the other. This feature of 12-LOX may impact ligand binding and catalysis, especially if the 2 conformations of the active site preexist in equilibrium and interconvert on a time scale comparable to substrate binding, as observed in allosteric enzymes.<sup>7</sup>

To further support the functional importance of different oligomeric states of 12-LOX, a fatty acid acyl-coenzyme A (CoA) molecule of unconfirmed nature is found in the active site in the open form of all 12-LOX oligomers, but resolved with confidence only in the tetramer and hexamer. The observation is physiologically relevant because fatty acid acyl-CoA derivatives inhibit platelet aggregation in a chain-, length-, and saturation-dependent manner through interference with P2Y1 and P2Y12 receptors.<sup>8</sup> It is possible that fatty acid acyl-CoA, especially oleyl-CoA identified by the authors from enzymatic assays, functions as endogenous inhibitor of 12-LOX and contributes to suppress platelet aggregation. Related to this issue is new structural information on the binding mode of ML355, a selective and potent inhibitor of human platelet 12-LOX<sup>9</sup> capable of preventing thrombus formation without impairing hemostasis.<sup>10</sup> The inhibitor recently concluded phase I clinical trials for the treatment of HIT and received fast-track designation by the Food and Drug Administration. It is conceivable that ML355 may also find applications as an anticancer and anti-metastatic agent. Previous docking and mutagenesis studies predicted that ML355 would penetrate the active site of 12-LOX, but the cryo-EM structure instead shows ML355 binding to an allosteric site at the entrance to the active site and only in the hexamer. The very high resolution of the cryo-EM structure also enables direct validation of detailed contacts responsible for formation of the complex by site-directed mutagenesis. The stage is now set for further optimization of ML355 and the development of new inhibitors of 12-LOX using structure-based approaches.

The work by Mobbs et al<sup>1</sup> broadens our understanding of 12-LOX at the structural level and will definitely impact future studies of this intriguing enzyme

as a target of antiplatelet and possibly anticancer therapy. New efforts should address the functional properties of each oligomeric state of 12-LOX, the role of the open and closed states of the active site in catalysis, and the biological consequences of inhibition by endogenous fatty acid acyl-CoA. Importantly, the structure of 12-LOX pushes the limits of resolution that can be achieved with cryo-EM when investigating proteins relevant to hemostasis and thrombosis. It sets a high bar for future studies, but also shows what is possible with application of this revolutionary technique. So, pass the 12-LOX!

**Conflict-of-interest disclosure:** The author declares no competing financial interests. ■

## REFERENCES

1. Mobbs JI, Black KA, Tran M, et al. Cryo-EM structures of human arachidonate 12S-lipoxygenase bound to endogenous and exogenous inhibitors. *Blood*. 2023;142(14):1233-1242.
2. Contursi A, Tacconelli S, Hofling U, et al. Biology and pharmacology of platelet-type 12-lipoxygenase in platelets, cancer cells, and their crosstalk. *Biochem Pharmacol*. 2022;205:115252.
3. Johnson EN, Brass LF, Funk CD. Increased platelet sensitivity to ADP in mice lacking platelet-type 12-lipoxygenase. *Proc Natl Acad Sci U S A*. 1998;95(6):3100-3105.
4. Yeung J, Tourdot BE, Adili R, et al. 12(S)-HETE, a 12-lipoxygenase oxylipin of

dihomo-gamma-linolenic acid, inhibits thrombosis via G $\alpha$ s signaling in platelets. *Arterioscler Thromb Vasc Biol*. 2016;36(10):2068-2077.

5. Kobe MJ, Neau DB, Mitchell CE, Bartlett SG, Newcomer ME. The structure of human 15-lipoxygenase-2 with a substrate mimic. *J Biol Chem*. 2014;289(12):8562-8569.
6. Aleem AM, Jankun J, Dignam JD, et al. Human platelet 12-lipoxygenase, new findings about its activity, membrane binding and low-resolution structure. *J Mol Biol*. 2008;376(1):193-209.
7. Di Cera E. Mechanisms of ligand binding. *Biophys Rev*. 2020;1(1):011303.
8. Manolopoulos P, Glenn JR, Fox SC, et al. Acyl derivatives of coenzyme A inhibit platelet function via antagonism at P2Y1 and P2Y12 receptors: a new finding that may influence the design of anti-thrombotic agents. *Platelets*. 2008;19(2):134-145.
9. Luci DK, Jameson JB 2nd, Yasgar A, et al. Synthesis and structure-activity relationship studies of 4-((2-hydroxy-3-methoxybenzyl)amino)benzenesulfonamide derivatives as potent and selective inhibitors of 12-lipoxygenase. *J Med Chem*. 2014;57(2):495-506.
10. He H, Adili R, Liu L, Hong K, Holinstat M, Schwendeman A. Synthetic high-density lipoproteins loaded with an antiplatelet drug for efficient inhibition of thrombosis in mice. *Sci Adv*. 2020;6(49):eabd0130.

<https://doi.org/10.1182/blood.2023021939>

© 2023 by The American Society of Hematology

## IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on [Graham et al](#), page 1248, and [Karschnia et al](#), page 1243

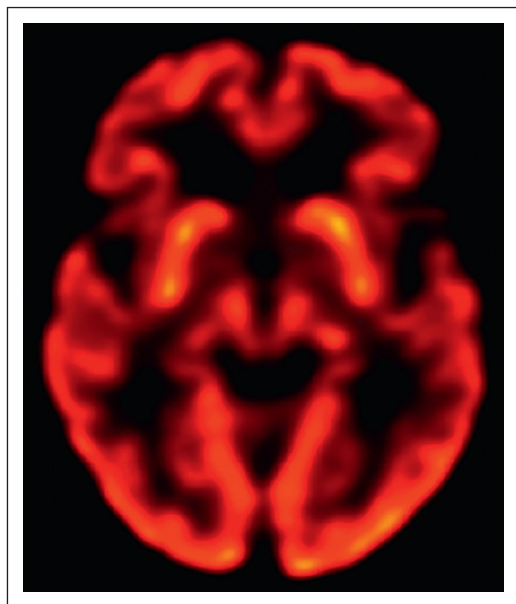
# BCMA-CAR T-cell treatment–associated parkinsonism

**Juliane Gust** | Seattle Children’s Research Institute and University of Washington

**In this issue of *Blood*, [Graham et al](#)<sup>1</sup> and [Karschnia et al](#)<sup>2</sup> provide new insights into the rare hypokinetic movement disorder (also termed movement and neurocognitive treatment emergent adverse events) that has been observed with BCMA-chimeric antigen receptor (CAR) T-cell treatment for multiple myeloma.**

A subacute to late-emerging hypokinetic movement disorder with many features of parkinsonism was first reported in 5 patients enrolled in the CARTITUDE-1

study for ciltacabtagene autoleucel (cilta-cel).<sup>3-5</sup> Patients presented weeks to months after CAR T-cell infusion with slowing of movements, limb rigidity,



Normal uptake in the basal ganglia on brain FDG-PET in a BCMA-CAR T-cell patient with reversible parkinsonism. See Figure 1G in the article by Graham et al that begins on page 1248.

micrographia, tremor, and other typical parkinsonian symptoms. These symptoms were not fully reversible even with aggressive immunomodulation. However, many of the patients died from other causes, making the true reversibility uncertain. Subsequent clinical trials of the same product reported a lower but nonzero rate of parkinsonism. Puzzlingly, no analogous cases had been published until now for idecabtagene vicleucel (ide-cel), although there was mention of grade 3 parkinsonism in the package insert. For the first time, Karschnia et al now report a newly emergent movement disorder in a patient treated with ide-cel. More details on the timing and evolution of the symptoms will be helpful to ascertain whether this case truly falls within the spectrum of cases seen with ciltacel.

Karschnia et al also examined risk factors for conventional immune effector cell-associated neurotoxicity syndrome (ICANS) in a mixed cohort of 76 patients treated with either ciltacel or ide-cel in a real-world setting or with not otherwise specified BCMA-CAR T cells as part of a clinical trial. Of these patients, 40.8% developed ICANS, which was higher than the incidence reported in most of the pivotal studies for ide-cel and ciltacel. Similar to what is seen with CD19-CAR T cells, cytokine release

syndrome was identified as a major risk factor. Hypoalbuminemia was also associated with higher ICANS risk.

Two of the BCMA-CAR patients with ICANS later went on to develop parkinsonism, one on day 22 after ide-cel and the other on day 19 after ciltacel. The latter patient is described in further detail by Graham et al, in a case that is notable for being the first report of fully reversible BCMA-CAR-associated movement disorder. There was a tight temporal correlation of emergence of neurologic symptoms and high counts of peripheral circulating CAR T cells. Symptoms completely resolved after CAR T cells were depleted with a single dose of cyclophosphamide. These findings suggest that there may be a causal relationship between the burden of circulating CAR T cells and parkinsonism symptoms. An on-target off-tumor activity against targets in the brain could be consistent with this picture, although the authors are careful to avoid any speculation that is not supported by their data. The fact that the symptoms were so readily reversible is encouraging, but also puzzling. If there is a destructive process at work, one would not expect such easy reversibility.

The overall gestalt of clinical examination findings in all patients reported to date is indeed most consistent with

parkinsonism, a clinical syndrome that occurs with disruption of dopaminergic signaling in the brain. This can be due to loss of substantia nigra dopaminergic neurons in Parkinson disease, but also due to a variety of other mechanisms such as acquired striatal injury from stroke or hypoxia or drugs that interfere with dopaminergic neurotransmission. In the few BCMA-CAR patients where it has been reported, dopamine transporter imaging has been normal and histopathology has shown no loss of substantia nigra dopaminergic neurons. No patients with BCMA-CAR-associated parkinsonism have responded to carbidopa/levodopa. Instead, there is some evidence that the problem lies in the striatum, which receives dopaminergic innervation from the substantia nigra. Although the patient in Graham et al had normal positron emission tomography using fludeoxyglucose imaging (FDG-PET) of the basal ganglia (see figure), the ide-cel patients in Van Oekelen et al<sup>5</sup> and in Karschnia et al had decreased uptake in the bilateral caudate nuclei. BCMA (=TNFRSF17) is detectable by RNA sequencing (RNA-seq) in the human striatum through development into young adulthood ([www.brainspan.org](http://www.brainspan.org)). Since this finding comes from bulk RNA-seq data sets, it is unknown what cell type this constitutes. BCMA is not detectable by single-cell RNA-seq in other brain regions in adults (<https://portal.brain-map.org/atlas-and-data/maseq/human-m1-10x>). In adult striatum, there is no BCMA RNA detectable by in situ hybridization, and no protein is convincingly detectable by immunohistochemistry.<sup>6</sup> Although this evidence is reassuring, we still cannot conclusively rule out low-level expression in normal adult brain.

The late onset of parkinsonism, multiple weeks to months after CAR T-cell infusion, may not be surprising. In other pathogenic processes, such as hypoxic injury or stroke to the basal ganglia, parkinsonism often emerges months to even years after the acute injury, presumably due to reorganization of neuronal circuits.<sup>7</sup> Thus it is possible that an insult to the striatum may have occurred in the setting of CRS and ICANS, with the movement disorder not emerging until later, and potentially also spontaneously resolving over time. The single report of successful remission of

parkinsonism after treatment with cyclophosphamide, although encouraging, should be treated with equipoise until further evidence becomes available.

Future investigations should continue to closely monitor patients for development of unusual toxicities. Timely expert clinical assessment is key, which can be an issue with patients receiving CAR T cells in a real-world setting. For BCMA-CAR patients with concern for movement disorder, this should include carefully documented serial neurologic examinations and advanced neuroimaging as directed by experts (such as FDG-PET and dopamine transporter imaging). As more sophisticated tools become available, we should reassess the presence of CAR targets in the brain. Efforts are underway to ensure more transparent case reporting, which will

hopefully reduce confusion about the true incidence of these rare toxicities.

*Conflict-of-interest disclosure:* The author declares no competing financial interests. ■

## REFERENCES

1. Graham CE, Lee W-H, Wiggin HR, et al. Chemotherapy-induced reversal of ciltacabtagene autoleucel-associated movement and neurocognitive toxicity. *Blood*. 2023;142(14):1248-1252.
2. Karschnia P, Miller KC, Yee AJ, et al. Neurologic toxicities following adoptive immunotherapy with BCMA-directed CAR T cells. *Blood*. 2023;142(14):1243-1248.
3. Berdeja JG, Madduri D, Usmani SZ, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. *Lancet*. 2021; 398(10297):314-324.
4. Cohen AD, Parekh S, Santomaso BD, et al. Incidence and management of CAR-T neurotoxicity in patients with multiple myeloma treated with ciltacabtagene autoleucel in CARTITUDE studies. *Blood Cancer J*. 2022;12(2):32.
5. Van Oekelen O, Aleman A, Upadhyaya B, et al. Neurocognitive and hypokinetic movement disorder with features of parkinsonism after BCMA-targeting CAR-T cell therapy. *Nat Med*. 2021;27(12):2099-2103.
6. Marella M, Yao X, Carreira V, et al. Comprehensive BCMA expression profiling in adult normal human brain suggests a low risk of on-target neurotoxicity in BCMA-targeting multiple myeloma therapy. *J Histochem Cytochem*. 2022;70(4):273-287.
7. Alarcón F, Zijlmans JCM, Dueñas G, Cevallos N. Post-stroke movement disorders: report of 56 patients. *J Neurol Neurosurg Psychiatry*. 2004;75(11): 1568-1574.

<https://doi.org/10.1182/blood.2023021860>

© 2023 by The American Society of Hematology