TO THE EDITOR:

BACH2 is a putative T-cell lymphoma tumor suppressor that may play a role in product-derived CAR T-cell lymphomas

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We read with great interest the recent article published in *Blood* by Micklethwaite et al, "Investigation of product-derived lymphoma following infusion of *piggyBac*-modified CD19 chimeric antigen receptor T cells."[1] In this article, the authors conducted a phase 1 clinical trial of *piggyBac* transposon–generated CD19-targeted chimeric antigen receptor (CAR) T cells in patients with relapsed or persistent B-cell lymphomas following allogeneic hematopoietic stem cell transplant. The authors describe the first 2 reported cases of T-cell lymphomas arising from CAR-modified T cells and perform detailed immunological, genomic, and transcriptomic analyses of these cells. Their work represents a highly important investigation into CAR T-cell safety.

The authors observed that the CAR lymphomas of both patients had insertions into introns 3 and 4 of *BACH2*, which were associated with mildly reduced expression of the gene. For several reasons, Micklethwaite et al argued that *BACH2* inactivation may not contribute to lymphomagenesis. First, they note an absence of previous reports implicating *BACH2* in T-cell lymphoma. Second, they contend that because *BACH2* is already expressed at low levels in untransformed memory T cells, further downregulation of *BACH2* levels by these insertions may not have a sufficient molecular effect to drive positive selection. Moreover, they note enrichment of retroviral insertion sites in the *BACH2* gene locus. These data suggest that recurrent *BACH2* insertions could thus result from the locus being a hotspot for insertion of foreign genetic material, rather than positive selection for *BACH2*-disrupted T cells.

However, in a paper recently published in *Blood* (Park et al[2]), we identify *BACH2* as a putative tumor suppressor in T-cell malignancies for the first time. In our study, we performed whole-genome sequencing in patients with cutaneous T-cell lymphoma (CTCL). We used previously published algorithms to identify single nucleotide variants, copy number variants, and translocation events.[3-5] The data, detailed methods, and description of samples are available in our paper. Our study was approved by the Northwestern University Institutional Review Board and was conducted in accordance with the Declaration of Helsinki.

Multiple lines of evidence support a tumor suppressor role for *BACH2* in CTCL. First, we performed an unbiased analysis for recurrent translocations that occur more often than expected by chance. This analysis identified *BACH2* as the most significantly affected gene (8 samples, $P_{adj} = 1.3 \times 10^{-7}$). These appear to be loss-of-function translocations, as they do not have a recurrent translocation partner. Moreover, the intronic breakpoint events occur in important *BACH2* enhancers[6,7]between the start codon and the transcriptional start site (introns 1-5). These structural variants are predicted to inactivate gene expression by uncoupling the promoter from the protein-coding messenger RNA sequences (Figure 1). As is typical for tumor suppressors, there are also additional deleterious mutations. In this case, there are 6 additional samples with deletions that affect part of or the entire gene and an additional nonsynonymous point mutation. In total, we identified *BACH2* alterations in 14% of CTCLs (Figure 1).

Like the breakpoints identified in CTCL, the CAR insertions observed by Micklethwaite et al occurred in enhancers in introns 3 and 4 (Figure 1). It is therefore not surprising that their insertion events lead to downregulation of gene expression in the malignant cells.[1] In Micklethwaite et al, both patients had mild downregulation of *BACH2* expression levels (patient 2: -0.17 $\log_2$ fold change; patient 8: -0.71 $\log_2$ fold change) in the CAR lymphoma as compared with the initial CAR product. Although others have observed that normal memory $CD4^+$ T cells express less *BACH2* than normal naive $CD4^+$ T cells,[8] modest decreases in BACH2 protein expression in T cells may be sufficient to cause clinical disease. Germline heterozygous *BACH2* mutations reduce BACH2 protein expression in $CD4^+$ T cells by only ~25%. Nonetheless, they are sufficient to drive a clinical syndrome characterized by immunodeficiency and autoimmunity.[9] The changes in T-cell immunophenotype can also be recapitulated by small interfering RNA reduction of BACH2 levels in healthy T cells and by haploinsufficiency of BACH2 in vivo in mice.[9]

BACH2 normally functions in T cells to limit expression of T-cell receptor–dependent transcriptional programs. It limits access of AP-1 transcription factors to their target DNA sequences.[10] In Jurkat cells, BACH2 inhibits T-cell receptor–dependent AP-1–mediated induction of interleukin-2, a proproliferative cytokine.[11] Of cancer relevance, the AP-1 pathway mediates drug resistance in CTCL[12] and promotes growth and survival of other T-cell lymphomas.[13]

Disruption of T-cell lymphoma tumor suppressors may improve CAR T-cell fitness without inducing malignant transformation. For example, lentivirally produced CAR T cells have been observed to have clonal expansion of cells with inactivation of *TET2*, another T-cell lymphoma tumor suppressor.[14,15] A phase 1 trial[16] utilizing T-cell receptor–transgenic T cells with CRISPR/Cas9 editing of the T-cell lymphoma tumor suppressor PD-1[17] was recently reported and did not detect malignant transformation. Notably, the

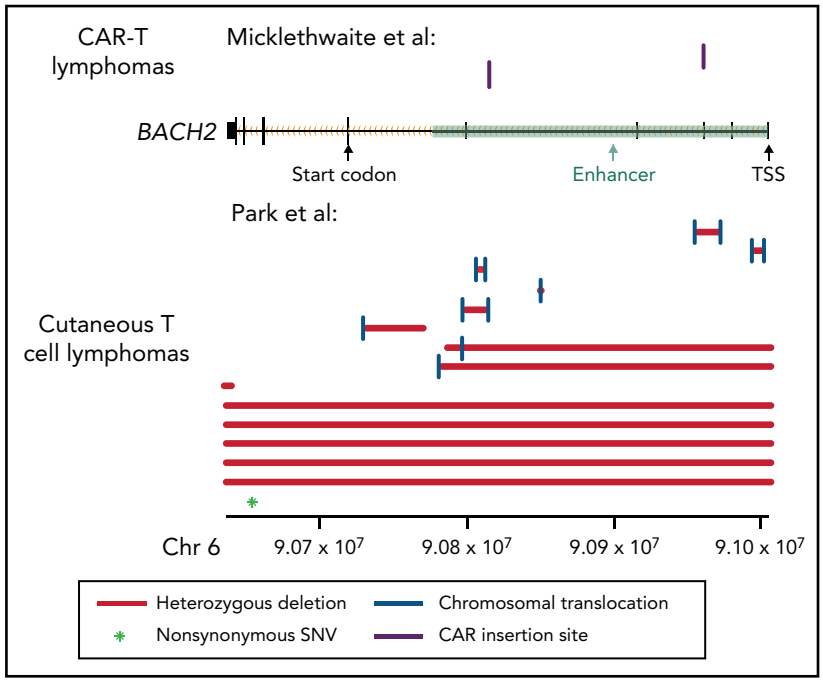

Figure 1. Mutation pattern consistent with a tumor suppressor role in BACH2. Alterations in the BACH2 gene locus in patients with CAR T-cell lymphomas (Micklethwaite et al[1]) and in CTCL (Park et al[2]). Black rectangles represent BACH2 exons. Coordinates shown use the hg19 genome build. SNV, single nucleotide variant; TSS, transcriptional start site.

follow-up time was insufficient to exclude CAR malignancies occurring up to 11.5 months following infusion as reported in the Micklethwaite et al trial.[1]

CAR T-cell manufacturers must balance the risks and benefits of increasing efficacy and/or risk of malignant transformation. Thus, it is important to identify genetic events that can plausibly induce lymphomagenesis and functionally validate their effects. Additional functional studies are required to elucidate whether insertions in *BACH2* had a causal impact on lymphomagenesis in the Micklethwaite et al trial. As noted by the authors, *BACH2* is a hotspot for both recurrent retroviral and piggyBac insertions in primary human $CD4^+$ T cells.[18] However, it remains unclear whether the recurrence rate of insertions in the *BACH2* locus was due to a preference for the insertion into this site or due to positive selection pressure for *BACH2* mutant T cells. Last, because the clinical phenotypes of CTCL and CAR T-cell lymphomas differ, it will be important to address whether there are context-dependent effects of genes such as *BACH2* or other tumor suppressors. If CAR T-cell lymphoma tumor suppressors are known, this information can be leveraged to screen genetically engineered T-cell products for those most likely to result in CAR product-derived cancers.

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Authorship

Contribution: J.D. and J.C. designed the project, analyzed the data, and wrote the manuscript.

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All data supporting this analysis are available in the article by Park et al.[2]

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TO THE EDITOR:

Antiplatelet drugs block platelet activation by VITT patient serum

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Vaccines are an important part of the response to the SARS-COV-2 global pandemic. Although rare, aggressive thrombotic events at unusual sites, with accompanying thrombocytopenia and bleeding with high mortality, have increasingly been reported in young, healthy individuals at 4 to 30 days after vaccination with the Oxford-AstraZeneca chimpanzee adenovirus-vectored ChAdOx1 nCoV-19 (AZD1222).[1,2] This syndrome of vaccine-induced immune thrombocytopenia and thrombosis (VITT) clinically resembles autoimmune heparin-induced thrombocytopenia (HIT), in which antibodies against platelet factor 4 (PF4) bind and cross-link to the platelet surface receptor FcγRIIA (CD32a), inducing platelet activation.[1-3] VITT after the first AZD1222 vaccination has a reported incidence of between 1 in 25 000 and 1 in 100 000.[2,4,5]

In this study, we investigated the effect of serum from patients with VITT on platelet activation monitored by light transmission aggregometry (LTA), assessing the ability of clinically available antiplatelet drugs and kinase inhibitors to prevent platelet aggregation in vitro. Blood collection from patients, healthy individuals after AZD1222 vaccination, and nonvaccinated healthy donors were authorized under research ethics approvals 15/NW/0079 and 20/HRA/1817 and Birmingham University Internal Ethical Review approval ERN_11-0175, respectively. Experimental procedures are detailed in the supplemental Information (available on the *Blood* Web site).

Patients (or their next of kin in the case of those patients who lacked capacity) gave informed consent for collection of their blood in line with ethical principles laid out in the Declaration of Helsinki.

The presentations of 7 patients with VITT are summarized in Table 1. All patients were Caucasian and under the age of 50 with no previous symptomatic COVID-19. Patients presented with thrombosis (6 patients with cerebral venous sinus thrombosis [CVST] and 1 patient with ischemic stroke) and thrombocytopenia 9 to 14 days after the first AZD1222 vaccination. Clinical investigation at the time of presentation revealed all patients had thrombocytopenia (range, 7-113 $\times$ 10^9 platelets per L), with massively elevated D-dimer (range, 6574-62 342 ng/mL) and low fibrinogen (range, <0.35-2.36 g/L) levels. Despite no prior heparin exposure, HIT screening (anti-PF4 IgG Immucor enzyme-linked immunosorbent assay) showed strong reactivity in all patients. Heparin-induced platelet activation (HIPA) assays in the 4 patients tested showed activation in response to patient serum that was reduced by low heparin concentrations and blocked by high ones. Similar findings are reported in other patients with VITT.[1,2] All patients received IVIg and the steroid dexamethasone, as recommended by VITT treatment guidelines,[6] and 2 patients received plasma exchange. Platelet counts improved over 1 to 4 days in all patients except 1 who died 24 hours after presentation. At the time of this writing, 3 patients had recovered and been discharged from the hospital with ongoing normal platelet counts, 1 patient remained in hospital, and 2 patients had died because of the sequelae of CVST and secondary intracerebral hemorrhage. In addition, 1 discharged patient,