G-CSF mobilization effect. The potential tumor purging effect of ixazomib in addition to its mobilizing activity would be highly desirable in patients with multiple myeloma. The clinical role of ixazomib as a rapid mobilizing agent in man is yet to be determined.

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Authorship
Contribution: A.G., M.P.R., L.E., and J.F.D. designed and analyzed the experiments and wrote the paper; A.G., M.P.R., M.S.H., K.K., E.C., and J.K.R. performed the animal study; and all authors discussed the results and commented on the paper.

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TO THE EDITOR:
False-positive results with select HIV-1 NAT methods following lentivirus-based tisagenlecleucel therapy
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Genetically reprogrammed T-cell therapy is a novel treatment approach being investigated in various hematologic malignancies, including relapsed and refractory pediatric B-cell acute lymphoblastic leukemia (B-ALL), multiple myeloma, diffuse large B-cell lymphoma, and chronic lymphocytic leukemia.1-3 This therapy is accomplished by ex vivo transduction of patient-derived T cells with a viral vector containing a chimeric antigen receptor (CAR). CARs are genetically engineered to contain an scFv domain targeting a tumor-associated protein, together with the CD3ζ domain of the T-cell receptor and costimulatory domain (eg, 4-1BB or CD28).4 Tisagenlecleucel contains a CD19-directed CAR with the 4-1BB costimulatory domain and uses lentiviral vector technology for gene transfer. Tisagenlecleucel is approved for the treatment of pediatric and young adult patients with B-ALL that is refractory or in second or later relapse.5 CTL119, another CAR T-cell therapy, contains a humanized anti-CD19 scFv domain and is under investigation for the treatment of chronic lymphocytic leukemia and pediatric acute lymphoblastic leukemia.6

Lentiviral vector technology, highly modified from HIV-1, is used to transduce human cells and induce stable, long-term transgene expression by integration into the host genome.5-8 Tisagenlecleucel and CTL119 use a self-inactivating lentiviral vector system in which the transgene is encoded on a transfer plasmid between viral long terminal repeats, whereas genes necessary for lentivirus assembly are encoded on separate vectors.9 Upon transduction, long terminal repeats are integrated into the host
genome with the interposed transgene and residual HIV-1 sequence. Envelope and packaging genes are not incorporated, preventing the development of replication-competent lentivirus, which has not been observed in any patient treated with tisagenlecleucel.

Current US Food and Drug Administration (FDA)—approved HIV tests detect antibodies to HIV, the HIV p24 antigen, and/or HIV-1 RNA (nucleic acid testing [NAT]). NAT uses polymerase chain reaction (PCR) or transcription-mediated amplification of a portion of the HIV genome. The exact amplicons of commercial tests are not publicly available; therefore, it is unknown if they amplify the retained portion of the HIV-1 genome present in patients treated with tisagenlecleucel or CTL119. Here, we report 4 cases of patients treated with tisagenlecleucel or CTL119 who had false-positive results with select HIV-1 NAT tests.

Patient 1 was a 21-year-old woman enrolled in a tisagenlecleucel clinical trial (NCT02374333) after a refractory first relapse of B-ALL. The patient’s apheresis product was HIV negative by Prism Antibody Testing (Abbott Laboratories, Abbott Park, IL [v3L68]) and Procleix Ulitro HIV-1 PCR (Hologic, Inc [v3]). The patient received a CTL119 infusion and achieved MRD-negative remission by day 28. Two months later, while the patient was in remission and being evaluated for SCT, COBAS TaqScreen MPX Test (Roche Molecular Systems, Branchburg, NJ [v2.0]) and COBAS Qualitative Test were positive. However, the Architect Test, chemiluminescence microparticle immunoassay, and Aptima HIV-1 RNA Qualitative Test (Aptima Test; Hologic, Inc, Marlborough, MA [v5]) results were negative. There were 408 copies per microgram of tisagenlecleucel transgene DNA in peripheral blood at the time of HIV-1 NAT testing.

Patient 3 was a 2-year-old girl enrolled in a CTL119 clinical trial (NCT02374333) after a refractory first relapse of B-ALL. Results of an Architect HIV Antigen Antibody Combo test (Architect Test; Abbott Laboratories, Wiesbaden, Germany [v4J27]) were negative at screening. The patient was infused with $2 \times 10^8$ tisagenlecleucel cells and achieved a minimal residual disease (MRD)–negative remission by day 28. In preparation for a stem cell transplant (SCT), planned due to early B-cell recovery, the patient had a positive COBAS AmpliPrep/COBAS TaqMan Qualitative HIV-1 test (COBAS Qualitative Test; Roche Molecular Diagnostics, Pleasanton, CA [v2.0]) 5 months after tisagenlecleucel infusion. A Roche COBAS AmpliPrep/COBAS Quantitative TaqMan HIV-1 Test (COBAS Quantitative Test [v2.0]) was negative 1 week later. Quantitative PCR of peripheral blood 2 weeks before HIV-1 NAT testing revealed 82 copies per microgram of tisagenlecleucel transgene DNA (lower limit of quantitation, 50 copies per microgram).

Patient 2 was a 13-year-old girl with a second relapse of B-ALL and was enrolled in a tisagenlecleucel clinical trial (NCT02374333). An Architect Test was negative at screening. The patient was infused with $1.9 \times 10^8$ tisagenlecleucel cells and achieved an MRD-negative remission by day 28. Two months later, while the patient was in remission and being evaluated for SCT, COBAS TaqScreen MPX Test (Roche Molecular Systems, Branchburg, NJ [v2.0]) and COBAS Qualitative Test were positive. However, the Architect Test result was negative at screening. The patient received a CTL119 infusion and achieved MRD-negative remission by day 28 but had early B-cell recovery and received a second CTL119 infusion on day 35. One month after reinfusion, the patient was evaluated for SCT and had positive COBAS Qualitative Test and COBAS Quantitative Test results. An Architect Test and Aptima Test were repeated within 1 week, and results were negative. There were 120 copies per microgram of tisagenlecleucel transgene DNA in peripheral blood 1 month before HIV-1 NAT testing.

Patient 4 was a 6-year-old girl with a second relapse of B-ALL enrolled in a tisagenlecleucel clinical trial (NCT02228096). An Architect Test result was negative at screening. The patient received an infusion of $3 \times 10^8$ tisagenlecleucel cells per kilogram and achieved an MRD-negative remission by day 28. Three months later, while still in remission, the patient was evaluated for SCT due to early B-cell recovery, and a COBAS Qualitative Test was positive. However, an Architect Test result was negative. There were 246 copies per microgram of transgene DNA in peripheral blood 2 weeks before HIV-1 NAT testing.

False-positive HIV-1 NAT tests have been observed following treatment with therapies that use lentiviral vectors. We have

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**Table 1. Selected HIV-1 NAT tests approved by the FDA**

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Test name</th>
<th>Method</th>
<th>Approved intended use for HIV-1</th>
<th>Patients tested postinfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Molecular Inc</td>
<td>Abbott RealTime HIV-1</td>
<td>Quantitative PCR</td>
<td>Patient monitoring</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Roche Diagnostic Systems</td>
<td>AmpliDx HIV-1 Monitor Test</td>
<td>Quantitative PCR</td>
<td>Patient monitoring</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Roche Molecular Diagnostics</td>
<td>COBAS AmpliPrep/COBAS TaqMan HIV-1 Test</td>
<td>Quantitative PCR</td>
<td>Patient monitoring</td>
<td>Patient 1 (P/N, v2.0) Patient 2 (P, v2.0) Patient 3 (P, v2.0) Patient 4 (P, v2.0)</td>
</tr>
<tr>
<td>Roche Molecular Systems</td>
<td>COBAS AmpliScreen HIV-1 Test v1.5</td>
<td>Qualitative PCR</td>
<td>Donor screening</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

N, negative; P, positive.
described several patients with positive COBAS Qualitative Test results following tisagenlecleucel or CTL119 infusion. All patients were HIV negative at screening prior to CAR T-cell therapy, and negative results were subsequently confirmed using HIV-1 NAT or antigen tests by another commercial vendor. In 2 patients, the Aptima Test was useful in discerning false-positive results. No patient developed replication-competent lentivirus as evaluated by vesicular stomatitis virus glycoprotein quantitative PCR; thus, these cases represent false-positive HIV-1 NAT test results.

Following a period of rapid expansion after infusion to peak levels of $\geq 5000$ copies per microgram of DNA, tisagenlecleucel and CTL119 transgene levels slowly decline, with the majority of patients having long-term persistence for $\leq 1$ year. False-positive HIV-1 NAT test results were seen with as few as 82 copies per microgram of tisagenlecleucel sequence DNA. The COBAS Qualitative Test can quantitate HIV with as few as 48 copies per microgram, and our findings suggest that the COBAS Qualitative Test can also detect very low tisagenlecleucel levels. Conversely, 2 of these patients had negative Aptima Test results shortly after the positive COBAS Qualitative Test results. Therefore, depending on the HIV-1 NAT-assay specificities (primer sequences, etc), some tests may give false-positive results, highlighting the importance of using a clinical laboratory diagnostic test in conjunction with its FDA-intended use (Table 1). Applicability of these findings to other lentiviral gene delivery systems, including other CARs, should be explored and will depend on the similarity of the specific sequence of transfer vector and primers used in the particular HIV-1 NAT kits.

Tisagenlecleucel and CTL119 induce B-cell aplasia by targeting CD19 on both malignant and normal B cells. The resulting hypogammaglobulinemia or agammaglobulinemia suggests that antibody detection-based HIV tests are unlikely to be reliable after tisagenlecleucel or CTL119 infusion, similar to that seen in other settings of hypogammaglobulinemia or agammaglobulinemia.12 Conversely, HIV-1 NAT tests may detect transgene sequences and produce false-positive results. Therefore, we recommend that either a combination antigen and antibody detection test (which would not detect the third-generation lentiviral vector that lacks these antigens, and which is recommended by the Centers for Disease Control and Prevention HIV testing algorithm) or the Aptima Test, which is FDA approved for HIV-1 diagnosis and which was negative in all patients tested here, be performed when HIV testing is indicated following tisagenlecleucel or CTL119 treatment. Similarly, consideration should be given to NAT tests that have been shown not to cross-react with the vector when performing HIV testing following other lentiviral-based therapy.

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Authorship

Contribution: T.W.L., S.L.M., M.C.M., K.L.D., J.K., and S.A.G. designed and performed the research, contributed vital new reagents or analytical tools, collected data, analyzed and interpreted data, wrote the manuscript, and read and approved the manuscript for submission; L.K.E., C.H.K., and P.A.W. designed the research, analyzed and interpreted data, wrote the manuscript, and read and approved the manuscript for submission; and A.M.C. contributed diagnostic interpretation and read and approved the manuscript for submission.

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