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BRIEF REPORT

False-Positive Human Immunodeficiency Virus Nucleic Acid Amplification Test After Chimeric Antigen Receptor T-Cell Therapy With Ciltacabtagene Autoleucel

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Chimeric antigen receptor (CAR) T-cell therapy has emerged as a novel therapeutic option for hematologic malignancies. Human immunodeficiency virus (HIV) nucleic acid amplification tests (NAATs) amplifying 5′ long terminal repeat and *gag* genes cross-react with lentiviral vector-based CAR T-cell products. Cross-reactivity between CAR T-cell products and HIV NAATs may lead to false-positive test results.

Keywords. CAR T-cell therapy; ciltacabtagene autoleucel; human immunodeficiency virus nucleic acid amplification test.

Chimeric antigen receptor (CAR) T-cell therapy has revolutionized the field of cancer immunotherapy, emerging as a promising new treatment option for patients with B-cell malignancies and relapsed refractory multiple myeloma [1]. Ciltacabtagene autoleucel is a new CAR T-cell product targeted against B-cell maturation antigen approved in 2022 by the United States Food and Drug Administration for the treatment of relapsed refractory multiple myeloma [2]. Human immunodeficiency virus (HIV) nucleic acid amplification tests (NAATs), when performed on CAR T-cell recipients, may lead to false-positive test results. This report illustrates one such case and discusses the biology of the cross-reactivity between HIV NAATs and CAR T-cell products. It emphasizes the need to consider the HIV NAAT and the vector used in the preparation of the CAR T-cell product while interpreting positive test results.

CASE REPORT

A 66-year-old woman was diagnosed with immunoglobulin G lambda multiple myeloma in 2016. She received 4 cycles of lenalidomide, bortezomib, and dexamethasone initially, followed by a peripheral blood autologous stem cell transplant. Her disease relapsed and was refractory to multiple lines of chemotherapy thereafter. She received her second peripheral blood autologous stem cell transplant in January 2022. Months later, she was considered a candidate for CAR T-cell therapy. She received ciltacabtagene autoleucel (CARVYKTI) CAR T-cell therapy after lymphodepleting chemotherapy in December 2022. On day +9, she developed cytokine release syndrome, which improved after treatment with tocilizumab and conservative management. An HIV NAAT done inadvertently, 41 days following CAR T-cell therapy, was positive and the copy number was below the lower limit of detection of the assay (<20 copies/mL). A fourth-generation HIV antigen/antibody assay was negative. An infectious diseases consultation was requested.

On review, she had no symptoms suggestive of acute HIV infection. She lived alone, with no history of sexual activity for many years, and she denied intravenous drug use. Physical examination was normal. HIV testing with fourth-generation antigen/antibody assay and NAAT done as a part of pre-CAR T-cell screening had been negative.

The fact that she had received CAR T-cell therapy with ciltacabtagene autoleucel, which uses a lentiviral vector, raised the possibility of a false-positive HIV result. Detroit Medical Center laboratory uses the Roche COBAS AmpliPrep/COBAS TaqMan HIV test version 2.0 to detect HIV RNA. This system amplifies and detects the genes 5′ long terminal repeat (LTR) and *gag* of HIV. These genes are shared by the lentiviral vector used in the preparation of CAR T cells. We confirmed our suspicion by repeating a HIV NAAT using the Abbott Alinity m HIV-1 Viral Load assay, which amplifies the 5′LTR and p31 integrase region of the *pol* gene, not shared by the lentivirus. No HIV RNA was detectable by this assay.

DISCUSSION

The preparation of CAR T cells involves genetically modifying T cells using a retroviral vector, enabling them to express chimeric antigen receptors that identify and destroy specific tumor cells. The commonly used retroviral vector is either a murine γ-retrovirus or lentivirus. In the preparation of ciltacabtagene

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Table 1. Summary of Available Evidence Demonstrating Cross-reactivity of Human Immunodeficiency Virus Nucleic Acid Amplification Tests With Chimeric Antigen Receptor T-Cell Products

Case Report	CAR T-Cell Product	Viral Vector	Cross-reacting HIV NAATs (Genes Amplified)	HIV NAATs With No Cross-reactivity (Genes Amplified)	Duration of Positivity After CAR T-Cell Therapy (Viral Load)
Ariza-Heredia et al [9] (1 patient)	Anti-CD19 CAR T cells	Lentivirus	Roche COBAS AmpliPrep/ COBAS TaqMan HIV test, version 2.0 (5′LTR + *gag*) Procleix Ultrio Assay (5′ LTR + *pol* [p31 integrase region])	Abbott RealTime HIV-1 (*pol* [p31 integrase region])	2 mo (74 copies/mL)
Laetsch et al [7] (4 patients)	Tisagenlecleucel	Lentivirus	Roche COBAS AmpliPrep/ COBAS TaqMan HIV test, version 2.0 (5′LTR + *gag*) COBAS TaqScreen MPX Test (5′LTR + *gag*)	Gen-Probe Aptima HIV-1 RNA Qualitative Assay (5′LTR + *pol* [p31 integrase region])	Case 1: 5 mo (82 copies/mL) Case 2: 2 mo (408 copies/mL) Case 3: 1 mo (120 copies/mL) Case 4: 3 mo (246 copies/mL)
Hauser et al [10] (3 patients)	Investigational CD22 CAR T cells	Lentivirus	Roche COBAS AmpliPrep/ COBAS TaqMan HIV test, version 2.0 (5′LTR + *gag*) Procleix Ultrio Assay (5′LTR + *pol* [p31 integrase region])	Abbott RealTime HIV-1 (*pol* [p31integrase region])	Case 1: 35, 37, 38, and 46 d (20, 78, 171, and 169 copies/mL, respectively) Case 2: 30 d (320 copies/mL) Case 3: 15 and 17 d (4091 and 419 copies/mL, respectively)
Villalba et al [5] (1 patient)	Investigational CAR T cells	Lentivirus	Roche COBAS AmpliPrep/ COBAS TaqMan HIV test, version 2.0 (5′LTR + *gag*) Procleix Ultrio Assay (5′ LTR + *pol* [p31 integrase region])	Hologic Aptima HIV-1 RNA Qualitative Assay (5′LTR + *pol* [p31 integrase region])	1 wk (383 copies/mL)
Pronier et al [8] (1 patient)	Idecabtagene vicleucel	Lentivirus	Abbott Alinity m HIV-1 assay (5′LTR pol [p31 integrase region]) and Cepheid Xpert HIV-1 Viral Load XC assay	Abbott Alinity m HIV-1 assay (5′LTR *pol* [p31 integrase region])	Day 11 (3.5 log copies/mL) Day 21 (2.18 log copies/mL) 3 mo (not detected)
Our case	Ciltacabtegene autoleucel	Lentivirus	Roche COBAS AmpliPrep/ COBAS TaqMan HIV test, version 2.0 (5′LTR + *gag*)	Abbott Alinity m HIV-1 assay (5′LTR *pol* [p31 integrase region])	41 d (<20 copies/mL)

Abbreviations: CAR, chimeric antigen receptor; HIV, human immunodeficiency virus; NAAT, nucleic acid amplification test.

autoleucel, a replication-incompetent, self-inactivating, lentiviral vector is used, which is genetically very similar to HIV. CAR T cells contain chromosomally integrated lentiviral DNA present within the cell; thus, HIV NAATs testing for nucleic acid in the plasma should not be able to detect it [3, 4]. However, this could be possible due to cell lysis resulting in circulating free DNA in the plasma, which is detected by highly sensitive polymerase chain reaction assays. One should thus be aware of the following chief considerations when interpreting HIV NAAT results in CAR T-cell recipients.

First, HIV belongs to the lentivirus subfamily of retroviruses and contains *gag*, *pol*, and *env* genes along with LTR regions (5′ and 3′ LTR). The commercial assays for nucleic acid amplification target these conserved genomic regions. The lentiviral vector used in the preparation of CAR T cells also shares the genes LTR and *gag* that are present in the genome of HIV. Thus, false-positive HIV NAAT results may be possible with assays that amplify the shared genes, but when tested with a different assay that exclusively amplifies the integrase region of the *pol* gene, false positivity is avoided. The information on genes amplified by the assays is not readily available. A review by Villalba et al summarizes the cross-reactivity of the available CAR T-cell products with the commercial HIV NAATs, along with details of the genes amplified [5].

Second, the virus vector used in CAR T-cell preparation needs to be considered. False-positive results are expected with CAR T-cell products using the lentiviral vectors. Similar risk has not been associated with murine γ-retrovirus as it is phylogenetically distantly related to HIV-1 and with differing gene structures [6]. Among the available CAR T-cell products, axicabtagene ciloleucel (YESCARTA) and brexucabtagene autoleucel (TECARTUS) use murine γ-retrovirus, for which cross-reactivity has not been demonstrated. Idecabtagene vicleucel (ABECMA), lisocabtagene maraleucel (BREYANZI), and tisagenlecleucel (KYMRIAH) use lentiviral vector. Although cross-reactivity is expected with all 3 lentiviral preparations, to date it has been reported only with idecabtagene vicleucel and tisagenlecleucel [7, 8].

Third, this clinical scenario may be encountered while evaluating for acute retroviral syndrome. In that case, patients will

have clinical signs, symptoms, and risk factors compatible with the syndrome. HIV antigen/antibody assay will be negative in the window period in true infections like CAR T-cell recipients. In the latter, cells transfected by lentiviral vector are incapable of producing p 24 antigen and serologic response. The viral load would be very high in true infection, in contrast to cross-reactivity to CAR T-cell therapy in which the copy numbers will be low.

Table 1 shows the 10 published cases and the present case, demonstrating cross-reactivity between HIV NAATs and CAR T-cell preparations used. The cross-reactivity was mainly observed by platforms using 5′LTR and *gag* genes and CAR T-cell preparations that used a lentiviral vector. Our case adds ciltacabtagene autoleucel to this list. Some HIV NAATs that amplify the 5′LTR may not cross-react with lentivirus-based CAR T-cell products. The sequence of 5′LTR genes amplified by these assays may not be shared by the 5′LTR portion of the lentivirus genes used in the preparation of CAR T cells. The study by Pronier et al [8] demonstrated cross-reactivity between idecabtagene vicleucel and Abbott Alinity m HIV-1 assay, whereas in our case there was no cross-reactivity between ciltacabtagene autoleucel and Abbott Alinity m HIV-1 assay. Thus, it is not easy to predict this cross-reactivity between specific CAR T-cell products and commercial HIV NAATs. The data for the portion of the lentiviral gene used in the preparation of CAR T cells or the segment of the gene amplified by the commercial NAATs are not available in the public domain. How long the false positivity lasts and how rapidly the decline in copy numbers occurs after CAR T-cell therapy remain unclear. In our case, the repeat test by Abbott Alinity m HIV-1 assay was negative after 55 days post–CAR T-cell therapy. Published reports indicate that it remained positive up to 5 months following CAR T-cell therapy.

False-positive HIV NAAT is possible in CAR T-cell recipients. While interpreting a positive test, one should pay attention to the assay used to perform the test and the vector used in the preparation of CAR T cells, along with clinical and risk factor evaluation. As newer CAR T-cell products continue to be introduced, manufacturers using lentivirus-based vectors need to be careful in their preparation as there has been a report of wild-type HIV derived from gene therapy with BB305 lentiviral vector used for β-thalassemia, 22 months after therapy, suggesting that long-term safety monitoring studies may be required for these products [11]. Precise genome editing tools may be used to develop novel virus-free methods for T-cell engineering to circumvent such problems [12].

Notes

Previous presentations. This case was presented at the annual Michigan Infectious Diseases Society Fellows' Dinner, 21 April 2023.

Patient consent. This report does not include factors necessitating patient consent. However, we did obtain a verbal consent from the patient for publication.

Potential conflicts of interest. All authors: No reported conflicts.

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