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1 Letter

2 Analytical interference with most current commercial HIV molecular
3 assays in patients treated by idecabtagene vicleucel, a recently
4 approved lentivirus-based chimeric antigen receptor T-cells (CAR T
5 cells) therapy.

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20 To the Editor

21 We report the case of a 65-year-old woman followed for multiple
22 myeloma diagnosed in 2007. After multiple lines of therapy and
23 relapses, she received idecabtagene vicleucel (a lentiviral-based
24 vector CAR T-cells immunotherapy). At day 9 post infusion, HIV-1,
25 HBV and HCV molecular testing were irrelevantly prescribed by the
26 clinician, without any particular reason justifying them. A positive
27 HIV-1 signal was detected by 2 commercial RT-PCR assays (Alinity m
28 HIV-1, Abbott; 2.8 log cp/ml and Xpert HIV-1 Viral Load XC, Cepheid;
29 3.3 log cp/ml). Pretherapeutic HIV serology (fourth and fifth
30 generation EIAs) and molecular testing were negative and no risk
31 factor for HIV infection was reported by the patient. At day 11 post
32 infusion, HIV serology remained negative while HIV-1 RNA was still
33 detected with Alinity m HIV-1 assay (3.5 log cp/ml) on a control
34 sample. At day 21, HIV-1 RNA was quantified at 2.18 log cp/ml (Alinity
35 m HIV-1 assay) and was no more detected 3 months after infusion.
36 Finally, we concluded to a false HIV-RT-PCR positive result, both
37 assays targeting the long terminal region (LTR), due to an interference
38 with the lentiviral-based vector LTR sequence used to manufacture
39 idecabtagene vicleucel therapy. Cross-reactions between
40 idecabtagene vicleucel and current commercial assays targeting the
41 LTR region were expected but had not been reported to date.

42 Eleven patients have received idecabtagene vicleucel therapy in our
43 center between March 2022 and January 2023. To better investigate
44 the initially observed interference (case #1), we retrospectively

45 tested all available patient's frozen samples (serum and/or plasma) at
46 different time points, according to the availability of samples in our
47 biocollection. In total, prior to CAR-T infusion, negative HIV
48 serological status and/or negative HIV-1 RNA were documented for
49 9/11 (82%) and 6/11 (54%) patients, respectively. HIV-1 viral loads
50 ranging from 1.72 to 4.59 log cp/ml were detected in plasmas taken
51 2 to 9 days post CAR-T infusion for 8 out of 8 patients, with Alinity m
52 HIV-1 assay (Table 1). For 3 tested samples, similar quantifications
53 were obtained with the Xpert® HIV-1 Viral Load XC assay.
54 Interestingly, HIV-1 RNA was not detected in 2 samples from 2
55 patients using an assay targeting only the integrase region (Abbott
56 RealTime HIV-VL), while HIV-1 RNA was detected but not quantified
57 for 2 samples from 2 patients with the transcription-mediated
58 amplification method Aptima® HIV-1 Quant Dx Assay assay, targeting
59 the LTR region. HIV-1 DNA (Generic HIV DNA cell, Biocentric) was
60 quantified from 5 whole blood from 4 patients. No correlation was
61 found between HIV RNA and CAR-T and non-CAR-T cells quantified by
62 flow cytometry on this small size (n=12 samples for 7 patients). Due
63 to limited data, the correlation between whole blood HIV DNA and
64 CAR T quantification by flow cytometry could not be assessed.

65 Chimeric antigen receptor T –cell immunotherapies are promising
66 therapeutic options in oncology. CAR T-cells are *ex vivo* genetically
67 engineered T-cells with a CAR, targeting a defined surface antigen on
68 malignant cells (CD19 for lymphoma or B-cell maturation antigen for
69 multiple myeloma). CAR T-cells manufacturing involves isolating the
70 patient's T-cells which are then modified by viral transduction, either

71 with gamma-retroviral or with lentiviral vectors, to express the CAR.
72 No interference is expected with gammaretroviral-based CAR-T and
73 current HIV-1 molecular assays. By contrast, interference between
74 tisagenlecleucel (CD19-targeted CAR-T cell therapy), a lentivirus-
75 based CAR-T, and different HIV-1 molecular assays targeting LTR, has
76 been previously described [1–4]. Cross-reactions between
77 idecabtagene vicleucel (B-cell maturation antigen-targeted CAR-T cell
78 therapy), another new lentivirus-based CAR-T, and new commercial
79 assays targeting the LTR region (as Alinity m HIV, Xpert® HIV-1 Viral
80 Load XC or Aptima® HIV-1 Quant Dx Assay) are expected but have not
81 been reported to date. Current guidelines recommend to screen HIV
82 serological status before CAR-T infusion and molecular HIV testing
83 should not be performed for non-HIV infected patient receiving
84 lentiviral based CAR-T. For patient not infected with HIV, one may
85 wonder if molecular tests performed on whole blood could not be
86 used in a roundabout way to monitor CAR-T evolution. Indeed, one
87 should expect a good correlation between the false signal generated
88 by LTR amplification and the level of circulating BCMA-CAR-T cells.
89 Noteworthy, Mika et al. showed a good correlation between PBMC
90 CD19-CAR quantified by digital-droplet PCR assay (primers designed
91 in the LTR) and measured by flow cytometry [5]. Patient living with
92 HIV (PLWH) have a higher risk of developing hematological
93 malignancies and are likely to benefit from these innovative
94 therapies. In the literature, only few case reports have been reported
95 to date. All concerned PLWH with diffuse large B-cell lymphoma and
96 treated with gamma-retroviral-based axicabtagene ciloleucel (CD19-

97 targeted CAR-T cell therapy), with no analytical interference concerns
98 [6]. For efficiently treated PLWH and eligible for lentivirus-based CAR-
99 T cell therapy, as idecabtagene vicleucel, performing molecular HIV
100 testing during the first months after infusion should be avoided as it
101 may falsely lead to a misdiagnosis of virological failure. Clinicians and
102 virologists need to know this interference for a good medical care of
103 patients.

104

105 **Conflict of interest**

106 The authors declare no competing interests.

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110 Charlotte Pronier, Anne Maillard and Vincent Thibault contributed to
111 the conception, analysis, drafting and revision of this work. Karl Stefic,
112 H el ene Le Guillou Guillemette and Mikael Roussel performed some
113 analysis and critical reading.

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ID Sex, age	Sample date	HIV Ag/Ab screening	HIV-1 RNA assays (copies/ml)				HIV-1 DNA Assay# Generic HIV DNA cell [Biocentric]	CAR T CD3+ (%)**	Non CAR T CD3+ (%)**
			Alinity m HIV-1 [Abbott]	Xpert HIV-1 Viral Load XC [Cepheid]	Aptima HIV-1 Quant Dx Assay [Hologic]	Abbott RealTime HIV-VL [Abbott]			
#1 ♀ 65	(D-6)		Not detected						
	(D-1)	negative							
	D0						0	100	
	D4		1 830			19 495			
	D9		632	1895			63	37	
	D11	negative	3 191						
	D21		150				30	70	
#2 ♀ 70	D0						0	100	
	D6		3 741	3470	< 30 D+	< 40 ND	791 019	85	15
	D28		397		< 60 ND*		60	40	
	D32		278						
	D35		102						
	D39		73						
#3 ♀ 53	(D-6)		ND						
	D0						0	100	
	D7		366				15	85	
#4 ♀ 70	(D-74)	negative							
	(D-1)		ND						
	D0						0	100	
	D2		70			5 215			
	D5		2167	708					
	D9		754		< 30 D+	< 80 ND*	67	33	
#5 ♀ 70	D13		476				75	25	
	(D-151)								
	(D-1)		ND						
#6 ♀ 54	D0						0	100	
	D9		38744						
#7 ♂ 66	(D-85)	negative							
#8 ♀ 55	(D-70)	negative							
#9 ♂ 63	(D-116)	negative							
	(D-85)	negative							
	D0		ND				0	100	
#10 ♂ 64	D7		2120				53	47	
	(D-96)	negative							
	(D-1)		ND						
	D0						0	100	
	D2		53			798			
#11 ♂ 68	D6		168				0	100	
	D13		624			860 538	0.22	99.78	
	(D-5)		ND						
#11 ♂ 68	D0						0	100	
	D2		120						
	D7		646				59	41	
	D10		324				64	36	

Accepted manuscript

Table 1 : summary of all performed assays for 11 patients treated by idecabtagene vicleucel.

*Sample date indicate the delay in days between plasma and CAR-T infusion. All samples collected before CAR-T infusion are in parentheses while all samples collected after CAR-T infusion are indicated in bold. * half sample dilution due to insufficient volume; ND : not detected; D+ : detected; # HIV-1 DNA assay was performed on whole blood and results are expressed in copies/10⁶ cell; ** CAR T CD3+ and non CAR T CD3+ percents were determined by cell flow cytometry ; all HIV-1 RNA assays use quantitative RT-PCR method except Aptima HIV-1 Quant Dx Assay; ; all HIV-1 RNA assays target both LTR and pol except Abbott RealTime HIV-VL which targets only pol.*

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