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References

1. Stock PG, Barin B, Murphy B, Hanto D, Diego JM, Light J, *et al*. **Outcomes of kidney transplantation in hiv-infected recipients.** *N Engl J Med* 2010; **363**:2004–2014.
2. Gathogo EN, Hamzah L, Hilton R, Marshall N, Ashley C, Harber M, *et al*., Post FA; UK HIV/Kidney Transplantation Study Group. **Kidney transplantation in HIV-positive adults: the UK experience.** *Int J STD AIDS* 2014; **25**:57–66.
3. Roland ME, Barin B, Huprikar S, Murphy B, Hanto DW, Blumberg E, *et al*., HIVTR Study Team. **Survival in HIV-positive transplant recipients compared with transplant candidates and with HIV-negative controls.** *AIDS* 2016; **30**:435–444.
4. Locke JE, James NT, Mannon RB, Mehta SG, Pappas PG, Baddley JW, *et al*. **Immunosuppression regimen and the risk of acute rejection in HIV-infected kidney transplant recipients.** *Transplantation* 2014; **97**:446–450.
5. Gathogo E, Jose S, Jones R, Levy JB, Mackie NE, Booth J, *et al*. **End-stage kidney disease and kidney transplantation in HIV-positive patients: an observational cohort study.** *J Acquir Immune Defic Syndr* 2014; **67**:177–180.
6. Gathogo E, Harber M, Bhagani S, Levy J, Jones R, Hilton R, *et al*., UK HIV Kidney Transplantation Study Group. **Impact of tacrolimus compared with cyclosporin on the incidence of acute allograft rejection in human immunodeficiency virus-positive kidney transplant recipients.** *Transplantation* 2016; **100**:871–878.
7. Hanaway MJ, Woodle ES, Mulgaonkar S, Peddi VR, Kaufman DB, First MR, *et al*., INTAC Study Group. **Alemtuzumab induction in renal transplantation.** *N Engl J Med* 2011; **364**:1909–1919.
8. Tan HP, Kaczorowski DJ, Basu A, Khan A, McCauley J, Marcos A, *et al*. **Living-related donor renal transplantation in HIV+ recipients using alemtuzumab preconditioning and steroid-free tacrolimus monotherapy: a single center preliminary experience.** *Transplantation* 2004; **78**: 1683–1688.

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Transient HIV-specific T cells increase and inflammation in an HIV-infected patient treated with nivolumab

Immune checkpoint inhibitors (ICIs) can reverse T-cell anergy and boost immune responses against tumours. Nivolumab, an ICI directed against programmed death-1 (PD-1), has become the new standard of care for second-line treatment of advanced nonsmall cell lung cancer (NSCLC) [1–3]. It is thought to be an even clever therapeutic option in HIV-infected patients [4], as the overexpression of PD-1 on exhausted HIV-specific T cells allows ICIs to reactivate PD-1^+ T cells and to improve HIV-specific immune responses [5–7].

So far, two case reports involving HIV-infected patients with melanoma treated with ICIs have shown encouraging safety results but distinct viral effects [8,9]. Upon ipilimumab treatment, $CD4^+$ and $CD8^+$ cell counts, as well as the cell-associated HIV-RNA transiently increased [9]. Upon pembrolizumab, no rebound of HIV viral load was observed [8]. The question of whether anti-PD-1 could be a tool for HIV cure is still pending. Here, we report for the first time the detailed immunovirological evolution upon nivolumab in an HIV-infected patient with lung cancer.

The $CD4^+$ and $CD8^+$ cell counts were analysed on an FC500 flow-cytometer. Phenotypic and functional analysis used a 5-laser-beam LSR-Fortessa cytometer on the CyPS platform. Intracellular-cytokine-staining assay used 15-mer peptides targeting HIV-Gag, reverse transcriptase and Nef and 9-mer Epstein–Barr virus (EBV) peptides [10,11]. The plasma IL-6 levels were quantified using the Simoa-Quanterix SIMOA HD-1 analyzer (Quanterix Corporation, Lexington, Massachusetts, USA). Plasma viral load was quantified using the Amplicor monitors assay [12]. HIV-1 DNA was amplified in *LTR* gene by real-time PCR [13].

A 53-year-old man, smoker, HIV-infected since 1993, was diagnosed with advanced NSCLC in May 2014. After a decompressive radiotherapy, six cisplatin/gemcitabine and four taxotere chemotherapy cures, nivolumab was started in September 2015. The patient was virally suppressed with $CD4^+$ and $CD8^+$ T-cell counts of 204 and 1142 cells/μl, respectively, upon abacavir, lamivudine and dolutegravir. Seven nivolumab injections were administered, causing Grade I hepatic toxicity. Tumoral stability was reported after six injections. Nivolumab was discontinued after the seventh injection due to disease progression and the patient finally passed away in June 2016.

During follow-up, no significant changes of ultrasensitive HIV viral load were observed, but a slight two-fold increase in HIV-cell-associated DNA levels (116 at D0, 213 copies/10^6 peripheral blood mononuclear cells at D30, Fig. 1a). The immunological follow-up showed a marked increase of IL-6 plasma levels peaking at D14

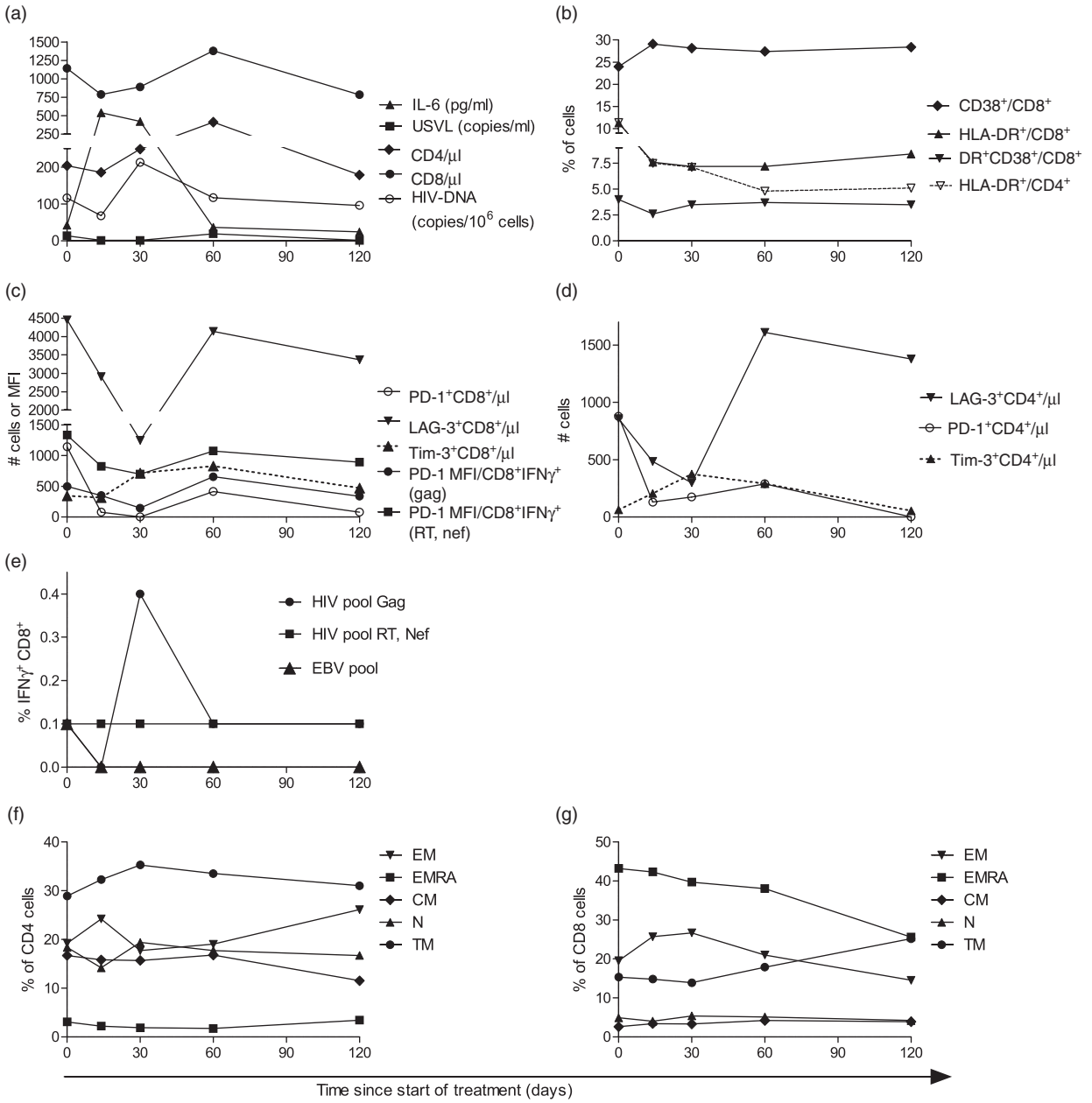

Fig. 1. Immunovirological modulation of antiprogrammed death-1 therapy in an HIV-infected patient treated for lung cancer. (a) $CD4^+$ cell count, IL-6 plasma levels, HIV-1 plasma viral load measured with ultrasensitive technique, and total HIV-DNA (DNA copies/million cells) through time. (b) Expression of activation markers on $CD4^+$ and $CD8^+$ peripheral T cells. (c) Immune check point expression on total $CD8^+$ T cells, on HIV Gag (pool 1)-specific T cells and on HIV RT/Nef (pool 2)-specific T cells. Results are expressed as mean fluorescence intensity for HIV-specific T cells or number of total immune check point positive $CD8^+$ T cells/µl. (d) Immune check point expression on total $CD4^+$ T cells. (e) Frequencies of $IFN\gamma^+CD8^+$ T cells specific for HIV Gag and RT/Nef and for EBV (optimal $CD8^+$ peptides). (f), (g) Percentages of $CD4^+$ (f) and $CD8^+$ (g) subpopulations of T cells: CM, central memory ($CD45RA^-CCR7^+CD27^+$); EBV, Epstein–Barr virus; EM, effector memory ($CD45RA^-CCR7^-CD27^-$); EMRA, RA-re-expressing effector memory T cells ($CD45RA^+CCR7^-CD27^-$); N, naïve ($CD45RA^+CCR7^+CD27^+$); TM, transitional memory ($CD45RA^-CCR7^-CD27^+$).

and returning to normal beyond D60. The $CD4^+$ and $CD8^+$ cell counts peaked at D60 to 413 and 1380 cells/µl, respectively, returning to baseline values at D120, together with a slight $CD4^+/CD8^+$ ratio increase (0.18–0.30 from D0 to D60). The $CD4^+$ and $CD8^+$ cell activation status showed discordant changes with a modest CD38 increase on $CD8^+$ T cells, whereas human leukocyte antigen – antigen D related on $CD4^+$ and $CD8^+$ T cells remained within normal values (Fig. 1b). The immune check point expression on total

$CD4^+$ and $CD8^+$ T cells displayed a marked decrease of both PD-1 and LAG3 at D30 (Fig. 1c, d). In contrast, Tim-3 expression increased on $CD4^+$ and $CD8^+$ T cells during follow-up and returned to baseline values at D120 (Fig. 1c, d). The almost undetectable IFNγ^+ Gag-specific $CD8^+$ T cells at baseline (below 0.1%) increased at D30 (0.4%) then returned to baseline values, whereas reverse transcriptase-, Nef-specific $CD8^+$ T cells remained very low (Fig. 1e). Furthermore, PD-1 expression was down-modulated on IFN-γ^+ Gag-specific $CD8^+$ T cells at D30 (Fig. 1c). Frequencies of IL-2^+ and TNF-α^+ HIV and EBV-specific T cells showed no significant modification (data not shown). Finally, the $CD4^+$ T cell differentiation status remained stable (Fig. 1f), whereas transitional-memory $CD8^+$ population increased (+64%) and RA-re-expressing effector-memory decreased (−41%) 4 months after treatment initiation (Fig. 1g).

We describe here the immunovirological effects of nivolumab in an HIV-infected patient. A slight increase in HIV-specific IFNγ^+CD8^+ cells occurred together with an increase in $CD4^+$ and $CD8^+$ cell counts, in the $CD8^+$ transitional-memory population and in IL-6 plasma levels, contrasting with a decrease of PD-1 expression on T cells. Taken together, these data suggest that nivolumab is successful at enhancing the capacities of HIV-specific $CD8^+$ transitional-memory cells to proliferate and to secrete cytokines [5,6,14], expanding the PD-1 low T-cell subset [15]. Those changes had no or little impact on HIV replication or reservoirs. The transient increase in inflammation has not been reported before and might result either from the PD-1/programmed death-ligand 1 (PD-L1) pathway disruption in immune cells or from a rapid HIV replication in tissues that would have immediately been controlled by the stimulated HIV-specific $CD8^+$ T cells. These first results are encouraging and remain to be confirmed in other HIV-patients treated with anti-PD-1/PD-L1-blocking antibodies.

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Conflicts of interest

There are no conflicts of interest.

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References

1. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, *et al*. **Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer.** *N Engl J Med* 2015; **373**:1627–1639.
2. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, *et al*. **Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer.** *N Engl J Med* 2015; **373**:123–135.
3. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, *et al*. **Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced nonsmall-cell lung cancer (KEYNOTE-010): a randomised controlled trial.** *Lancet* 2016; **387**:1540–1550.
4. Guihot A, Cadranel J, Lambotte O, Lavole A, Autran B, Spano JP. **Biological follow-up of patients with HIV treated with anti-PD-1 or anti-PD-L1 for nonsmall cell bronchial carcinoma: a task group proposal.** *Rev Mal Respir* 2016; **33**:419–421.
5. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, *et al*. **PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression.** *Nature* 2006; **443**:350–354.
6. Trautmann L, Janbazian L, Chomont N, Said EA, Gimmig S, Bessette B, *et al*. **Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction.** *Nat Med* 2006; **12**:1198–1202.
7. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, *et al*. **Enhancing SIV-specific immunity in vivo by PD-1 blockade.** *Nature* 2009; **458**:206–210.
8. Davar D, Wilson M, Pruckner C, Kirkwood JM. **PD-1 blockade in advanced melanoma in patients with hepatitis C and/or HIV.** *Case Rep Oncol Med* 2015; **2015**:737389.

9. Wightman F, Solomon A, Kumar SS, Urriola N, Gallagher K, Hiener B, *et al*. **Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma.** *AIDS* 2015; **29**:504–506.
10. Guihot A, Dupin N, Marcelin AG, Gorin I, Bedin AS, Bossi P, *et al*. **Low T cell responses to human herpes virus 8 in patients with AIDS-related and classic Kaposi sarcoma.** *J Infect Dis* 2006; **194**:1078–1088.
11. Le Corre N, Thibault F, Pouteil Noble C, Meiffredy V, Daoud S, Cahen R, *et al*. **Effect of two injections of nonadjuvanted influenza A H1N1pdm2009 vaccine in renal transplant recipients: INSERM C09-32 TRANSFLUVAC trial.** *Vaccine* 2012; **30**:7522–7528.
12. Calin R, Hamimi C, Lambert-Niclot S, Carcelain G, Bellet J, Assoumou L, *et al*. **Treatment interruption in chronically HIV-infected patients with an ultralow HIV reservoir.** *AIDS* 2016; **30**:761–769.
13. Avettand-Fenoel V, Chaix ML, Blanche S, Burgard M, Floch C, Toure K, *et al*. **LTR real-time PCR for HIV-1 DNA quantitation in blood cells for early diagnosis in infants born to seropositive mothers treated in HAART area (ANRS CO 01).** *J Med Virol* 2009; **81**:217–223.
14. Appay V, Dunbar PR, Callan M, Klenerman P, Gillespie GM, Papagno L, *et al*. **Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections.** *Nat Med* 2002; **8**:379–385.
15. Blackburn SD, Shin H, Freeman GJ, Wherry EJ. **Selective expansion of a subset of exhausted CD8 T cells by alphaPD-L1 blockade.** *Proc Natl Acad Sci U S A* 2008; **105**:15016–15021.

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HIV cure strategies: response to ignore the central nervous system at your patients' peril

We read with interest the recent article published in *AIDS* by Gama *et al*. [1], highlighting evidence for a significant central nervous system (CNS) reservoir in simian immunodeficiency virus macaque models despite effective long-term peripheral viral suppression by antiretroviral therapy. This was followed by an informative editorial by Spector and Rappaport [2], cautioning those involved in HIV cure research not to overlook this important viral reservoir. Future cure strategies, which test the impact of interventions on measures of viral reservoir in peripheral body compartments, may not assume that they have the same efficacy in the CNS and thereby mitigate the effectiveness of HIV cure interventions.

Although we acknowledge and fully agree with this potential lack of efficacy for HIV cure strategies if sanctuary sites are overlooked, we would like to highlight additional potential perils facing HIV cure strategists with respect to the CNS; namely adverse CNS outcomes that may include toxicities of HIV cure therapies, direct immune-mediated CNS pathogenesis or the impact of viral reactivation on the brain [3].

Mechanisms of negative outcomes on the CNS and neuronal tissue due to cure strategies could include, first, adverse effects on brain function secondary to the removal or elimination of latently infected neuronal cells with crucial function for brain health, such as microglial cells and astrocytes [4]. Second, neuronal damage from either drug utilized during cure research strategies or neuronal damage from viral proteins, the expression of which may be upregulated during cure treatments. An example being the gene upregulation resulting from histone deacetylase (HDAC) inhibitor use. Finally, a further adverse outcome, which could be a catastrophic event, is immune reconstitution inflammatory syndromes occurring in the CNS compartment. This could occur due to cytokine storms caused by immunotherapeutic agents modifying neuroinflammatory responses, or immune activation following viral rebound and blips caused by HDAC inhibitors (and similar agents) or viral rebounds associated with antiretroviral treatment interruptions. Cases of HIV encephalopathy associated with viral rebound and cerebrospinal fluid viral escape are well described in the literature [5]. In addition, cure approaches that include the use of therapeutic HIV vaccines that induce HIV-specific $CD8^+$ cytotoxic cells have the potential to trigger $CD8^+$-mediated encephalitis [6].

To date and to our knowledge, no significant adverse effects on CNS function have been observed in HIV cure trials, but limited data are available. One small study has reported on the effects of HIV-latency-reversing agents on CNS parameters in HIV-positive participants with no adverse impact on cerebrospinal-fluid neuroinflammatory or degenerative soluble biomarkers observed [7]. Such results are reassuring. However, one should be weary of the results from other fields such as cancer studies, from which some of the agents used in HIV cure strategies originate. The syndrome of chemotherapy-related cognitive dysfunction or 'chemobrain' is being increasingly recognized with the use of modern oncological treatments, although the pathophysiology of this condition remains elusive [8].

For HIV cure strategists and researchers, consideration of and monitoring for CNS adverse events within HIV cure studies will be crucial. Monitoring for CNS adverse events is challenging given the closed anatomical sanctuary site of the brain and the complexity of monitoring nervous system function. Brain biopsies are clearly not possible, and repeated cerebrospinal fluid examinations are costly and not practical for every study. However, monitoring clinical parameters such as cognitive function and patient-related outcome measures of cognitive health, coupled with the monitoring of sensitive peripheral markers of neuronal integrity, such as highly sensitive plasma neurofilament light protein [9], and noninvasive neuroimaging could be practical approaches to consider.

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