Immunovirological evolution in HIV-infected patients treated with anti-PD-1 therapy

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ABSTRACT (Updated)

Background: Immune checkpoint-blocking antibodies can reverse T cell anergy, boost immune responses against tumors, and has become the new standard of care for second-line treatment of advanced non-small cell lung cancer (NSCLC). 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 clinical experience in 12 HIV-infected patients (pts) treated with the anti-PD-1 antibody nivolumab, and the detailed immunovirological evolution in two pts with lung cancer.
Methods: Twelve HIV-infected pts (10 male, 1 female, 1 transgender) received nivolumab as a second line therapy for NSCLC. Ultrasensitive plasma viral load (US-VL), HIV-cell associated DNA, phenotypic and functional T cell analysis, intracellular-cytokine-staining assay against 15-mer HIV peptides, and plasma IL-6 levels were quantified at different time points (D0, D14, D30, D60, D120) in two patients.
Results: In 12 pts, best tumoral response was partial response (n=3), stability (n=4), or progression (n=5). All plasma HIV viral load were undetectable at D0, and one blip was observed at D45 (86 copies/mL). CD4 and CD8 cell counts were not significantly modified upon nivolumab (median 338 and 830/mm^3 at D0; 336 and 570/mm^3 after 4 nivolumab injections, respectively). In pt#1, US-VL was undetectable and stable, but a slight 2-fold increase in HIV-cell associated DNA levels was observed (116 at D0 vs 213 copies/10^6 PBMC at D30). IL-6 plasma levels peaked at D14 (539 pg/mL) and returned to normal beyond D60. In pt#11, a drastic drop of the HIV-DNA in PBMC was observed after 9 nivolumab injections (369 to 30 copies/10^6 PBMC). In both patients, PD-1 expression on total CD4 and CD8 T cells decreased at D30 and reached again pre-treatment values. HIV-specific IFNg+CD8+ cells increased from 0.1% at D0 to 0.4% (gag) at D30 in pt #1, and from 0.31 to 1.31% (RT+nef) of CD8 T cells at D120 in pt#11.
Conclusions: Our data suggest that nivolumab is well tolerated in HIV-infected patients, is successful at enhancing the capacities of HIV-specific CD8 TM cells to proliferate and to secrete cytokines, expanding the PD-1low T cell subset, with promising results on HIV reservoirs.

INTRODUCTION

The PD-1/PD-L1 axis was shown to be of utmost importance for T-cell exhaustion, a state of immune dysfunction encountered in cases of chronic infection or cancer[1] . Anti-PD-1 immune checkpoint inhibitors (ICI) have become the new standard of care for treatment of advanced non-small cell lung cancer (NSCLC) following platinum-based doublet chemotherapy[2-3]. In patients living with HIV/AIDS (PLWHA), NSCLC is the most common non-AIDS-related malignancy and the leading cause of cancer-related death. In this population, ICIs are thought to be an attractive therapeutic option, as chemotherapy has proven to produce poor efficacy and higher toxicity than in the general population[4] .
In HIV-infected individuals, total CD4 and CD8 activated T cells express high PD-1 levels during HIV infection. PD-1 expression on HIV-specific T-cells is associated with T-cell exhaustion and disease progression. PD-1 is significantly upregulated on CD8 specific T cells. Blockade of the PD-1/PD-L1 pathway would increase HIV-specific CD4 and CD8 T-cell function[5,6]. Those results were confirmed in vivo in macaques[7] and humanized mice models[8] . However, the ICI safety profile and efficacy have not yet been evaluated in PLWHAs because they were excluded from clinical trials. It is therefore of importance to accumulate some safety data in this population[9]. Furthermore, the effect of this immunotherapy on the HIV reservoirs need to be further characterized.

PATIENTS CHARACTERISTICS

Patient#	Sex	Age (years)	Tabacco	Pack-years	Cancer diagnosis	Cancer stage	Pathology	molecular biology	First line treatment	HIV diagnosis	HBV/HCV co-infection	CD4/mm3	VL (copies/mL)	HAART
1	M	53	SMOKER	NA	2014	IV	SCC	WT	CIS GEM ; DOC	1993	0	364	<20	ABC 3TC DTG
2	T	46	SMOKER	20	nov-15	IV	ADC	WT	CARBO PEM	2005	NA	660	<20	FTC TDF EFV
3	M	51	EX SMOKER	62	juin-14	IV	ADC	WT	CARBO PEM ; GEM ; ERLO	1995	VHC	700	<20	FTC TDF rLPV
4	F	64	EX SMOKER	10	dec-2015	IV	ADC	WT	CARBO PEM BEVA	2005	0	350	<20	EVG COBI TDF FTC
5	M	61	SMOKER	45	déc-14	IIIB	ADC	WT	CARBO PEM	1994	0	294	<20	FTC TDF ATV
6	M	77	EX SMOKER	25	oct-14	IIIB	ADC	WT	CARBO PEM ; DOC ; GEM	1985	NA	214	<20	3TC RPV DTG
7	M	69	NA	NA	mars-14	IVa	SCC	NA	CIS GEM	1997	0	596	53	RPV rDRV RAL
8	M	69	EX SMOKER	30	oct-14	IV	SCC	NA	NA	1992	0	190	<20	ETR rDRV
9	M	40	SMOKER	25	may-2016	IV	ADC	WT	CARBO PEM BEVA	2003	VHC	313	<20	FTC TDF RPV
10	M	72	EX SMOKER	40	may-2016	IVb	ADC	KRAS	CARBO PEM	1980	0	424	34	FTC EVR RAL
11	M	51	EX SMOK	40	May-2015	IIIa	NA	WT	CIS PEM	1995	Former HBV	206	<20	FTC TDF DTG
12	M	54	SMOKER	70	Oct-2014	IV	ADC	NA	CARBO PEM BEVA	1993	0	NA	<20	DRV DTG

M, male; F, female; T, transgender; SCC, squamous cell carcinoma; ADC, adenocarcinoma, CIS, cisplatin; GEM, gemcitabin; DOC, docetaxel; CARBO, carboplatin; PEM, pemetrexed; ERLO, erlotinib; BEVA, bevacizumab; NA, not available; VL, Viral load; HAART, highly active antiretroviral therapy; ABC, abacavir; 3TC, lamuvudine; FTC, emtricitabine ; DTG, dolutegravir; TDF, tenofovir; EFV, efavirenz; rLPV, lopinavir+ritonavir; EVG, eviltegravir; COBI, cobisistat; ATV, atazanavir; RPV, rilpivirine; rDRV, darunavir+ritonavir; RAL, raltegravir; ETR, etravirin

RESULTS

1) Clinical and biological evolution upon nivolumab

Patient#	First nivolumab infusion	CD4/mm3 after 4 injections	VL after 4 infusions	# Infusions administered	Tumor response after 4 infusions	Toxicity	Intercurrent infection	Alive /Dead	Nivolumab in progress (april-17)
1	23/09/2015	202	<20	7	S	hepatitis - grade 1	no	dead	no
2	31/03/2016	657	<20	6	DP	no	no	NA	NA
3	13/01/2016	404	<20	8	S	no	no	dead	no
4	28/09/2016	307	<20	9	S	no	no	alive	yes
5	20/01/2016	270	<20	23	PR	no	no	alive	yes
6	15/04/2016	327	<20	4	S	no	no	dead	no
7	30/08/2016	351	<20	3	PR	hypereosinophilia	yes	alive	no
8	24/05/2016	110	<20	3	DP	no	no	alive	no
9	22/09/2016	346	<20	4	DP	no	no	dead	no
10	05/10/2016	400	<20	5	DP	no	no	alive	no
11	15/12/2016	214	86	12	PR	no	no	alive	yes
12	20/04/2017	NA	NA	4	DP	no	no	alive	no

VL, viral load; S, stability; DP, disease progression; PR, partial response; NA, not available; Patient #11 : missing data

RESULTS (Continued)

Figure 1. CT scan thoracic findings before and after nivolumab treatment (Patient #8)
A : before nivolumab B : after 5 nivolumab injections

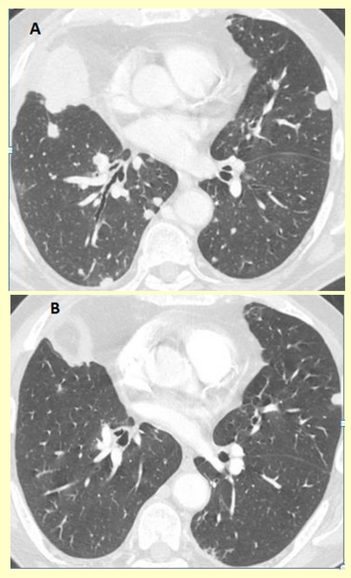
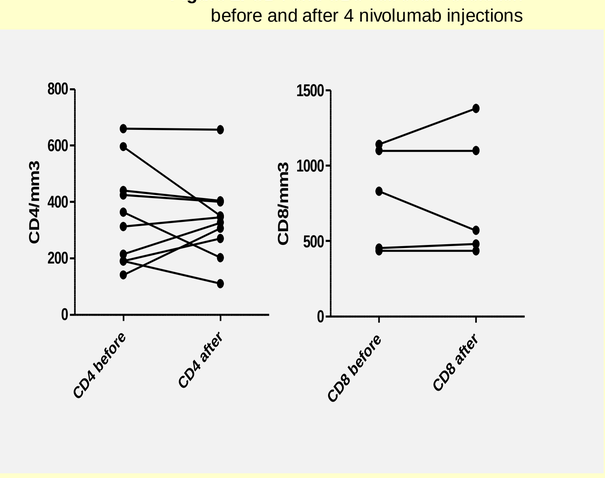


Figure 2. CD4 and CD8 cell count evolution before and after 4 nivolumab injections



2) Detailed immunovirological evolution in two patients with cancer treated with nivolumab

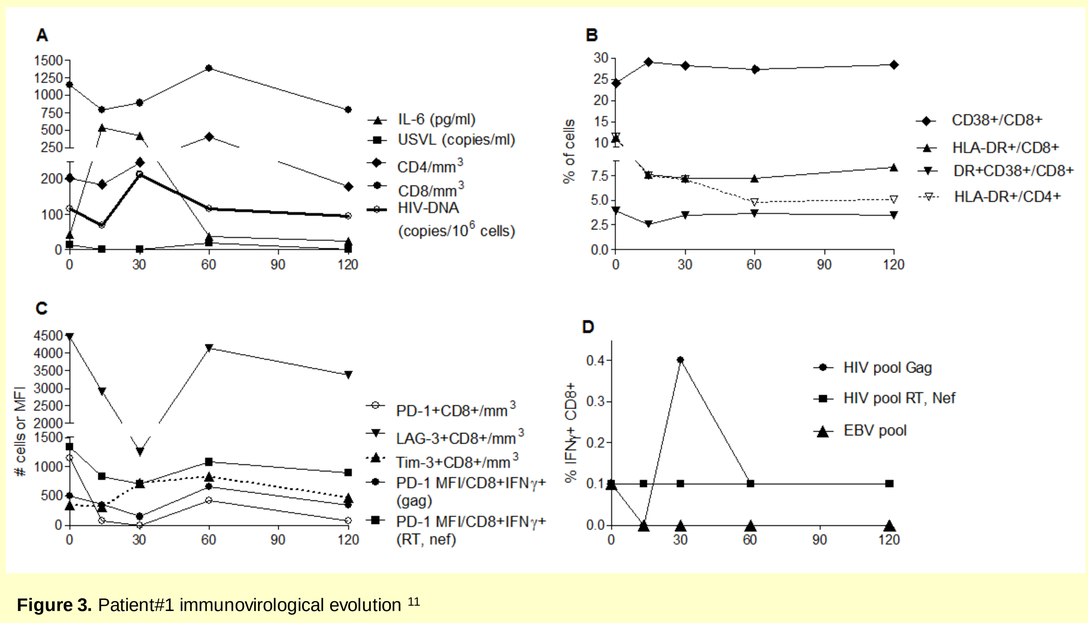


Figure 3. Patient#1 immunovirological evolution [11]

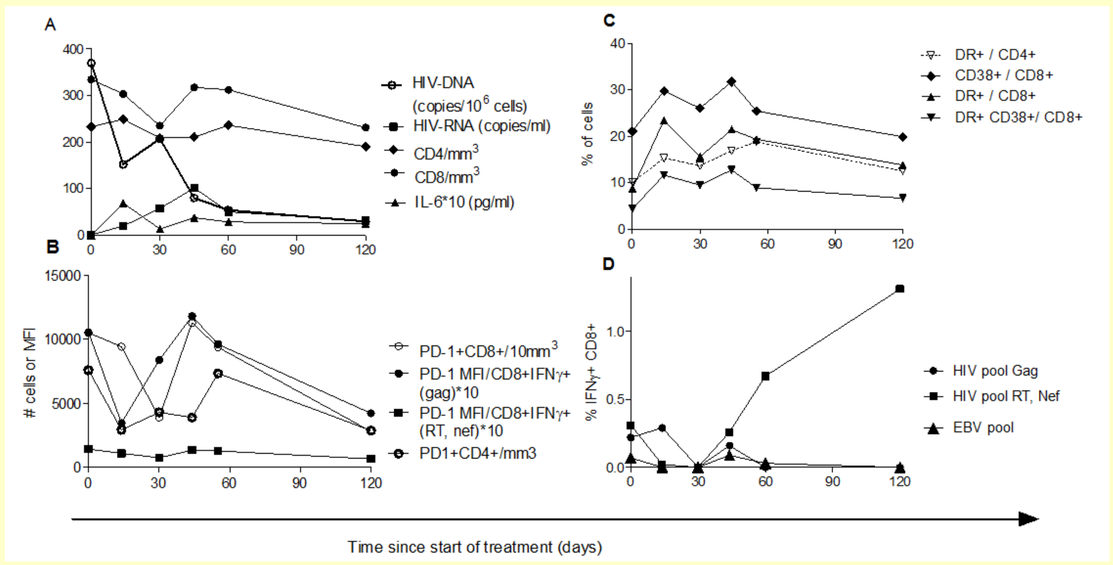


Figure 4. Patient#11 immunovirological evolution

DISCUSSION / CONCLUSION

Nivolumab treatment had a favorable clinical outcome in our patients' series (7/12 disease control – 3 Partial response and 4 Stability), with no significant clinical side effects except 1 case of neurosyphilis, and with no or minimal effect on HIV viral load nor CD4/CD8 cell counts.

Nivolumab is successful at enhancing the capacities of HIV-specific CD8 cells to proliferate and to secrete cytokines expanding the PD-1low T cell subset [10].

Those changes had no impact on HIV replication or reservoirs in one patient, but had drastic impact in another patient.

The transient increase in inflammation has not been reported before and might result either from the PD-1/PD-L1 pathway disruption in immune cells, or from a rapid HIV replication in tissues [11].

Those 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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METHODS

CD4 and CD8 cell counts. CD4 and CD8 T-cell counts were done at baseline, day (D) 14, D30, D60 and D120 on fresh whole blood by flow cytometry, using Cyto-Stat tetraCHROME™ reagents (Beckman-Coulter, Hyaley, Florida). CD4 T cells were defined as CD3+CD4+ lymphocytes, and CD8 T cells as CD3+CD8+ lymphocytes. Sample acquisition with Flow-Count Fluorospheres® was performed on a FC500 flow cytometer (Beckman Coulter). The laboratory values in 101 healthy HIV-seronegative individuals were as follows (median [percentiles 10th-90th]): CD4/CD8 ratio 1.9 [1.2-3.1], CD4 T cell count 716 [510-1037]/mm3, CD8 T cell count 401 [258-615]/mm3.
Phenotypic T cell characterization. The phenotypic characterization of T-cells was determined at all time points on cryopreserved PBMC by flow cytometry, using the following monoclonal antibodies: anti-CD3, CD4, CD8, CD38, CD45RA, CCR7, CD27, HLA-DR, and PD1 (BD Biosciences, San Jose, CA, USA); anti-Tim-3 (Biolegend, San Diego, CA, USA); anti-LAG-3 (R&D Systems, Minneapolis, MN, USA) and anti-CD45 (Beckman Coulter, Villepinte, France). All flow cytometry analyses were performed on a 5-laser-beam LSR-Fortessa device (Becton-Dickinson, Franklin Lakes, New Jersey, USA) on the CyPS platform (UPMC), and Flowjo software (Treestar, Inc., Ashland, Oregon, USA).
Intracellular-cytokine-staining. Intracellular-cytokine-staining (ICS) assay was performed (Lecorre et al) at all time points by incubating cryopreserved PBMCs with 18 pools of 15-mer synthetic peptides targeting Gag (pool 1), Reverse Transcriptase and Nef (pool 2) from HIV. Medium alone or Staphylococcal enterotoxin B (SEB) were used as negative or positive controls, respectively. In addition, five pools of 9-mer peptides covering the most common HLA restricted epitopes from EBV were tested. Staining was performed with anti-CD3, CD4, CD8, interferon gamma (IFN-γ), interleukin-2 (IL-2), tumour necrosis factor alpha (TNF-α) and PD1 (BD Biosciences, San Jose, CA, USA) and live-Dead Fixable Aqua (Life Technologies) monoclonal antibodies. Percentages are presented after subtraction of the background observed with medium alone for each cytokine. All flow cytometry analyses were performed on a 5-laser-beam LSR-Fortessa device (Becton-Dickinson, Franklin Lakes, New Jersey, USA) on the CyPS platform (UPMC).
Cytokine quantification. The quantification of plasma IL-6 levels were done at all time-points using the emerging technology for single-molecule array (Simoa) detection with a fully automated immunochemistry platform, the Quanterix SIMOA HD-1 analyzer (Quanterix Corporation, Lexington, MA).
Plasma HIV-1 RNA ultra sensitive quantification. Plasma viral load (pVL) was quantified using the Amplicor monitors assay (Cobas Taqman 2.0, Roche Diagnostics, Basel Switzerland). Below the standard cutoff, the assay indicates the qualitative detection of HIV-1 RNA in the range of 1 to 20 copies/mL (ref Calin R et al. AIDS 2016).
HIV-cell associated DNA quantification. Total DNA was extracted using the Magnapur Total Nucleic® (Roche) technology. The HIV-1 DNA was amplified in LTR gene by real-time PCR as previously described (ref. (Avettand-Fènoël V et al J Med Virol 2009). The quantification standard is based on the 8E5 cell line containing 1 copy of the HIV provirus per cell. Results are reported either as actual numbers of copies/10^6 cells or as a threshold value of detection (<66 copies/10^6 cells) when cell HIV-DNA was not detected.