HHS Public Access

Author manuscript

*AIDS*. Author manuscript; available in PMC 2015 July 06.

Published in final edited form as:

*AIDS*. 2015 February 20; 29(4): 504–506. doi:10.1097/QAD.0000000000000562.

# Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma

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Long-lived latently infected resting $CD4^+$ T cells are the main reason why current antiretroviral therapy (ART) is unable to cure HIV infection [1]. Recent work has suggested that the expression of immune checkpoint markers, such as programmed death-1 (PD1), may play a role in viral persistence on ART via either suppression of virus transcription and/or reduced HIV-specific T cell activity [2,3], but the role of cytotoxic T lymphocyte antigen 4 (CTLA-4 or CD152) in HIV persistence on ART is not clear.

Ipilimumab (Yervoy, Bristol-Myers Squibb, New York, New York) is a human immunoglobulin G1 antibody to CTLA-4 that inhibits binding of CTLA-4, expressed on activated T cells and regulatory T cells (Tregs), to its ligands CD80 and CD86. The drug is used to treat metastatic melanoma and has been associated with multiple changes in immune function thought to enhance antitumor T cell function [4].

In HIV-infected individuals, CTLA-4 expression on $CD4^+$ T cells correlates with HIV disease progression [5], and loss of HIV-specific $CD4^+$ T cell function can be reversed *in vitro* by CTLA-4 blockade [5–7]. In a simian immunodeficiency virus (SIV) macaque model, CTLA-4 blockade led to an increase in T-cell activation and viral replication [8]. Here, we describe changes in the HIV reservoir in an HIV-infected patient on ART who received ipilimumab for the treatment of metastatic melanoma.

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Author contributions: C.M., R.G., and S.R.L. designed the study. S.K., N.U., and C.M. conducted the study. F.W., A.S., K.G., B.H., and S.P. contributed to laboratory-based investigations. F.W., S.K., C.M., and S.R.L. wrote the manuscript. All authors reviewed and approved the final manuscript.

**Conflicts of interest**

There are no conflicts of interest.

At initiation of ipilimumab treatment in October 2013 for disseminated melanoma, the patient was a 51-year-old man diagnosed with HIV in 1986 and with a $CD4^+$ nadir of 159 cells/μl in 1995. He was on ART since 1996 and plasma HIV RNA was less than 400 copies/ml from 2004 and less than 20 copies/ml from July 2012 (Fig. 1a). He received four doses of ipilimumab 3 mg/kg given at three-weekly intervals.

Whilst receiving ipilimumab, there was no overall change in plasma HIV RNA as measured by the Roche viral load assay [lower limit of detection (LLOD) = 20 copies/ml; Fig. 1c]. Using a sensitive single-copy HIV RNA assay (SCA) (LLOD = 0.3 copies/ml) [9], there was a cyclical decrease in plasma HIV RNA following each infusion and an overall decline from 60 to 5 copies/ml (Fig. 1c). Given more frequent sampling was performed with the SCA, we believe that longitudinal changes over time were best assessed with this assay.

There was an increase in $CD4^+$ T cells after each infusion (overall change from 610 to 900 cells/μl) (Fig. 1b). This increase was predominantly in total memory (Fig. 1d) and effector memory $CD4^+$ T cells (Fig. 1e). Postinfusion increases in $CD4^+$ T-cell activation were seen as measured by human leukocyte antigen-DR and CD38 and CCR5 expression (Fig. 1f). There were transient increases in $CD8^+$ T cells following the second and third infusions, but no overall change in $CD8^+$ T cell activation (Fig. 1g).

Cell-associated unspliced HIV RNA in sorted $CD4^+$ T cells was quantified with increases observed following the first and second infusions, with a maximum change from baseline of 19.6-fold (Fig. 1h). The changes in cell-associated unspliced HIV RNA was greater than those recently reported, following the administration of the histone deacetylase inhibitors vorinostat [10,11] or panobinostat [12], or following disulfiram [13].

There was no change in cell-associated HIV DNA (Fig. 1i), but any change in the small proportion of cells with HIV DNA containing inducible proviruses [14] may not have been detectable with the assays used here.

Acknowledging the limitations deriving from this being a single case, we speculate the increase in cell-associated unspliced RNA could have been due to mechanisms, including an increase in HIV RNA transcription secondary to blocking the inhibitory effects of CTLA-4 on T cell transcription, similar to that described following ex-vivo anti-PD1 treatment of $CD4^+$ T cells from HIV-infected patients on ART [15]; redistribution or expansion of effector memory $CD4^+$ T cells that may have a higher ratio of cell-associated HIV RNA to HIV DNA [16] (Satish Pillai, San Francisco, UCSF, San Francisco, California, personal communication); or redistribution or expansion of activated T cells including Tregs. The increase in cell-associated unspliced HIV RNA and decline in SCA was intriguing, perhaps mediated by elimination of latently infected $CD4^+$ T cells that were induced to express viral antigens. But the rapidity of the decline in SCA makes this somewhat unlikely.

Blockade of CTLA-4 with ipilimumab in an HIV-infected patient on ART had significant effects on the total number and phenotype of $CD4^+$ T cells and induced a profound increase in cell-associated unspliced HIV RNA with onset after the first dose and was associated with subsequent decline in plasma HIV RNA. Further studies are warranted to determine if

ipilimumab could play a role in eliminating latently infected cells in HIV-infected patients on ART.

## Acknowledgments

We acknowledge the participation and commitment of the case study participant. The assistance of Linda Dayan in data extraction is acknowledged. The authors acknowledge helpful discussions and comments from Steve Deeks, Rafick Sekaly and Nicolas Chomont.

Funding: This work was supported in part by the Australian National Health and Medical Research Council (APP1042654, Dora Lush Postgraduate Scholarship APP607230). S.R.L. is an NHMRC Practitioner Fellow and S.R.L., S.P. and B.H., are supported by the National Institutes of Health Delaney AIDS Research Enterprise (U19 A1096109). The authors gratefully acknowledge the contribution to this work of the Victorian Operational Infrastructure Support Program received by the Burnet Institute.

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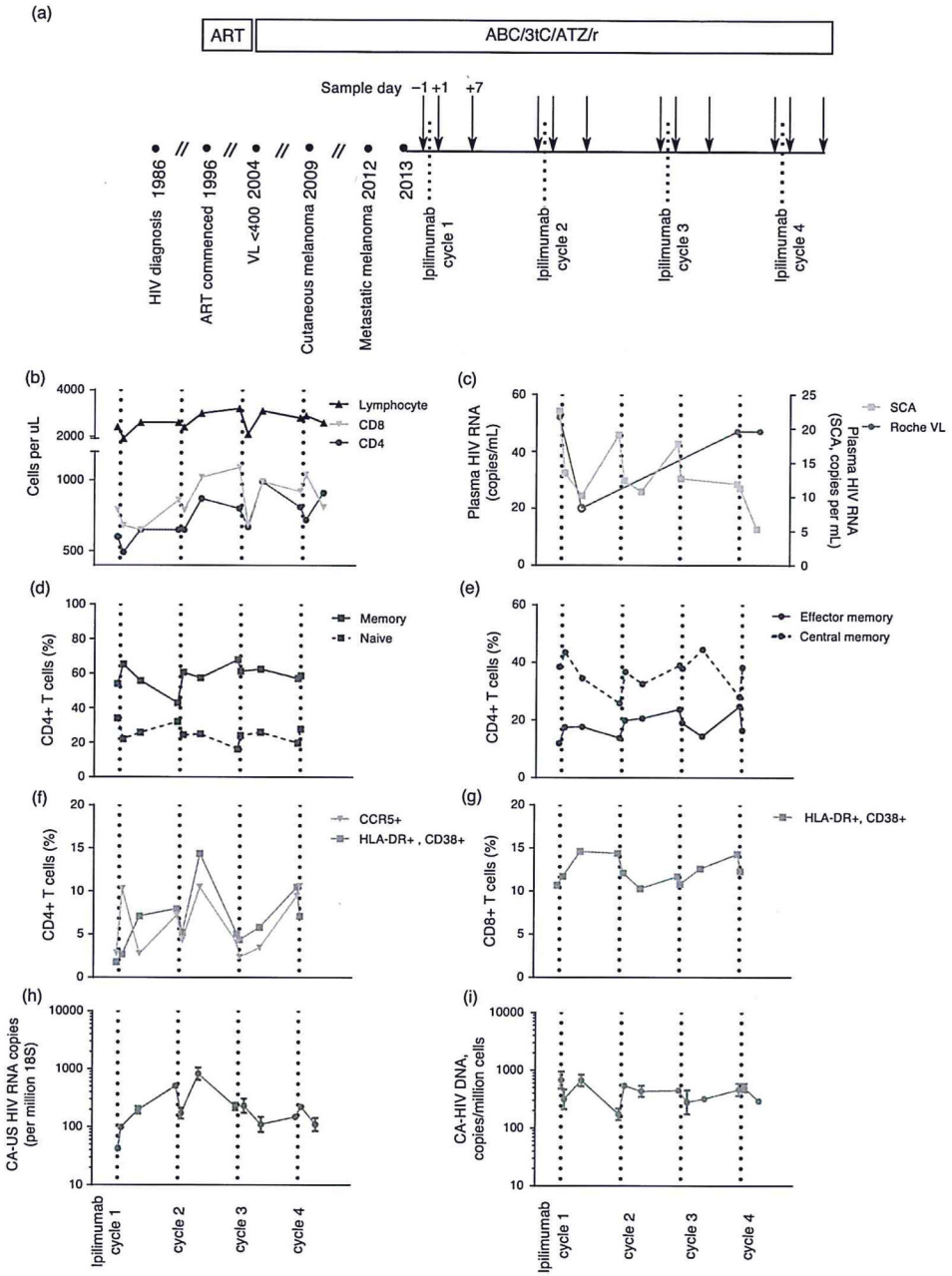


**Fig. 1. Clinical details and changes and impact of ipilimumab on virological and immunological parameters**

(a) An HIV-infected patient on ART who developed metastatic melanoma involving brain, right axilla, mesentery, and small bowel, all managed with surgical resection, underwent four cycles of ipilimumab treatment (dashed lines). The protocol for collection of plasma and peripheral blood mononuclear cells (PBMCs) for assessment of the HIV reservoir was approved by the Human Research and Ethics Committee, Royal Prince Alfred Hospital, Sydney, Australia, and the patient provided written informed consent. Plasma and PBMCs were collected at day −1, +1, and +7 for each cycle (arrows). Changes over the course of

treatment are shown for (b) total lymphocyte, and $CD4^+$ and $CD8^+$ T cells quantified by flow cytometry; (c) plasma HIV RNA measured by the Roche RT-PCR viral load assay (red line, open circles indicate sample below LLOD = 20 copies/ml) and single-copy assay (SCA) (green circles; LLOD = 0.3 copies/ml); (d) the percentage of memory (squares, solid line) and naïve (squares, dashed line); (e) effector memory (circles, solid line) and central memory (circles, dashed line) $CD4^+$ T cells and activation markers HLA-DR and CD38 (purple) and CCR5 (pink line) on (f) $CD4^+$ and (g) $CD8^+$ T cells. (h) Cell-associated (CA) unspliced (US) HIV RNA and (i) HIV DNA were quantified in sorted $CD4^+$ T cells using RT-PCR. The LLOD for both assays was 10 copies per million cell equivalents. ART, antiretroviral therapy; LLOD, lower limit of detection; RT, real-time.

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**Title:**
Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma

**Date:**
2015-02-20

**Citation:**
Wightman, F., Solomon, A., Kumar, S. S., Urriola, N., Gallagher, K., Hiener, B., Palmer, S., Mcneil, C., Garsia, R. & Lewin, S. R. (2015). Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma. AIDS, 29 (4), pp.504-506. https://doi.org/10.1097/QAD.0000000000000562.