

Supplementary Materials for  
**Pembrolizumab induces HIV latency reversal in people living with HIV and cancer on antiretroviral therapy**

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**The PDF file includes:**

Tables S1 to S4

**Other Supplementary Material for this manuscript includes the following**

Data file S1

MDAR Reproducibility Checklist

**Table S1. Participant baseline characteristics, treatments and cancer outcomes**

ID	Age (years)	CD4 <sup>+</sup> T cells (cells/mL)	CD4:CD8 Ratio	HIV RNA >LLD	ART	Cancer	Prior Cancer Therapy	Cycles Pembro (n)	Best Response to Pembro
1	71	477	0.90	NO	RAL, 3TC, ABC,	Bladder	Chemo	10	SD
2	58	271	0.71	NO	DRV, EVG/c, FTC, TAF	HCC	Chemo, TKI	11	SD
3	53	298	0.32	NO	EFV, FTC, TAF, ABC	Lung	Chemo	35	CR
4	47	132	0.41	NO	DTG, FTC, TAF	PEL	Chemo, mAb, IMiD, XRT	13	PR
5	59	144	0.56	NO	NVP, FTC, TAF	Anal	Chemo, XRT	6	SD
6	54	184	0.72	NO	RPV, FTC, TDF	Anal	Chemo, XRT	5	PD
7	50	220	0.26	NO	EFV, FTC, TDF	Tonsil	mAb	2	SD
8	68	164	0.47	NO	DTG, ABC, 3TC	Anal	Chemo, XRT, IMiD	2	PD
9	46	249	0.50	NO	MVC, FTC, TAF	SCC Skin	Chemo, XRT	4	SD
10	53	204	0.21	NO	DTG, FTC, TDF	DLBCL	Chemo, mAb, ACST	7	SD
11	53	382	1.61	NO	RPV, FTC, TDF	Lung	Surgery only	2	PD
12	56	343	0.68	NO	EFV, FTC, TDF	Anal	Chemo, XRT	1	PD
13	58	698	0.82	NO	DRV/r, DTG, FTC, TDF	KS	Chemo, XRT	6	SD
14	61	226	0.84	YES	DRV/c, FTC, TAF	Cholangiocarcinoma	Chemo	4	PD
15	62	600	1.97	NO	EFV, FTC, TDF	Pancreatic	Chemo	3	PD
16	43	228	0.39	NO	DTG, DRV/r, FTC, TDF	PEL	Chemo, mAb, XRT	6	SD
17	77	226	0.33	NO	DTG,3TC	Anal	Chemo	2	NA
18	73	321	1.83	YES	RAL, FDC, TDF	Prostate	Endo, Chemo, PARPi	6	SD
19	48	211	0.28	NO	DTG, ATZ/c, TDF	KS	Chemo, XRT	30	PR
20	61	210	0.48	NO	DTG, FTC, TDF	KS	Chemo, IMiD, XRT	5	PD
22	59	374	1.65	NO	EVG/c, FTC, TAF	KS	Chemo, TKI, mAb, IMiD, XRT	2	SD
23	58	495	0.48	NO	DTG, FTC, TDF	KS	Chemo, IMiD, XRT	4	SD
24	66	351	0.35	NO	DTG, ABC, 3TC	Adenocystic Carcinoma	Chemo	35	SD
25	42	468	1.33	NO	DTG, FTC, TAF	KS	Chemo, XRT	30	PR
26	71	536	0.94	NO	EVG/c, FTC, TAF	Bladder	Chemo	6	SD
27	52	557	0.34	YES	DRV/c, DTG, ABC	DLBCL	Chemo, mAb	15	PR
28	53	153	0.52	NO	DTG, FTC, TAF	SCC Skin	Chemo	2	PD
29	39	152	0.47	NO	EVG/c, FTC, TAF	Anal	Chemo	3	SD
30	49	966	0.87	NO	BIC, FTC, TAF	SCC Skin	None	2	PD
31	62	104	0.35	NO	DTG, ETR, DRV/r	Osteosarcoma	XRT, Chemo	2	NA
32	45	195	0.35	NO	DTG, FTC, TAF	Breast	Chemo	1	NA
33	47	187	1.61	NO	DRV/c, ABC, 3TC	Anal	XRT, Chemo	3	PD

LLD, lower limit of detection of the commercial HIV assay; Pembro, pembrolizumab; ART, antiretroviral therapy; 3TC, lamivudine; ABV, abacavir; ATV, atazanavir; BIC, bictegravir; /c, combined with cobicstat; DTG, dolutegravir; DRV, darunavir; EFV, efavirenz; EVG, elvitegravir; FTC, emtricitabine; ETR, etravirine; MVC, maraviroc; NVP, nevirapine; /r combined with ritonavir; RAL, raltegravir; RPV, rilpivirine. TAF, tenofovir alafenamide; TDF, tenofovir disoprofil fumarate; HCC, hepatocellular carcinoma; PEL, primary effusion lymphoma; KS, Kaposi sarcoma; DLBCL, diffuse large B-cell lymphoma; SCC, squamous cell carcinoma; Chemo, chemotherapy; Endo, endocrine therapy; IMiD, immune modulatory derivative of thalidomide (lenalidomide, pomali domide); mAb, monoclonal antibody (rituximab); TKI, tyrosine kinase inhibitor, PARPi, PARP inhibitor; XRT, radiation therapy; ASCT, autologous stem cell transplant; CR, complete response; PR, partial response, SD, stable disease; PD, progressive disease; NA, not available or not evaluable.

**Table S2. Pairwise comparison of HIV parameters quantified at baseline and 8 days following administration of the initial dose of pembrolizumab.**

<b>Baseline</b>									
	<b>Plasma HIV RNA</b>		<b>US HIV RNA</b>		<b>MS HIV RNA</b>		<b>HIV DNA</b>		
	<b><math>\rho</math></b>	<b>P</b>	<b><math>\rho</math></b>	<b>P</b>	<b><math>\rho</math></b>	<b>P</b>	<b><math>\rho</math></b>	<b>P</b>	
<b>Plasma HIV RNA</b>	1	na							
<b>US HIV RNA</b>	0.42	0.049	1	na					
<b>MS HIV RNA</b>	0.039	0.87	0.084	0.73	1	na			
<b>HIV DNA</b>	0.28	0.15	0.17	0.43	0.14	0.53	1	na	
<b>Day 8</b>									
	<b>Plasma HIV RNA</b>		<b>US HIV RNA</b>		<b>MS HIV RNA</b>		<b>HIV DNA</b>		
	<b><math>\rho</math></b>	<b>P</b>	<b><math>\rho</math></b>	<b>P</b>	<b><math>\rho</math></b>	<b>P</b>	<b><math>\rho</math></b>	<b>P</b>	
<b>Plasma HIV RNA</b>	1	na							
<b>US HIV RNA</b>	0.52	0.013	1	na					
<b>MS HIV RNA</b>	0.22	0.38	0.77	0.75	1	na			
<b>HIV DNA</b>	0.15	0.48	0.22	0.26	0.13	0.56	1	na	

US, unspliced; MS, multiply spliced. US HIV RNA, MS HIV RNA, and HIV DNA were measured on sorted CD4<sup>+</sup> T cells.  $\rho$ = Spearman correlation co-efficient; P= p-value; na, not applicable.

**Table S3. Effects of anti-PD1 on HIV parameters according to CD4:CD8 strata.**

HIV Assay	Comparison	CD4:CD8 < 0.5	CD4:CD8 ≥ 0.5	P (interaction) <sup>a</sup>
		<i>Fold-change (95%CI)</i>	<i>Fold-change (95%CI)</i>	
Plasma HIV RNA	D8 versus D1	0.39 (0.15, 1.01)	2.53 (0.99, 6.50)	0.053
Unspliced HIV RNA	D8 versus D1	1.00 (0.73, 1.36)	2.05 (1.41, 2.99)	0.004
HIV DNA	D2 versus D1	0.65 (0.52, 0.81)	0.92 (0.77, 1.11)	0.017
HIV DNA	D8 versus D1	0.86 (0.70, 1.05)	0.83 (0.63, 1.09)	0.8
Unspliced HIV RNA / HIV DNA	D8 versus D1	1.37 (0.91, 2.07)	2.44 (1.69, 3.54)	0.041
<i>Tat/rev</i> Induced Limiting Dilution Assay	EOT<C7 versus D1	0.71 (0.45, 1.11)	0.80 (0.63, 1.02)	0.63
<i>Tat/rev</i> Induced Limiting Dilution Assay	C7 versus D1	1.20 (0.95, 1.53)	1.85 (1.39, 2.48)	0.025

Estimates from a mixed-effects negative binomial regression model are shown stratified by baseline CD4:CD8 ratio. <sup>a</sup>Wald test of multiplicative interaction term indicating strength of evidence that the fold-change estimates differ between strata of CD4:CD8 ratio. D, Day; EOT, end of treatment; C, cycle.

**Table S4. Antibodies used in cell sorting for single genome sequencing.**

Antibody	Supplier	Catalog number	RRID
Anti-CD3 Phycoerythrin (PE)	BD Biosciences	555340	RRID: AB_395746
Anti-CD4 PE-Texas Red	Invitrogen	MHCD0417	RRID: AB_1473480
Anti-CCR7 Alexa Fluor 488	BioLegend	353205	RRID: AB_10918624
Anti-CD27 Brilliant Violet711	BD Biosciences	563167	RRID: AB_2738042
Anti-CD45RA PE-Cy7	BD Biosciences	337167	RRID: AB_647424