

SUPPLEMENTARY MATERIAL

Immune Reconstitution During the First Year of Antiretroviral Therapy of HIV-1-Infected Adults in Rural Burkina Faso

Fabrice Tiba¹, Frans Nauwelaers², Siaka Traoré³, Boubacar Coulibaly³, Thierry Ouedraogo³, Adama Compaoré³, Hans-Georg Kräusslich¹ and Thomas Böhler^{*1}

¹Department of Infectious Diseases, Virology, University of Heidelberg, INF324, D-69120 Heidelberg, Germany

²BD Biosciences, Erembodegem, Belgium

³Centre de Recherche en Santé de Nouna, Nouna, Burkina Faso

Supplementary Table 1. Immunological and Virological Parameters in HIV-1 Infected Subjects at Baseline, i.e., Before Starting HAART

	IR (A)	NIR (B)	VR (C)	NVR (D)
n =	33	15	25	17
CD4 ⁺ T-cell count (cells/ μ l)	179 (41-318)	202 (7-316)	214 (13-340)	146 (49-265)
HIV-1 plasma viral load (\log_{10} copies/ml)	5.7 (4.3-6.4)	4.9 (4.3-6.0)*	5.8 (4.9-6.0)	5.7 (5.1-7.0)
CD45RA ⁺ CCR7 ⁺ (% of CD4 ⁺ T-cells)	10 (3-32)	12.5 (3-28)	16 (3-32)	13 (3-32)
CD45RA ⁺ CCR7 ⁺ CD4 ⁺ T-cell count (cells/ μ l)	23 (2-77)	18 (1-42)	18 (2-79)	28 (1-102)
CD45RA ⁺ CCR7 ⁺ (% of CD8 ⁺ T-cells)	4 (1-9)	4 (0.4-14)	4 (1-9)	4 (3-9)
CD45RA ⁺ CCR7 ⁺ CD8 ⁺ T-cell count (cells/ μ l)	25 (2-54)	6 (0.08-14)	14 (0.2-52)	28 (1-102)
CD95 ⁺ (% of CD4 ⁺ T-cells)	97 (85-99)	97 (84-99)	96 (83-99)	98 (91-99)
CD95 ⁺ CD4 ⁺ T-cells (cells/ μ l)	134 (37-306)	186 (7-305)	191 (12-315)	146 (47-239)
CD95 ⁺ (% of CD4 ^{neg} T-cells)	98 (91-99)	96 (93-99)	97 (89-99)	99 (94-99)
CD95 MFI on CD95 ^{dim} CD3 ⁺ CD4 ⁺ cells	16 (8-33)	22 (4-50)	19 (6-39)	26 (7-52)
CD95 MFI on CD95 ^{bright} CD3 ⁺ CD4 ⁺ cells	307 (164-655)	349 (129-426)	279 (160-381)	337 (180-427)*
CD95 ⁺ CD4 ^{neg} T-cells (cells/ μ l)	978 (501-1980)	532 (87-1762)	778 (489-1836)	855 (312-1981)
CD95 MFI on CD95 ^{bright} CD3 ⁺ CD4 ^{neg} cells	126 (76-189)	141 (68-205)	125 (72-189)	150 (110-204)
CD38 ⁺ (% of CD8 ⁺ T-cells)	97 (90-99)	98 (84-98)	95 (87-99)	99 (94-99)
CD38 ⁺ CD8 ⁺ T-cells (cells/ μ l)	978 (501-1980)	688 (254-1756)	831 (546-1747)	1011 (377-1979)
CD38 MFI on CD38 ⁺ CD3 ⁺ CD8 ⁺ cells	296 (149-827)	276 (184-890)	278 (126-701)	509 (241-890)*
CD38 ⁺ (% of CD8 ^{neg} T-cells)	90 (81-95)	82 (75-94)	87 (80-96)	93 (82-96)
CD38 ⁺ CD8 ^{neg} T-cells (cells/ μ l)	160 (40-243)	145 (6-258)	193 (34-274)	140 (43-206)
CD38 MFI on CD38 ⁺ CD3 ⁺ CD8 ^{neg} cells	339 (209-655)	314 (205-781)	313 (205-667)	457 (231-781)

Median values are shown with their 10th - 90th centile in parentheses. Patients with complete immunological follow-up (n=48) were grouped according to response to treatment: IR = good immunologic response, NIR = immunologic non-response. Patients with complete virologic follow-up (n=42) were divided into the following groups: VR = good virologic response, NVR = virologic non-response. The Mann-Whitney U-test was used to compare the IR vs NIR and VR vs NVR groups, respectively; asterisks indicate statistically significant differences: *p<0.05. Abbreviations: MFI = Median fluorescence intensity.

Supplementary Table 2. Immunological and Virological Parameters in HIV-1 Infected Subjects After 12 Months of HAART

	IR (A)	NIR (B)	VR (C)	NVR (D)
n =	33	15	25	17
CD4 ⁺ T-cell count (cells/ μ l)	418 (173-562)	254 (82-405)**	398 (151-576)	322 (185-563)
HIV-1 plasma viral load (\log_{10} copies/ml)	1.6 (1.6-3.6)	1.6 (1.6-2.0)	undetectable	2.0 (1.7-4.8)
CD45RA ⁺ CCR7 ⁺ (% of CD4 ⁺ T-cells)	19 (4-38)	15 (4-26)	17 (3-38)	18 (5-37)
CD45RA ⁺ CCR7 ⁺ CD4 ⁺ T-cell count (cells/ μ l)	72 (11-172)	30 (3-38)	41 (3-179)	72 (10-161)
CD45RA ⁺ CCR7 ⁺ (% of CD8 ⁺ T-cells)	12 (2-22)	6 (5-7)	16 (2-28)	7 (3-14)
CD45RA ⁺ CCR7 ⁺ CD8 ⁺ T-cell count (cells/ μ l)	95 (23-258)	25 (22-28)*	125 (19-318)	68 (26-158)
CD95 ⁺ (% of CD4 ⁺ T-cells)	89 (75-96)	89 (69-98)	88 (70-98)	88 (78-99)
CD95 ⁺ CD4 ⁺ T-cells (cells/ μ l)	312 (140-481)	135 (67-242)**	307 (102-480)	289 (102-487)
CD95 ⁺ (% of CD4 ^{neg} T-cells)	87 (73-98)	83 (74-97)	85 (71-94)	92 (69-97)
CD95 ⁺ CD4 ^{neg} T-cells (cells/ μ l)	742 (342-1841)	634 (312-1316)	846 (297-1492)	703 (363-1911)
CD95 MFI on CD95 ^{dim} CD3 ⁺ CD4 ⁺ cells	12 (5-19)	9 (8-10)	12 (7-18)	10 (5-21)
CD95 MFI on CD95 ^{bright} CD3 ⁺ CD4 ⁺ cells	150 (77-276)	78 (53-100)**	153 (63-278)	110 (70-316)
CD95 MFI on CD95 ^{bright} CD3 ⁺ CD4 ^{neg} cells	74 (43-119)	48 (34-135)	69 (35-103)	85 (36-137)
CD38 ⁺ (% of CD8 ⁺ T-cells)	87 (67-96)	73 (62-95)	83 (60-98)	86 (64-95)
CD38 ⁺ CD8 ⁺ T-cells (cells/ μ l)	842 (404-1846)	395 (261-848)	846 (263-1524)	672 (405-1868)
CD38 MFI on CD38 ⁺ CD3 ⁺ CD8 ⁺ cells	75 (50-186)	102 (88-218)	65 (40-196)	84 (56-180)
CD38 ⁺ (% of CD8 ^{neg} T-cells)	78 (68-86)	67(67-77)	73 (61-87)	78 (69-83)
CD38 ⁺ CD8 ^{neg} T-cells (cells/ μ l)	302 (132-497)	65 (63-174)***	258 (102-437)	297 (101-577)
CD38 MFI on CD38 ⁺ CD3 ⁺ CD8 ^{neg} cells	153 (83-262)	102 (89-218)	127 (82-210)	166 (83-280)

Median values are shown with their 10th - 90th centile in parentheses. Patients with complete immunological follow-up (n=48) were grouped according to response to treatment: IR = good immunologic response, NIR = immunologic non-response. Patients with complete virologic follow-up (n=42) were divided into the following groups: VR = good virologic response, NVR = virologic non-response. The Mann-Whitney U-test was used to compare the IR vs NIR and VR vs NVR groups, respectively; asterisks indicate statistically significant differences: *p<0.05; **p<0.01; ***p<0.001. Abbreviations: MFI = Median fluorescence intensity.

Supplementary Table 3. Immunological and Virological Parameters in HIV-1 Infected Subjects at Baseline and After 12 Months of HAART

	IR and VR	IR but NVR	VR but NIR	NIR and NVR
n =	19	14	6	3
At baseline				
CD4 ⁺ T-cell count (cells/ μ l)	202 (13-412)	178 (63-251)	177 (7-316)	122 (7-256)
HIV-1 plasma viral load (\log_{10} copies/ml)	5.3 (4.3-6.2)	5.8 (5.0-6.4)	5.1 (4.4-5.9)	5.8 (5.5-5.9)
CD45RA ⁺ CCR7 ⁺ (% of CD4 ⁺ T-cells)	25 (1-32)	18 (8-35)	9 (3-27)	11 (9-13)
CD45RA ⁺ CCR7 ⁺ CD4 ⁺ T-cell count (cells/ μ l)	31 (2-87)	18 (4-75)	13 (5-43)	6 (1-10)
CD45RA ⁺ CCR7 ⁺ (% of CD8 ⁺ T-cells)	4 (1-10)	3 (1-7)	3 (0.4-7)	4 (0.5-9)
CD45RA ⁺ CCR7 ⁺ CD8 ⁺ T-cell count (cells/ μ l)	12 (0.2-50)	33 (1-108)	3 (0-18)	8 (7-9)
CD95 ⁺ (% of CD4 ⁺ T-cells)	97 (83-99)	99 (93-100)*	99 (92-100)	99 (99-100)
CD95 ⁺ CD4 ⁺ T-cells (cells/ μ l)	195 (11-407)	175 (58-248)	208 (46-311)	119 (5-247)
CD95 ⁺ (% of CD4 ^{neg} T-cells)	96 (83-100)	98 (92-100)	97 (94-100)	99 (99-100)
CD95 MFI on CD95 ^{dim} CD3 ⁺ CD4 ⁺ cells	24 (7-43)	24 (5-155)	19 (8-59)	23 (9-33)
CD95 MFI on CD95 ^{bright} CD3 ⁺ CD4 ⁺ cells	298 (159-442)	330 (204-427)	375 (215-523)	337 (264-425)
CD95 ⁺ CD4 ^{neg} T-cells (cells/ μ l)	959 (489-1970)	1156 (515-1989)	747 (87-1762)	267 (260-381)
CD95 MFI on CD95 ^{bright} CD3 ⁺ CD4 ^{neg} cells	126 (71-194)	151 (96-214)	160 (103-189)	159 (124-239)
CD38 ⁺ (% of CD8 ⁺ T-cells)	96 (90-99)	98 (91-99)	92 (84-96)	93 (92-94)
CD38 ⁺ CD8 ⁺ T-cells (cells/ μ l)	830 (499-1881)	1150 (498-1987)	1200 (688-1756)	315 (254-377)
CD38 MFI on CD38 ⁺ CD3 ⁺ CD8 ⁺ cells	217 (123-1125)	509 (154-886)*	239 (184-276)	495 (259-781)
CD38 ⁺ (% of CD8 ^{neg} T-cells)	88 (81-95)	93 (82-95)	95 (91-99)	98 (98-99)
CD38 ⁺ CD8 ^{neg} T-cells (cells/ μ l)	181 (14-248)	157 (57-231)	200 (144-258)	61 (6-115)
CD38 MFI on CD38 ⁺ CD3 ⁺ CD8 ^{neg} cells	292 (192-749)	482 (221-738)**	209 (205-313)	552 (449-890)
After 12 months of HAART				
CD4 ⁺ T-cell count (cells/ μ l)	418 (162-653)	399 (187-562)	248 (96-405)	205 (82-296)
HIV-1 plasma viral load (\log_{10} copies/ml)	undetectable	1.9 (1.8-5.2)	undetectable	1.7 (1.6-2.0)
CD45RA ⁺ CCR7 ⁺ (% of CD4 ⁺ T-cells)	17 (2-38)	23 (5-38)	14 (3-25)	16 (4-36)
CD45RA ⁺ CCR7 ⁺ CD4 ⁺ T-cell count (cells/ μ l)	69 (3-208)	72 (16-170)	35 (30-34)	20 (20-38)
CD45RA ⁺ CCR7 ⁺ (% of CD8 ⁺ T-cells)	16 (2-28)	8 (2-17)*	11 (7-16)	10 (3-14)
CD45RA ⁺ CCR7 ⁺ CD8 ⁺ T-cell count (cells/ μ l)	126 (19-319)	70 (26-159)	95 (28-128)	82 (25-160)
CD95 ⁺ (% of CD4 ⁺ T-cells)	87 (70-97)	88 (78-99)	89 (74-95)	90 (79-100)
CD95 ⁺ CD4 ⁺ T-cells (cells/ μ l)	398 (150-570)	439 (162-555)	154 (87-242)	184 (61-295)
CD95 ⁺ (% of CD4 ^{neg} T-cells)	86 (74-96)	92 (67-97)	83 (74-92)	92 (87-97)
CD95 MFI on CD95 ^{dim} CD3 ⁺ CD4 ⁺ cells	12 (8-19)	10 (8-22)	8 (8-9)	10 (6-22)
CD95 MFI on CD95 ^{bright} CD3 ⁺ CD4 ⁺ cells	155 (114-260)	156 (74-304)	58 (53-64)	92
CD95 MFI on CD95 ^{bright} CD3 ⁺ CD4 ^{neg} cells	72 (56-106)	74 (37-125)	102 (64-135)	97
CD38 ⁺ (% of CD8 ⁺ T-cells)	87 (60-98)	88 (65-95)	62	94
CD38 ⁺ CD8 ⁺ T-cells (cells/ μ l)	842 (315-1549)	849 (427-1871)	261	395
CD38 MFI on CD38 ⁺ CD3 ⁺ CD8 ⁺ cells	65 (39-309)	83 (56-205)	74	125
CD38 ⁺ (% of CD8 ^{neg} T-cells)	77 (63-88)	79 (65-95)	67	77
CD38 ⁺ CD8 ^{neg} T-cells (cells/ μ l)	310 (124-582)	274 (134-436)	64	63
CD38 MFI on CD38 ⁺ CD3 ⁺ CD8 ^{neg} cells	133 (80-230)	166 (91-281)	89	267

*p<0.01 and **p<0.001.

Median values are shown with their 10th - 90th centile in parentheses. Patients (n=42) are grouped according to response to treatment: IR = good immunologic response, VR = good virologic response, NIR = immunologic non-response, NVR = virologic non-response. MFI = Median fluorescence intensity. Mann-Whitney U-test was used to compare the IR and VR vs. IR but NVR group.

Supplementary Table 4. Analysis of Individual Treatment Responses to HAART in Patients with Virological Treatment Failure During 12 Months of HAART

Parameters	M0	2 weeks	M1	M3	M6	M9	M12
VL	5.987478	3.754425	ND	2.09691	3.439964	2.591065	1.78533
CD4	7	ND	50	52	60	80	82
% Naive CD4	2.5	1.84	2.72	1.03	ND	1.62	3.51
% Naive CD8	0.46	1.48	1.25	1.37	3.6	3.2	7.01
MFI CD95dimCD4+	4.18	6.79	4.53	8.35	8.35	15.75	8.94
MFI CD95dimCD4-	12.1	8.9	8.66	5.7	6.49	8.54	12.19
MFI CD38CD8+	889.65	777.37	577.72	858.21	162.53	121.88	124.09
MFI CD38CD8-	780.87	922.24	511.66	713.7	201.69	406.79	218.7
Parameters	M0	2 weeks	M1	M3	M6	M9	M12
VL	5.901261	3.792392	ND	2.706718	6.364538	3.371437	2.665581
CD4	59	ND	114	114	73	175	185
% Naive CD4	10.93	2.28	4.67	19.62	12.06	ND	10.37
% Naive CD8	1.06	1.46	1.7	1.92	1.01	ND	3.42
MFI CD95dimCD4+	20.81	18.94	12.19	7.43	7.43	ND	22.17
MFI CD95dimCD4-	11.76	21.97	11.34	17	20.35	ND	13.64
MFI CD38CD8+	805.84	264.16	417.92	86.6	291.64	ND	60.16
MFI CD38CD8-	482.61	271.39	302.32	132.16	187.69	ND	191.1
Parameters	M0	2 weeks	M1	M3	M6	M9	M12
VL	5.237391	3.196453	ND	1.995635	1.863323	<1,591065	2.178977
CD4	132	ND	213	217	191	162	314
% Naive CD4	18.2	5.3	4.7	13.32	ND	8.9	29.19
% Naive CD8	3.88	3.54	4.77	6.74	8.37	6.15	7.99
MFI CD95dimCD4+	6.26	13.76	13.1	8.58	9.65	ND	6.15
MFI CD95dimCD4-	6.49	13.7	10.65	12.86	18.19	ND	15.96
MFI CD38CD8+	495.81	339.82	179.43	72.99	65.52	49.58	224.68
MFI CD38CD8-	421.7	299.61	213.83	86.99	93.9	72.34	226.71
Parameters	M0	2 weeks	M1	M3	M6	M9	M12
VL	5.815216	3.408749	ND	2.217484	<1,591065	2.08636	2
CD4	198	ND	275	260	235	270	322
% Naive CD4	20.24	13.33	10.96	2.92	7.65	ND	19.27
% Naive CD8	7.43	2.3	2.29	4.62	5.42	ND	ND
MFI CD95dimCD4+	72.34	20.17	13.4	8.47	8.1	ND	11.76
MFI CD95dimCD4-	14.99	16.7	13.46	10.84	7.18	ND	3.29
MFI CD38CD8+	ND	327.81	213.83	144.6	130.97	ND	160.98
MFI CD38CD8-	ND	294.27	228.76	189.38	105.54	ND	179.86
Parameters	M0	2 weeks	M1	M3	M6	M9	M12
VL	5.409466	3.402605	ND	2.245513	<1,591065	<1,591065	1.94939
CD4	231	ND	219	244	248	249	360
% Naive CD4	5.44	4.64	5.35	13.48	7.11	ND	8.96
% Naive CD8	1.61	11.69	ND	22.96	4.22	ND	2.44
MFI CD95dimCD4+	17.7	16.91	16.7	10.37	10.37	ND	12.63
MFI CD95dimCD4-	20.44	20.08	19.63	14.14	9.37	ND	6.32
MFI CD38CD8+	ND	68.54	78.44	58.56	54.25	ND	79.15
MFI CD38CD8-	ND	93.9	101.82	108.92	94.75	ND	110.9

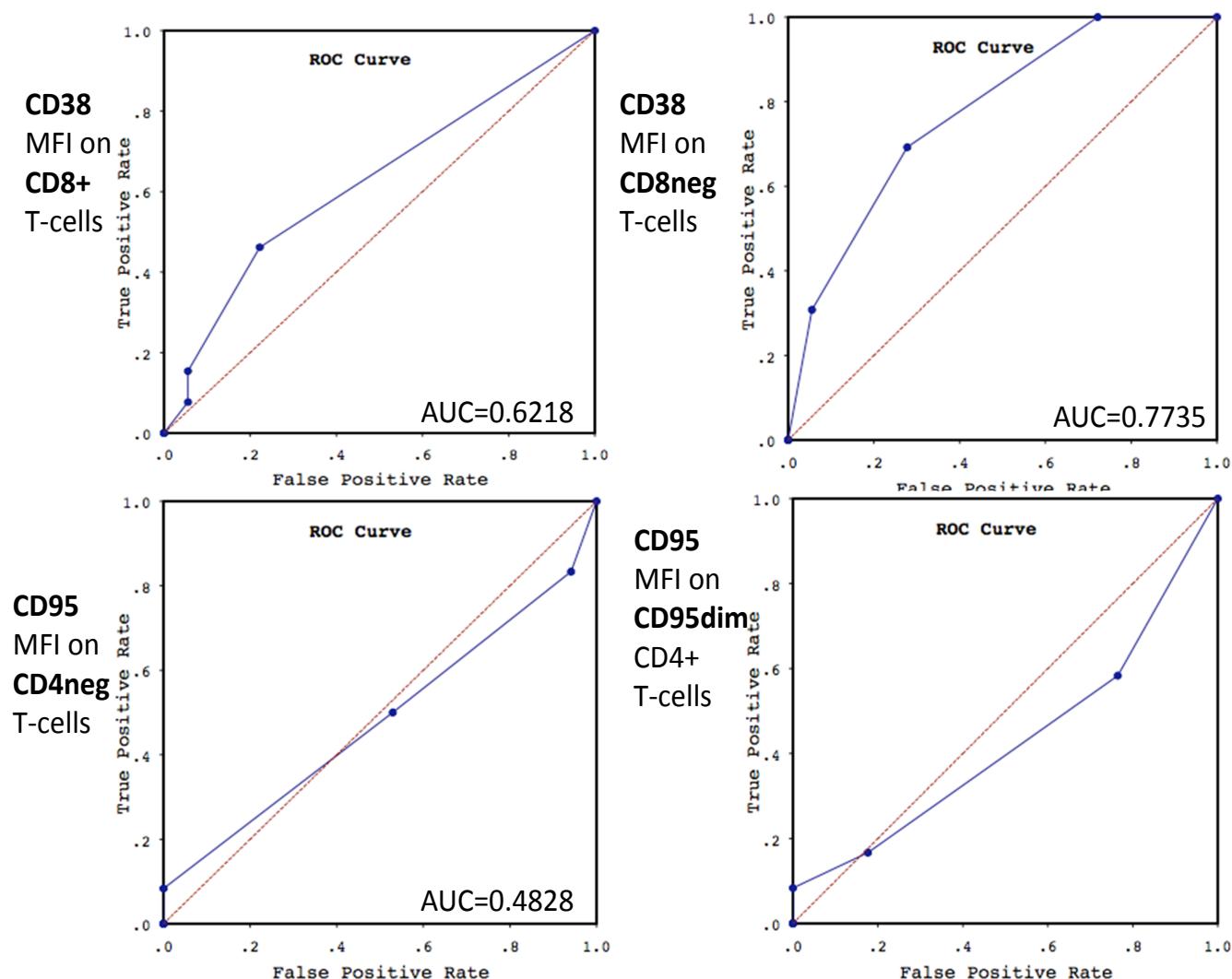
ND = not determined

VL= viral load (log10 copies/ml)

M= months of treatment

MFI = median fluorescence intensity

Longitudinal data from 5 patients with CD4 counts ranging from 7 to 231 cells μl^{-1} at baseline are shown. Abbreviations: M= months of treatment; MFI = median fluorescence intensity; ND = not determined; VL= viral load (log10 copies/ml).



Supplementary Fig. (1). Receiver operating characteristics (ROC) curves of median fluorescence intensity of activation markers on peripheral blood T-cells show the lack of usefulness of these parameters as indicators of poor virologic treatment response at 12 months of HAART. ROC curves were generated using the MS Excel template of Marley W. Watkins available at <http://www.public.asu.edu/~mwwatkin/Software/ROCanalysis.xls>. Abbreviation: AUC = area under the ROC curve.