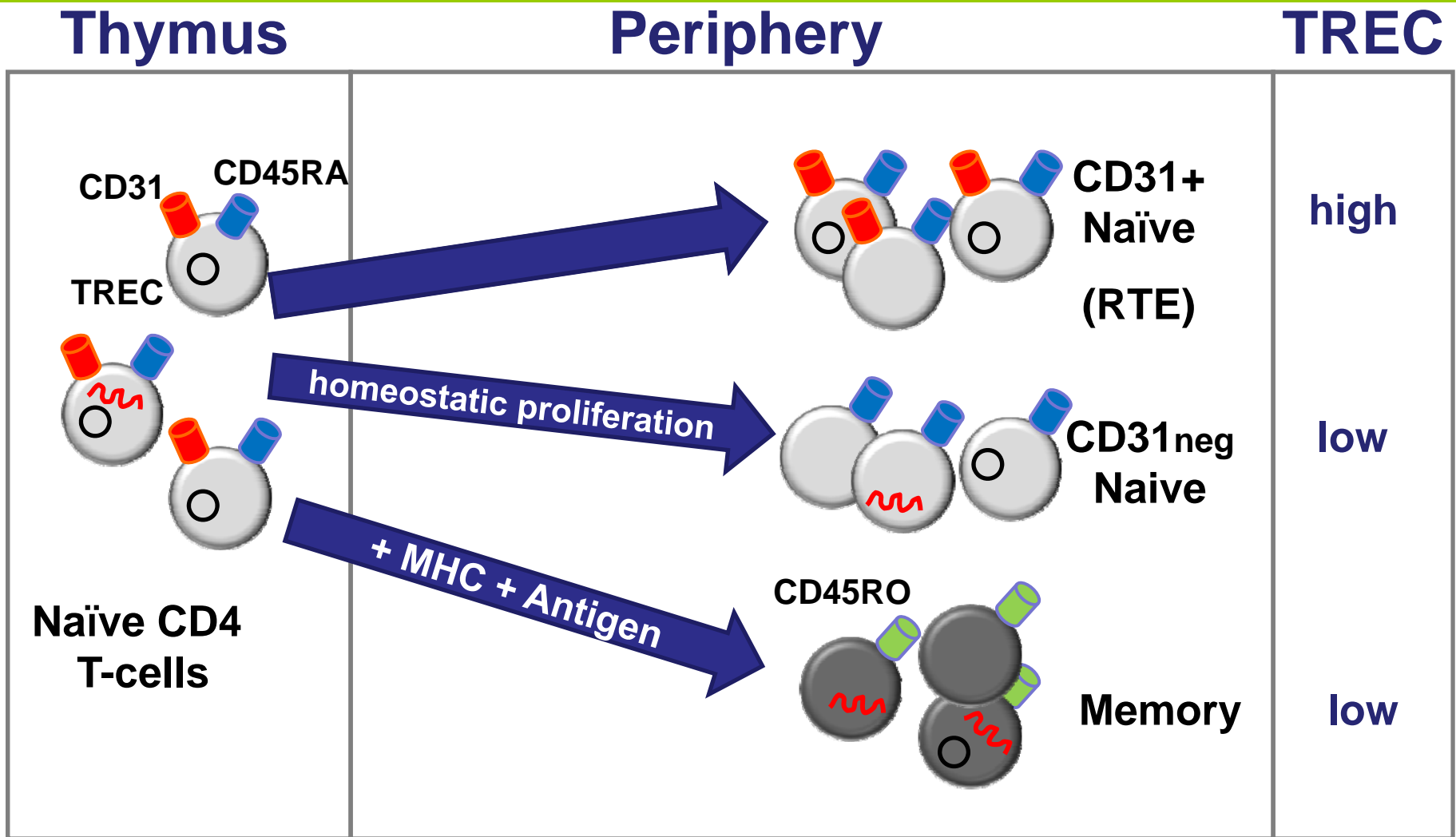

No Decay in HIV-1 infected Naïve CD4+ T-cells Following Antiretroviral Therapy (ART): a Persistent Long Term Reservoir.

Fiona Wightman, Ajantha Solomon, Jennifer Hoy, Paul Gorry,
Suzanne Crowe, Nitin Saksena,
Paul U Cameron, Sharon R Lewin.

Background

- Latent infection in resting memory CD4+ T-cells represent a major barrier to eradication of HIV
 - Finzi, Science 1997; Chun PNAS 1997; Chomont, Nature Med, 2009
- Latent infection of transitional memory T-cells may also be important in patients with CD4 T-cells < 500 cells/ul. This reservoir may be replenished via IL-7-driven proliferation
 - Chomont, Nature Med, 2009
- Naïve T-cells are latently infected in vivo but at a lower frequency than memory cells
 - Schnittman, PNAS 1990; Ostrowski, J. Virology 1999; McBreen, J. Virology 2001; Brenchley, J Virol, 2004; Chomont, Nature Med 2009
- Since naïve T-cells have a long half life and are under the control of IL-7 driven homeostasis, they may represent a small and possibly expanding reservoir in HIV-infected patients receiving ART.

Model of naïve T cell homeostasis



Aim

- To determine the changes in CD31+ and CD31- naïve T cells following ART
- To determine the role of CD31+ and CD31- naïve T cells as a persistent reservoir in patients on ART.
- To determine the phylogenetic relationship between viruses identified in naïve T-cell subsets, memory cells and plasma prior to and following ART

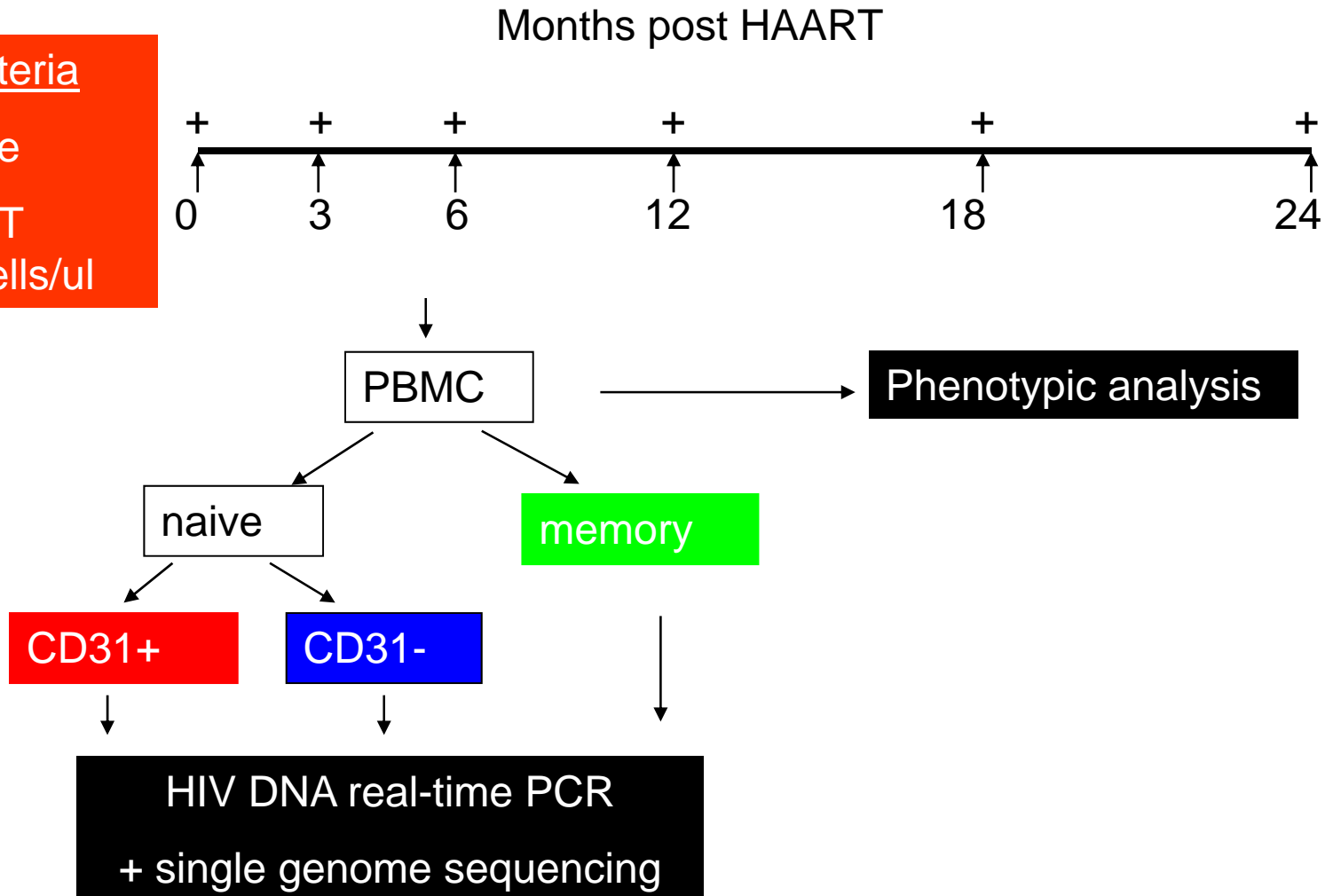
Patient recruitment and study design

Inclusion criteria

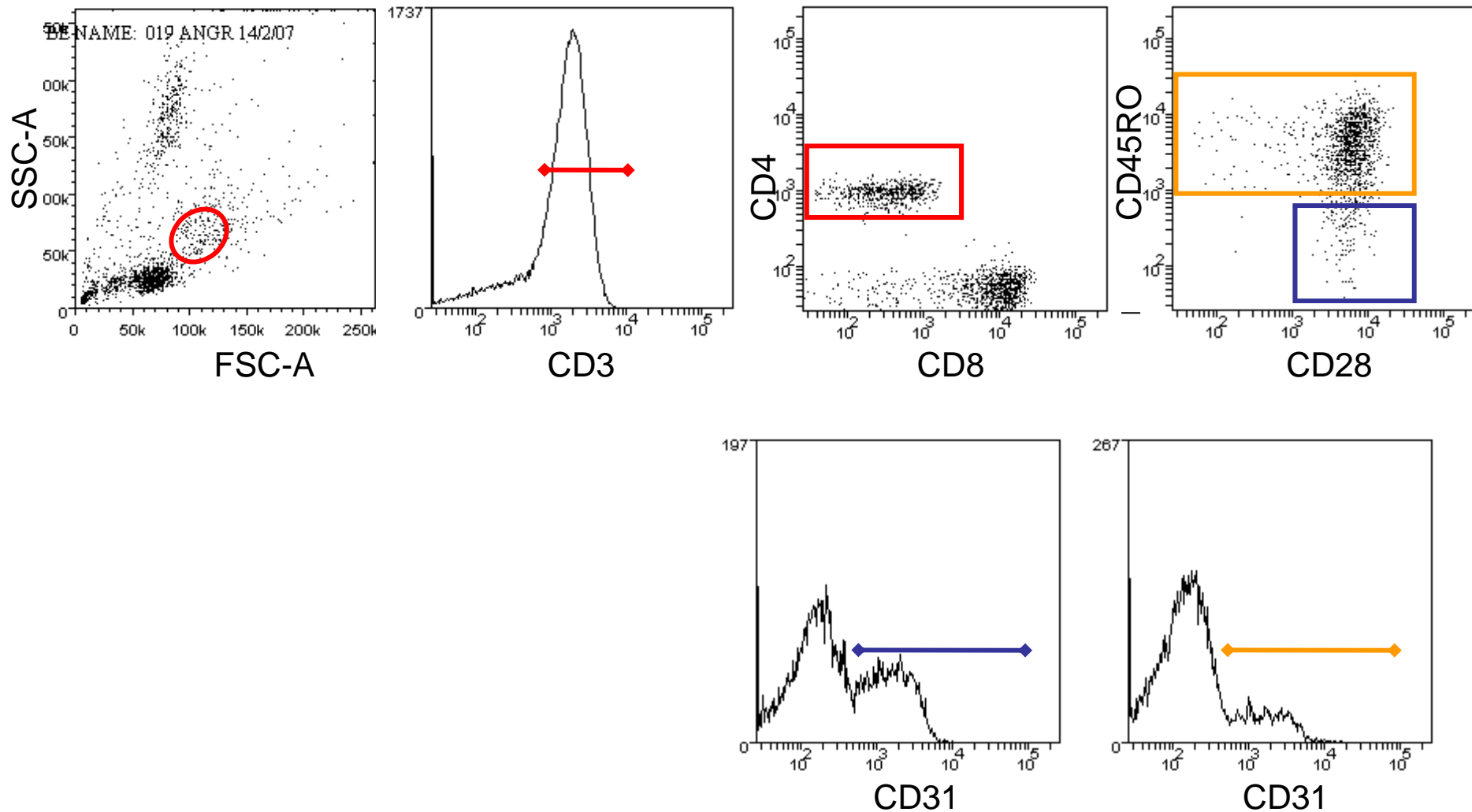
Single centre

Naïve to ART

CD4 > 350 cells/ul



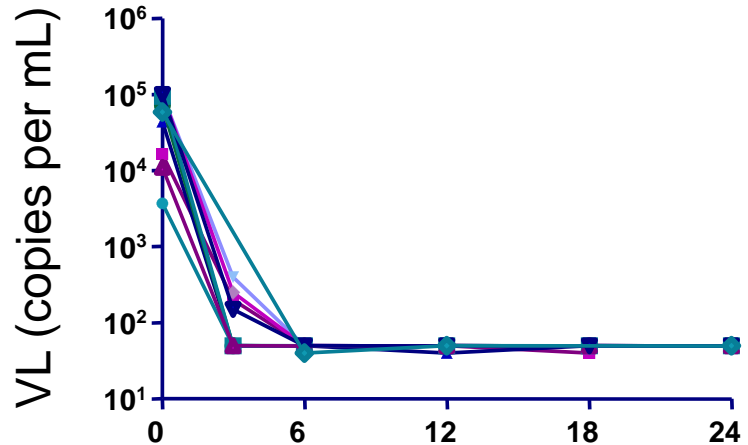
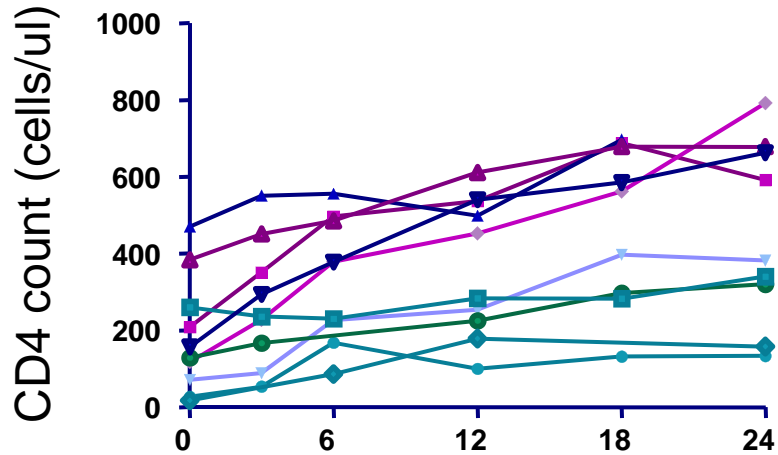
Phenotyping analysis: naïve and memory CD4 T-cell subsets



Patient demographics: enrolment

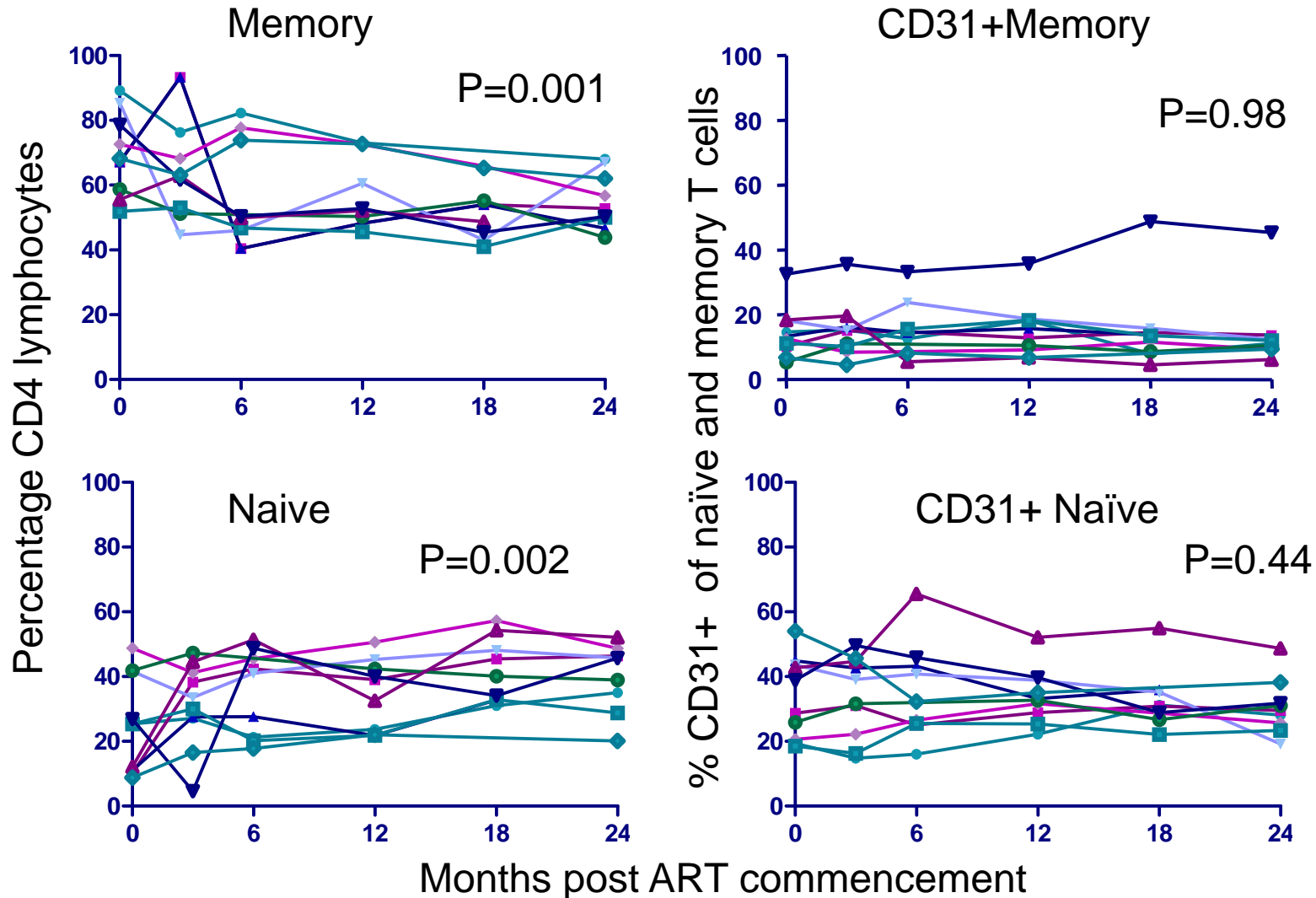
	Age (years)	Gender	Pre-ART CD4 count (cells/ μ l)	Pre-ART Viral Load (copies/ml)	Antiretroviral regimen
1	43	Male	27	3700	Zidovudine, 3TC, Nevirapine,
2	45	Male	209	16500	Abacavir, 3TC, Efavirenz, Atazanavir/r
3	42	Male	470	44100	Nevirapine, 3TC, Zidovudine
4	71	Male	71	>100000	Zidovudine, Abacavir, 3TC, Atazanavir
5	41	Male	118	>100000	Tenofovir, FTC, Nevirapine
6	41	Male	129	71700	Tenofovir, FTC, Efavirenz
7	37	Female	260	94600	Tenofovir, FTC, Lopinavir/r
8	25	Male	384	11100	Abacavir, 3TC, Efavirenz,
9	52	Male	157	>100000	Abacavir, 3TC, Lopinavir/r
10	40	Male	18	58600	Tenofovir, FTC, Efavirenz,
Median (IQR)	31 (41-46)		143 (60-291)	65150 (15150- 10000)	

Response to ART

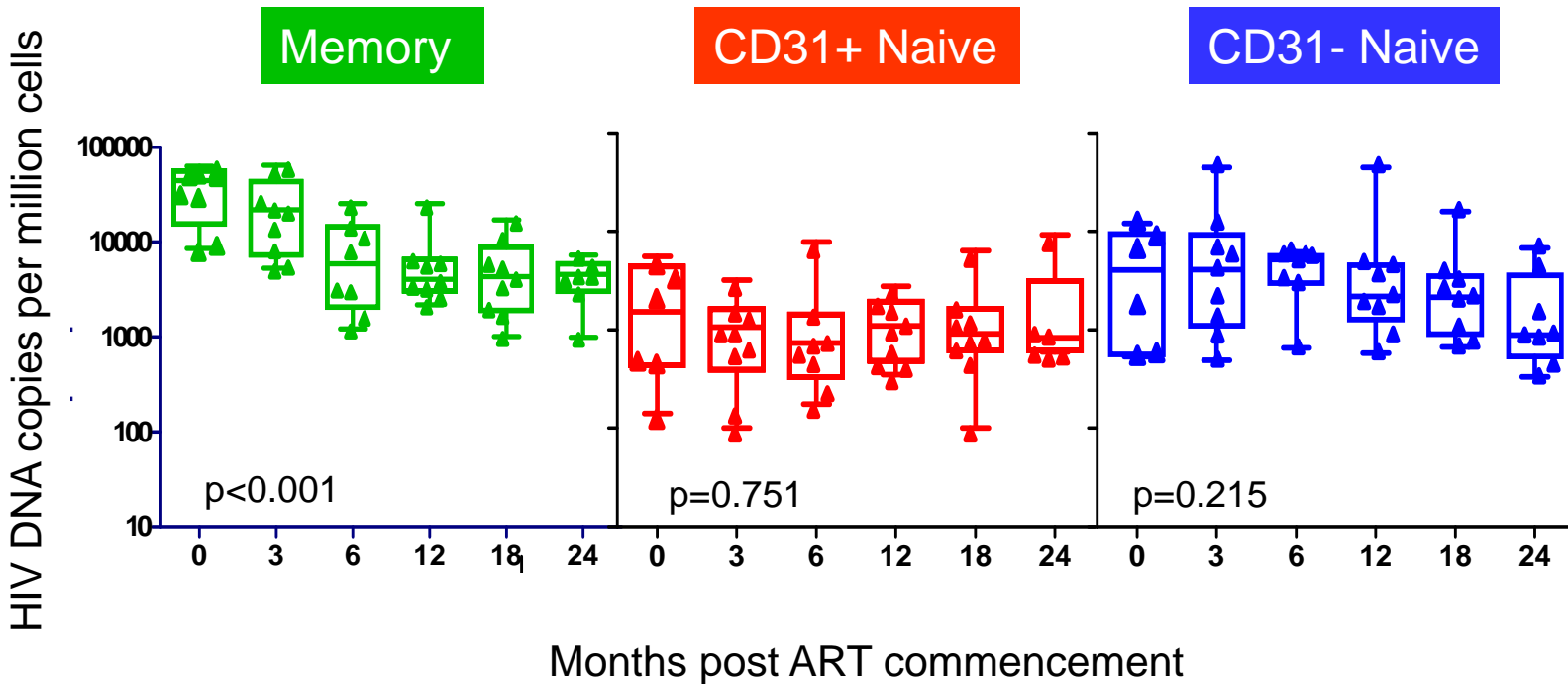


Months post ART commencement

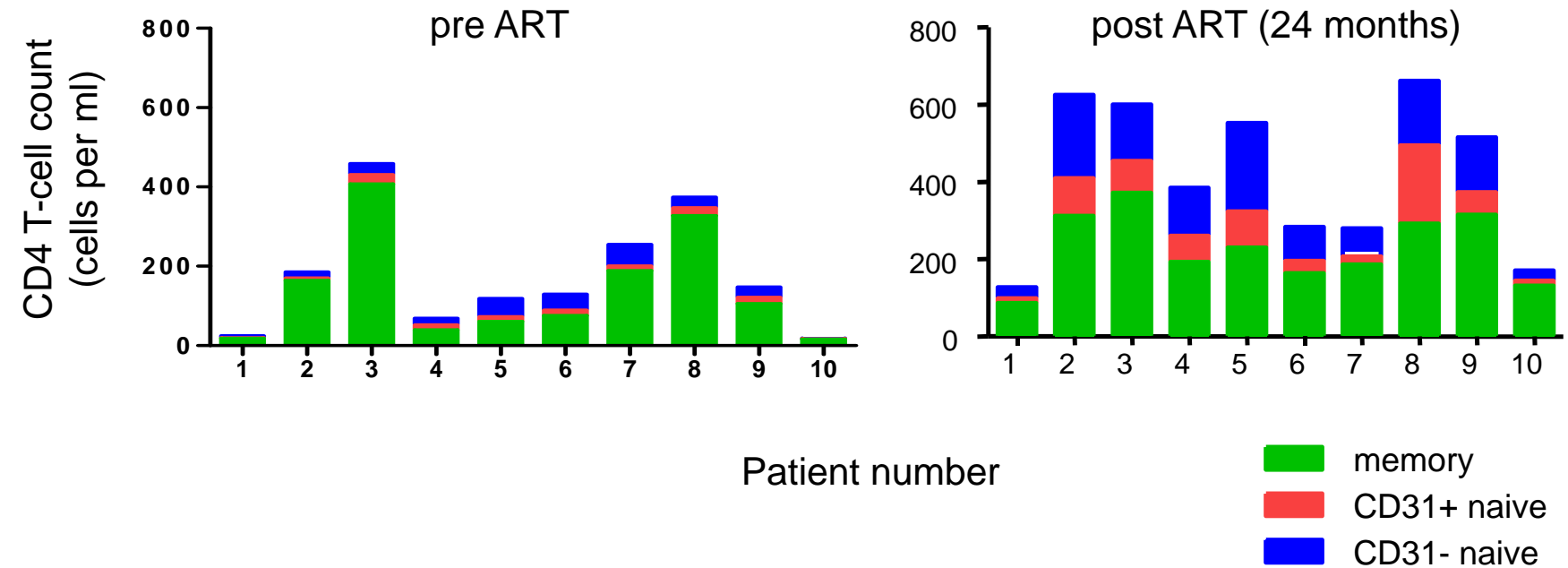
No significant change in proportion of CD31+ naïve T-cells post ART



No significant decline in HIV DNA content in CD31+ or CD31- naïve T-cells

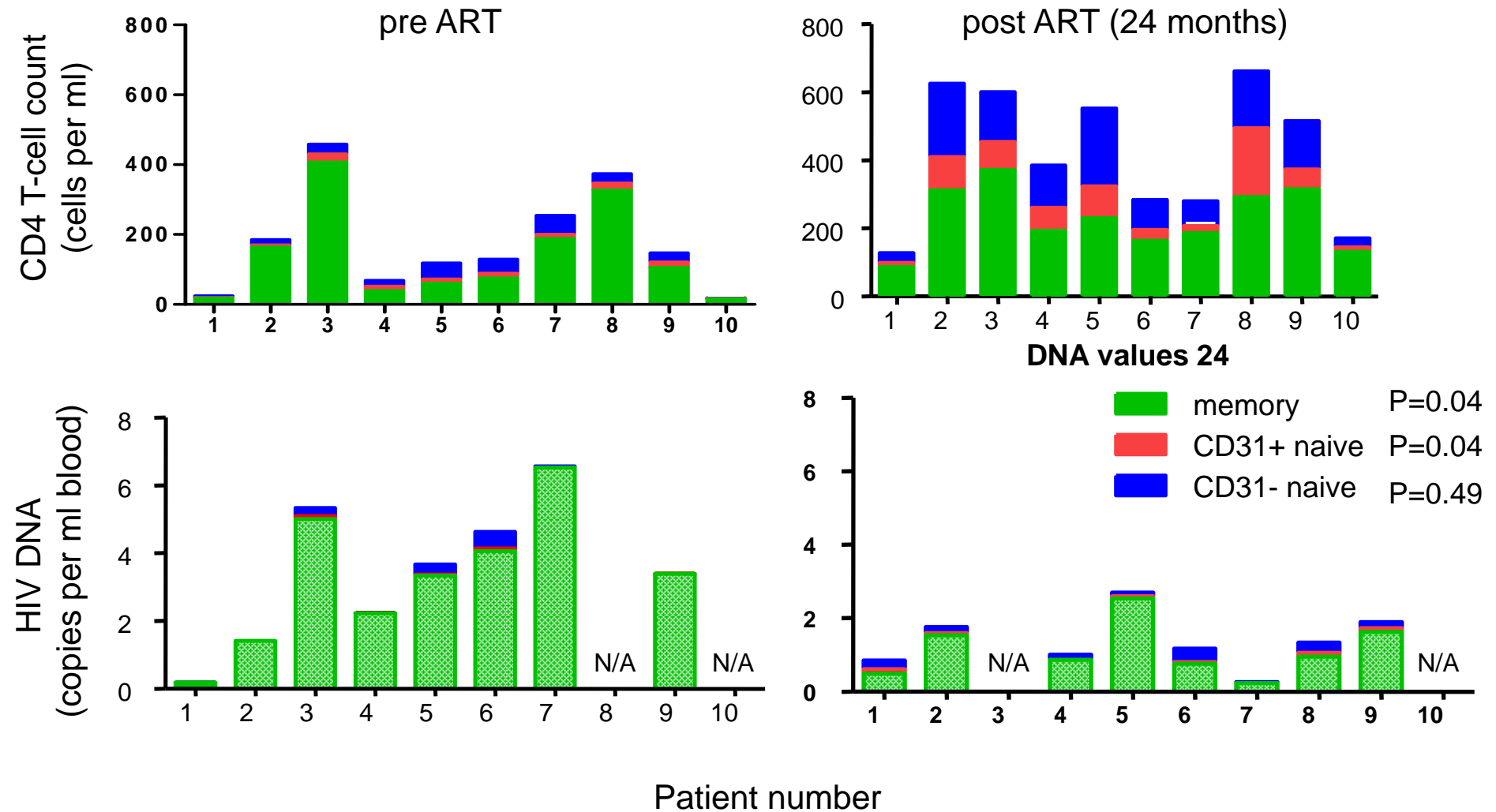


Decrease in absolute numbers of infected memory T-cells but no change in infected naïve T-cells

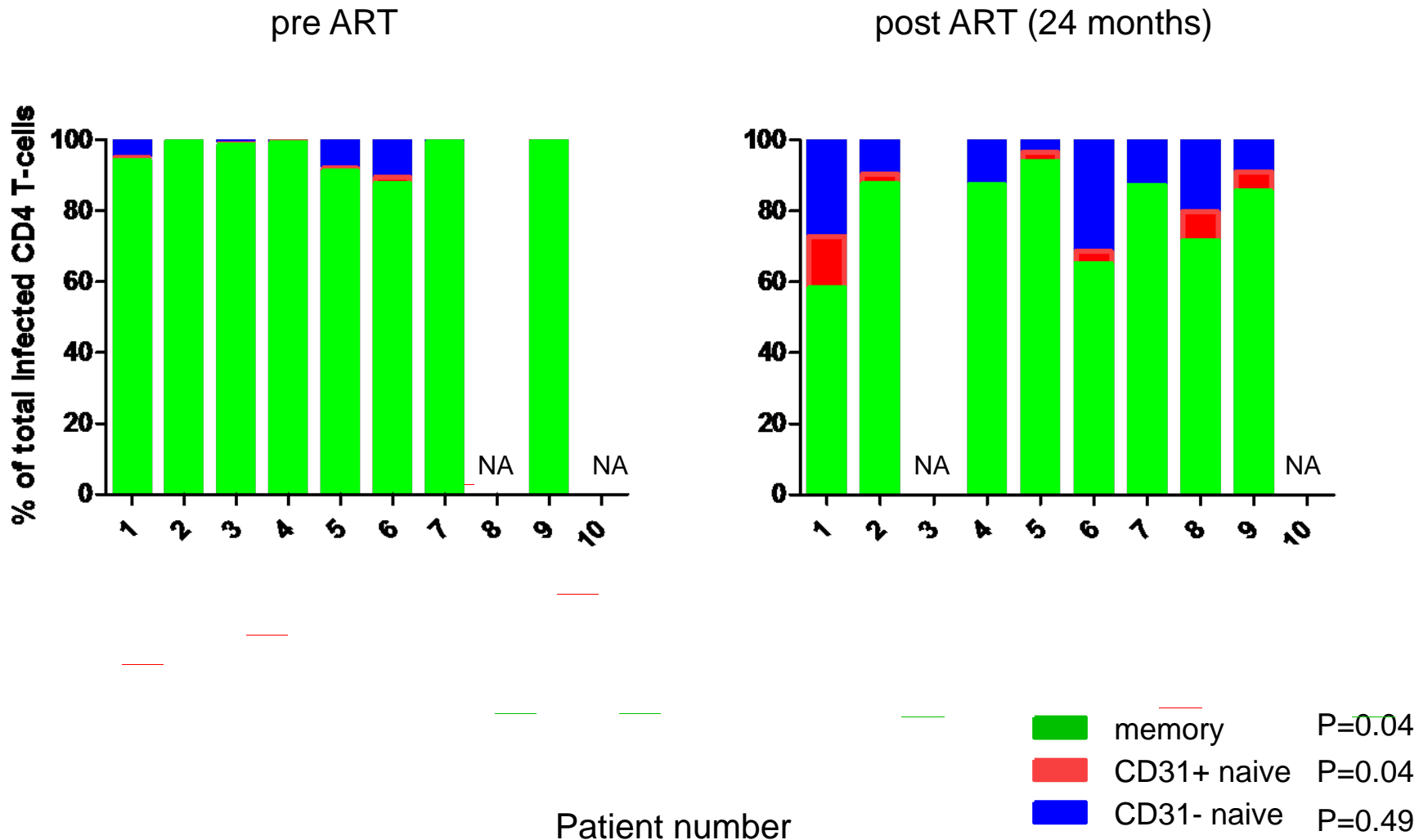


$$\frac{\text{HIV DNA}}{10\text{E6 cells}} \times \frac{\text{CD4 cell subset count}}{\mu\text{l}} \times 10\text{E6}$$

Decrease in absolute numbers of infected memory T-cells but no change in infected naïve T-cells

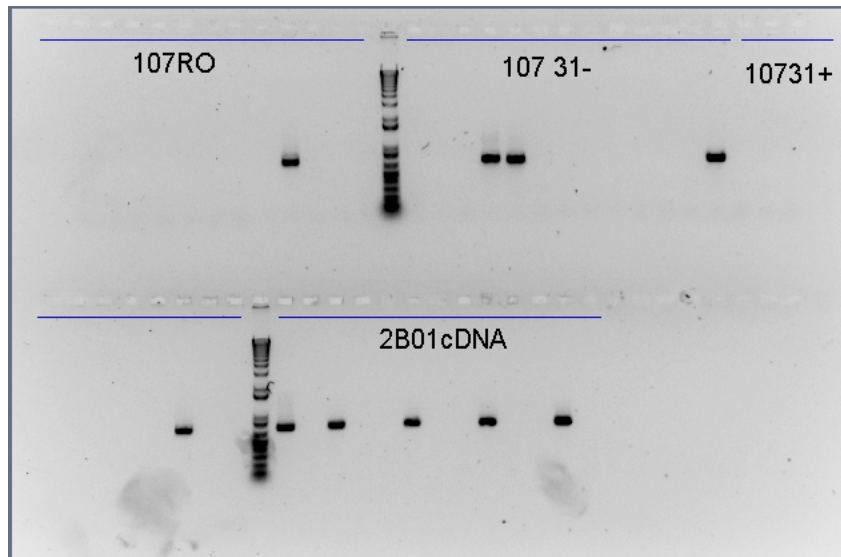
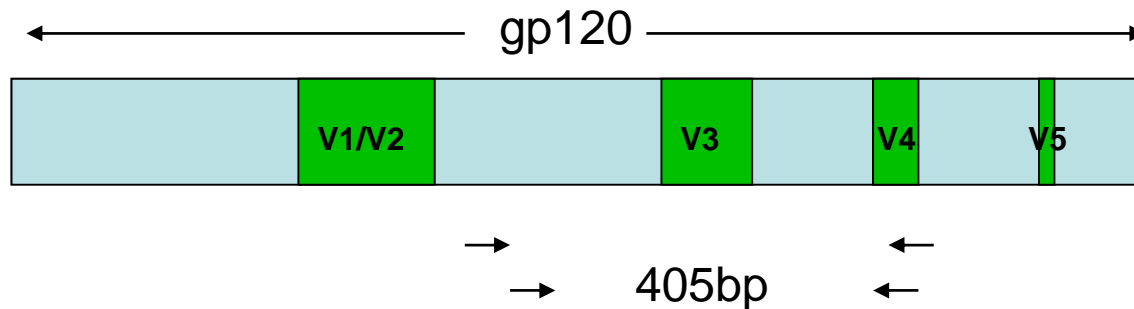


Infected naïve T-cells account for an increased proportion of reservoir on ART



Single copy PCR amplification of virus from naïve T-cells, memory T-cells and plasma

Nested PCR amplification of C2-C3 of HIV-1 gp120

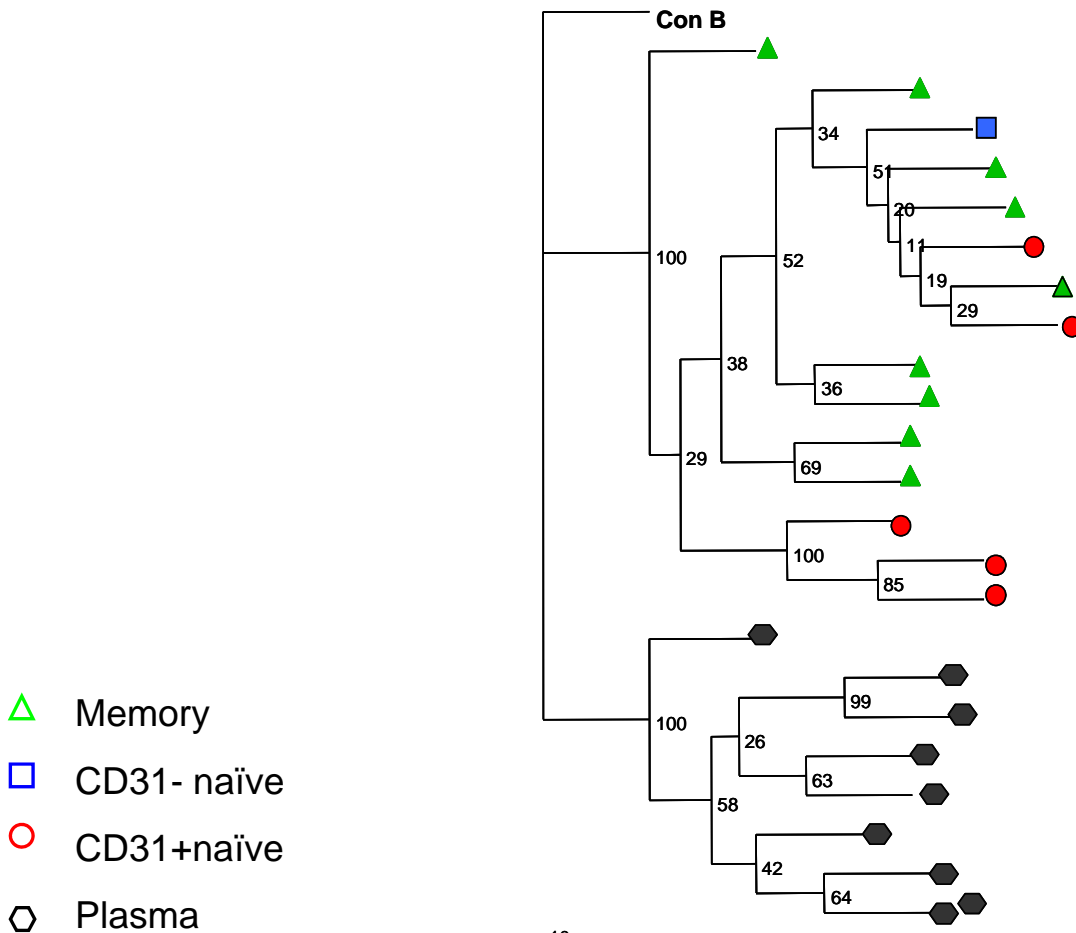


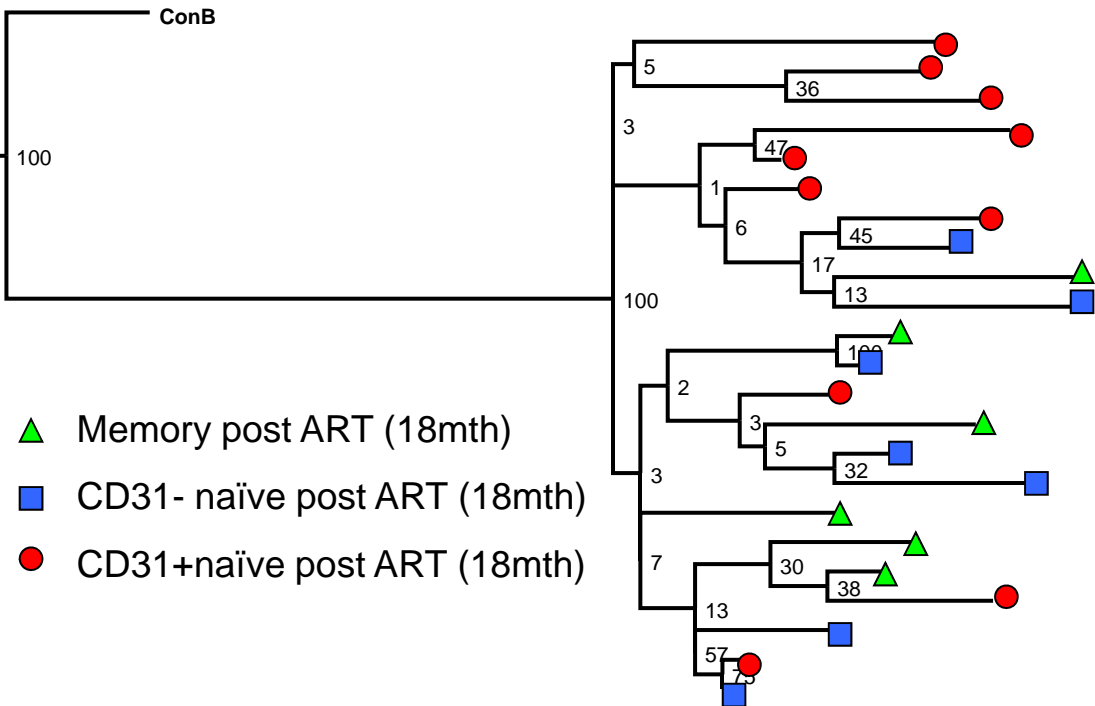
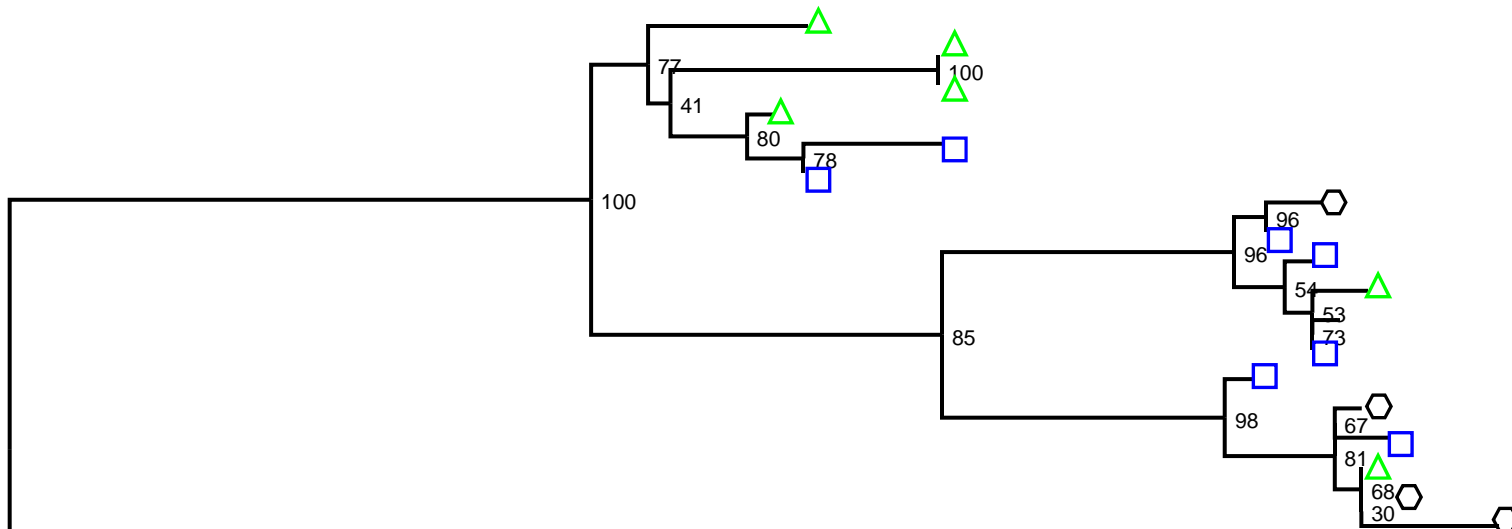
Template diluted 10-fold

Nested PCR amplification of HIV-1 env C2-C3 region

Amplification from a single-copy template most likely if <30% reaction PCR positive

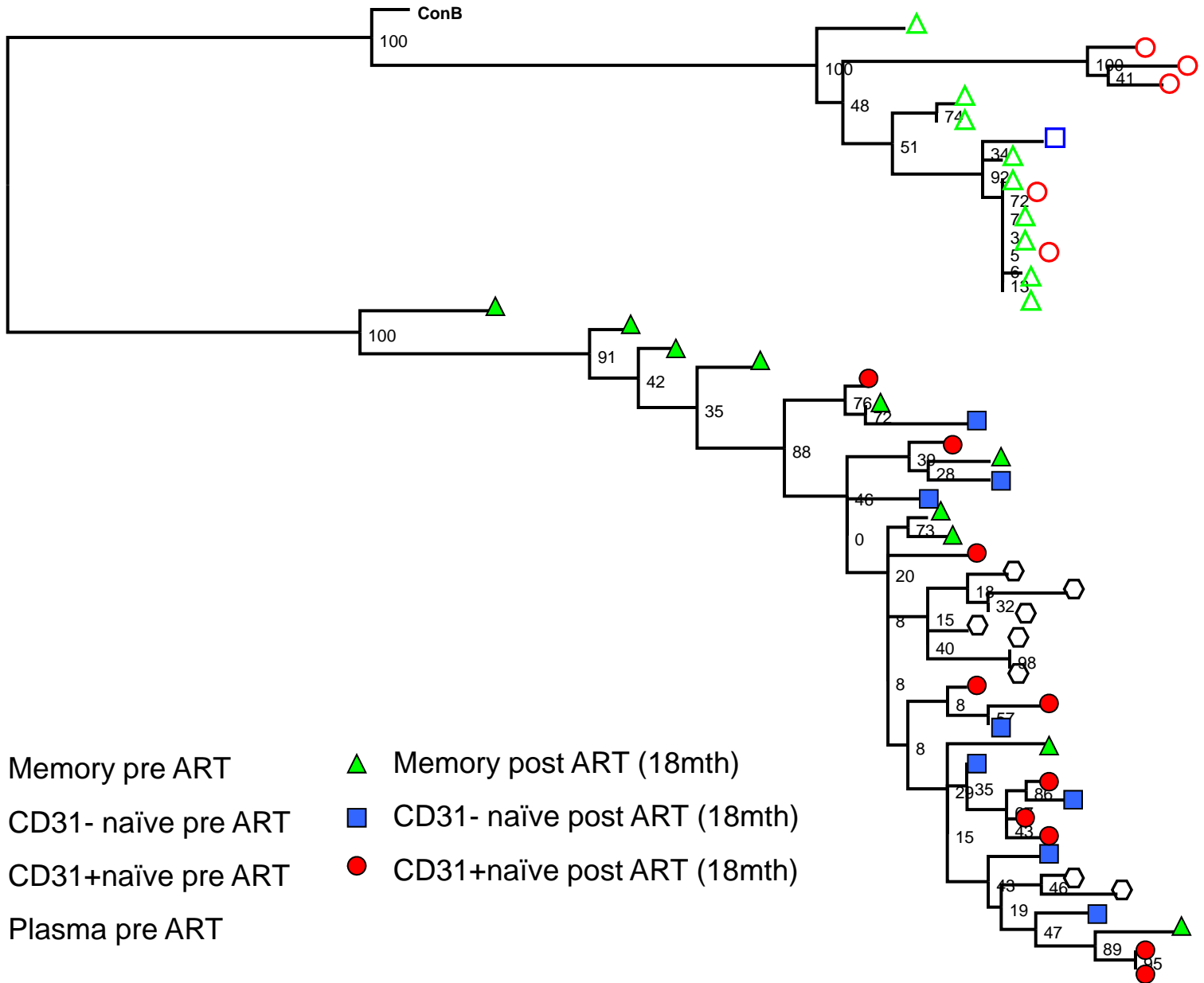
Phylogenetic analysis: no compartmentalisation of memory and naïve





- △ Memory pre ART
 - CD31- naïve pre ART
 - CD31+naïve pre ART
 - Plasma pre ART
- ▲ Memory post ART (18mth)
 - CD31- naïve post ART (18mth)
 - CD31+naïve post ART (18mth)

0.03



△ Memory pre ART

□ CD31- naïve pre ART

○ CD31+naïve pre ART

⬡ Plasma pre ART

▲ Memory post ART (18mth)

■ CD31- naïve post ART (18mth)

● CD31+naïve post ART (18mth)

0.04

Summary

- Following ART, there was an increase in total naïve T-cells but no change in the percentage of CD31+ and CD31- naïve T-cells consistent with increased thymic output and homeostatic proliferation post ART.
- There was no significant decrease in the concentration of HIV DNA in naïve T-cells post ART in contrast to memory T-cells
- The absolute number of infected memory T-cells decreased on ART but there was little change and possible increase in absolute number of infected naïve T-cells
- Phylogenetic analysis of patients pre and on ART showed no evidence of viral compartmentalisation within memory and naïve CD4 T-cell subsets suggesting a common source of infection or ongoing transfer between compartments.

Limitations

- Small study size
- Only total HIV DNA quantified
- No assessment of integrated HIV DNA or replication competent virus
- Relatively short follow up of 24 months
- Study ongoing

Implications

- Naïve CD4 T-cells represent a stable reservoir in patients on effective antiretroviral therapy
- The stability of the infected naïve T cells, indicate that elimination of this reservoir by specific strategies that target infected naïve T cells, e.g. IL-7 should be evaluated.

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