SPREAD OF HIV-DNA IN CD4+ T-CELLS SUBSETS DEPENDS ON ART INITIATION TIMING

Abs. 346

GANTNER Pierre[1,2], BARNIG Cindy[2,3], PARTISANI Marialuisa[4], BECK-WIRTH Geneviève[5], FALLER Jean-Pierre[6], MARTINOT Martin[7], MOHSENI-ZADEH Mahsa[7], CHENEAU Christine[4], BATARD Marie-Laure[4], FISCHER Patricia[4], URING-LAMBERT Béatrice[2], BAHRAM Siamak[2], REY David[4], FAFI-KREMER Samira[1,2].

1 Virology laboratory, Hôpitaux Universitaires de Strasbourg, Strasbourg, France. 2 Strasbourg University, INSERM, UMR-S U1109, F-67000 Strasbourg, France. 3 Physiology, Hôpitaux Universitaires de Strasbourg, Strasbourg, France.
4 Le Trait d'Union, HIV-infection care center, Hôpitaux Universitaires de Strasbourg, Strasbourg, France. 5 Internal Medicine Department, HIV-infection care center, GHR- Mulhouse Sud Alsace, Mulhouse, France.
6 Department of Infectious Diseases, Hôpital Nord Franche Comté, Belfort, France. 7 Internal Medicine and Rheumatology Department, Hôpital Civil de Colmar, Colmar, France.

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Corresponding author:
Pierre Gantner, Laboratoire de Virologie, 1, rue Koeberlé, 67000 Strasbourg, France. *pierre.gantner@chru-strasbourg.fr*

Background

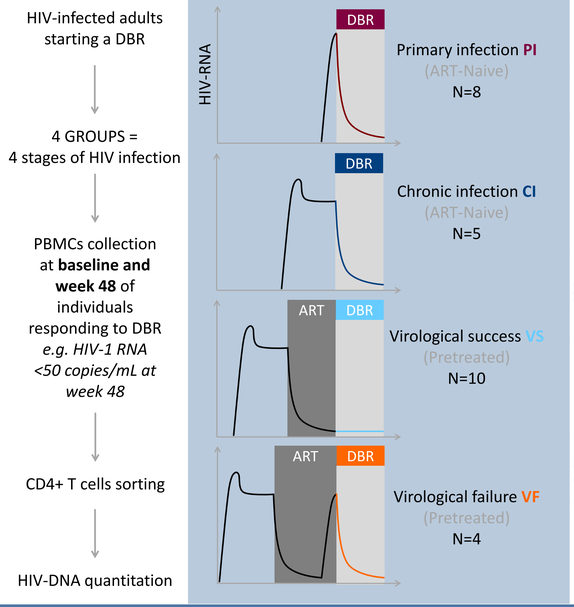
HIV-1 viral persistence is mostly attributed to long-lasting resting CD4+ T cells, even when antiretroviral therapy (ART) is used to prevent active replication.
Although, much is known about the size variation of HIV reservoirs on ART, there is a need for clinical investigations of CD4+ T cell specific subsets that serve as hotspots for viral long-term persistence.
Objective: Analyze the dynamics of HIV-DNA levels in CD4+ T-cell subsets in individuals starting successful dolutegravir-based regimen (DBR).

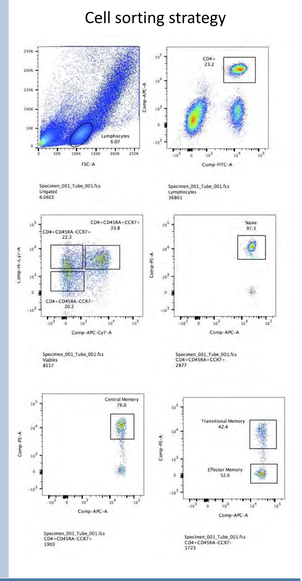
Methods

The **DRONE study** was a multicenter prospective longitudinal study including patients starting a dolutegravir-based regimen (NCT02557997).
1/ In the present substudy, we enrolled **27 participants** divided into four groups:
- ART-naïve individuals who initiated treatment with a DBR during **acute infection** (**AI**, n=8)
- ART-naïve individuals who initiated treatment with a DBR in **chronic infection** (**CI**, n=5)
- ART-experienced individuals who started a DBR while in **virological success** (VS, n=10)
- ART-experienced individuals who started a DBR while in **virological failure** (**VF**, n=4)

2/ Peripheral blood mononuclear cells (PBMCs) were collected at baseline and week 48 of successful DBR.
3/ CD4+ T cells were sorted on FACS Aria (BD Biosciences) in effector memory (TEM), transitional memory (TTM), central memory (TCM) and naïve (TN).
4/ Total HIV-DNA was quantitated by LTR real-time PCR assay with the Generic HIV-DNA CELL (Biocentric).

Study flowchart





Statistical analysis

We used a Bayesian approach to compare HIV-DNA levels changes over follow-up.
Posterior distribution parameter were estimated using program JAGS within the rjags package in R version 3.1.1.
Results are expressed as the **probability (Pr) of observing a difference** between 2 timepoints/groups/subsets:
- *In HIV-DNA*, **greater than 0.25 log copies/million cells** (standard deviation of the PCR assay)
- *In the cell contribution to the pool of HIV-infected cells percentage*, **greater than 10%**

Results

1/ HIV-DNA burden declined in all groups and all subsets after 48 weeks of treatment (Pr>52%), except in the VS group for TTM, TCM and TN cells (*Figure 1*).

2/ Contribution to HIV-DNA burden, both at baseline and week 48, was mainly from TTM cells in acute infections and from TCM cells in the other groups (*Figure 2*).

HIV-DNA burden in CD4+ T cell subsets analysis

Figure 1. Dynamic of the HIV-DNA burden in CD4+ T cells subsets and PBMCs between baseline and week 48 of dolutegravir-based regimen.

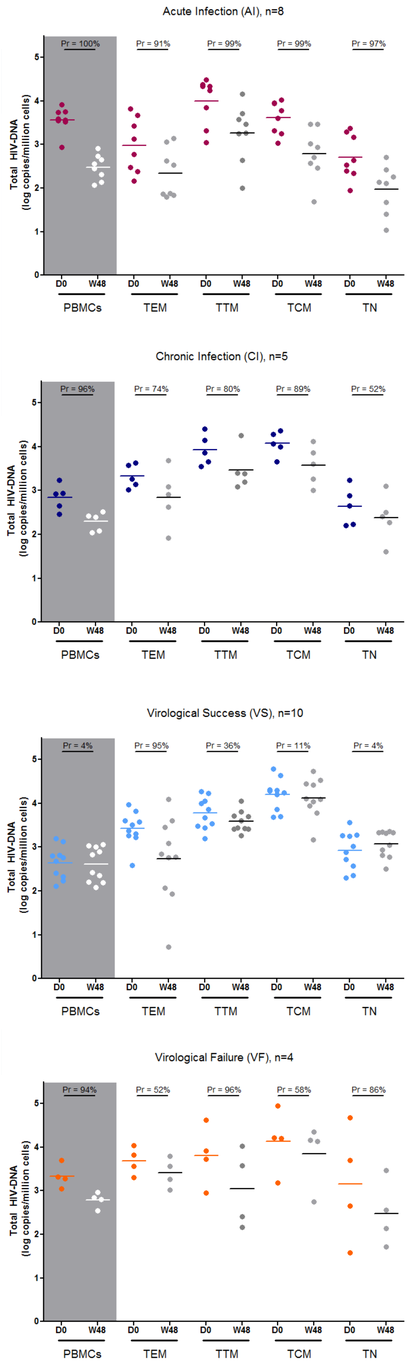
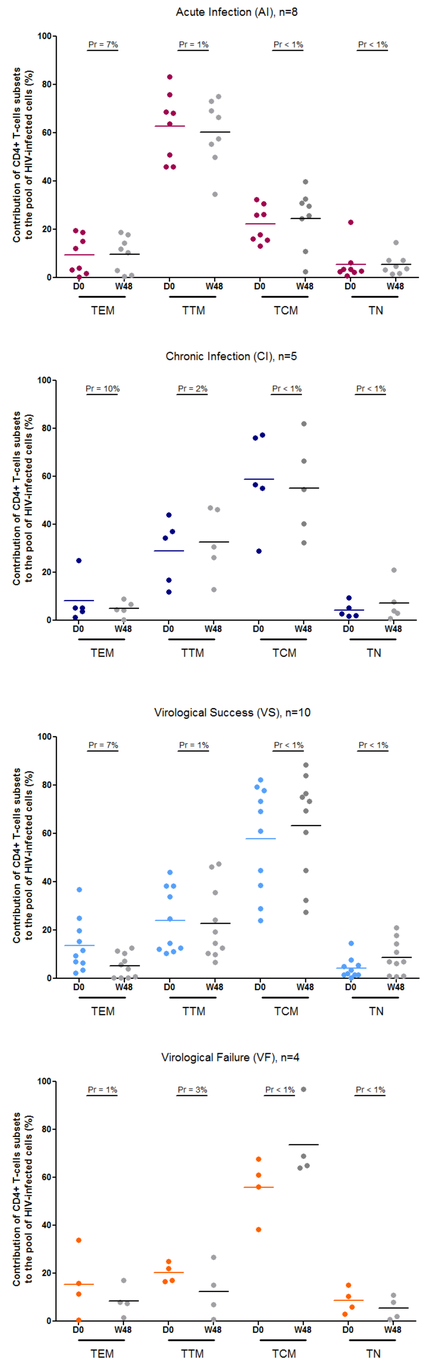


Figure 2. Dynamic of contribution to the pool of HIV-infected CD4+ T cells between baseline and week 48 of dolutegravir-based regimen.



* *TEM: effector memory, TTM: transitional memory, TCM: central memory, TN: naïve CD4+ T cells.*

Patients' characteristics

All patients (*Table 1*) achieved or maintained HIV-RNA < 40 copies/mL up to 48.

Table 1. Baseline demographics and biological parameters on dolutegravir-based regimen

Group *	n	Male	Age (years)	MSM	Time since Infection (days)	Virological control (years)	HIV-1 subtype B	ABC/3TC Backbone	Baseline			Week 48		
									HIV-RNA (log_{10} copies/mL)	CD4 T cells (/mm³)	HIV-DNA (log_{10} copies/10^6 PBMCs)	HIV-RNA (log_{10} copies/mL)	CD4 T cells (/mm³)	HIV-DNA (log_{10} copies/10^6 PBMCs)
PI	8	7/8	37	6/8	51	/	6/8	0/8	5.8	469	3.6	1.6	650	2.4
CI	5	4/5	30	4/5	226	/	4/5	5/5	4.0	440	2.9	1.6	698	2.4
VS	10	10/10	55	5/10	/	11	7/10	8/10	1.6	616	2.7	1.6	622	2.6
VF	4	4/4	43	3/4	/	/	3/4	3/4	3.1	387	3.3	1.6	425	2.8

** Results are expressed as number of individuals or median.*

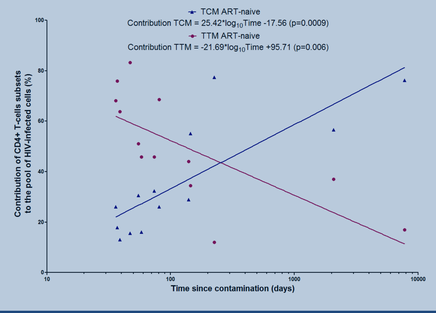
Reduction magnitude and predictors

1/ In terms of reduction magnitude of HIV-DNA levels, there was a:
- >20% reduction in **all subsets** from the **AI** group
- >10% reduction in **all subsets** from the **CI** and **VF** groups
- 5% reduction in **TEM cells** from the **VS** group.

2/ Contribution to HIV-DNA burden at baseline was mainly from TTM cells in acute infections and from TCM cells in the other groups.
At baseline and for ART-naive, there was an association between TCM and TTM infection levels with **time since HIV infection** (*Figure 3*):
- TTM cells were early infected
- whereas TCM cells were lately infected

but no association with absolute CD4+ T cell count.

Figure 3. Correlation between the contribution of TCM and TTM cells to the HIV reservoir and time since HIV infection. →



Discussion

1/ Our key finding is that when patients were viremic at baseline (AI, CI, VF groups), a DTG-based treatment decreased HIV-DNA burden across all subsets. However, when patients were aviremic and already on suppressive ART at baseline (VS group), switching to DTG-based regimen resulted in a decrease in TEM but not in other subsets nor in total PBMCs.

Hypothesis: This suggests that compared to previous regimens, a dolutegravir switch has some specific impact on TEM cells but not on the other subsets, that should further be investigated (i.e. with other switching ART-regimen).
TEM cells represents the last step of immune response upon antigenic stimulation, we could hypothesize that dolutegravir helps reducing immune activation by cell depending mechanism, thus leading to reduced infection level of TEM cells.

2/ HIV-DNA distribution remained stable on follow-up with TTM cells being the main contributors to HIV-DNA burden in acutely infected individuals and TCM cells in the other groups.

Hypothesis: As TTM cell reservoir is fueled by differentiation and proliferation of TCM cells due to immune activation, these results suggests that when ART is initiated in acute infection, uninfected TCM cells are protected from *de novo* infections and the pool of infected TTM cells is dramatically reduced by immune activation decrease on early ART. These findings strongly support the view that early ART might limit the size of the viral reservoir and improve the prospects for a functional HIV cure.

Conclusions

By comparing the changes in HIV-DNA levels in TEM, TTM, TCM and TN cells from individuals on successful dolutegravir-based regimen over 48 weeks, we demonstrated that the distribution of HIV reservoir strongly depends on the clinical and therapeutic stages of HIV infection at ART initiation.
Early treated patients had the highest probability of reduction.
In aviremic participants switching to a dolutegravir based regimen, reduction was limited to TEM cells.