

# **Effect of Associating an Immunosuppressive Therapy Mycophenolate Mofetil (MMF)+HAART during STI and Holding the MMF Drug after Definitive Interruption of HAART on Viral Replication.**

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# *OBJECTIVES*

- To study the effect of associating **MMF+HAART** and holding MMF after cycles of STI on plasma and tonsillar tissue **viral replication** (TTVL).
- The **PK** of MMF and the effect on **lymphocyte subsets** and proliferative responses (**LPR**) were also assessed.

# Methods

## ALLOCATION TO (N=17):

abacavir + efavirenz + nelfinavir

300 mg/12h

600mg/24h

1250mg/12H

After 1 year of treatment patients had to be randomized (Day 0) to

**Study arm I:** Abacavir (300 mg/12h)+ Efavirenz (600 mg/d)+Nelfinavir (1250 mg/12h) + MMF 250 mg/12h. (N=9)

**Study arm II:** Abacavir (300 mg/12h)+ Efavirenz (600mg/d)+ Nelfinavir (1250 mg/12h) (N=8)

# *Methods*

- After 4 months, HAART was discontinued (day 120) holding MMF in the MMF group. HAART was reintroduced during 4 months if viral load was  $> 200$  c/ml after 3 months of stop.

# *Methods*

- Plasma viral load and tonsillar tissue viral load
- An immunologic study (including **CD4+ and CD8+ T cell subsets** (including annexin and Ki67+ cells) and lymphoproliferative responses (**LPR**) to mitogens, and specific antigens (including HIV-1 antigens) were performed

# *Methods*

- The pharmacokinetic profile of MPA (area-under-the-curve,  $AUC_{0-12h}$ ) was carried out with blood samples (EDTA) collected at 0 (predose), 20,40 min and at 1,2,4,6,8,10 and 12 hours after MMF morning dose.
- The  $AUC_{0-12h}$  were analyzed at 7, 28 and 120 days post MMF treatment.
- Plasma concentrations of MPA were analyzed by a validated and previously reported HPLC method.

# ***Methods***

- The efficacy of MMF treatment was evaluated by the capacity of the patient sera to inhibit the response of T cell line (CEM).
  - Proliferation Cultures in the Presence of Patients' Sera: CEM cells were resuspended in RPMI-1640 containing 10% heat-inactivated FCS.  $2 \cdot 10^8$  cells/L were seeded in 96-well microtiter plates (TPP) and 100ml of patients' sera were added.  $^3\text{H}$ -Thymidine was added at 24h after the culture and incorporated thymidine was measured in a Beckman Scintillation Counter 24h later.
  - In some experiments normal human PBMC activated with PHA+IL-2 or PMA+IL-2 were used as proliferating cells to test the inhibitory capacity of patients' sera. Proliferation was measured by  $^3\text{H}$ -Thymidine uptake at 24hr.
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# ***RESULTS***

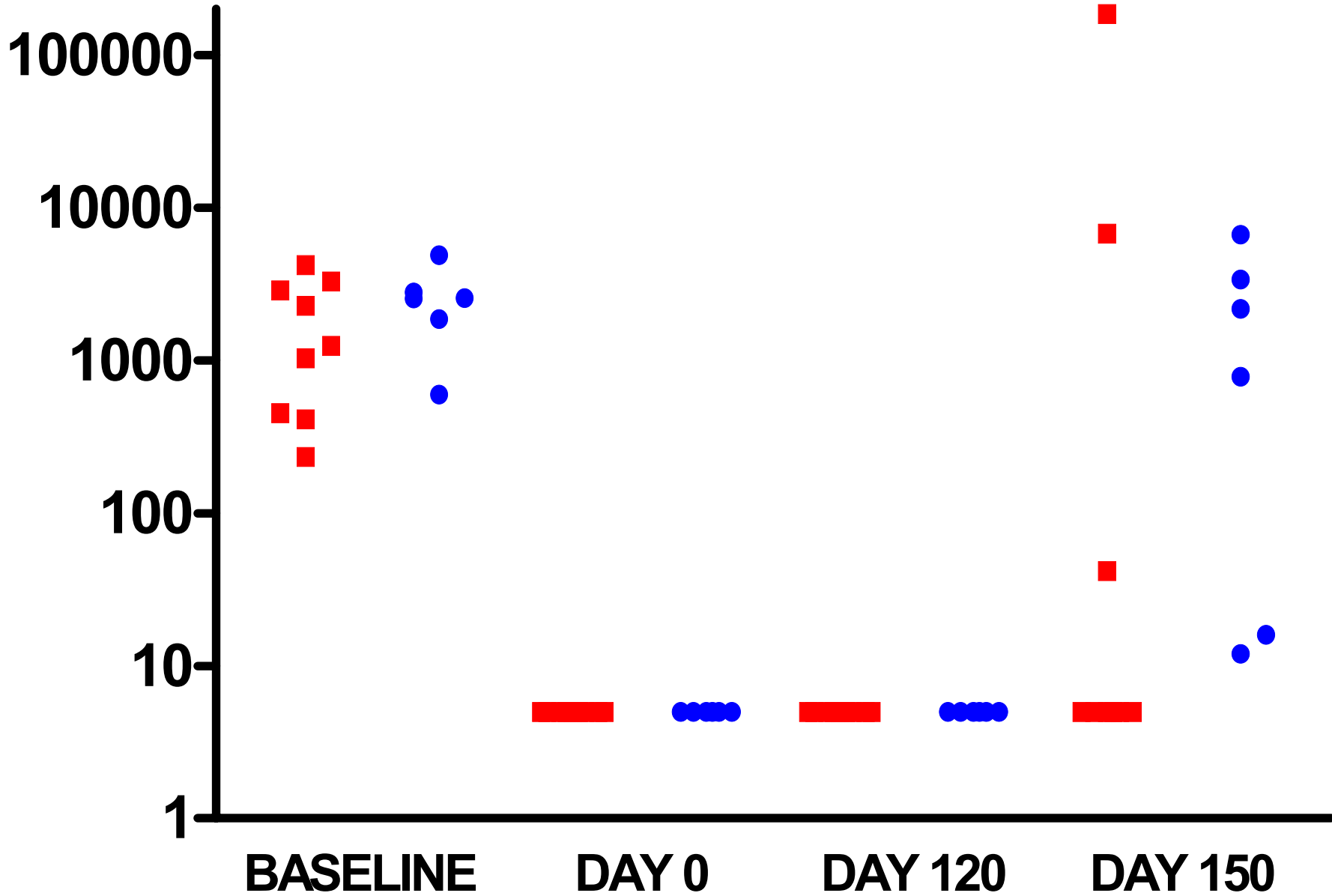
	<b>No.</b>	<b>%</b>
<b>RECRUITMENT TOTAL No</b>	<b>17</b>	
<b>Lost for follow-up</b>	<b>2</b>	<b>12</b>
<b>Age (median)</b>	<b>36</b>	
<b>MALES</b>	<b>9</b>	<b>53</b>
<b>DRUG ADDICTS</b>	<b>4</b>	<b>24</b>
<b>MEAN BASELINE VL</b>	<b>3.18</b>	
<b>MEAN BASELINE CD4+</b>	<b>702</b>	

# *RESULTS*

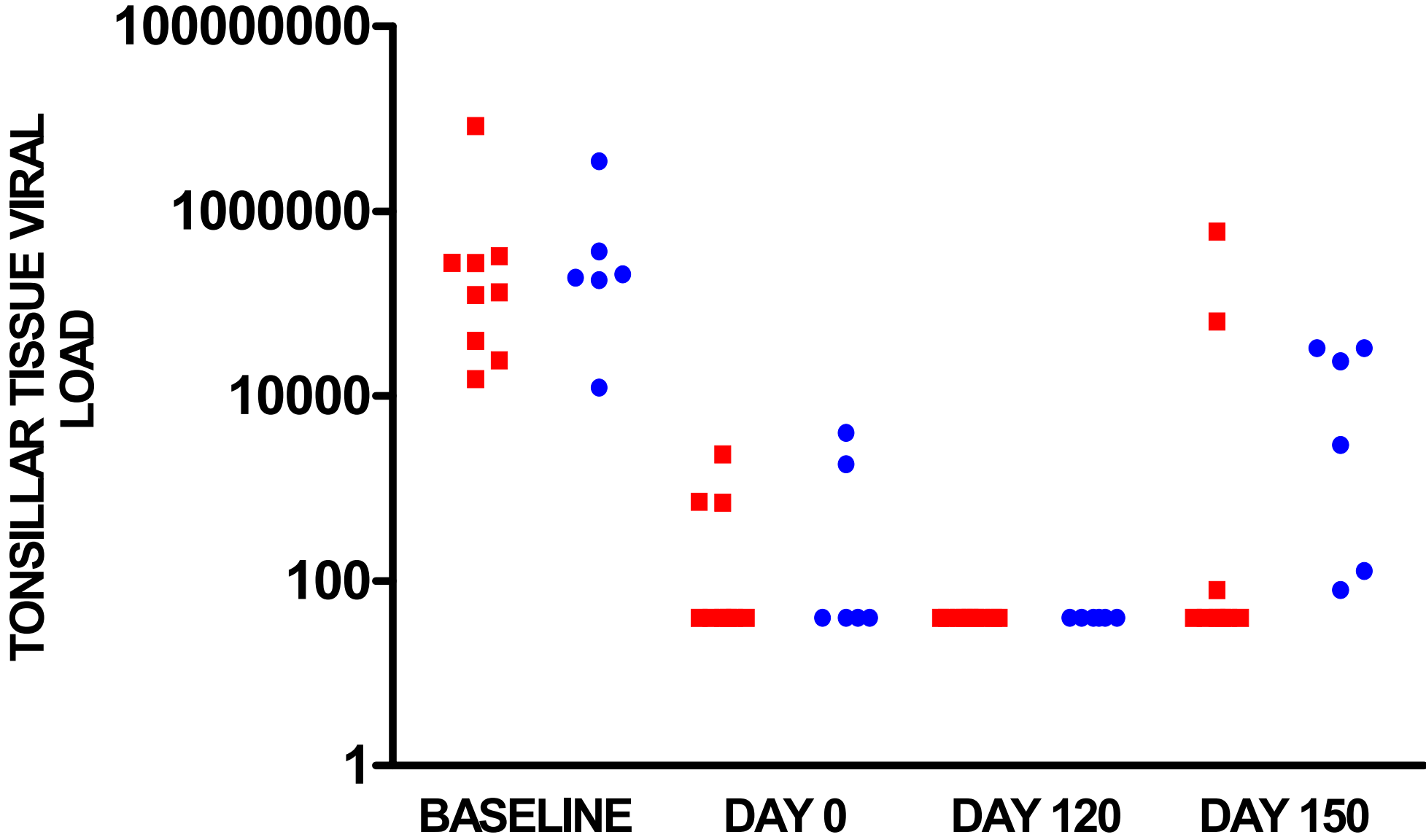
- **At day 120** all the patients had an undetectable level of PVL (<5c/ml) and TTVL (<40 c/mg of tissue).
- **At day 150**, PVL and TTVL increased above detectable level in 3/9 patients of MMF group and in all 6 patients of the ART group (p=0.01).

PLASMA VIRAL LOAD

- MMF
- ART



- MMF
- ART



# *RESULTS*

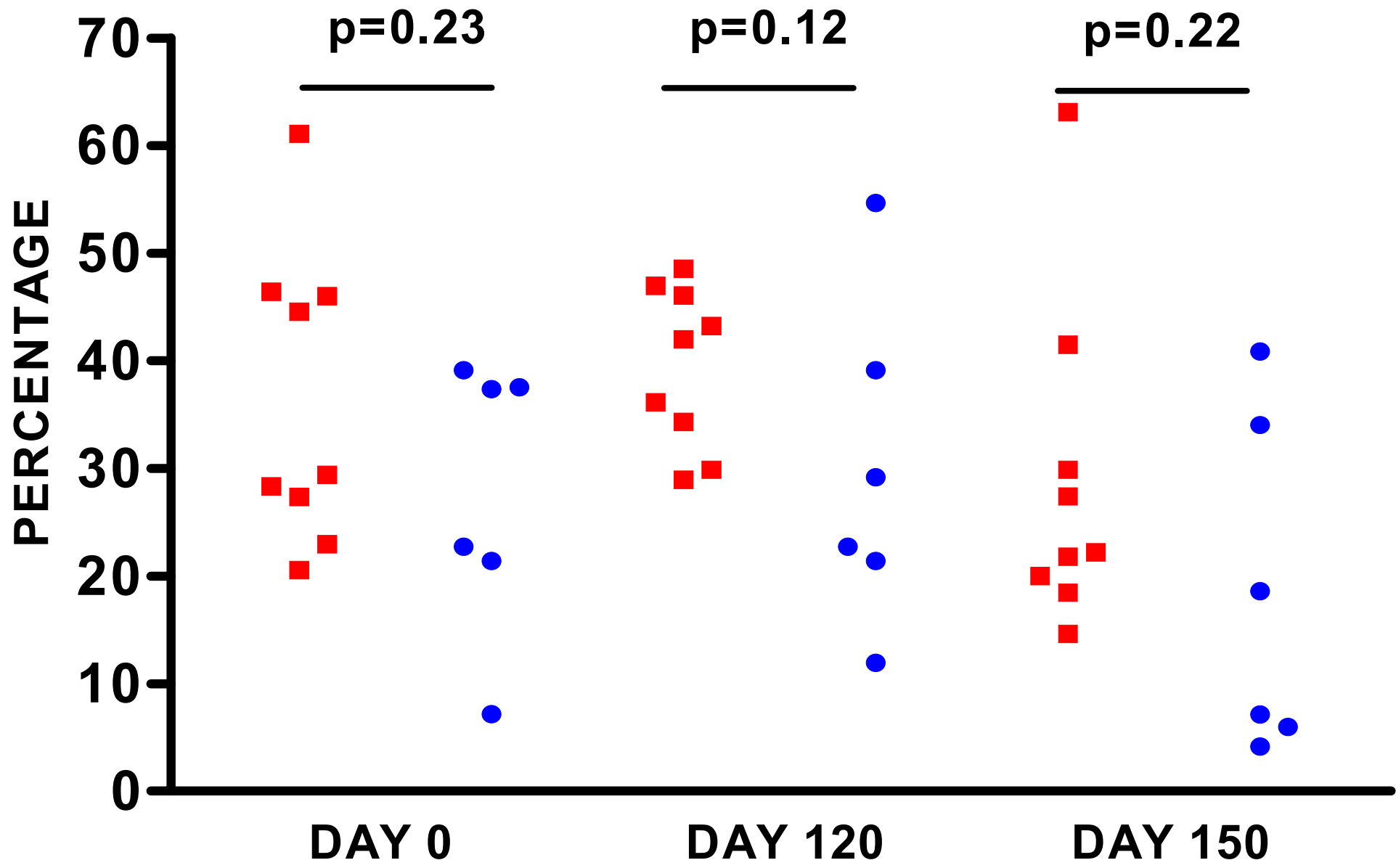
- Five out of 9 patients in MMF group vs 5/6 patients of ART group had to perform a **2<sup>nd</sup> cycle of STI** due to increase of VL >200 c/ml.
- There was a trend to have a **VL set-point** lower in MMF group than in ART group [median: 326 vs 1432 c/ml, respectively p= 0.10].

# *RESULTS*

- **At day 120**, there was no differences between MMF and ART groups neither in lymphocyte subset tested (CD4+, CD8+, naive and memory, CD8+CD28+ and CD8+CD38+ T cells) nor in LPR.
- **Anexin** levels did not change over all the time-point tested.
- After last cycle of STI, there was an increase of **Ki67+ cells**, without difference between MMF and ART groups.

■ MMF  
● ART

# ANEXIN



# Ki67+ CELLS

- MMF
- ART

p=0.02

10.0

p=0.19

p=0.55

7.5

5.0

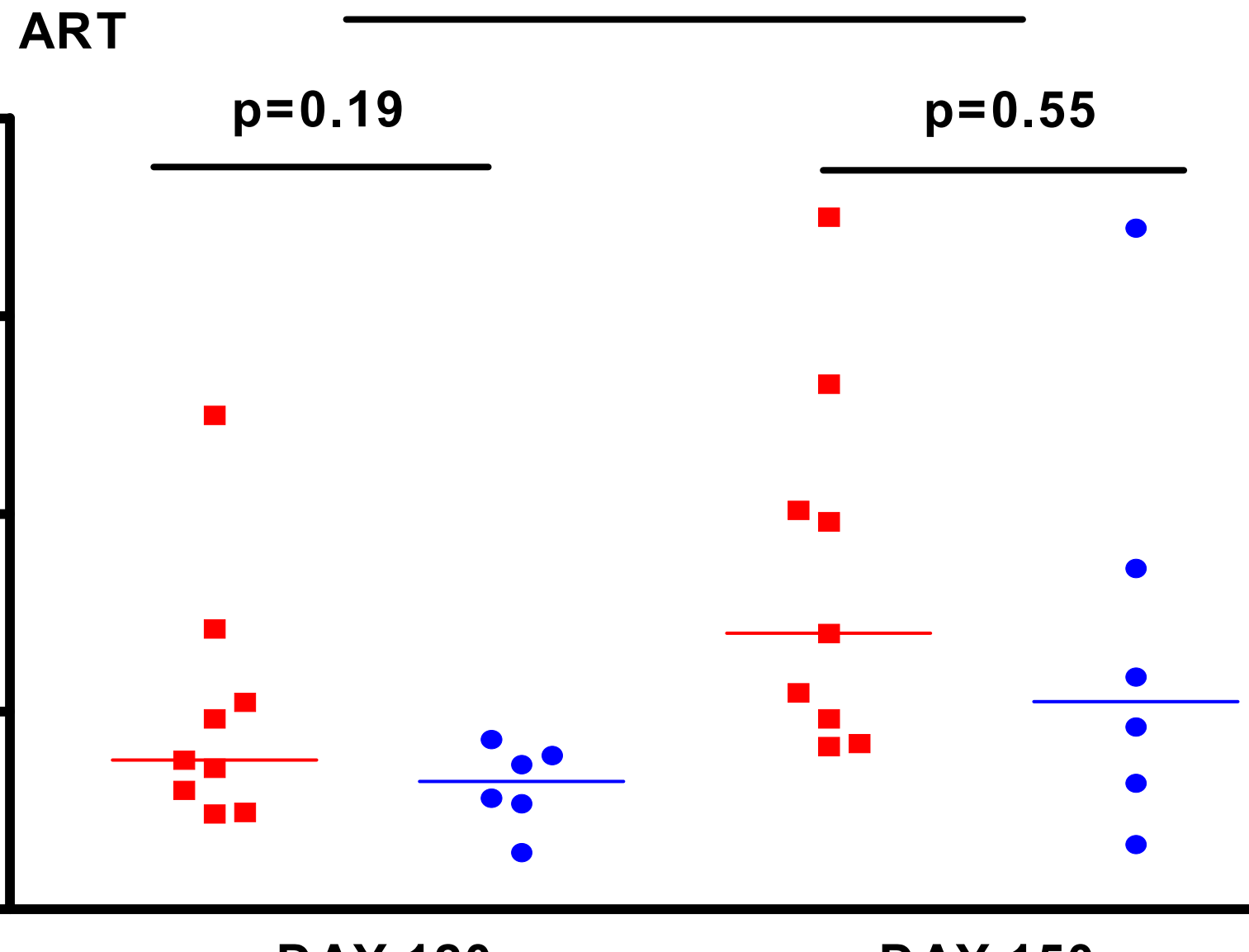
2.5

0.0

PERCENTAGE

DAY 120

DAY 150



# RESULTS

- The median for **AUC<sub>0-12h</sub>** was around 10 mg\*hr/L
- CEM response** decreased to 45% on all time-points in 50% of the patients, in the other half of the patients this decreased was maintained only 4 hours

MPA Plasma levels

	All curves Mean ± St. N=35	Day 7 Mean ± St N=9	Day 28 Mean ± St N=9	Day 120 Mean ± St. N=9	Day 150 Mean ± St N=8.
<b>Cmin</b>	0.81 ±0.98	1.05 ±1.36	0.68 ±0.20	0.51 ±0.17	1.03 ±1.48
<b>Cmax</b>	2.99 ±1.66	2.71 ±1.89	2.73 ±1.86	2.65 ±0.72	3.97 ±1.83
<b>Tmax</b>	1.23 ±0.71	1.36 ±1.17	1.33 ±0.50	1.11 ±0.55	1.08 ±0.39
<b>AUC</b>	9.26 ±2.68	10.09 ±4.22	9.75±2.51	7.89 ±1.47	9.31 ±1.12

# *CONCLUSIONS*

- Associating **MMF + HAART** and holding MMF after cycles of STI delayed the viral load rebound both in plasma and tonsillar tissue and improve the control of viral replication without HAART.
- This effect does not seem mediated neither by a decrease in **cell turn-over** nor by an increase in **apoptosis**.

# ***CONCLUSIONS***

- **The use of 0.250 g/12h will divide population in to at least two groups:**
  - **1. Group that maintain inhibitory capacity over 60% (40% CEM response) during all the dose interval**
  - **2. Group that maintain inhibitory capacity only during 0 to 4 hr post dose.**
- **It seems correct to postulate that the first group will have a GTP depletion more evident and completed that the second one.**