The Effect of Antiretroviral Protease Inhibitors on P-glycoprotein Expression on CD4+ and CD8+ Subpopulations of Lymphocytes

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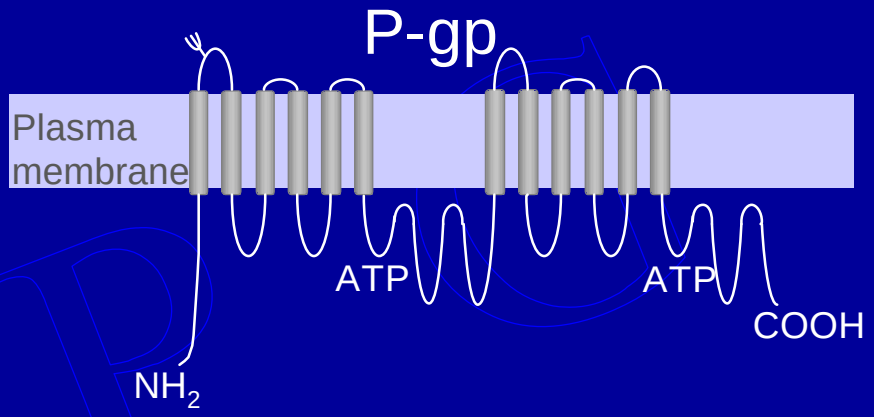
THE UNIVERSITY *of* LIVERPOOL

LIVERPOOL HIV PHARMACOLOGY GROUP
LHPG

Transport Proteins

P-glycoprotein (P-gp)

- ATP-dependent transport
- MDR-1 gene
- Drug efflux



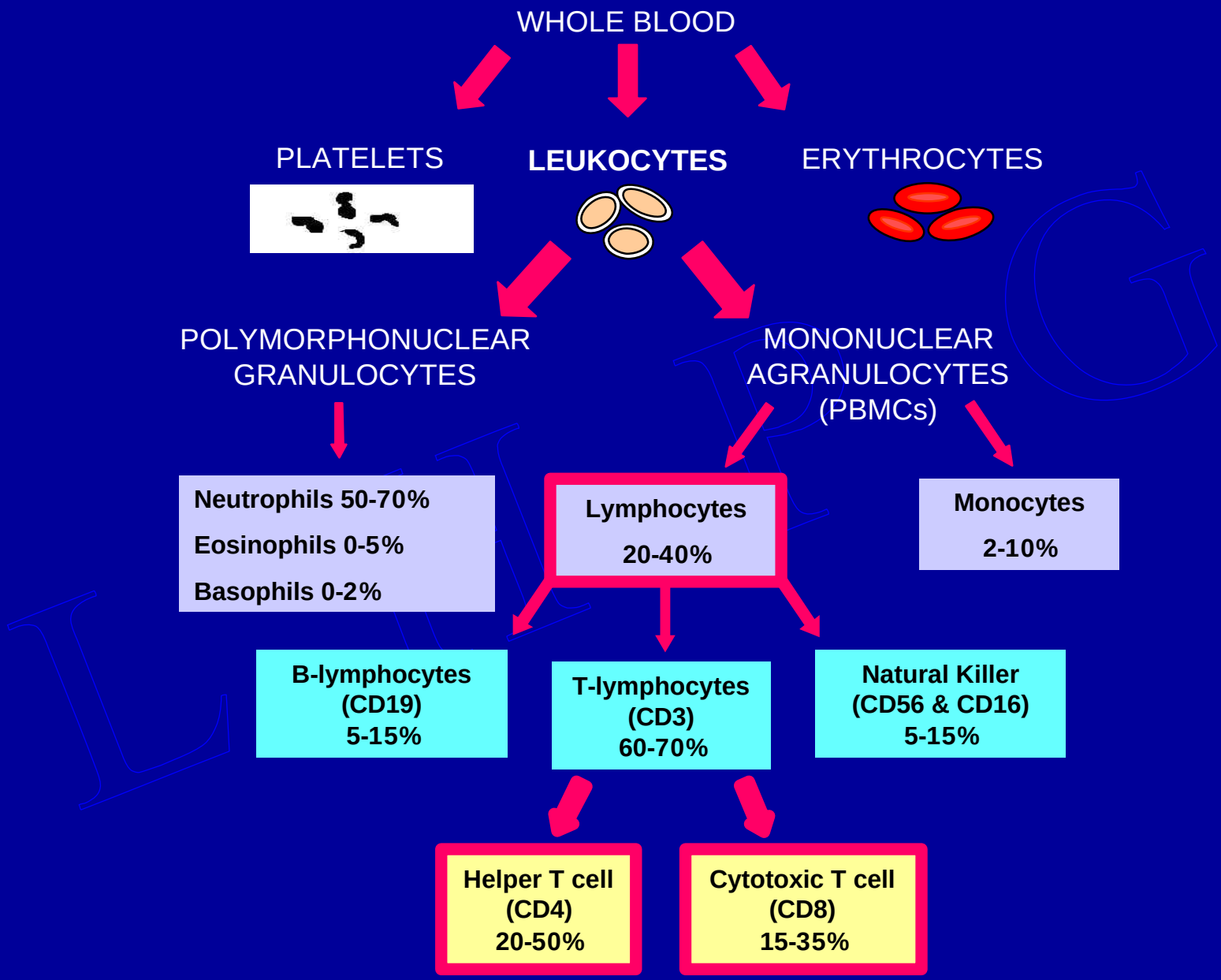
Transport Proteins

Importance in HIV infection

- Multidrug resistance: barrier - *intestine, liver, brain*
- Broad substrate specificity similar to CYP3A4
- Protease inhibitors (PIs) are substrates and inhibitors
- P-gp expression on subsets of lymphocytes

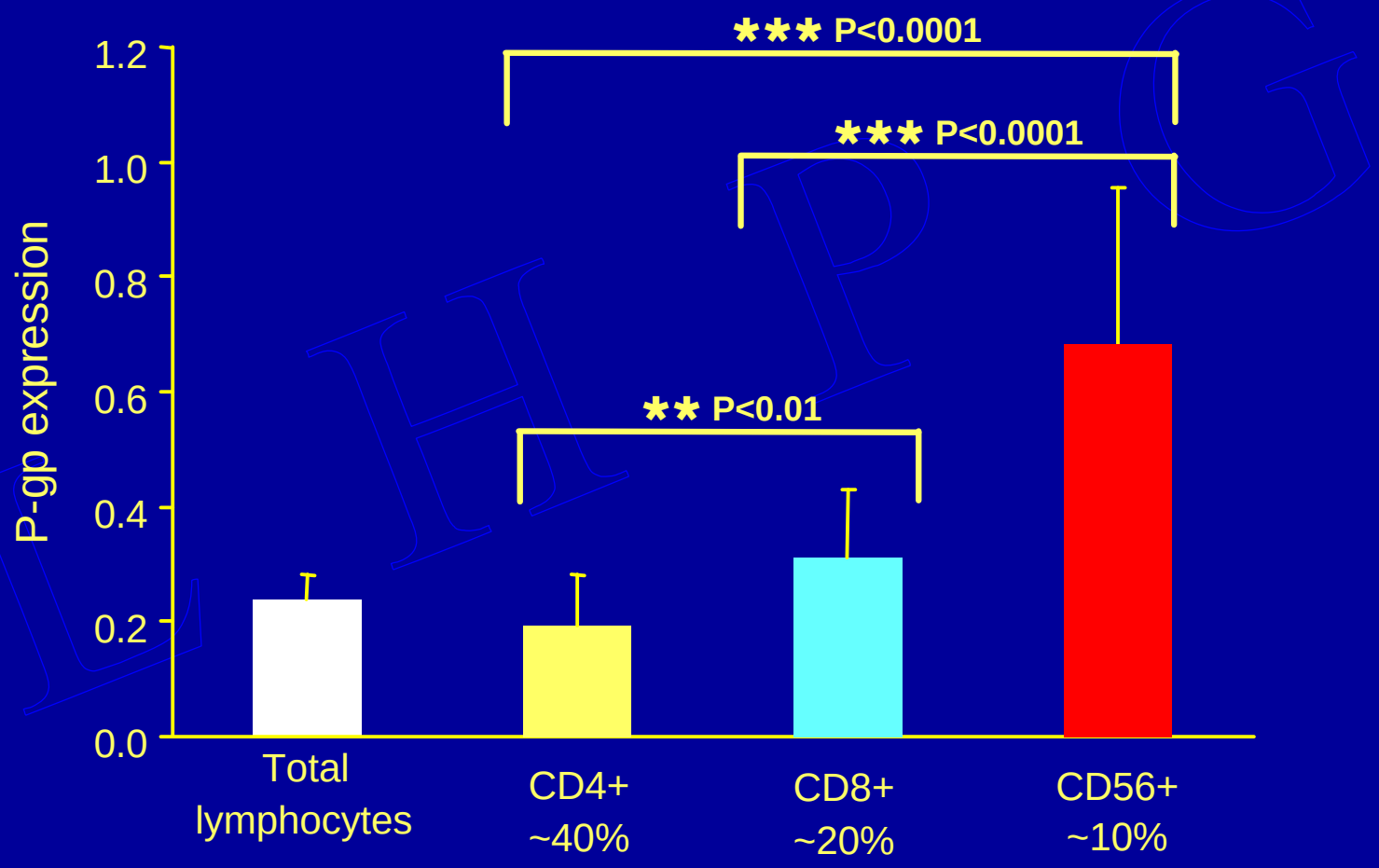
  - *reduced PI intracellular concentration*

Cell Subsets of Lymphocytes



Basal P-gp expression on lymphocytes

- Differential P-gp expression (n=15): CD56+>CD8+>CD4+
- Dual colour flow cytometry



J. of Immunol. Methods 2003

Question 1

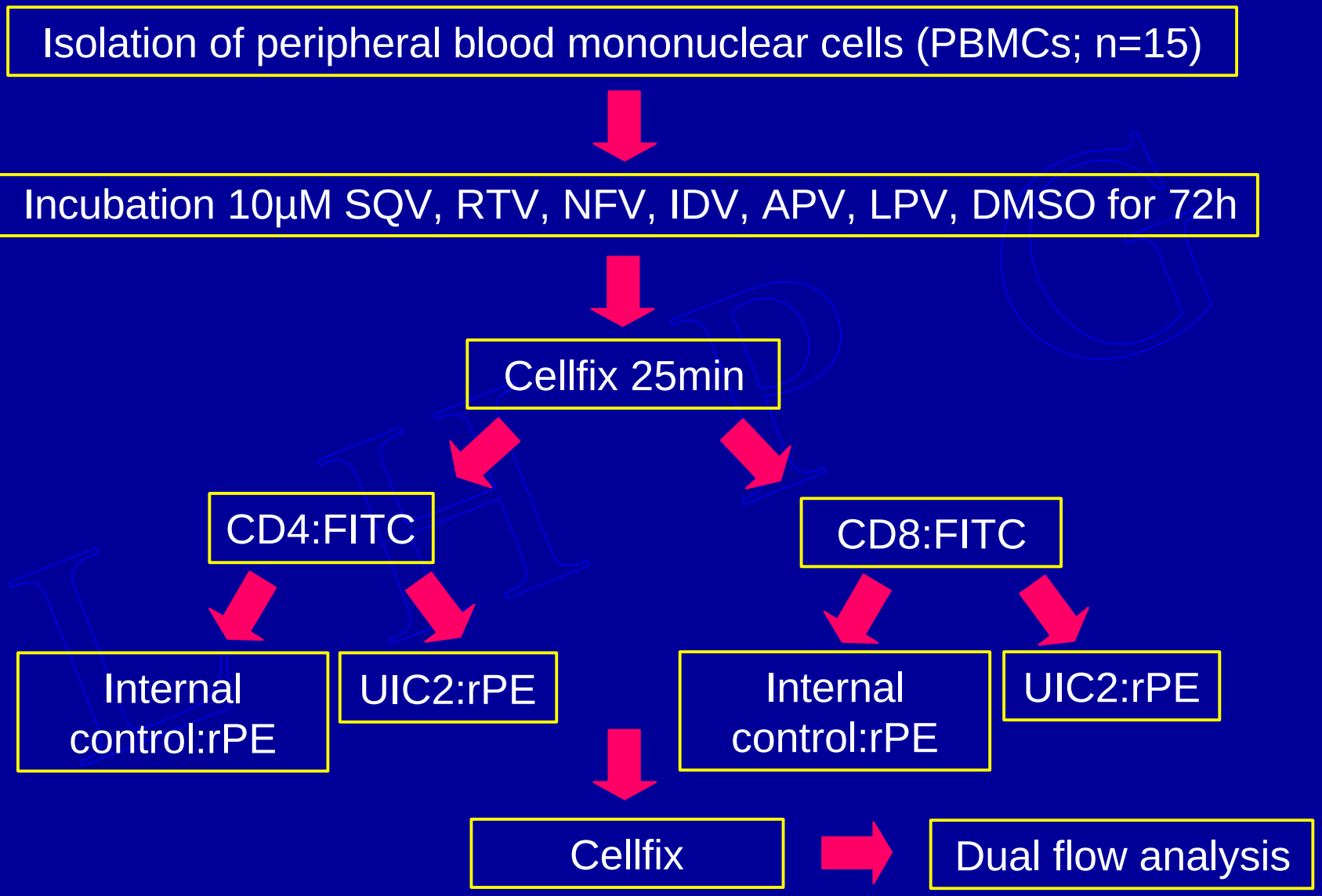
What is the effect of protease inhibitors on the expression of P-gp ***in vitro*** on total lymphocytes, CD4+ and CD8+ subpopulations from healthy volunteers?

Previous Studies: The effect of PIs on P-gp expression

In vitro

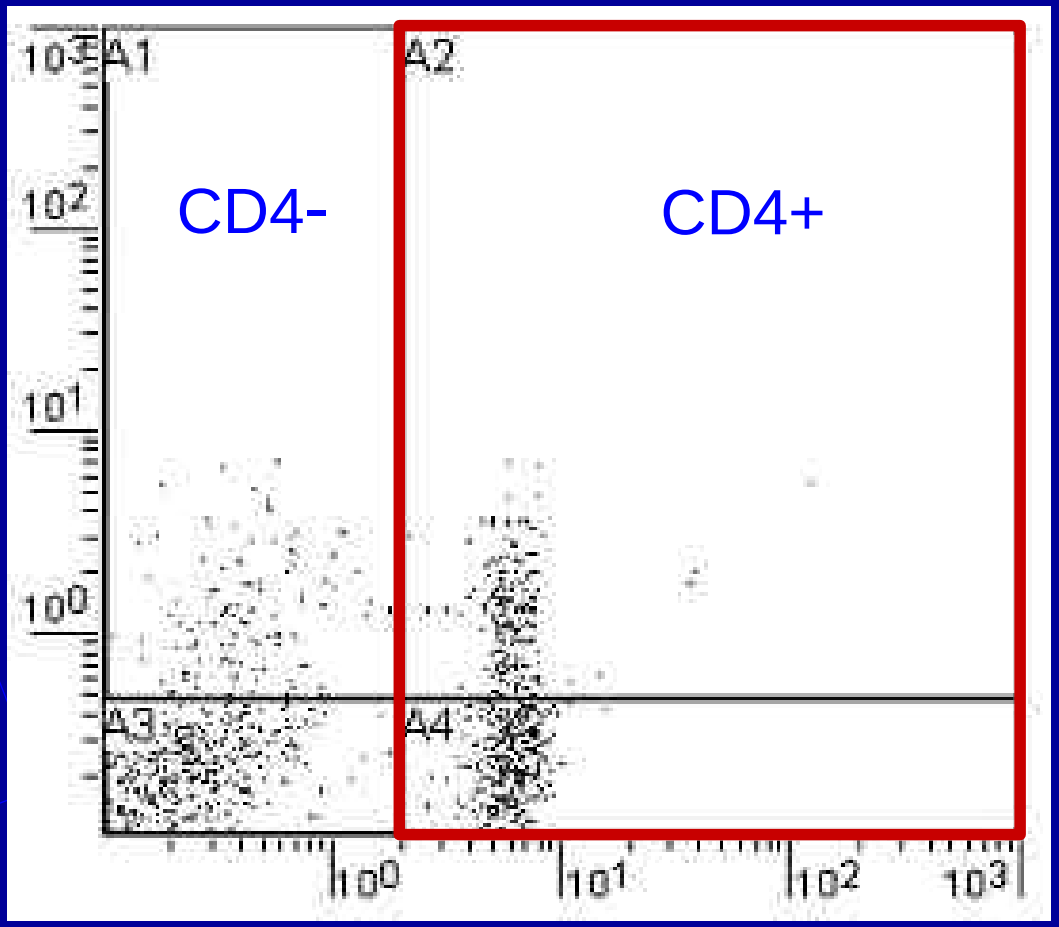
- Perloff *et al*., 2000: PIs increased P-gp expression in cell lines
- Huang *et al*., 2001: Increased intestinal P-gp in animal models treated with APV and NFV
- Dussault *et al*., 2001: PIs are ligands for SXR, a master regulator of xenobiotic clearance including MDR-1
- Chandler *et al*., 2002: PIs increase PBMC P-gp in a concentration dependent manner

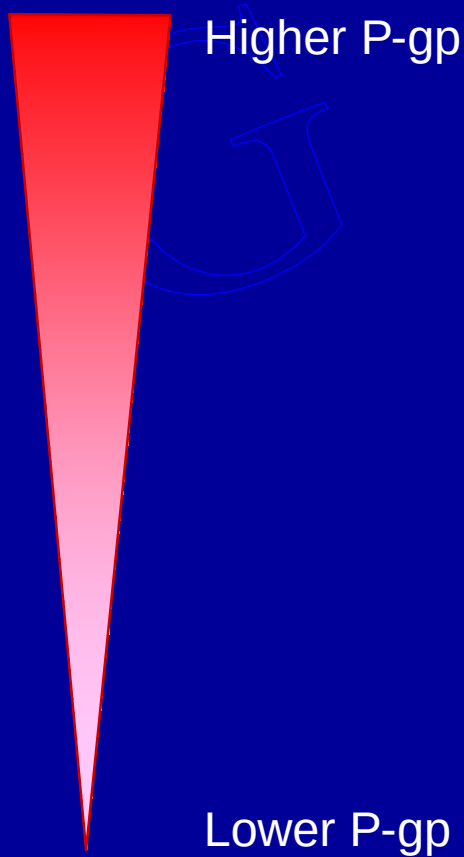
Method: PI incubation with PBMCs



Method: Dual Colour Flow Cytometry

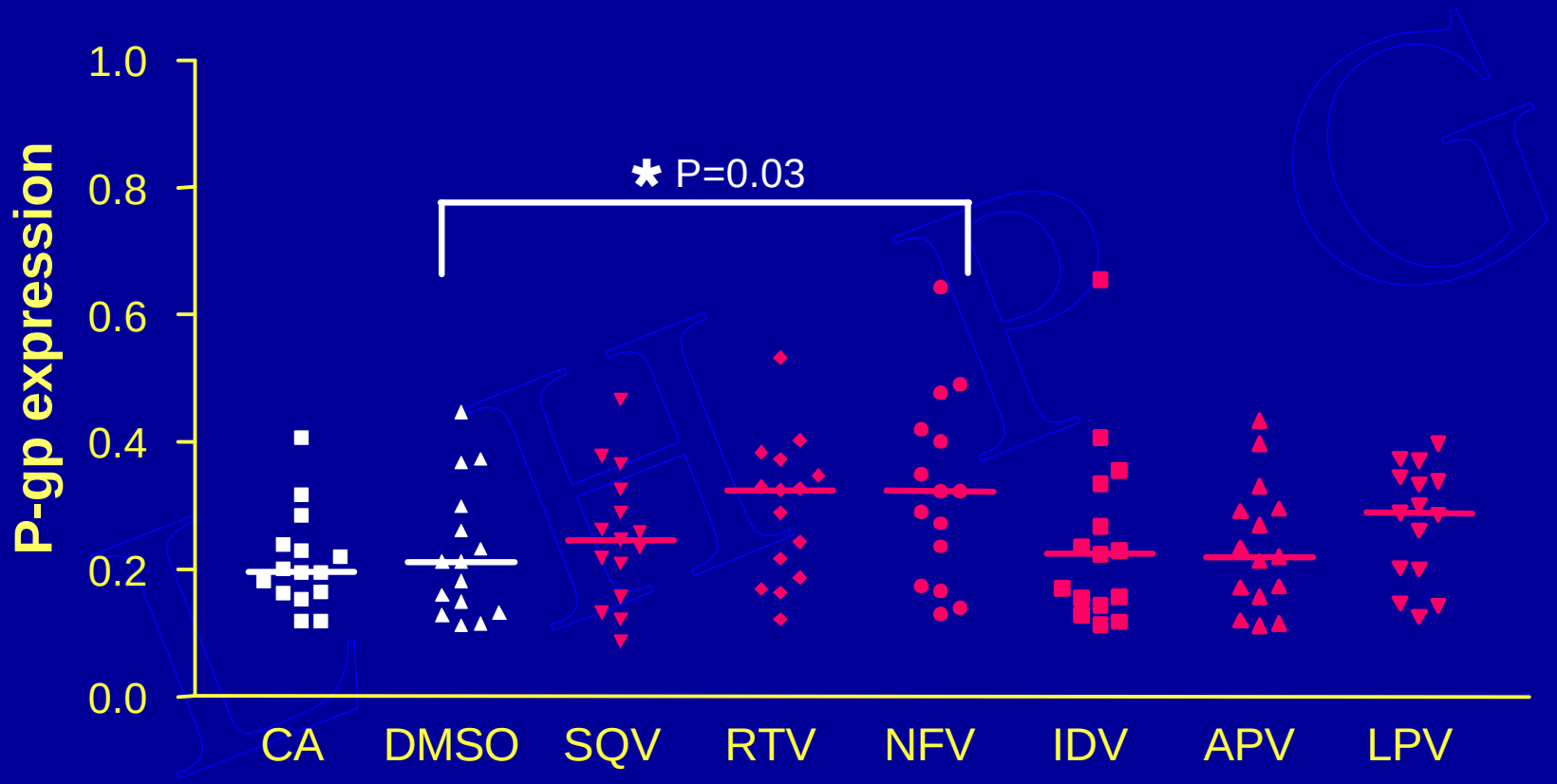
- Detect CD4+ or CD8+: FITC conjugated antibodies
- P-gp expression UIC2: PE conjugated antibody





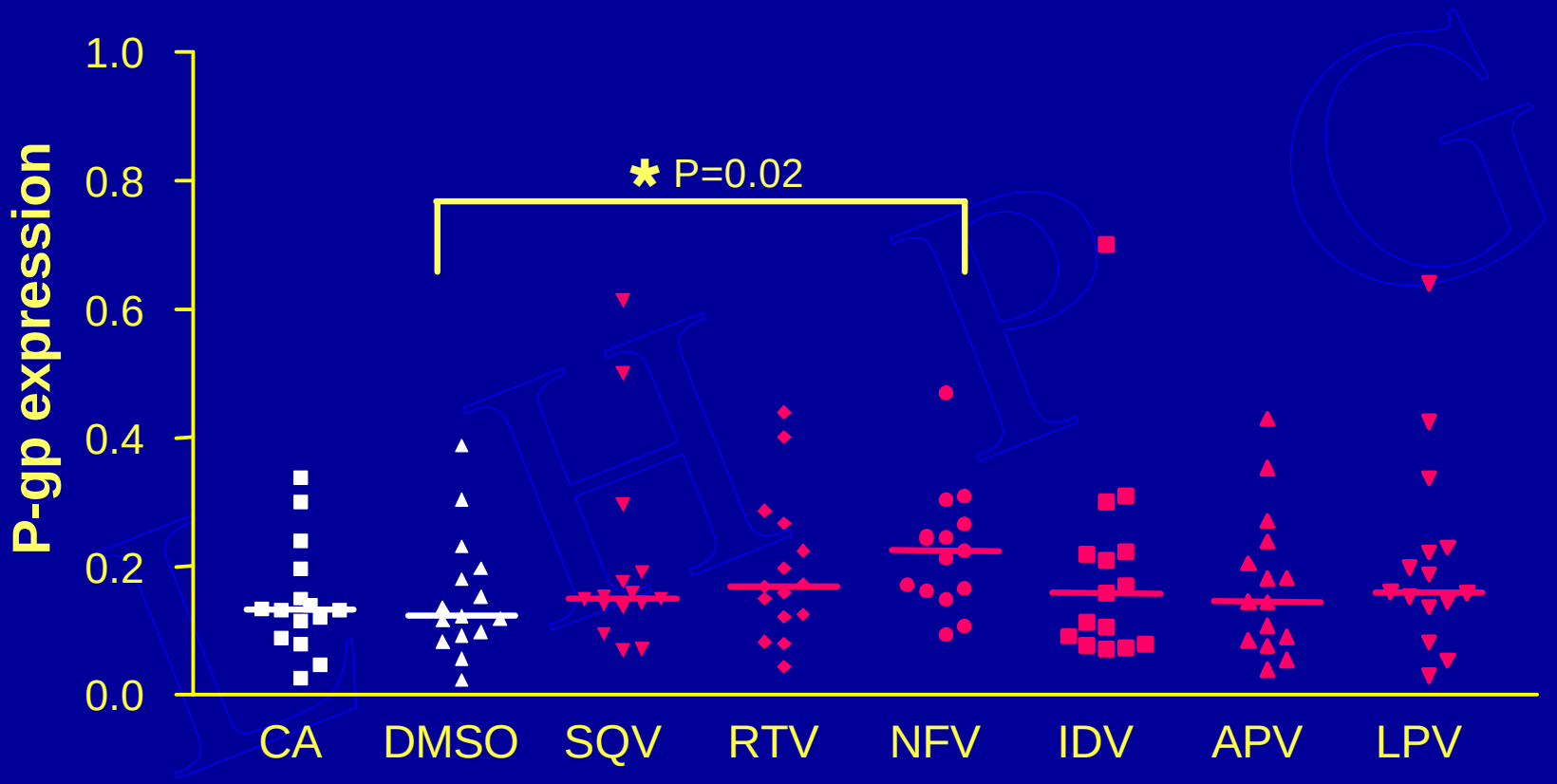
Results: PI effects on lymphocyte P-gp

- **Total lymphocytes:** n=15



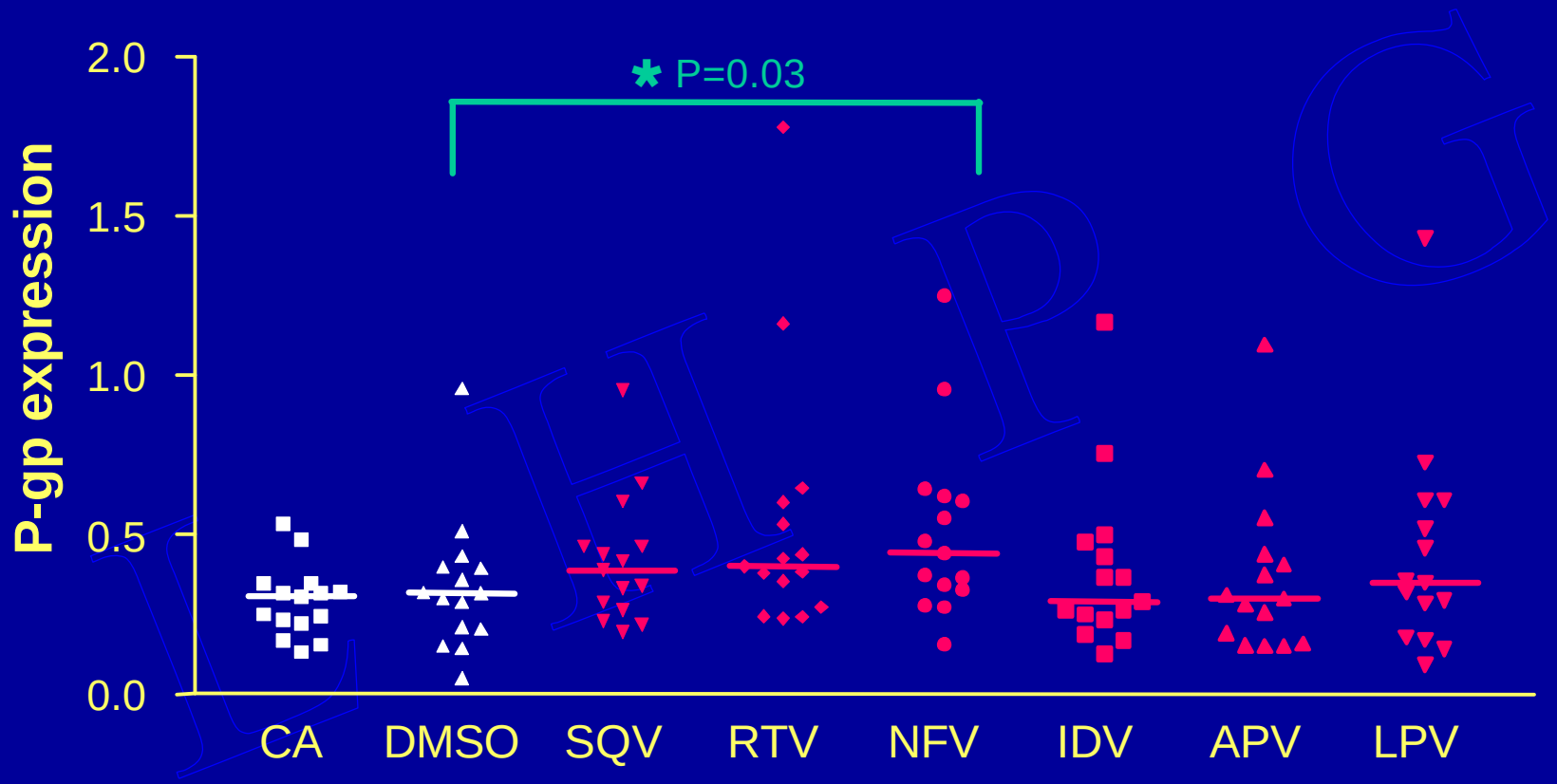
Results: PI effects on lymphocyte P-gp

- **CD4+ lymphocytes:** n=15



Results: PI effects on lymphocyte P-gp

- **CD8+ lymphocytes:** n=15

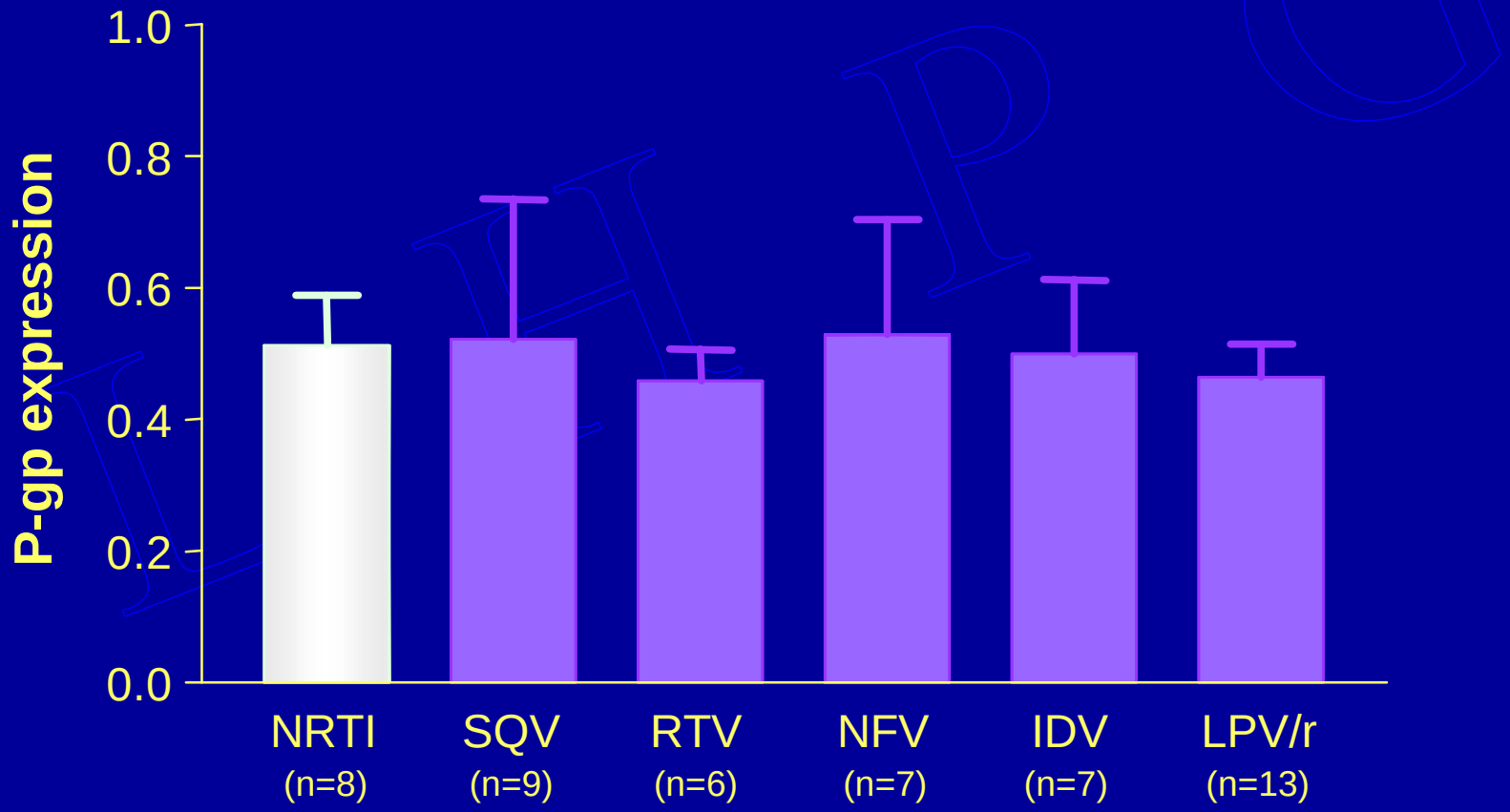


Question 2

What is the impact of protease inhibitors on the expression of P-gp on total lymphocytes ***in vivo***?

Results: PI-containing regimens and lymphocyte P-gp expression

- HIV infected patients (viral load ≤50 copies/ml; n=50)
- Regimens: 3 NRTIs (control)
  2 NRTIs + PI

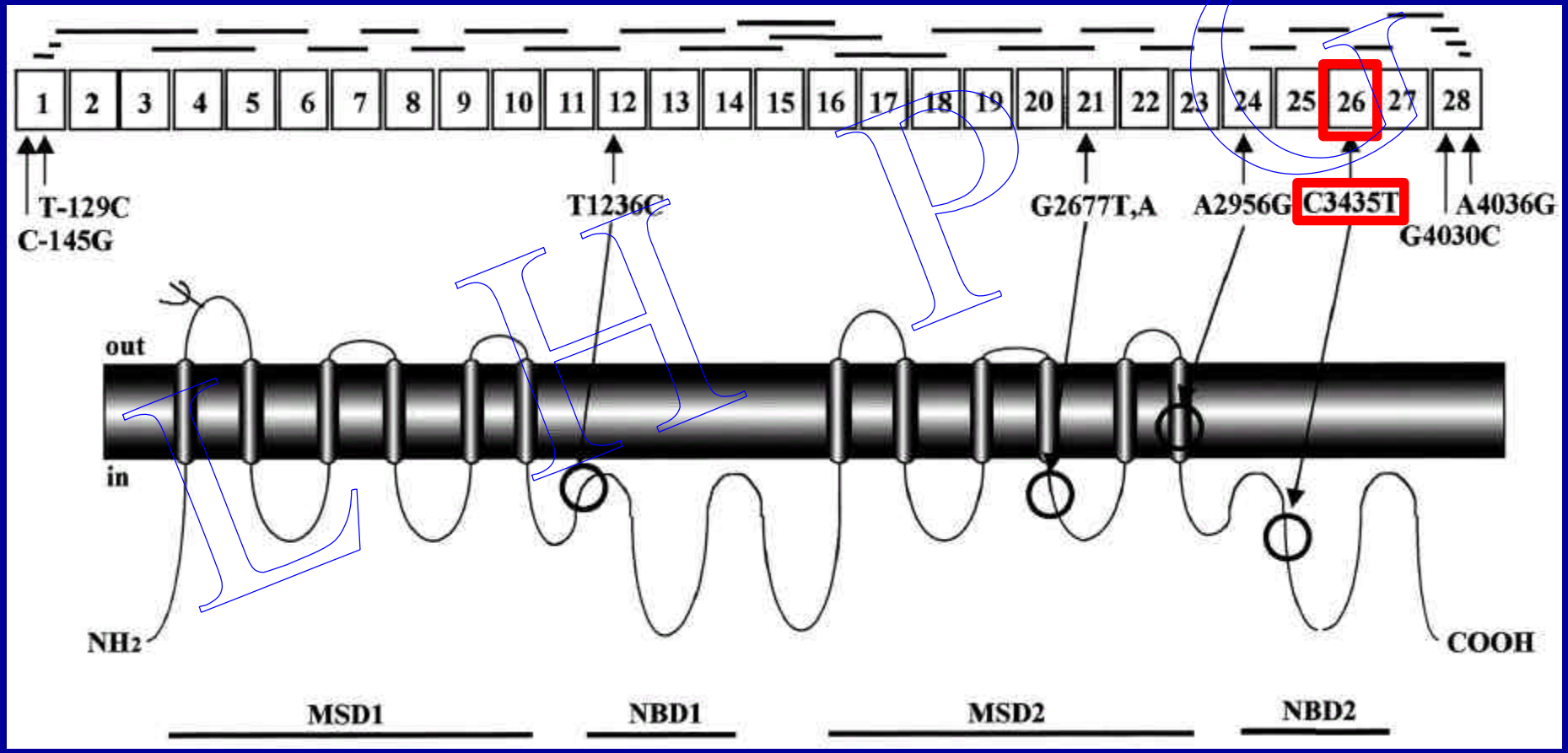


Question 3

Is there any relationship between change in P-gp expression *in vitro* following NFV incubation and **C3435T** mutation in the MDR-1 gene?

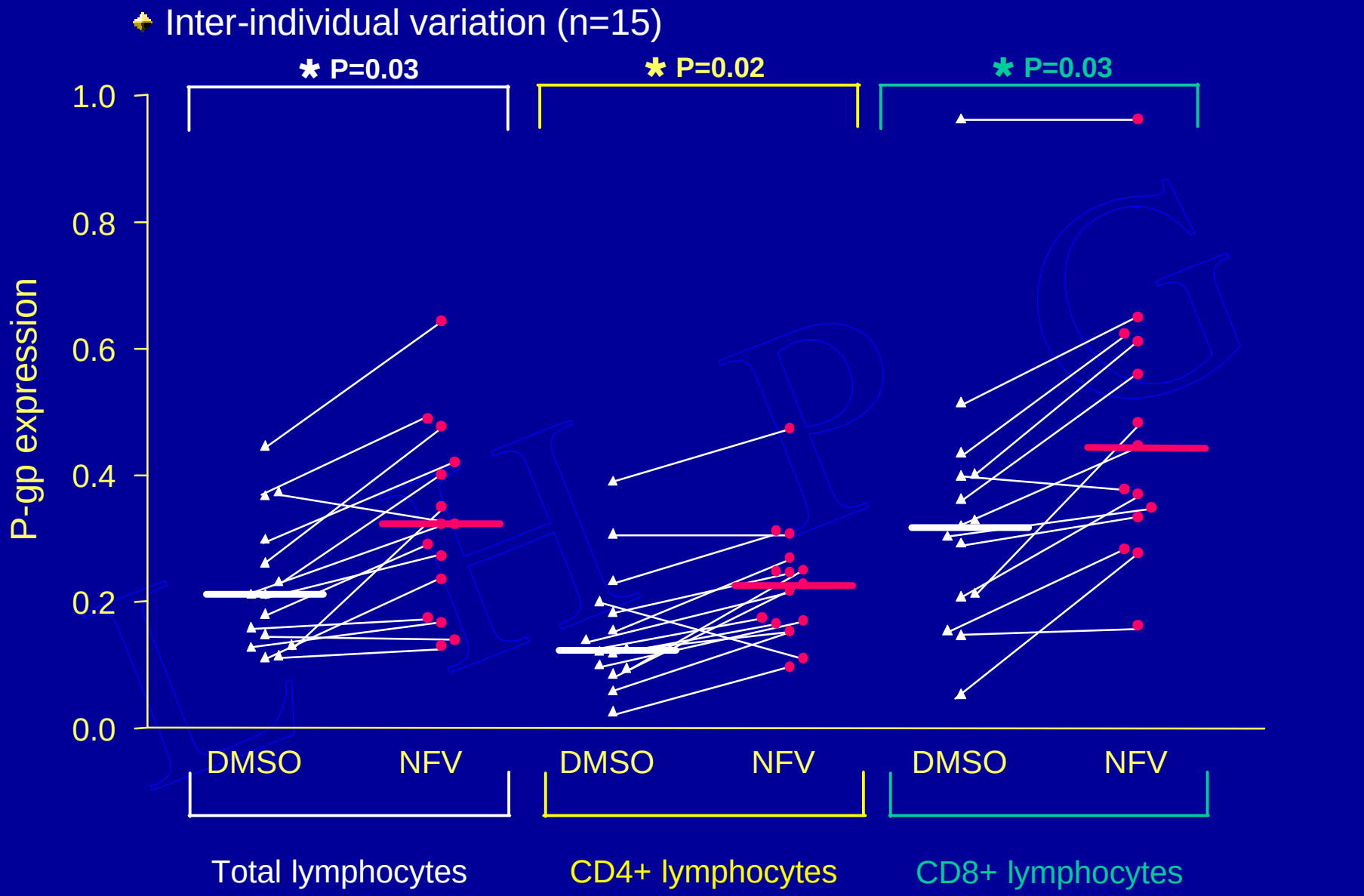
MDR-1 Polymorphisms

- Chromosome 7; 28 exons
- 28 SNPs at 27 positions
- C3435T (exon 26) linked to phenotypic consequences
- Synonymous
- Linkage - haplotypes



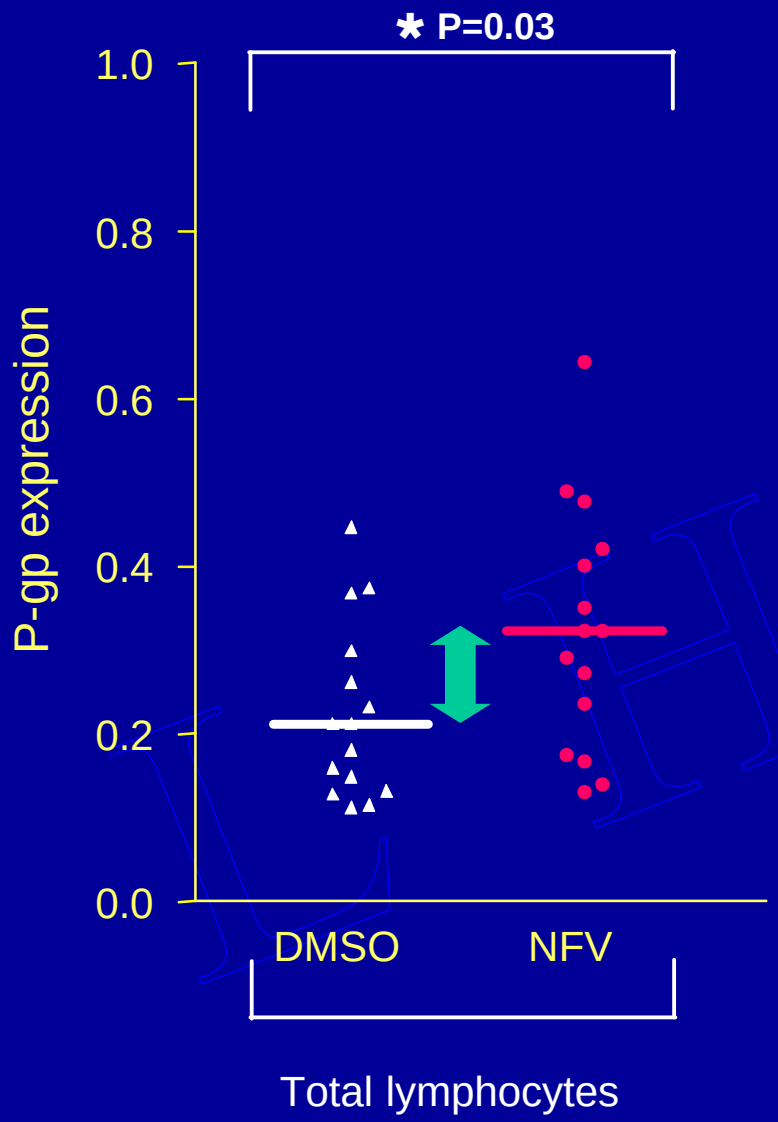
Tanabe et al., 2001

Variation in lymphocyte P-gp following NFV incubation



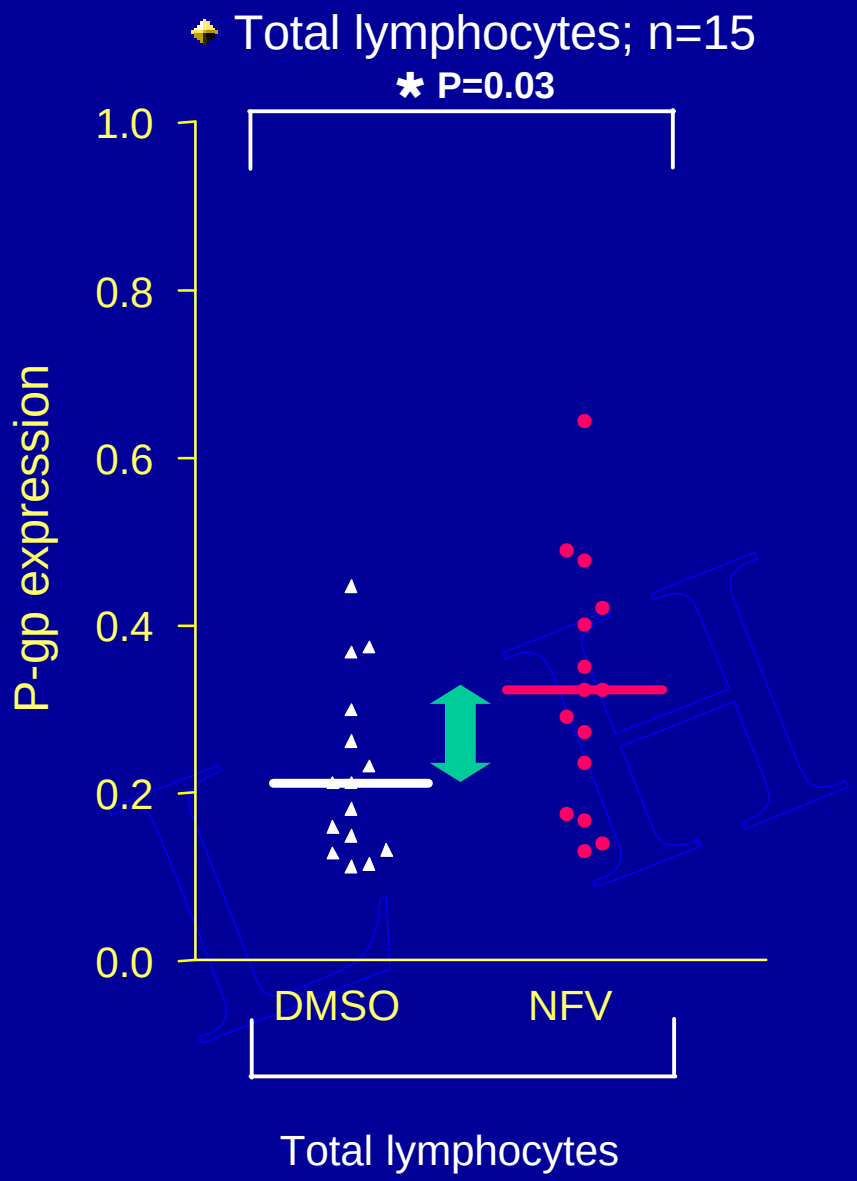
Change in lymphocyte P-gp following NFV incubation

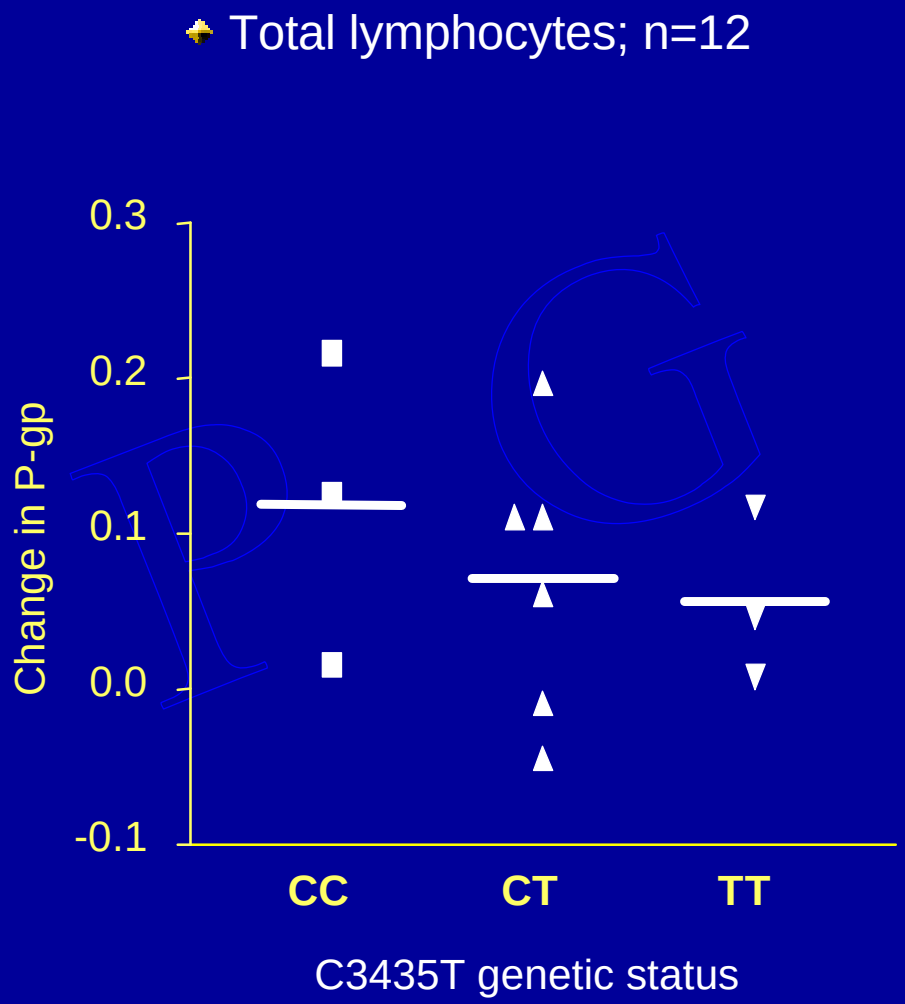
- Total lymphocytes; n=15



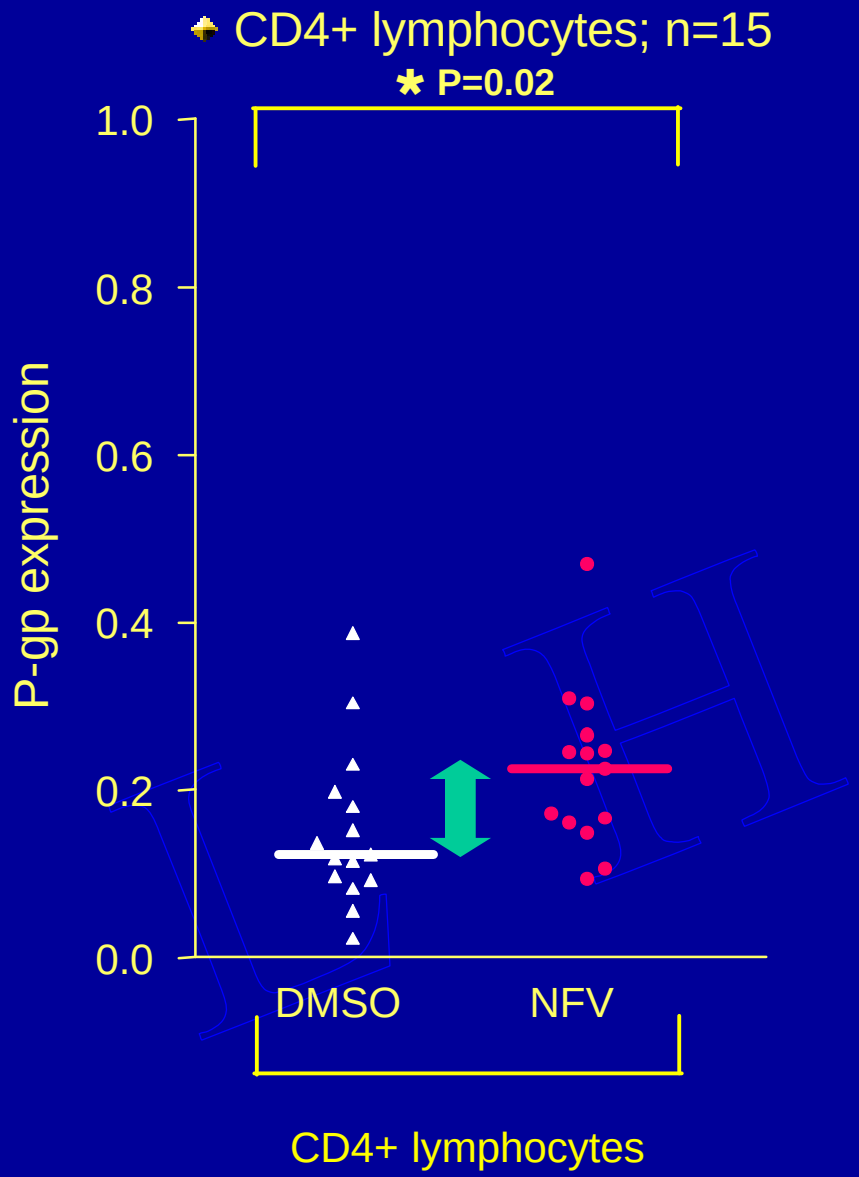
- Relationship of change in P-gp to C3435T genetic status

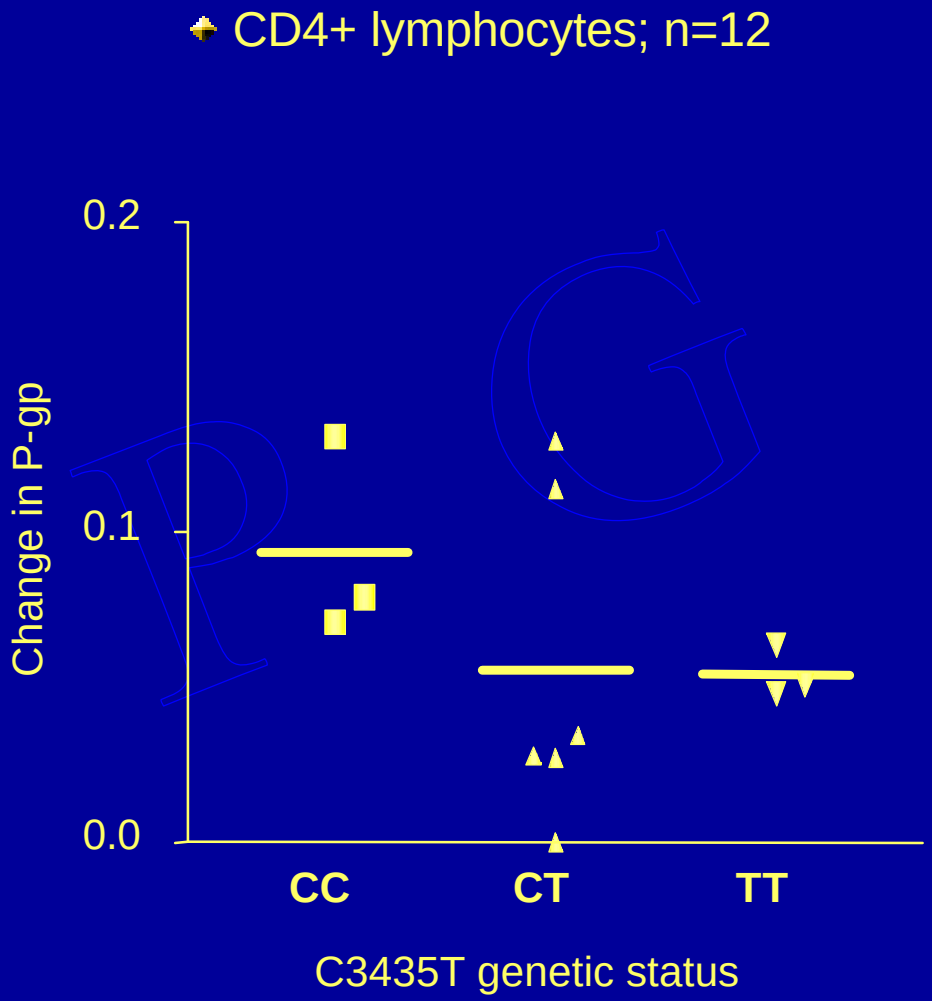
Change in lymphocyte P-gp following NFV incubation



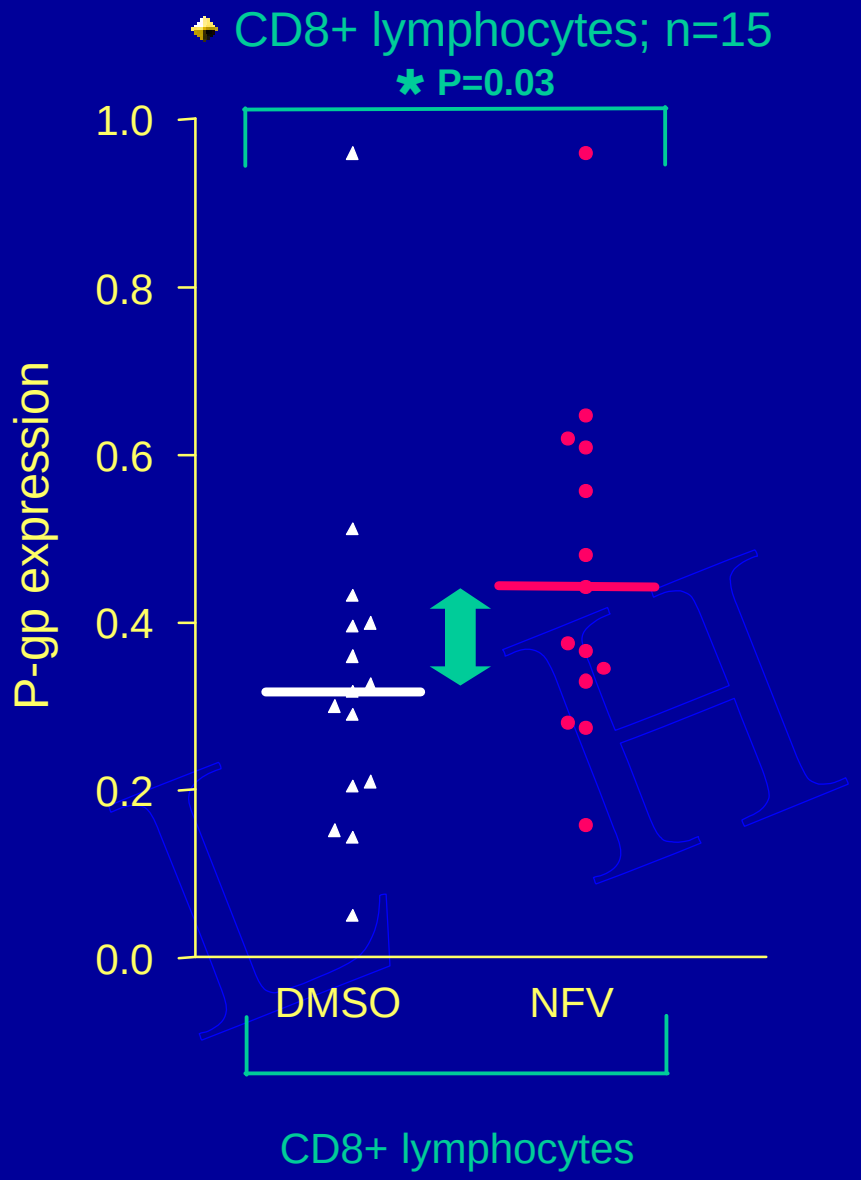


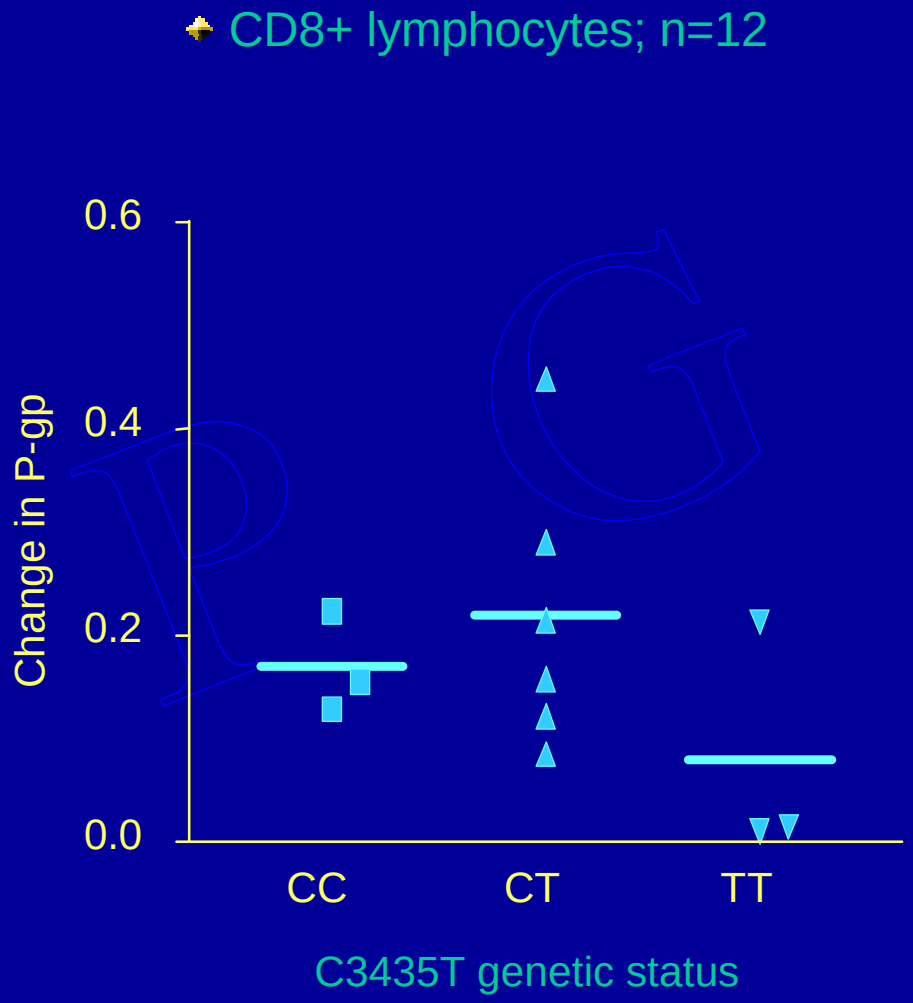
Change in lymphocyte P-gp following NFV incubation





Change in lymphocyte P-gp following NFV incubation





Conclusion

Effect of PIs on lymphocyte P-gp expression

- P-gp on CD8+ > P-gp on CD4+
- *In vitro:* No differences in P-gp on total, CD4+ or CD8+ lymphocytes were observed following SQV, RTV, IDV, APV or LPV incubation

  NFV showed a small inducing effect
- *In vivo:* No PI containing regimen resulted in a change in P-gp expression

Conclusion

C3435T genetic status and change in lymphocyte P-gp expression

- No relationship was observed between the various C3435T genetic groups and change in P-gp expression on lymphocytes, CD4+ or CD8+ cells following NFV incubation
- Require additional samples to see if there is any clear relationship between P-gp expression and genotype

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