Management of HCMV infections in haematopoietic stem cell recipients

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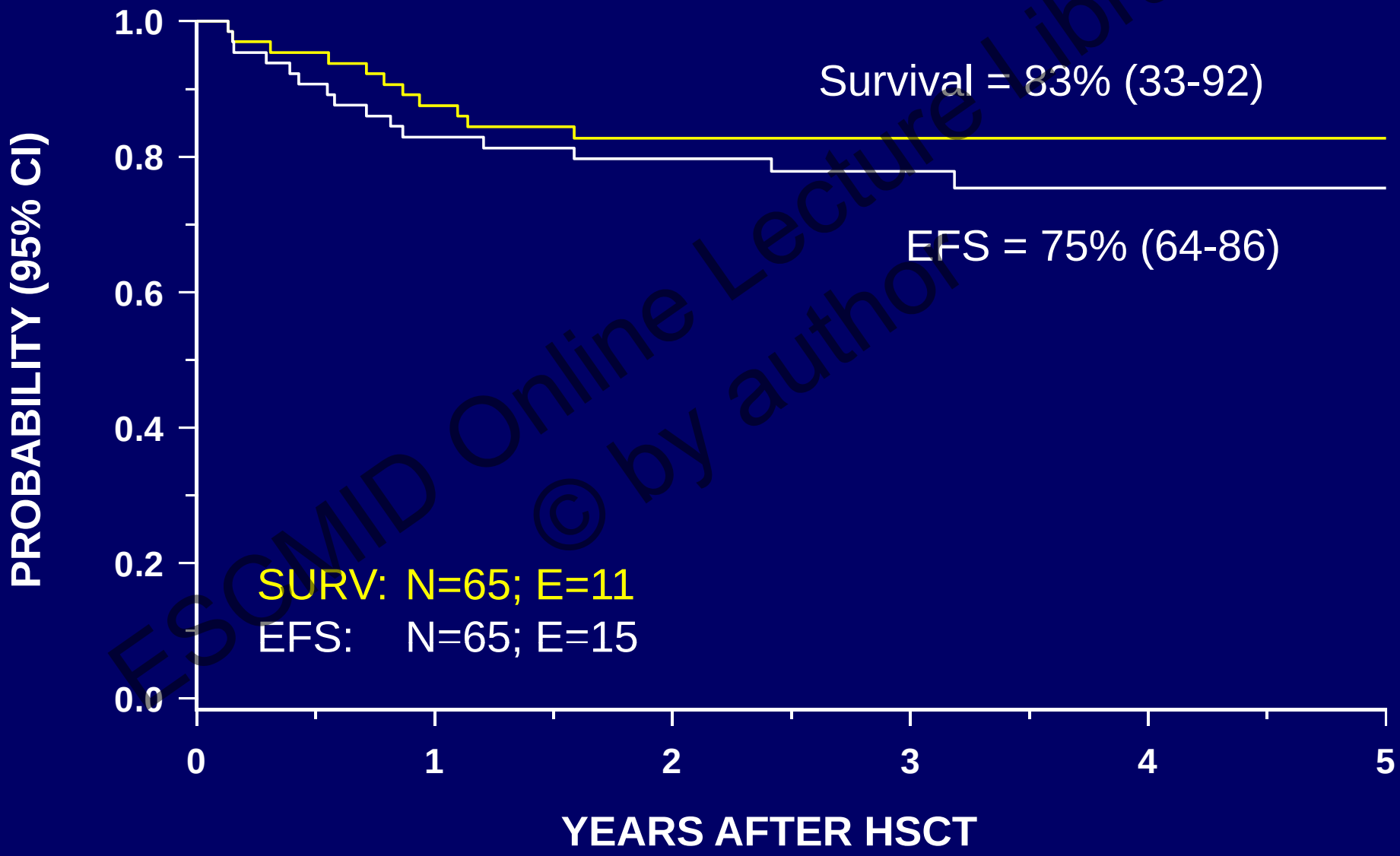
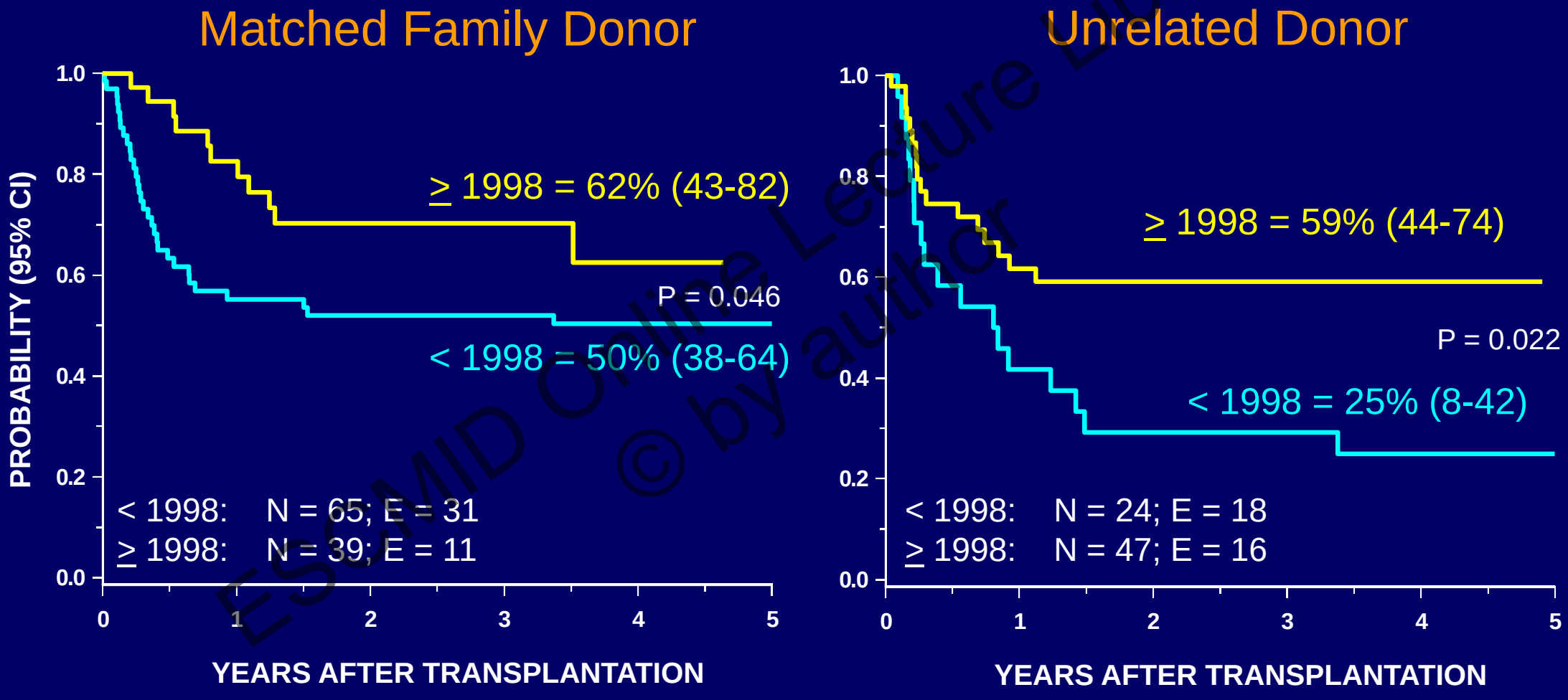
Overall Survival and EFSin children with AML given HSCT from a compatible sibling

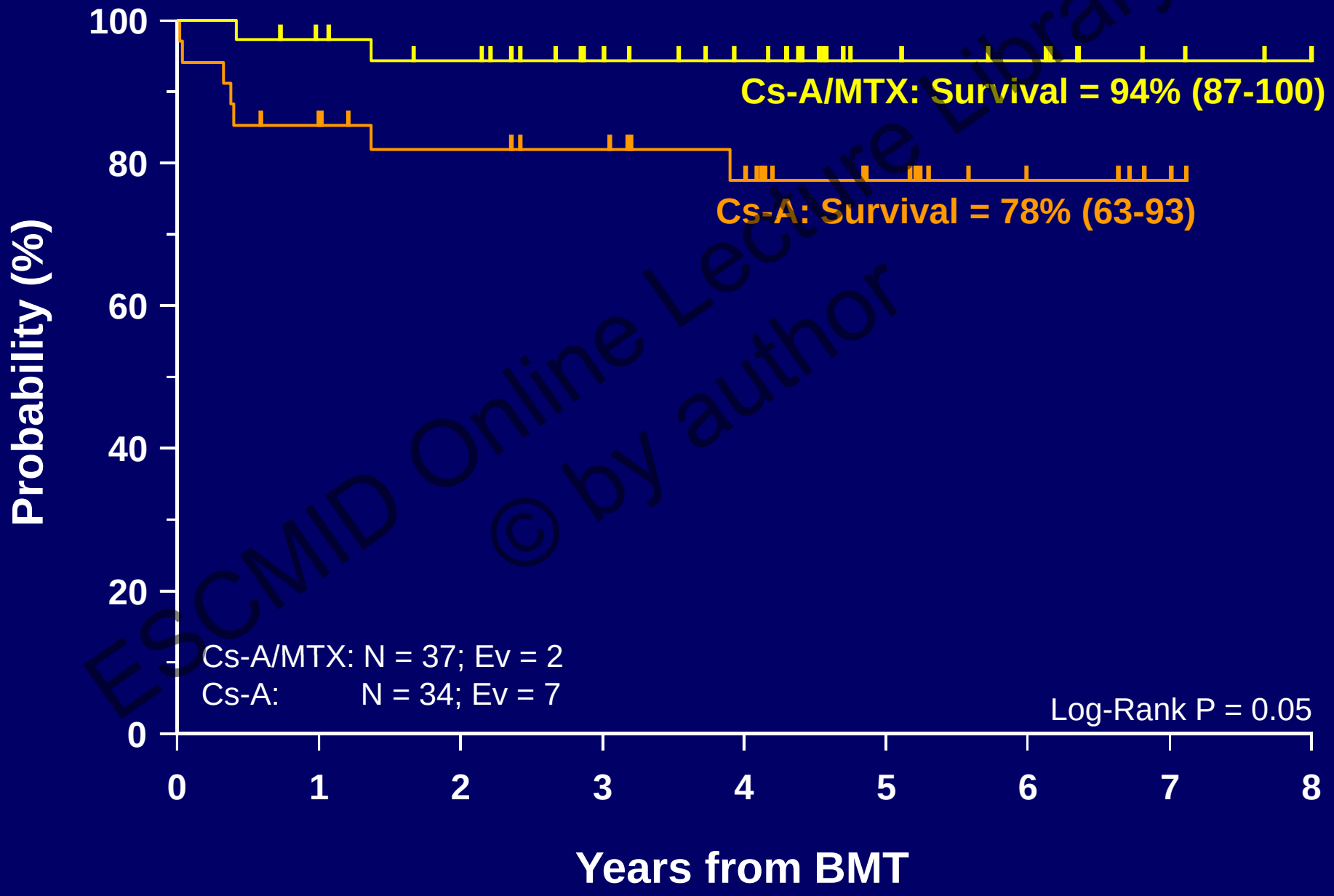


ALL in 2nd CR
DFS by Year of Transplantation

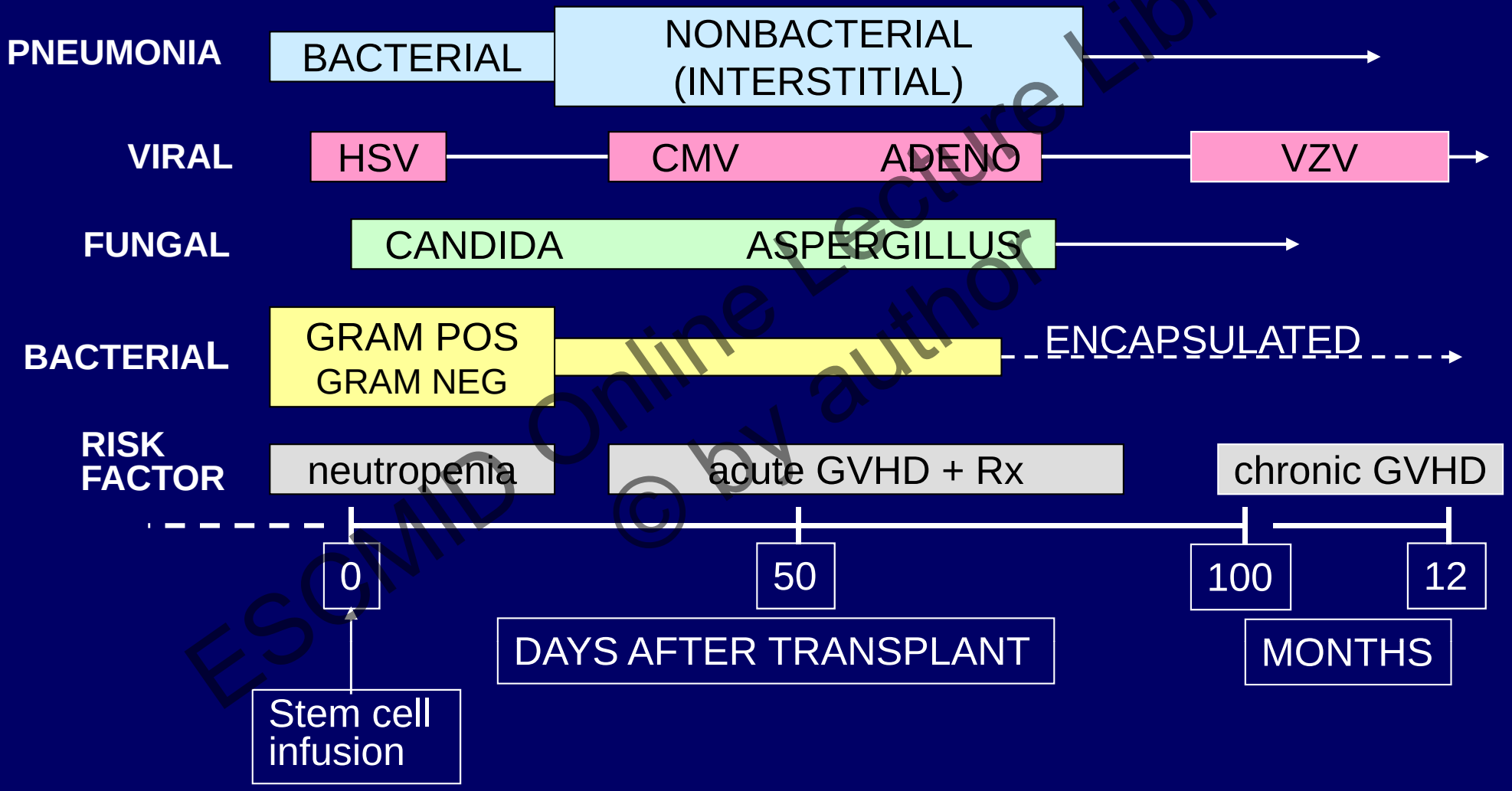


Locatelli F, et al. Leukemia, 2003

Survival probability in children with SAA given HSCT from a compatible sibling



F. Locatelli *et al.* Blood 2000; 96:1690-97.



Frequency of CMV-Disease

Transplantation	Frequency
Kidney-Tx	8%
Liver-Tx	29%
Heart-Liver-Tx	39%
Allo-HSCT	10% - 50%

HCMV in HSCT

- Human cytomegalovirus (HCMV) infection is a well known cause of morbidity and mortality in hematopoietic stem cell transplantation (HSCT) recipients
- HCMV-seropositive patients, regardless of the donor status, have a high incidence of HCMV infection and HCMV viremia and antigenemia have been demonstrated to be important predictors of HCMV disease.

Factors influencing the risk of developing HCMV reactivation after HSCT

- Serological status of the patient
- Serological status of the donor
- Source of stem cell employed
- T-cell depletion of the graft
- GVHD prophylaxis
- GVHD occurrence and therapy
- Patient’s immune recovery

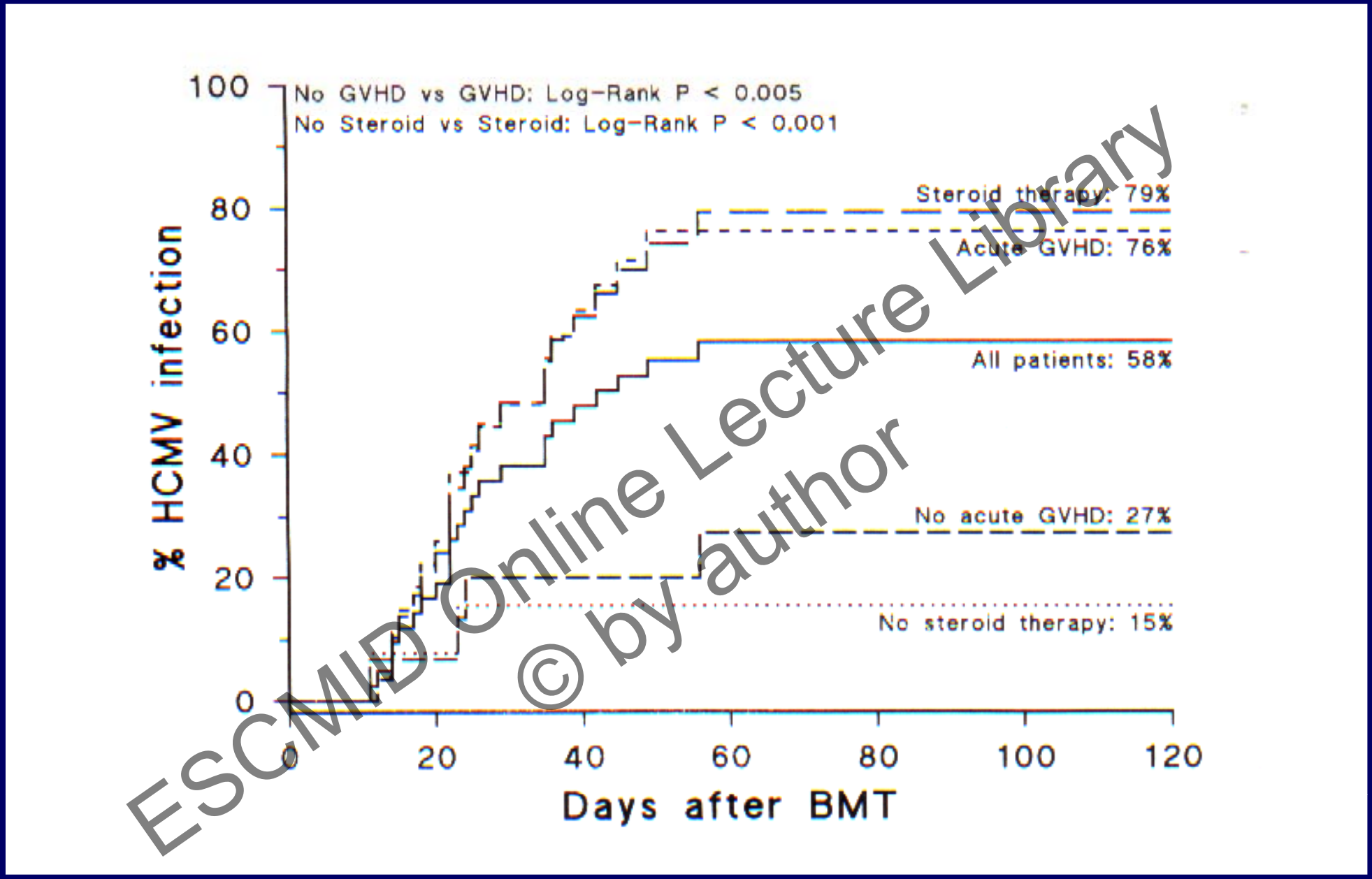


Fig. 1. Cumulative probability of overall HCMV infection (antigenaemia) and according to acute GVHD occurrence or steroid treatment.

F. Locatelli et al. Br J Haematol 1994; 88:64-71.

HCMV in HSCT

- In the 80’s, antiviral treatment was started only upon appearance of clinical symptoms of HCMV infection (deferred or symptomatic therapy), this leading to a high incidence of fatal events.
- Subsequently, pre-emptive (or pre-symptomatic) therapy, i.e. administration of antiviral drugs upon detection of HCMV to treat only patients undergoing viral infection and thus at risk of developing overt disease, was utilized.

Milestones in monitoring and treatment of HCMV infections in transplant recipients at IRCCS Policlinico San Matteo, Pavia

1985 1990 1995 2000 2005 2008

Conventional virus isolation

Development of rapid assays (Antigenemia, Viremia, DNAemia, RNAemia)

Development of assays for HCMV-specific T-cell immunity

Clinical evaluation of DNAemia and T-cell immunity

Clinical evaluation of antigenemia and viremia

Development of automatic DNA extraction and realtime PCR

Clinical evaluation of RNAemia and DNAemia

Symptomatic treatment

Preemptive treatment

Antigenemia-guided

DNAemia-guided

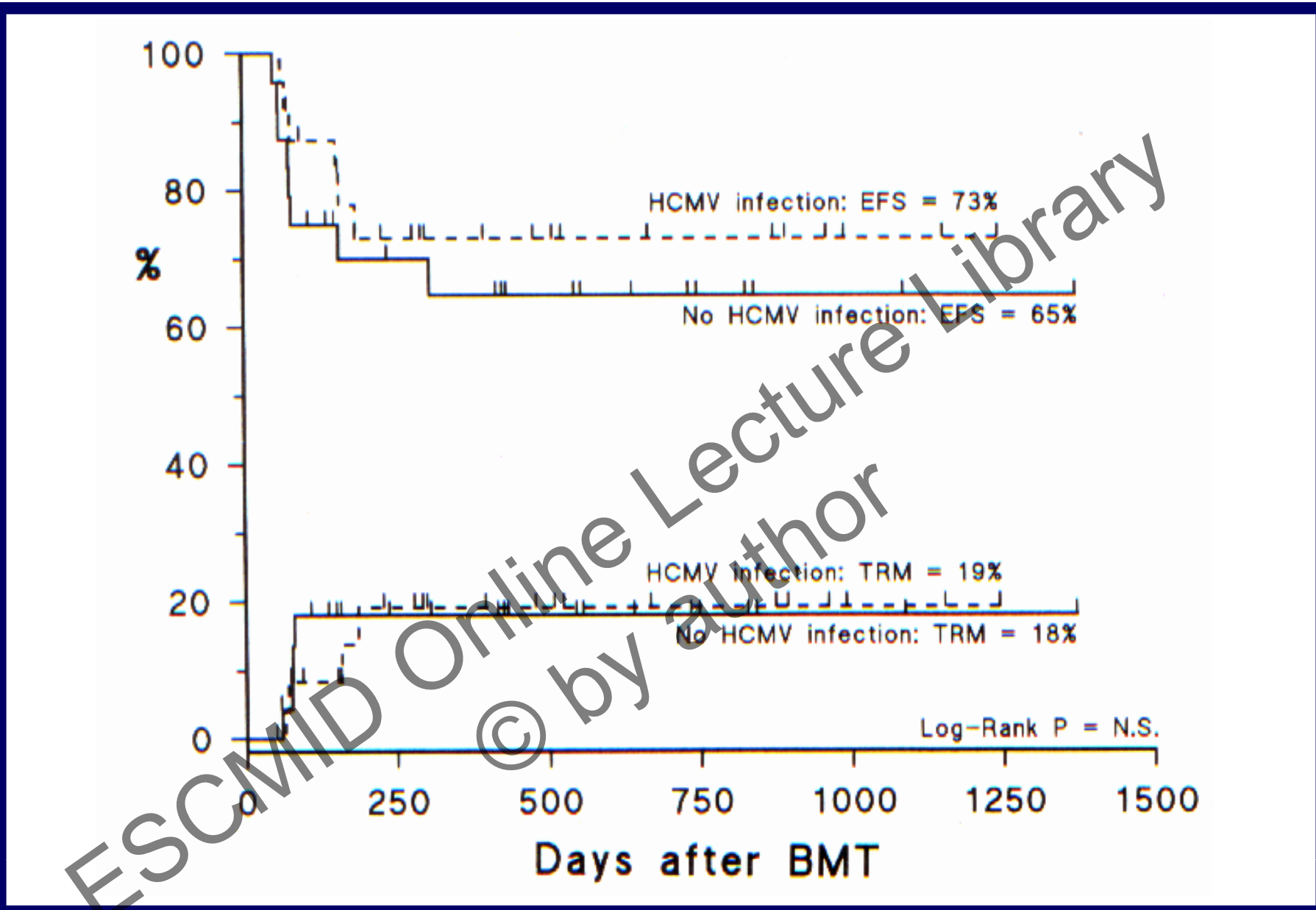


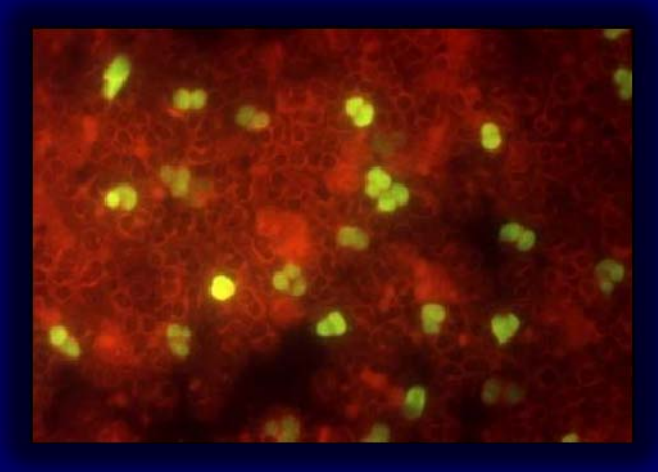
Fig. 3. Kaplan-Meier estimate of event-free survival (EFS) and transplant-related mortality (TRM) in children with and without HCMV infection (antigenaemia).

F. Locatelli et al. Br J Haematol 1994; 88:64-71.

Pros and Cons of antigenemia as guiding parameter for preemptive treatment in HSCTR

- **Pros**
  - most widely used assay for guiding preemptive therapy in SOTR and HSCTR
  - simple to perform
  - inexpensive

- **Cons**
  - lack of standardisation, automation and objectivity of test results
  - indirect marker of viral replication;
  - not applicable in HSCTR pts before graft take



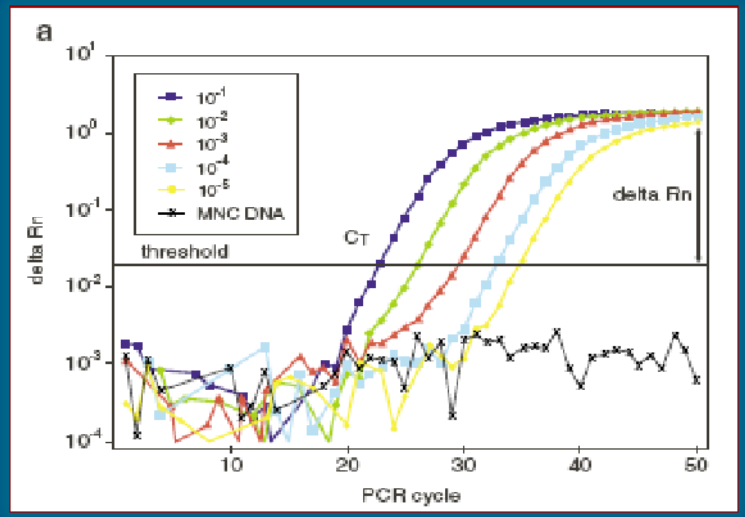
Pros and Cons of DNAemia (real-time PCR format)

Pros

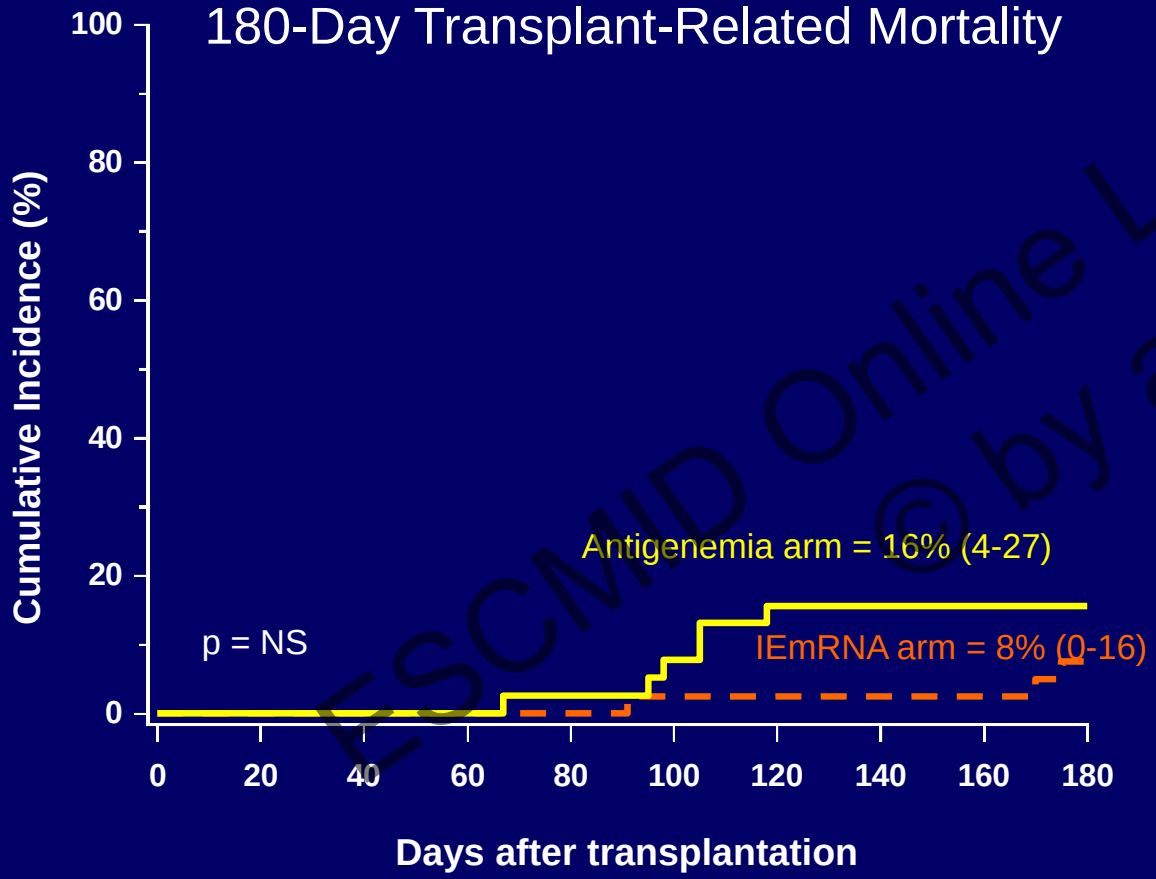
- wide dinamic range (>7 $\log_{10}$)
- negligible risk for carry-over contamination
- standardisable, semi-automated and objective assay
- direct expression of viral replication
- quantitative
- sensitive

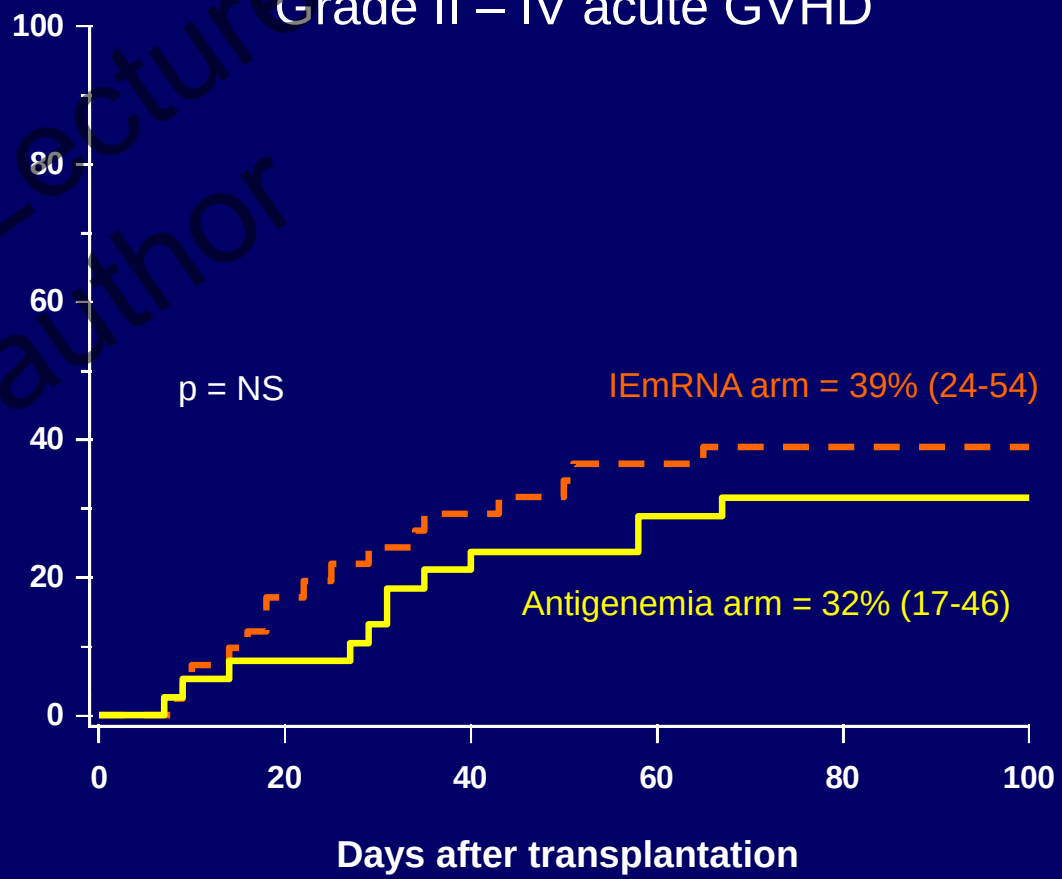
Cons

- need for consensus threshold levels



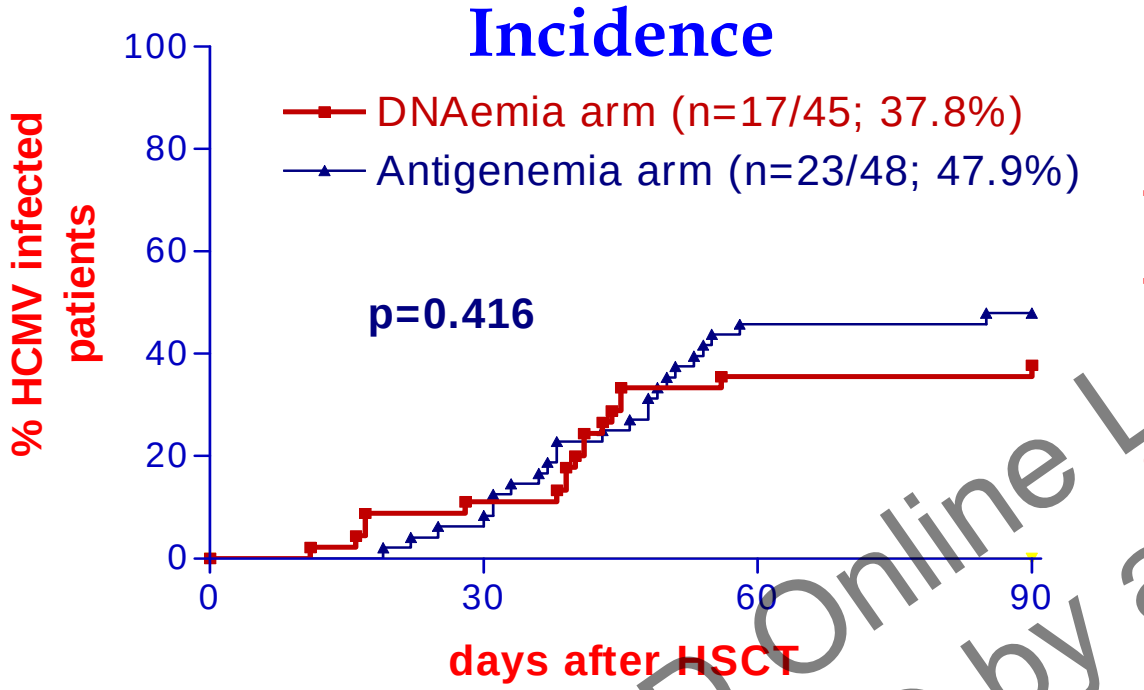
Human cytomegalovirus immediate early-mRNAemia *vs* pp65-antigenemia for guiding pre-emptive therapy in children and young adults given HSCT

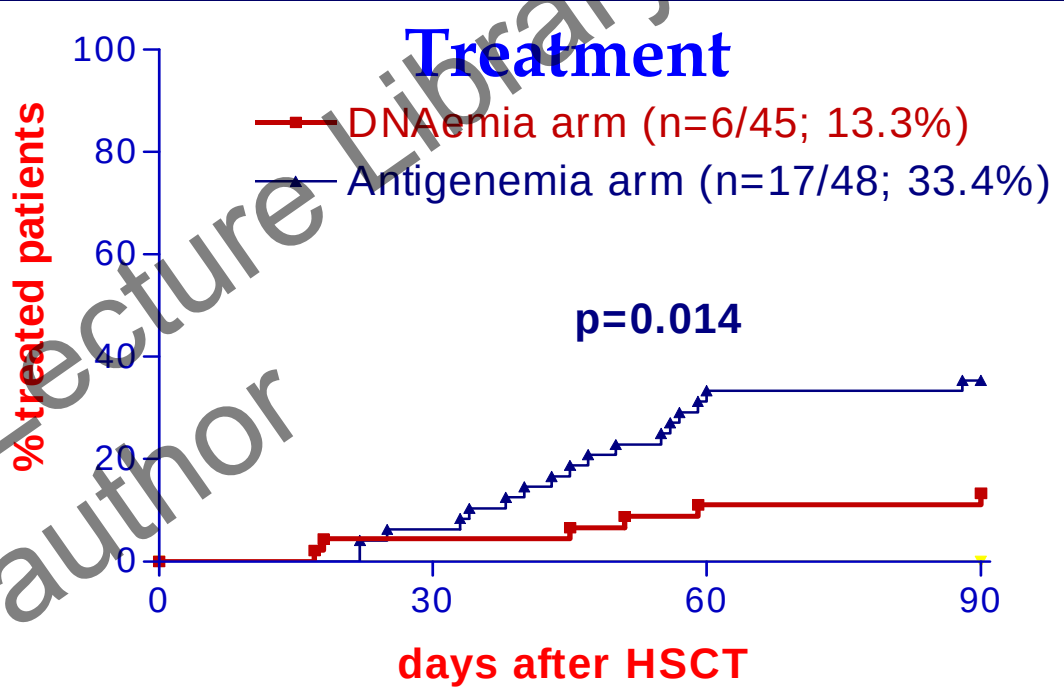




Gerna G, et al. Blood 2003

Antigenemia vs DNAemia a prospective open-labled randomized trial: treatment of HCMV infection in the two randomization arms (HSCTR).

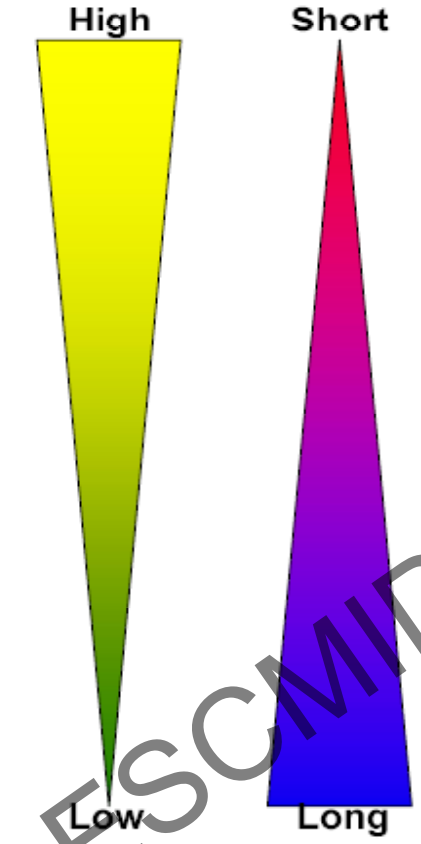




Cut-offs
DNAemia: 10,000 copies/ml blood
Antigenemia: first confirmed pp65-pos/10^5 PBLs

Lilleri et al, Blood 2008

HCMV viral load to start pre-emptive therapy



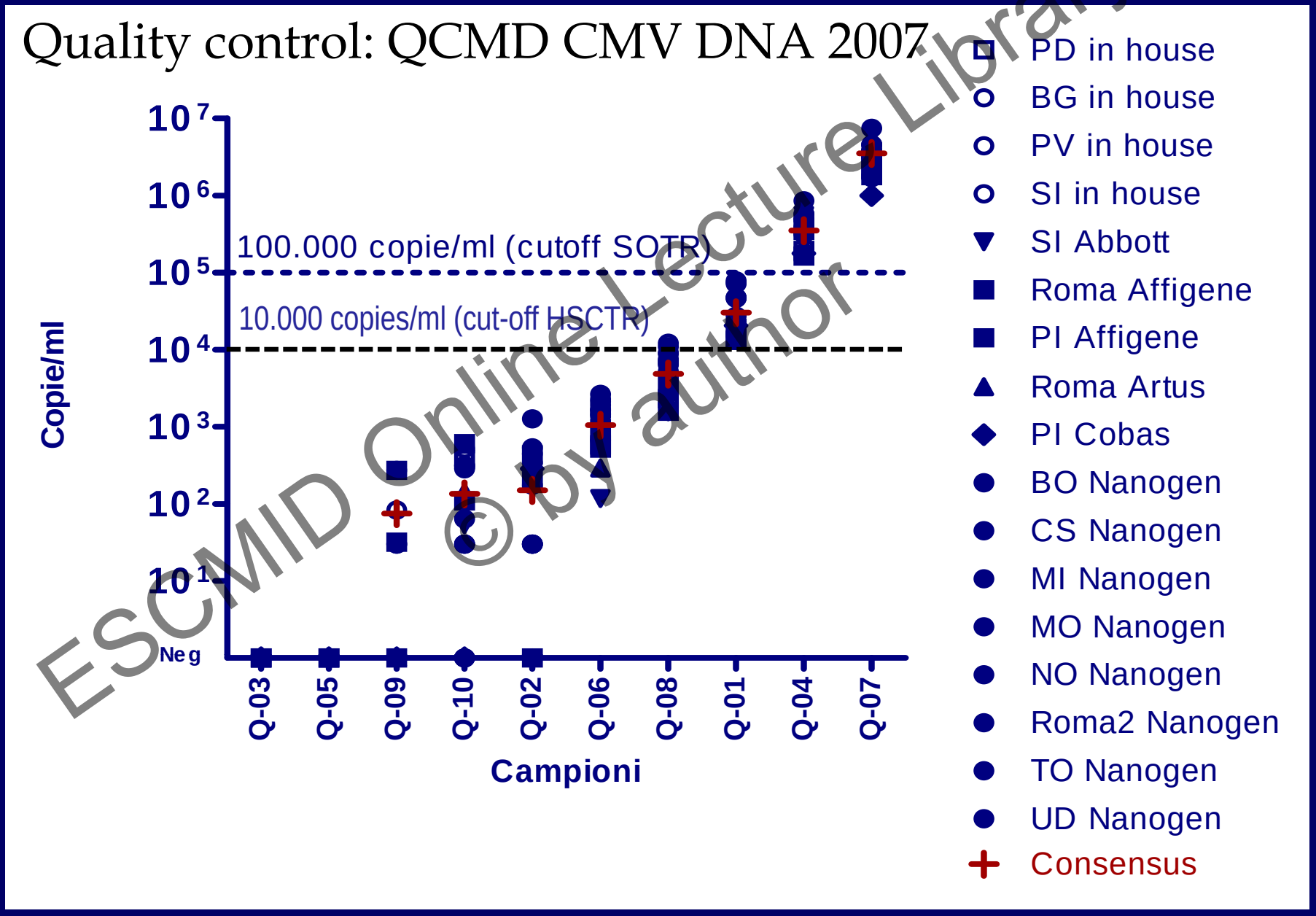
Immuno-suppression	CMV doubling time	Risk Groups	CMV Plasma DNA Level to Start PET at FHCRC*	CMV Whole Blood DNA Level to Start PET at Karolinska Institute**
		Cord blood	Any level	1000 copies
		Allograft - High-dose steroids+ - T cell depletion - Anti-T cell antibodies - CD34 selection	> 100 copies/mL	1000 copies
		Allograft - Low dose steroids - No T cell depletion or anti T cell antibodies	> 500 copies/mL > or 5-fold ⇑ †	1000 copies
		Allograft - after day 100	> 1000 copies/mL > or 5-fold ⇑ †	1000 copies if GVHD Other individual assessment based on ⇑

* Assays performed weekly or twice weekly (highest risk); limit of detection 25 copies/mL
+ 1 mg per kg of prednisone or higher
† If initial level is less than threshold
** Assays performed weekly, limit of detection 50 copies/mL

Figure 1. CMV viral load to start preemptive therapy (PET) used at the FHCRC in Seattle, WA, and the Karolinska Institute, Stockholm, Sweden.

Boeckh and Ljungman, Blood 2009

Standardizzation of HCMV DNAemia

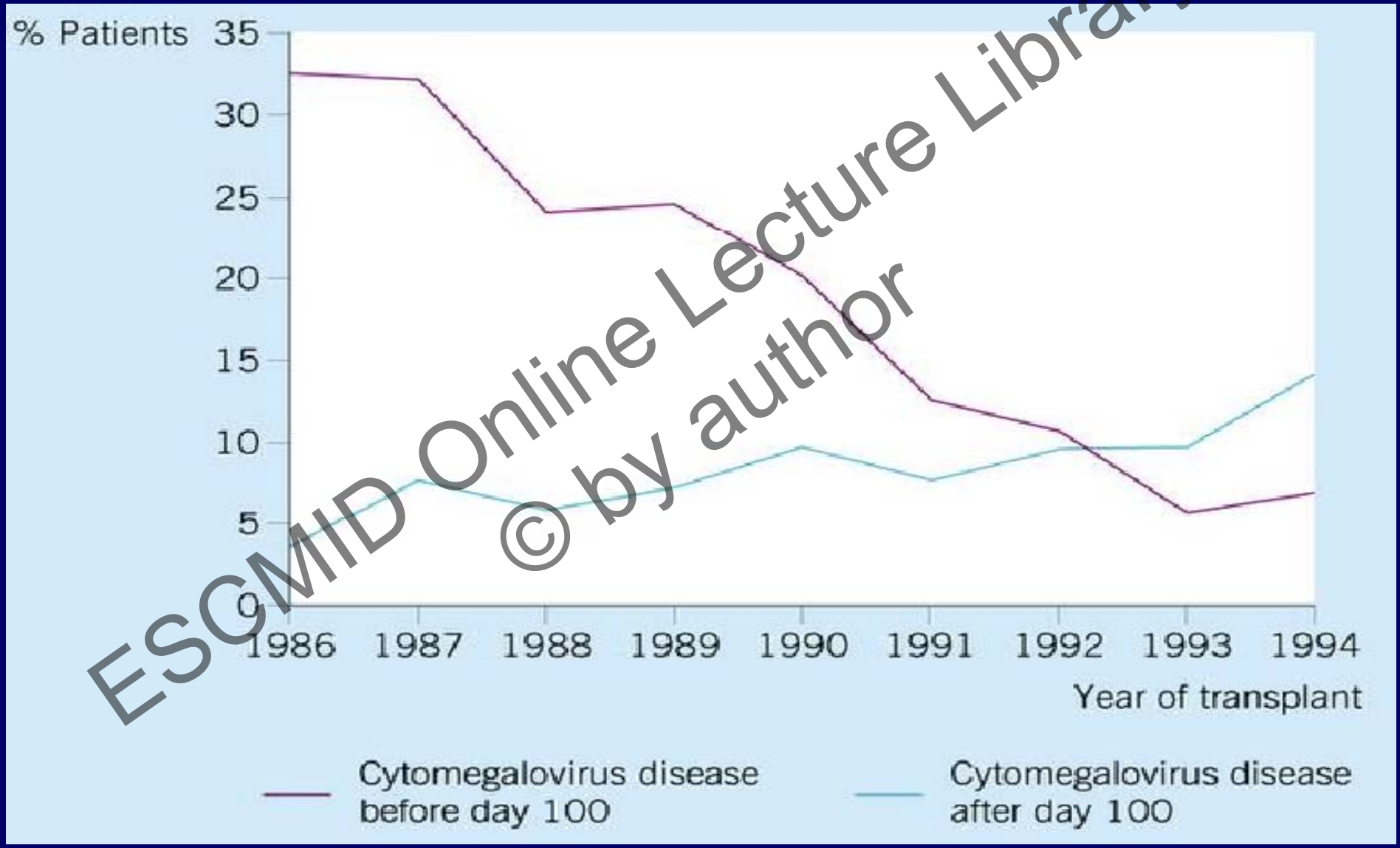


HCMV in HSCT

- Prophylaxis, i.e. administration of anti-HCMV drugs to all transplanted patients for a median time of 3 months or 100 days after transplantation, has been employed to prevent HCMV disease in HSCT recipients.
- Major drawbacks of such an approach are treatment of a substantial proportion of patients not at risk for disease, drug toxicity, occurrence of late (after discontinuation of prophylaxis) HCMV disease (mainly pneumonia), and low cost-effectiveness.

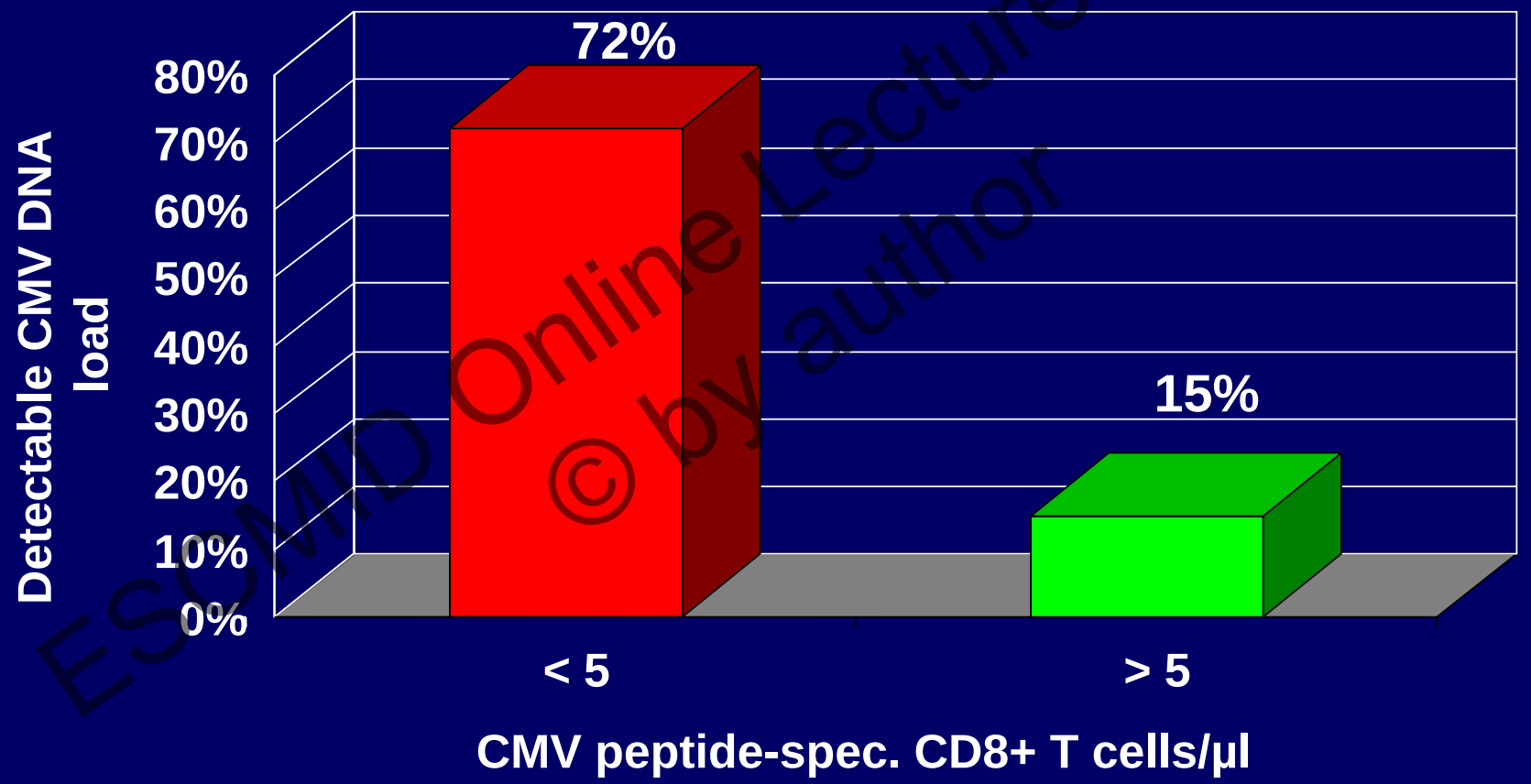
Incidence of HCMV-disease after alloHSCT

HCMV-sero+ Patients (n=1458)



R. Bowden, „Infectious Diseases“ 2000

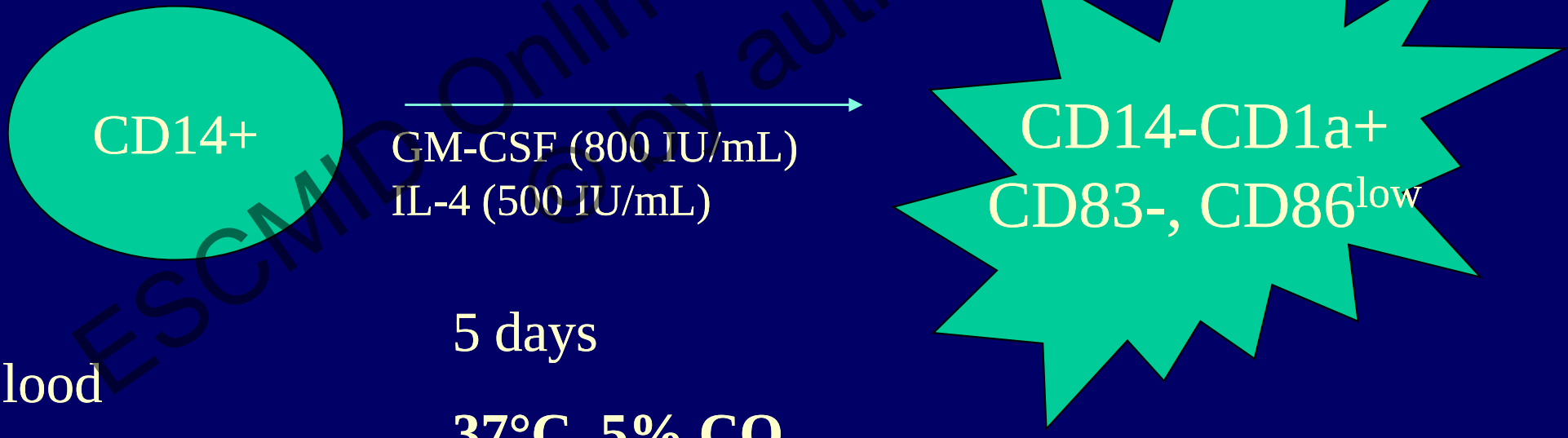
CMV-DNAemia and CMV-peptide specific CD8+ T cells



Hebart/Einsele Blood 2002

Simultaneous quantification of HCMV-specific CD4+ and CD8+ effector T cells.

1. Generation and culture of monocyte-derived immature DC



2. Infection of immature DC with HCMV (VR1814, MOI 10)

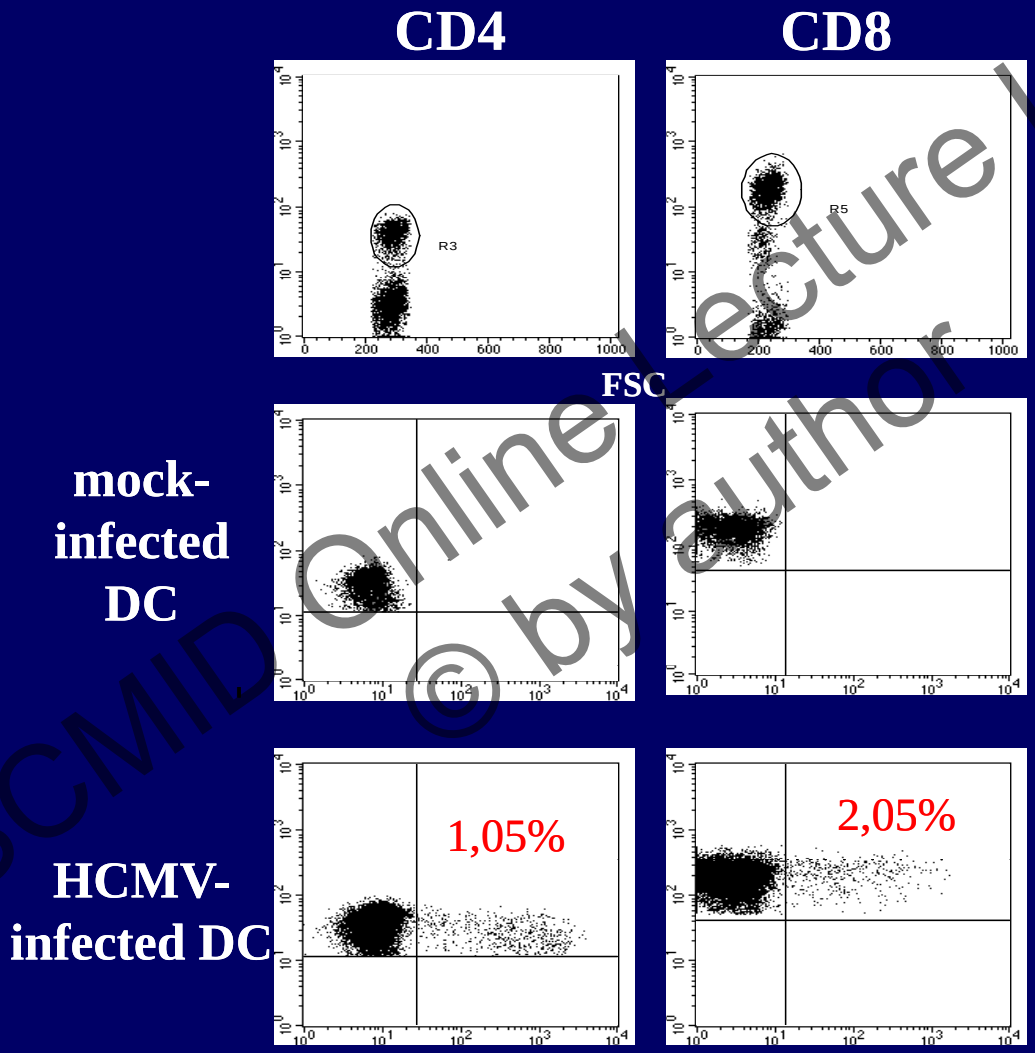
24 h

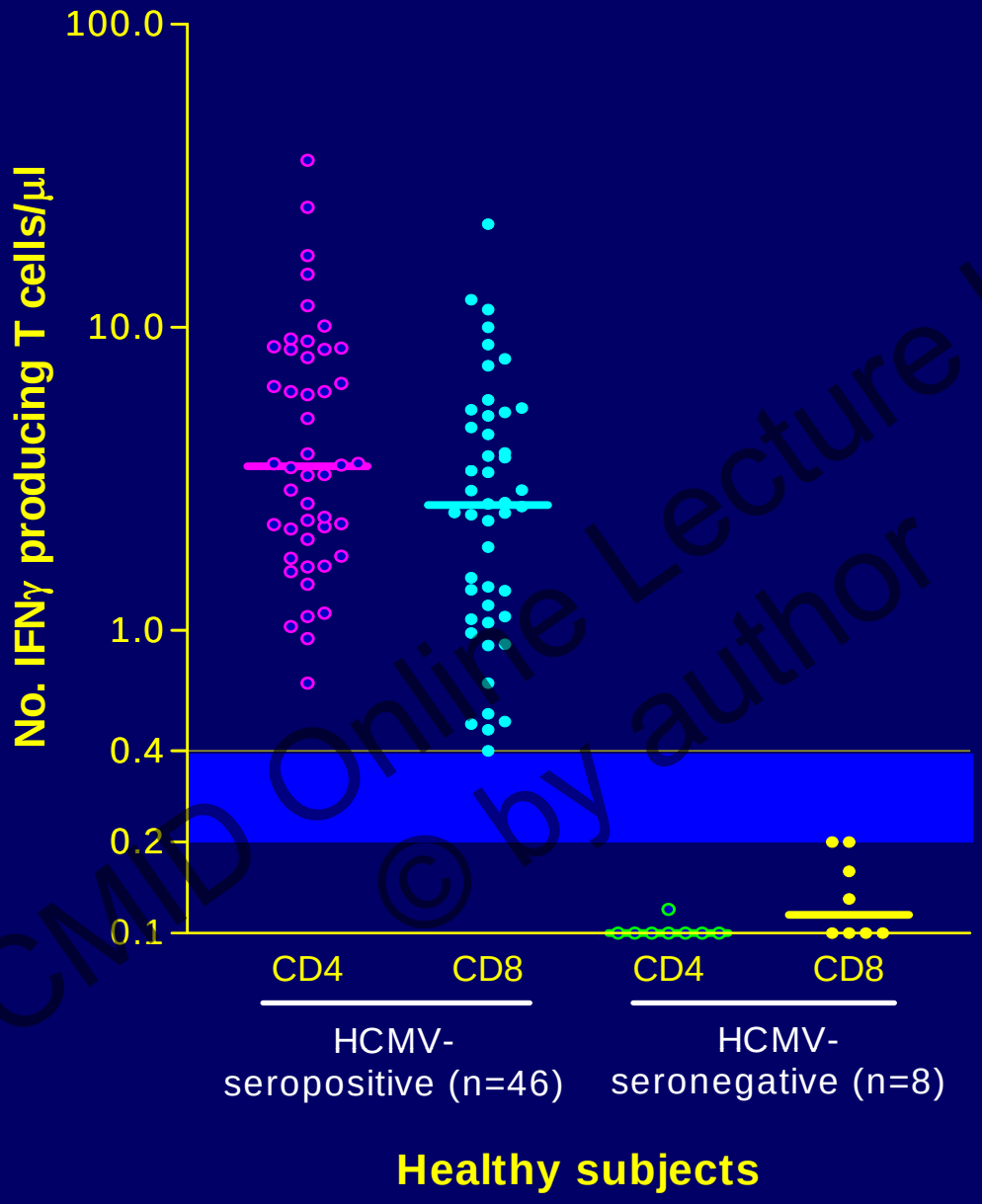
3. Co-culture of infected imDC and PBMC + Brefeldin A

overnight

4. Intracellular flow cytometry analysis of HCMV-specific CD4+ and CD8+ INFγ–producing T cells

Percent of INFγ-producing $CD8^+$ and $CD4^+$ T cells in HCMV-seropositive donor

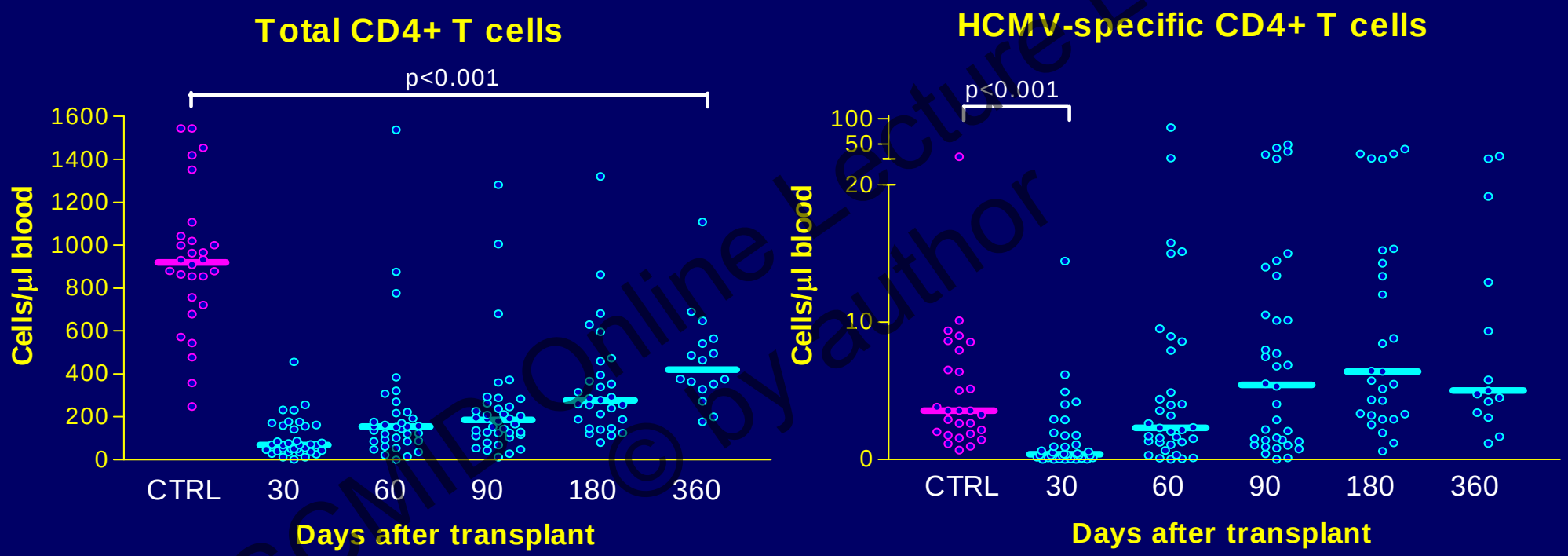




HCMV-specific CD4+ and CD8+ T cells in HCMV-seronegative and seropositive healthy subjects

HCMV-seropositive HSCT recipients (n=39):

Total and HCMV-specific CD4+ T cell counts

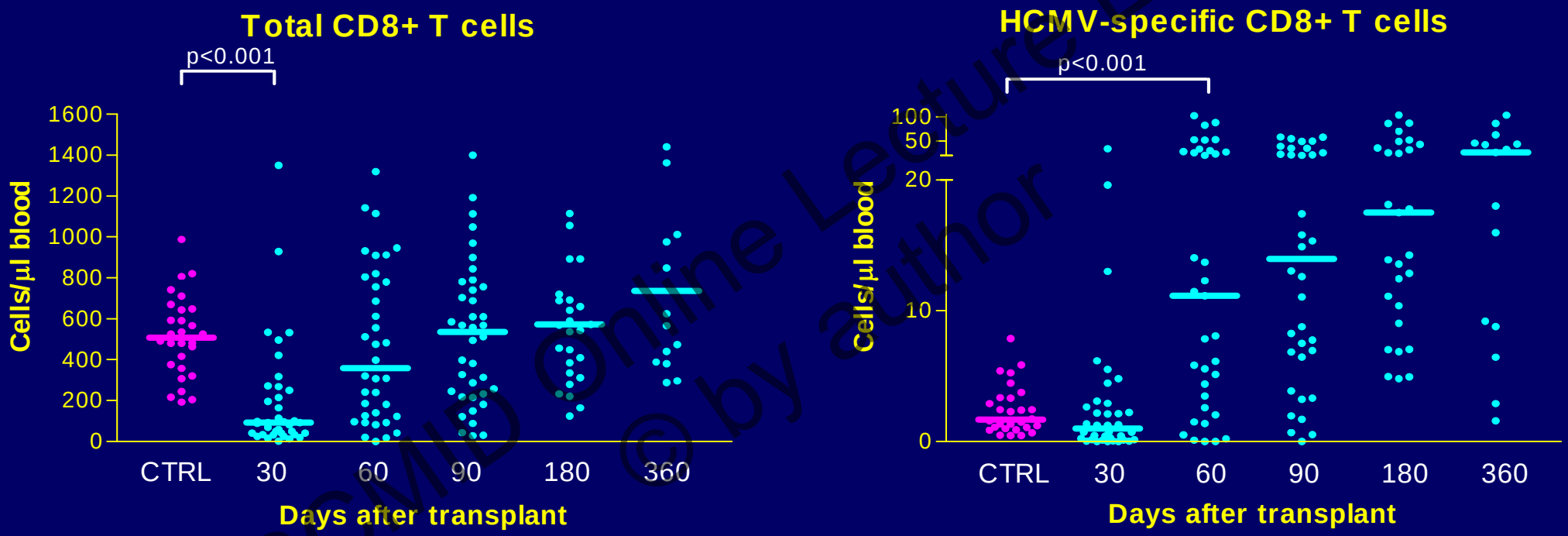


Total CD4+ T cell count increases over time, however remaining lower than that of controls.

HCMV-specific T cell count reaches levels similar to controls from day +60.

HCMV-seropositive HSCT recipients (n=39):

Total and HCMV-specific CD8+ T cell counts



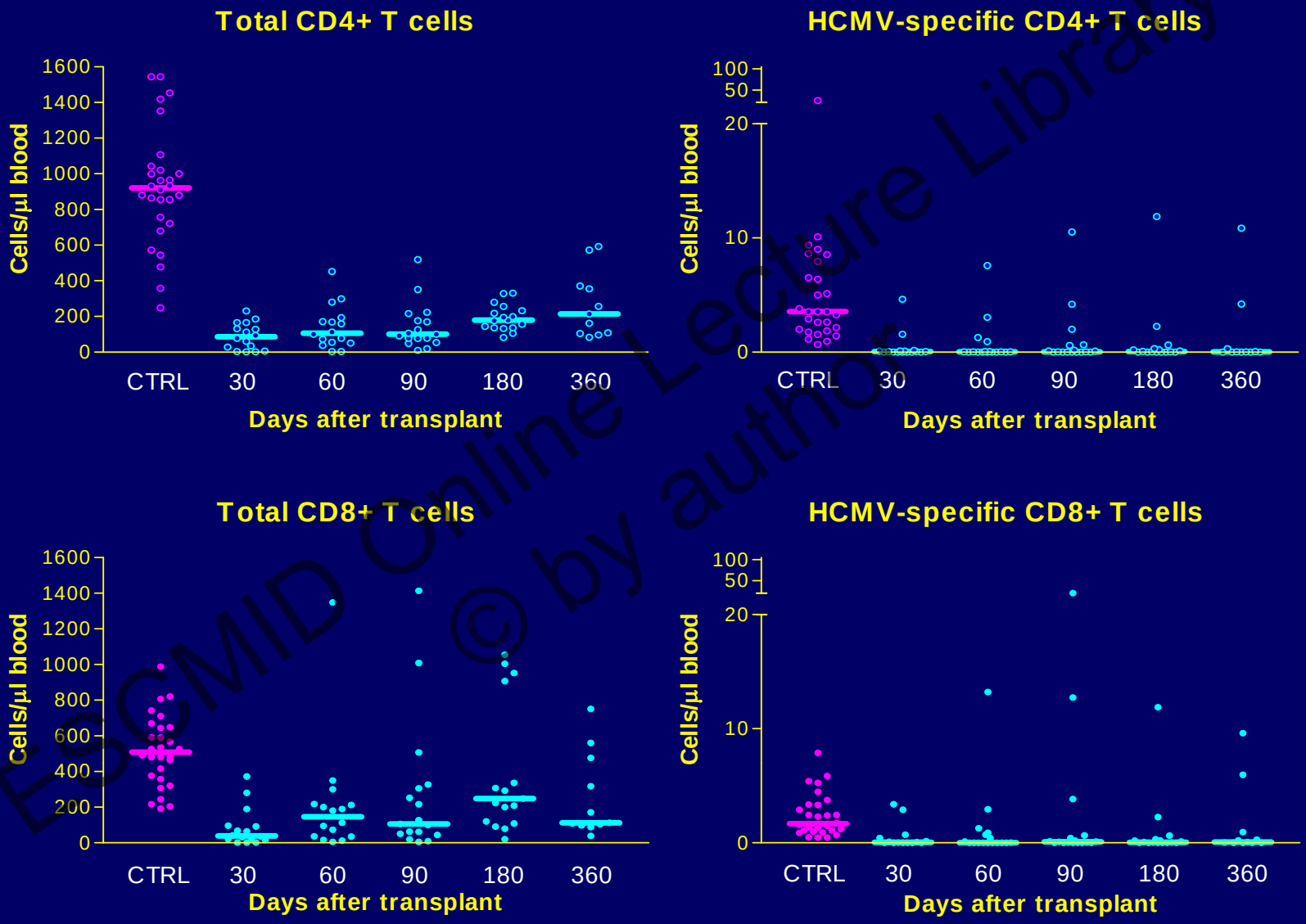
Total CD8+ T cell count reaches levels similar to controls from day +60.

HCMV-specific T cell count reaches levels higher than controls from day +60.

Thus, all seropositive recipients reconstituted HCMV-specific T-cell immunity within 6 months after transplantation

HCMV-seronegative HSCT recipients from seropositive donors (n=17):

Total and HCMV-specific CD4+ and CD8+ T cell counts



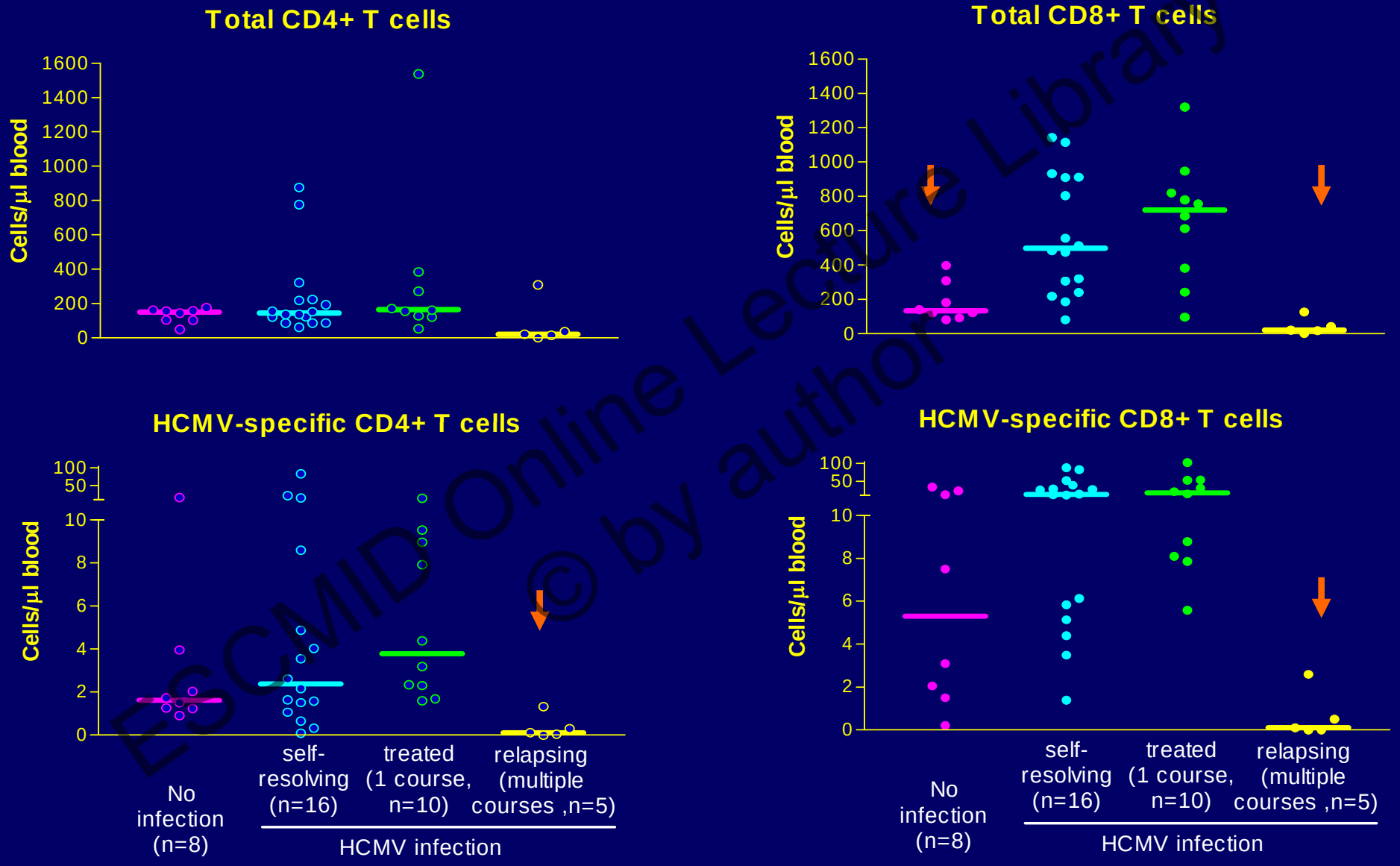
Virus-specific T cell counts are lower than those of HCMV-seropositive patients

Thus, despite all receiving graft from HCMV seropositive donors (100%), as many as 75% seronegative recipients did not show specific T-cell immune response

No significant difference in HCMV-specific T cell reconstitution during time was found according to:

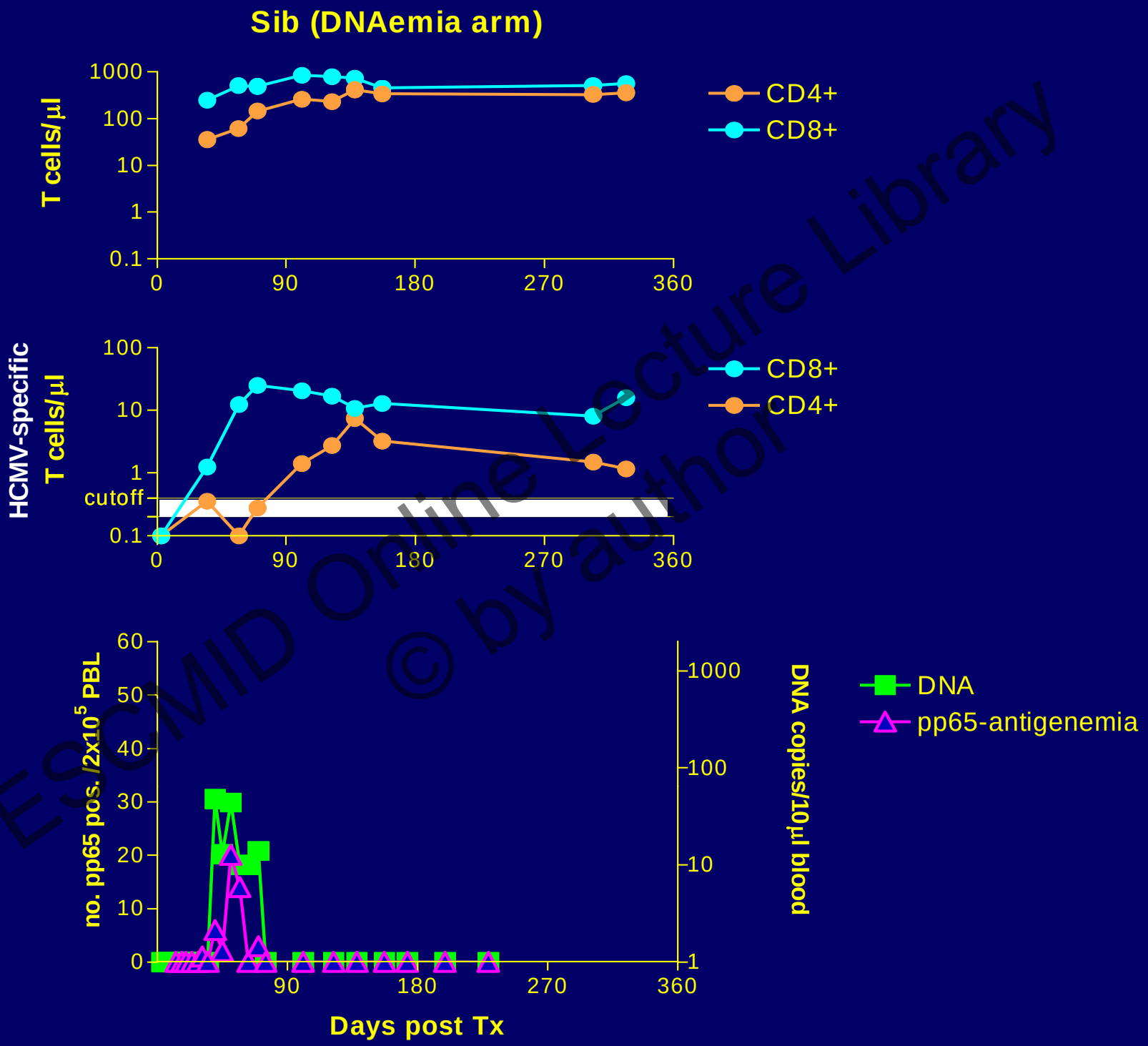
- Underlying disease (malignant/non-malignant)
- Conditioning regimen (TBI/non-TBI based)
- Donor type (sibling/unrelated/Haploidentical T cell-depleted)
- Donor HCMV serostatus

HCMV T cell immune response 60 days after transplantation and control of viral infection in 39 HCMV seropositive HSCT recipients



Single GCV course: 7 (5-16) days of treatment.
Multiple GCV courses: 49 (31-82) days of treatment.

↓ Significantly lower with respect to other groups



CONCLUSIONS

A simultaneous quantification of HCMV-specific CD4+ and CD8+ T cells in HSCT patients is achieved in a single test run

Effective HCMV-specific T cell immunity can promptly develop after HSCT (regardless of donor type, pre-existing immunity or T-cell depletion)

In seropositive recipients latent virus may be the major antigenic drive for rapid reconstitution of T cell compartment

Transfer of virus from seropositive donors to seronegative recipient and/or virus-specific memory T-cell expansion seem to be a rare event

Future studies will be conducted to correlate antiviral intervention to reconstitution of HCMV-specific T cell immune response