

Basic aspects of cytomegalovirus subversion and immune control

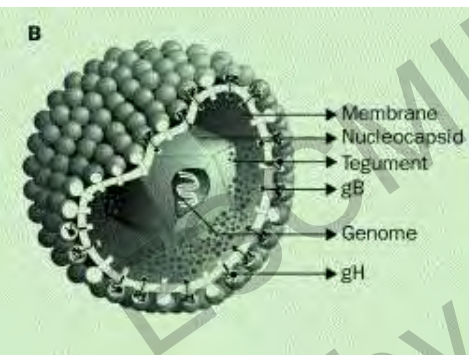
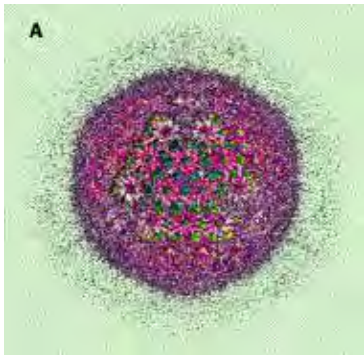
Ineke J.M. ten Berge
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outline



- Immune response against CMV
- CD8⁺ effector T cell compartment
 - Maintenance of latency
 - Immunopathology
- CD8⁺ microarray data
- Peripheral Blood versus Lymph Node compartment
- Clonal Heterogeneity of CMV specific T cells

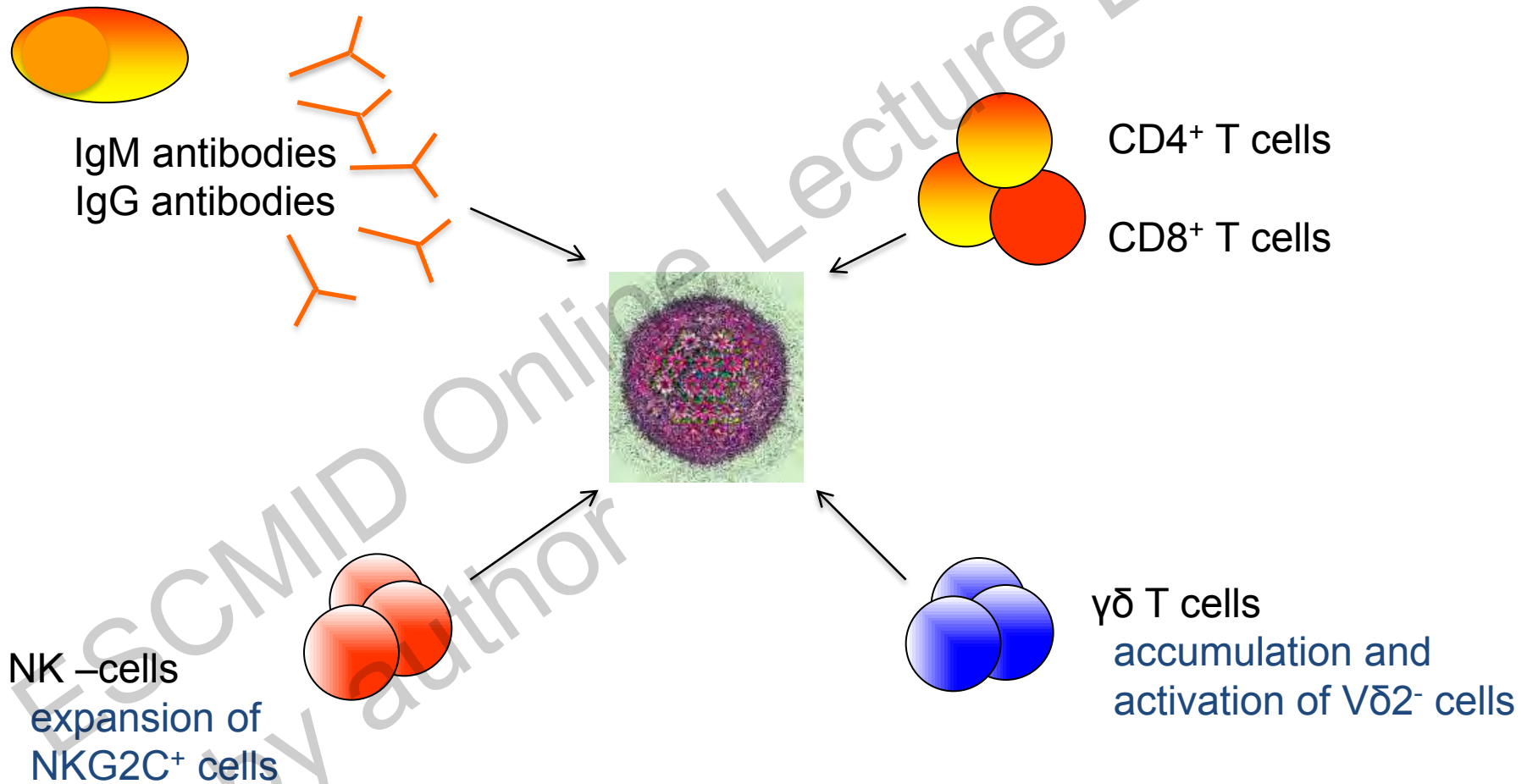
Human Cytomegalovirus (CMV)



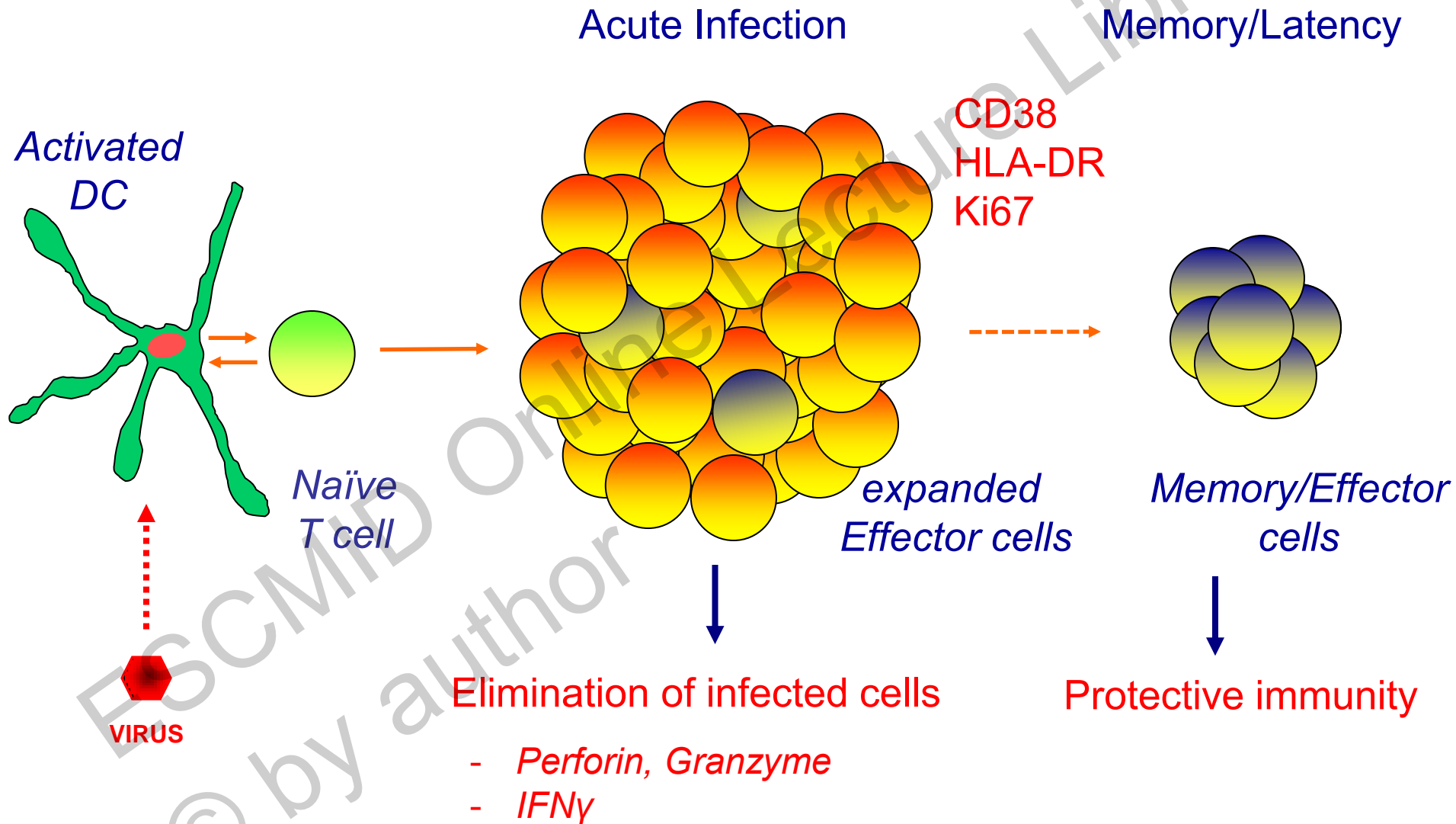
IE genes
late genes → pp65

- persistent β -herpesvirus
- affects approximately 75% of healthy individuals
- main tropism: white blood cells, endothelial cells
- transmission by body fluids
- in healthy individuals often asymptomatic
- from species origination on CMV has been within the human population (mutual adaptation)

Immune response against CMV

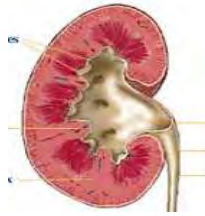


Activation of T cells by viruses



Immune response against CMV infection in recipients of kidney allografts

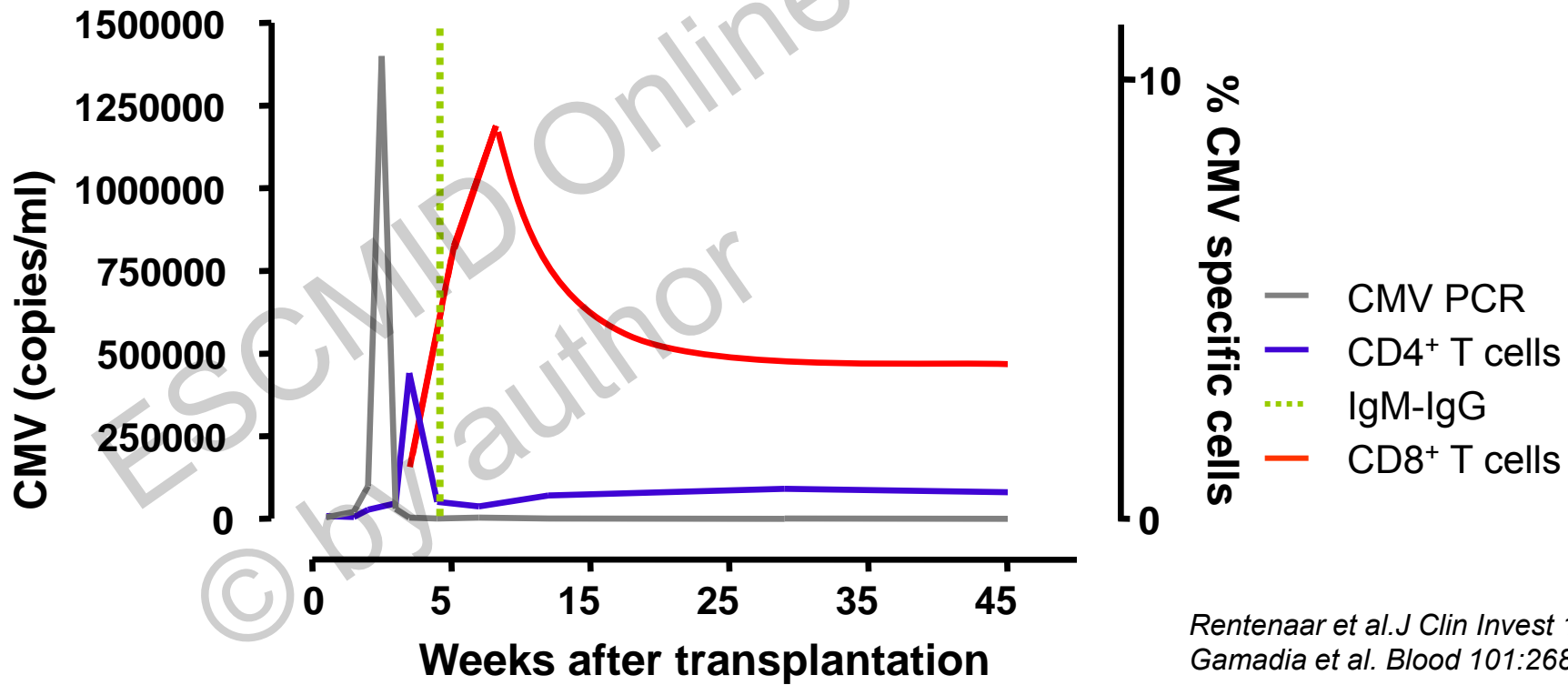
CMV+



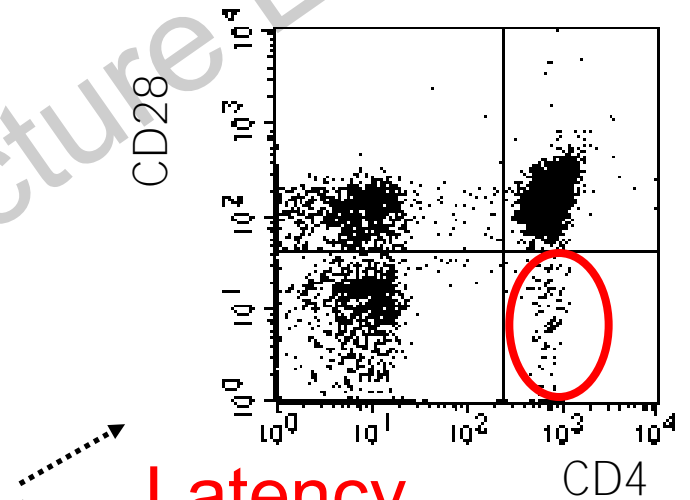
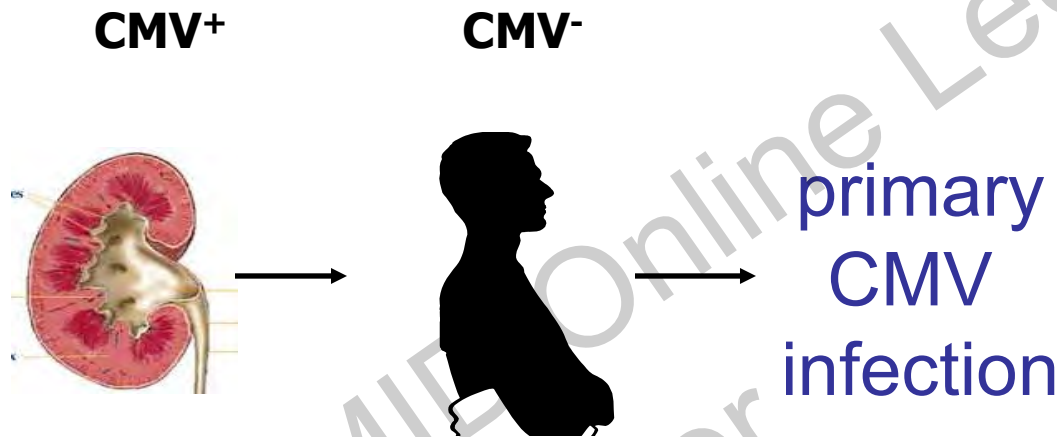
CMV-



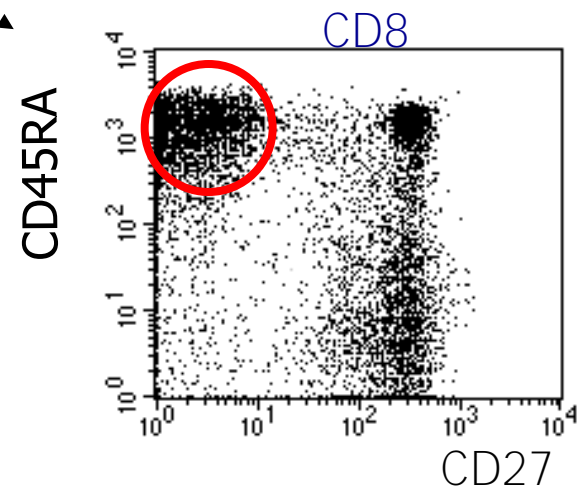
Primary
CMV
infection



CMV infection leaves a fingerprint in the CD4⁺ and CD8⁺ T cell subset

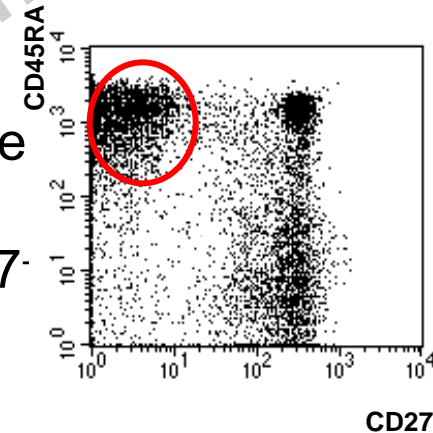


Latency



CD8⁺CD45RA⁺CD27⁻ T cells properties

- Resting T cells with low proliferation and low death rate
- Characteristic phenotype: CD45RA⁺CD27⁻CD28⁻CCR7⁻
- Inducible expression of IFN γ and TNF α
- Constitutive expression of perforin, granzymes A and B; direct cytolytic activity: effector-type cells
- Population increases with age and in situations of immunosuppression
- **These cells maintain latency to immune-evasive CMV**

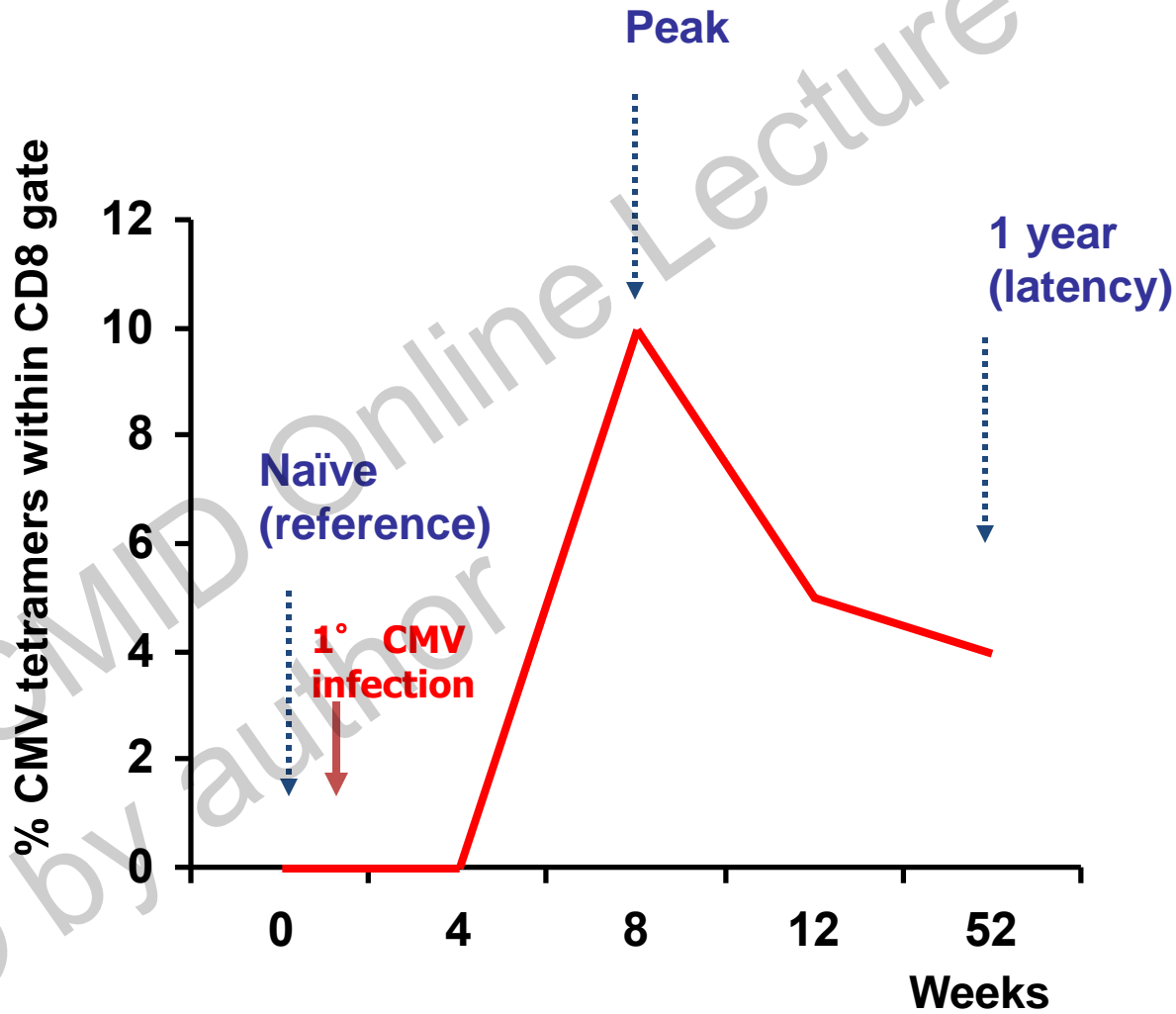


Gamadia, Blood 98:754, 2001

van Leeuwen, J Immunol 169:5838, 2002

Gamadia et al., Blood 101:2686, 2003

Gene expression of CMV specific CD8⁺ T cells microarray analyses

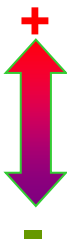


Genes regulated in CMV specific CD8⁺ T cells during acute and latent infection

**Activation
Molecules**

**Differentiation
markers**

Fold change	CD38	Ki67	CD127	CD28	CD27
naïve	1.0	1.0	1.0	1.0	1.0
peak	37.0	23.1	-36.3	-2.8	2.2
1 year post inf	3.0	2.2	-7.9	-14.1	-1.6



Genes regulated in CMV specific CD8⁺ T cells during acute and latent infection

Activation Molecules

Differentiation markers

Effector Molecules

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Perf	GrB	IFN γ
1.0	1.0	1.0
10.3	51.2	38.8
12.6	49.4	42.4



Key features of CMV specific CD8⁺ T cells are installed during the primary response

Activation Molecules

Differentiation markers

Chemokine Receptors

Effector Molecules

Fold change	CD38	Ki67	CD127	CD28	CD27	CCR7	CX3CR1	Perf	GrB	IFN γ
naïve	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
peak	37.0	23.1	-36.3	-2.8	2.2	-29.1	83.5	10.3	51.2	38.8
1 year post inf	3.0	2.2	-7.9	-14.1	-1.6	-26.6	94.4	12.6	49.4	42.4



Genes regulated in CMV specific CD8⁺ T cells during acute and latent infection

Strong upregulation of CX3CR1

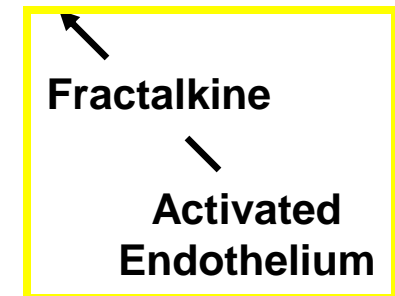
Activation Molecules

Differentiation markers

Chemokine Receptors

Effector Molecules

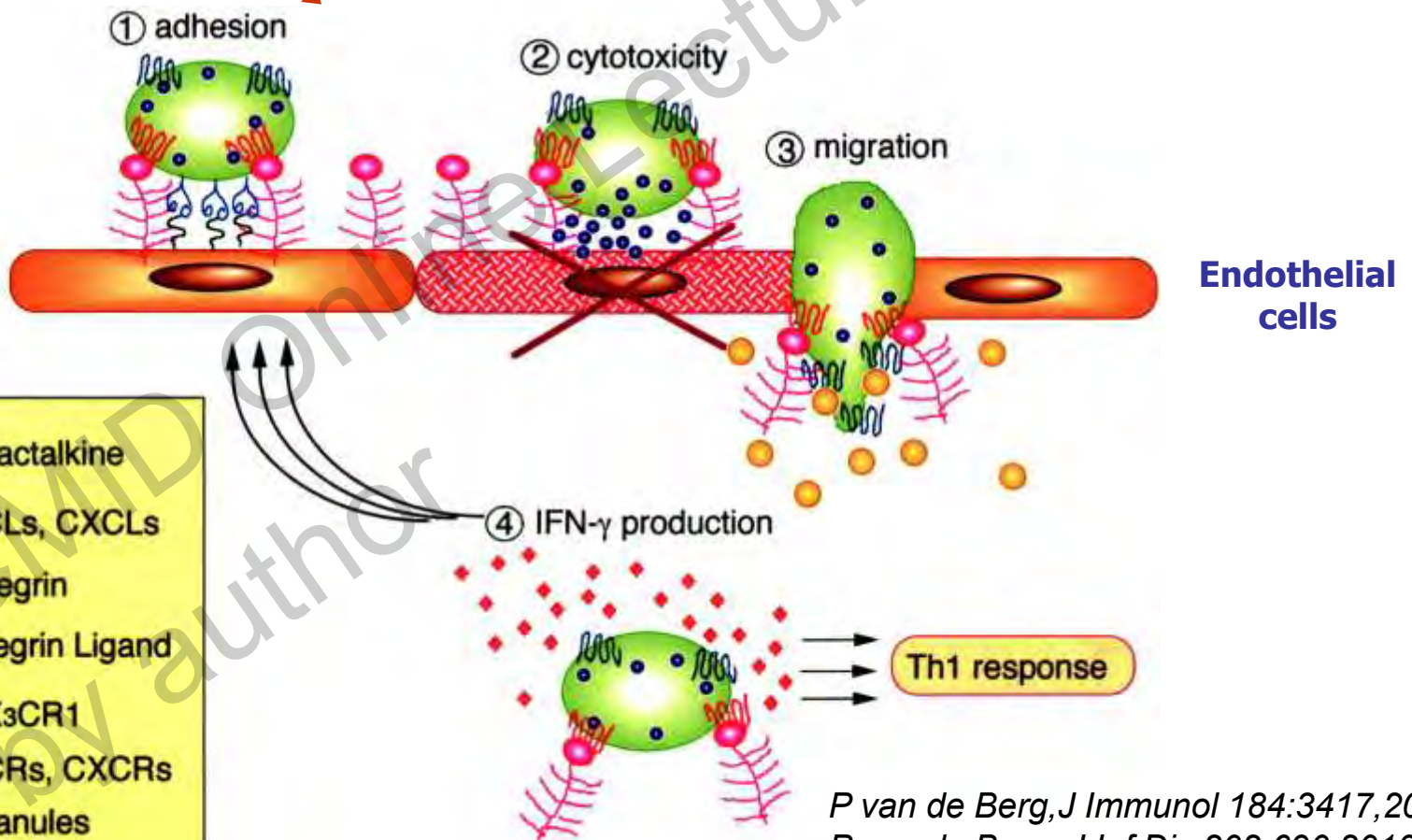
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1 year post inf	3.0	2.2	-7.9	-14.1	-1.6	-26.6	94.4	12.6	49.4	42.4











CMV reactive T cells and vascular damage

CMV infection → ↑↑ nr of CD8⁺CX3CR1⁺Teff cells

↓
Th1 response
↓
IFN γ , TNF α
↓
→



-  Fractalkine
-  CCLs, CXCLs
-  Integrin
-  Integrin Ligand
-  CX3CR1
-  CCRs, CXCRs
-  Granules
-  IFN- γ

Umehara, *Art Thromb Vasc Biol* 2004

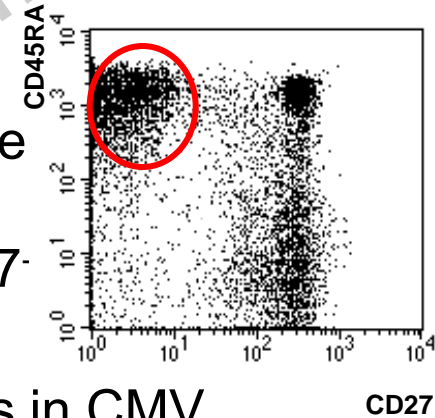
P van de Berg, J Immunol 184:3417, 2010

P van de Berg, J Inf Dis 202,690, 2010

P van de Berg Clin Vacc Imm 2012

CD8⁺CD45RA⁺CD27⁻ T cells properties

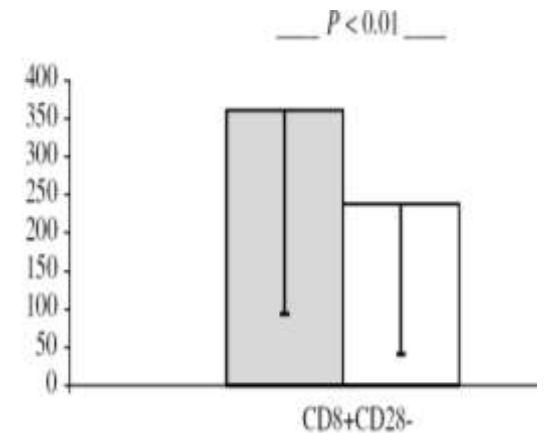
- Resting T cells with low proliferation and low death rate
- Characteristic phenotype: CD45RA⁺CD27⁻CD28⁻CCR7⁻
- Gene transcription governing a number of key features in CMV specific CD8⁺ T cells is already installed during the primary response and is stable over time
- Population increases with age and in situations of immunosuppression
- **These cells maintain latency to immune-evasive CMV**



CD8⁺CD27⁻ T cells: a double edged sword

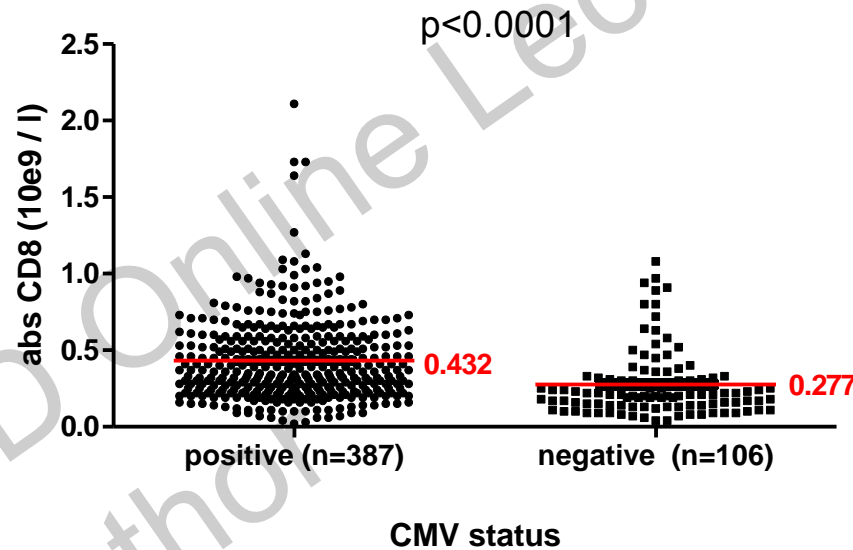
These CD8⁺CD28⁻CD27⁻ CMV specific cytotoxic effector T cells are also associated with immunopathology

- This same population is increased in atherosclerotic vascular disease
- An active role of these effector T cells in the progression of atherosclerotic vascular disease is very likely
- A high amount of CD8⁺CD28⁻CD27⁻ cytotoxic effector T cells was found to be predictive for mortality in the elderly (“immunosenescence”)

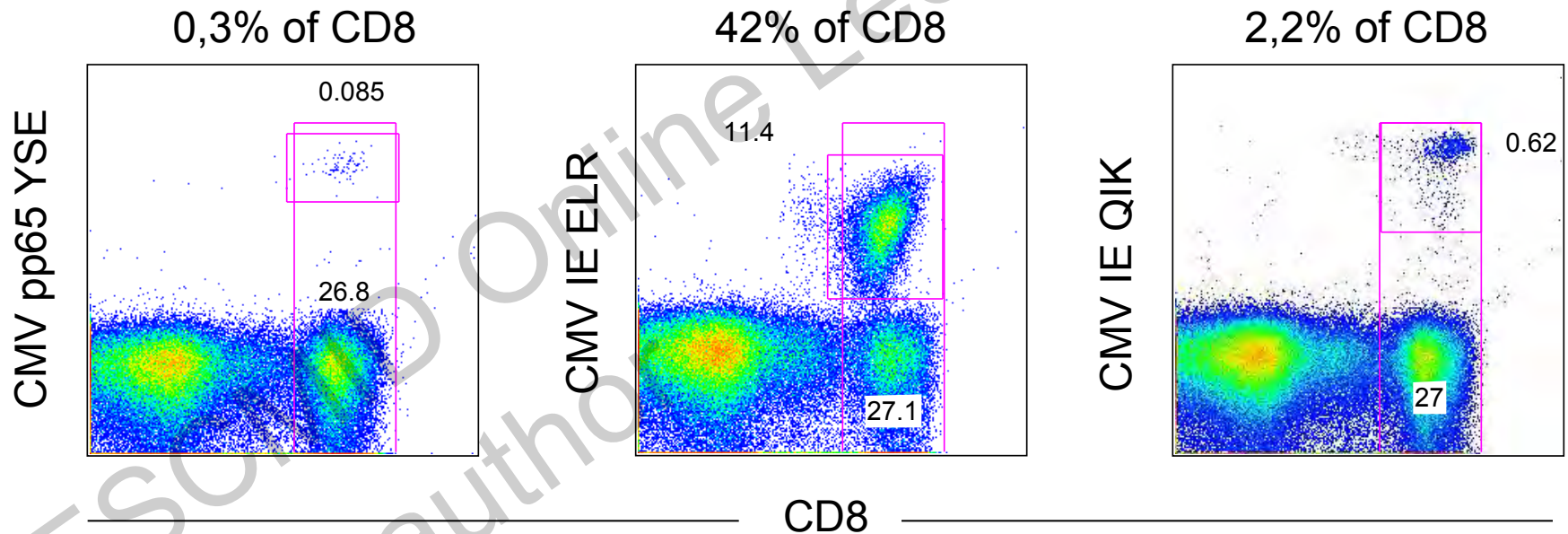


J Intern Med
254:472, 2003

CMV seropositive individuals have more circulating total CD8+ T cells

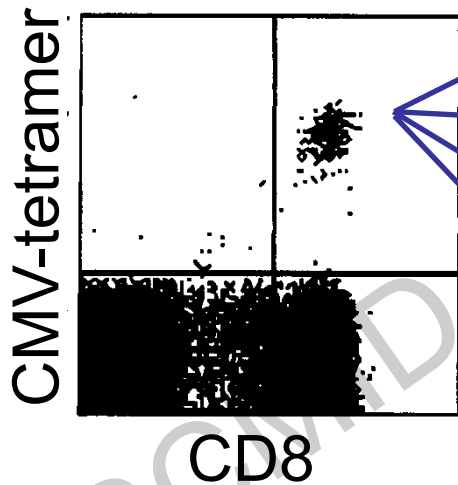


High percentage of circulating CD8+ T cells can be directed against just one CMV epitope



CMV specific CD8⁺ T cell repertoire

Questions



- I. Is the circulating pool related to lymph node populations ?
- II. Does CMV infection limits immunological space?
- III. What about clonal diversity ?
- IV. What about (possible) clonal evolution in time ?

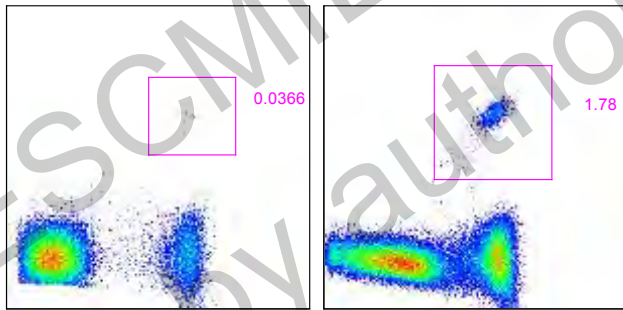
Materials & Methods



Pre-transplantation

Isolation of CMV-tetramer-positive CD8 T cells

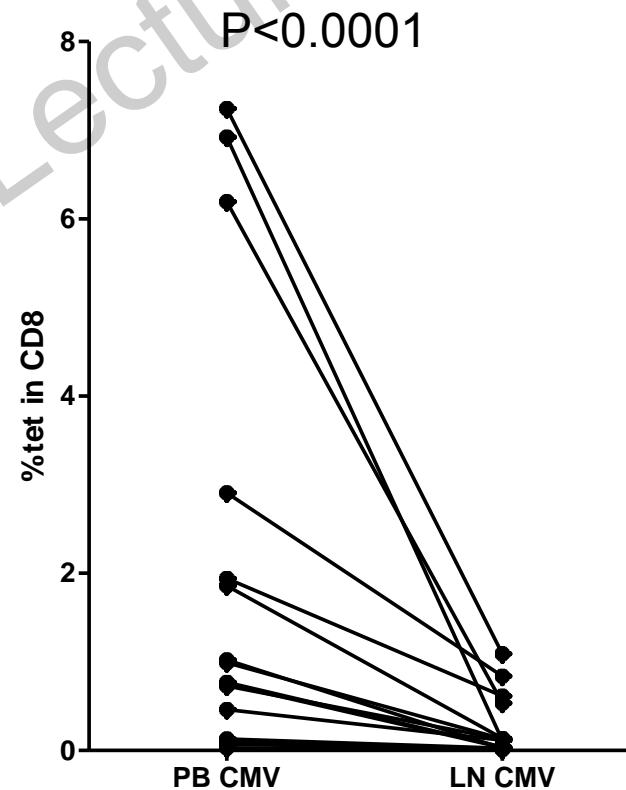
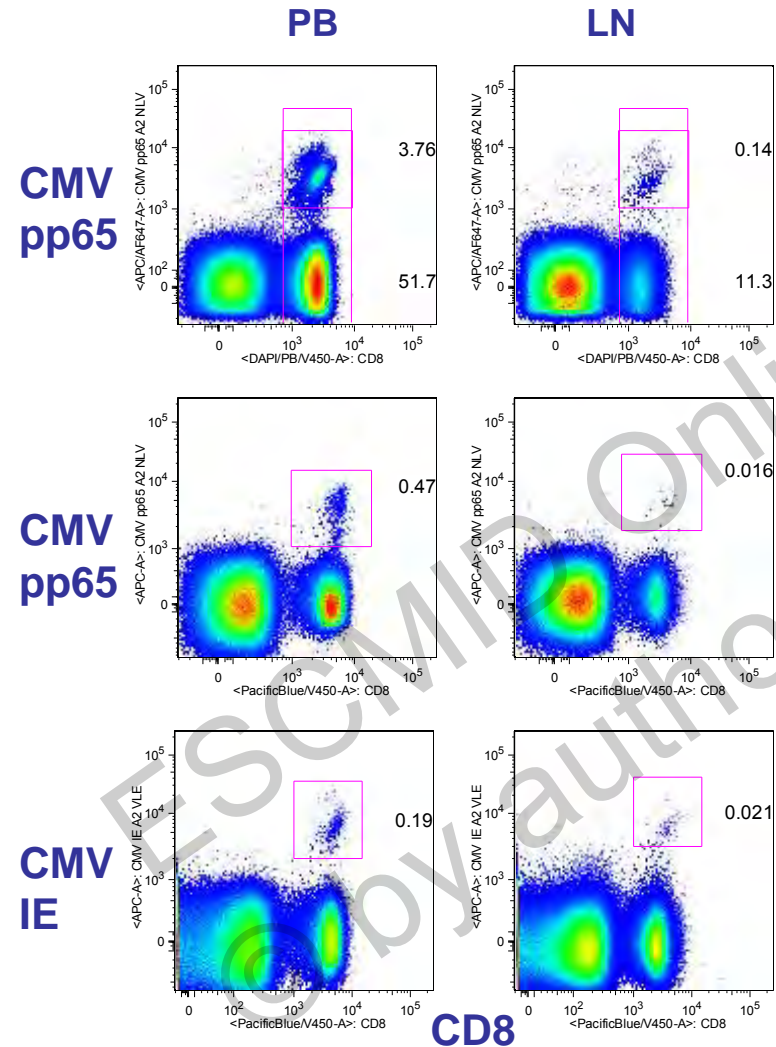
CMV-tetramer



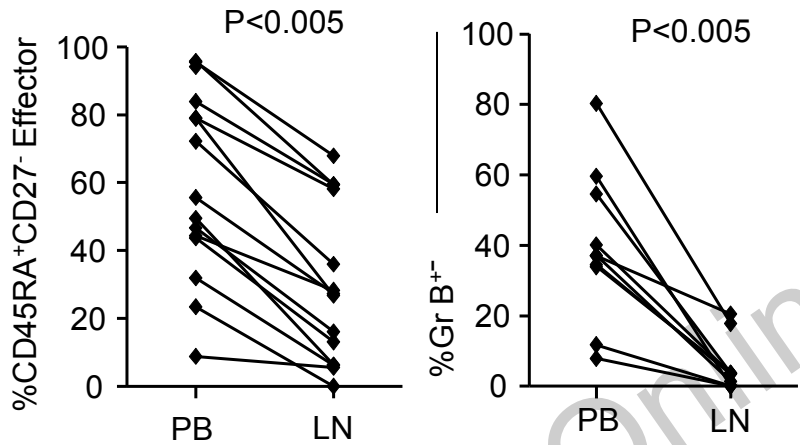
LN

PB

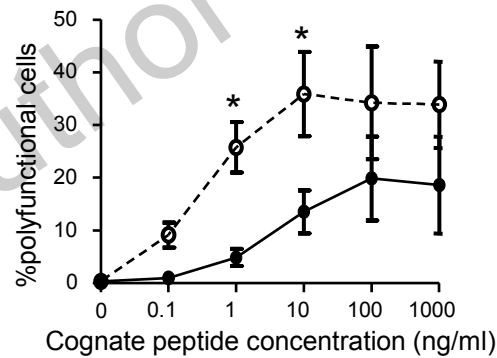
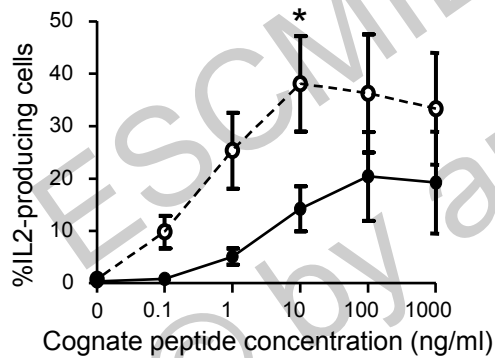
CMV specific CD8 T cells are less dominant in lymph nodes than in peripheral blood



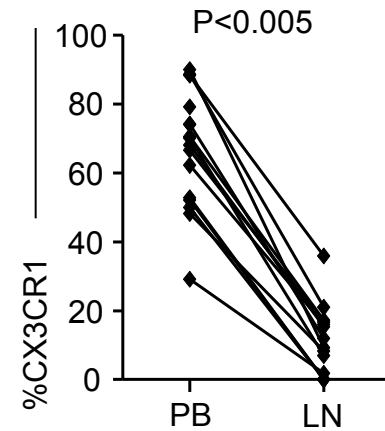
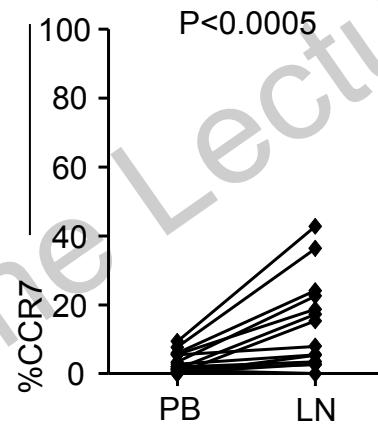
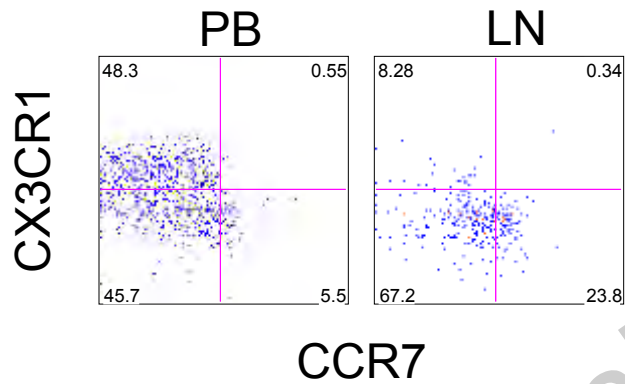
CMV specific LN cells resemble memory cells



- PB CMV-specific CD8 T cells
- LN CMV-specific CD8 T cells



CMV specific LN cells have a unique homing potential



CCR7 → lymph nodes / spleen (CCL19 and 21)

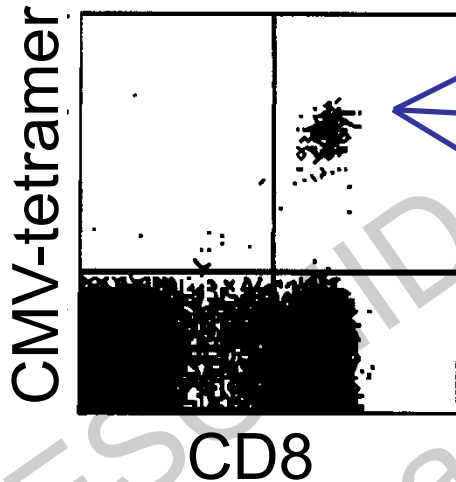
CX3CR1 → stressed endothelium / inflamed tissue (Fractalkine)

Conclusions

- The frequency of CMV-specific T cells in LN is relatively low, which may be due to expression of specific chemokine receptors (CX3CR1), that direct these cells to (stressed) endothelium
- The differentiation status of CD8⁺ CMV-specific T cells is different between circulation and lymph nodes (LN)
- CMV-specific T-cells do not limit immunological space in LN

CMV specific CD8⁺ T cell repertoire

Questions

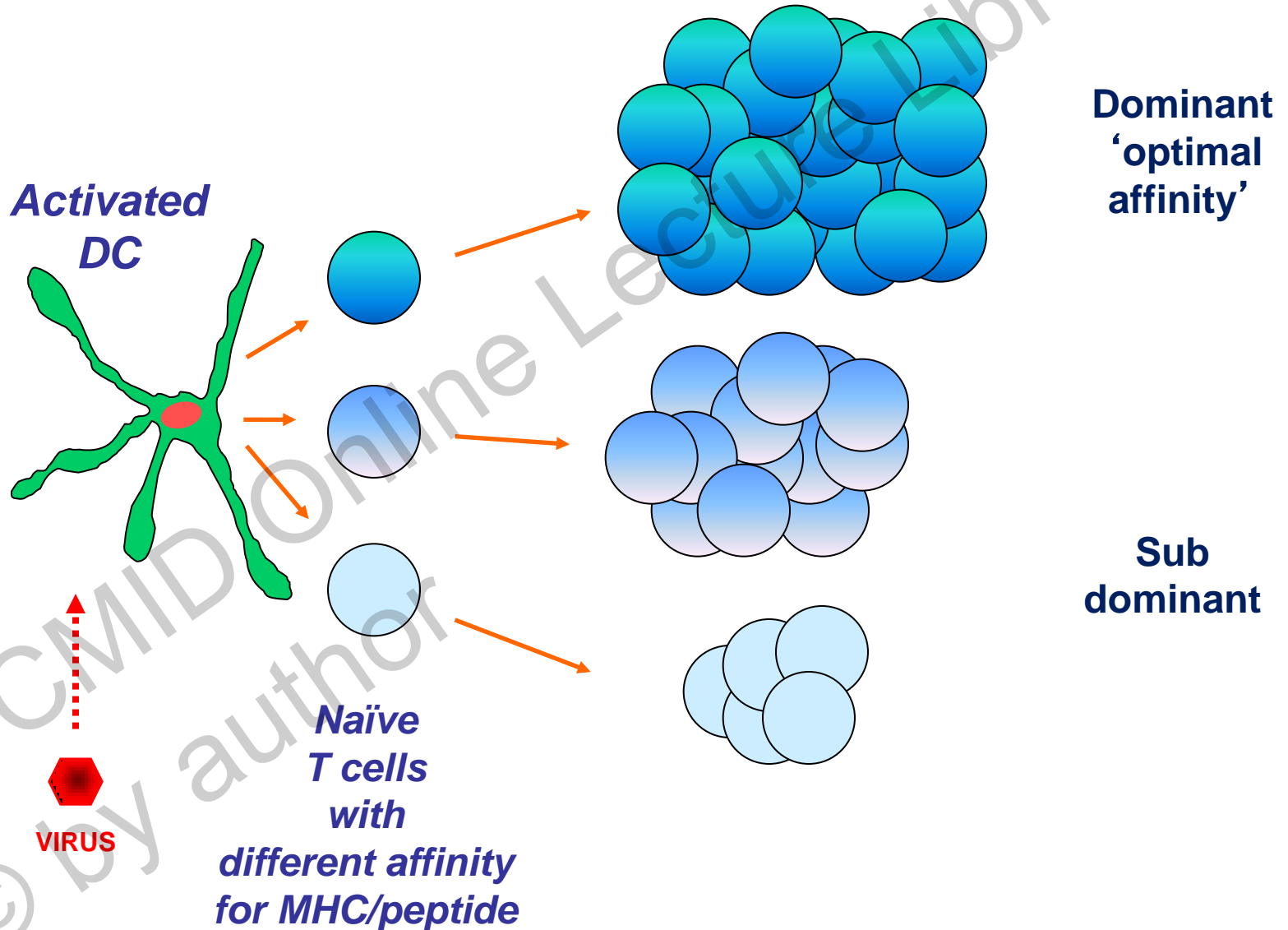


I. Is the circulating pool related to lymph node populations ?

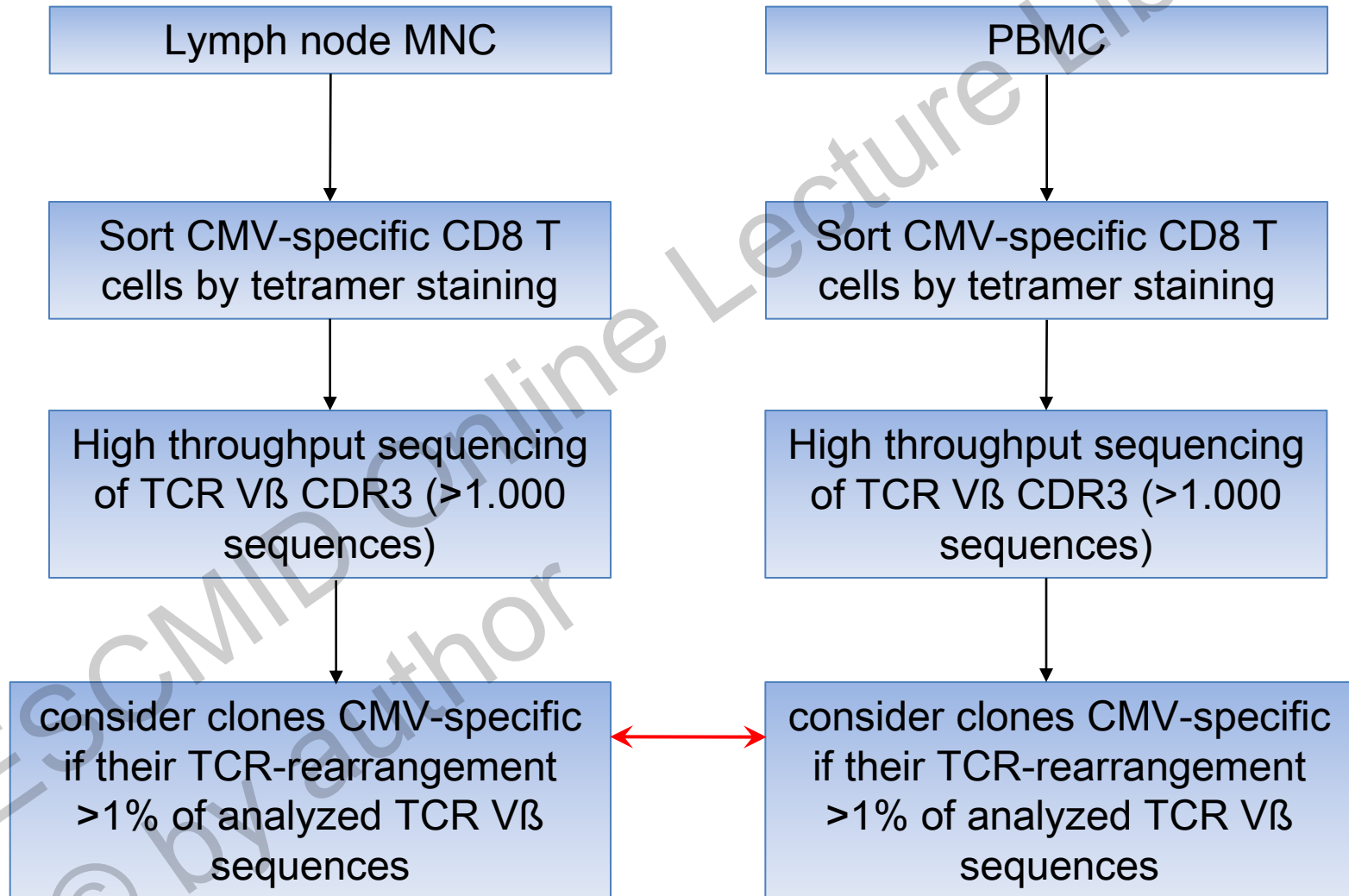
II. What about clonal diversity ?

III. What about (possible) clonal evolution in time ?

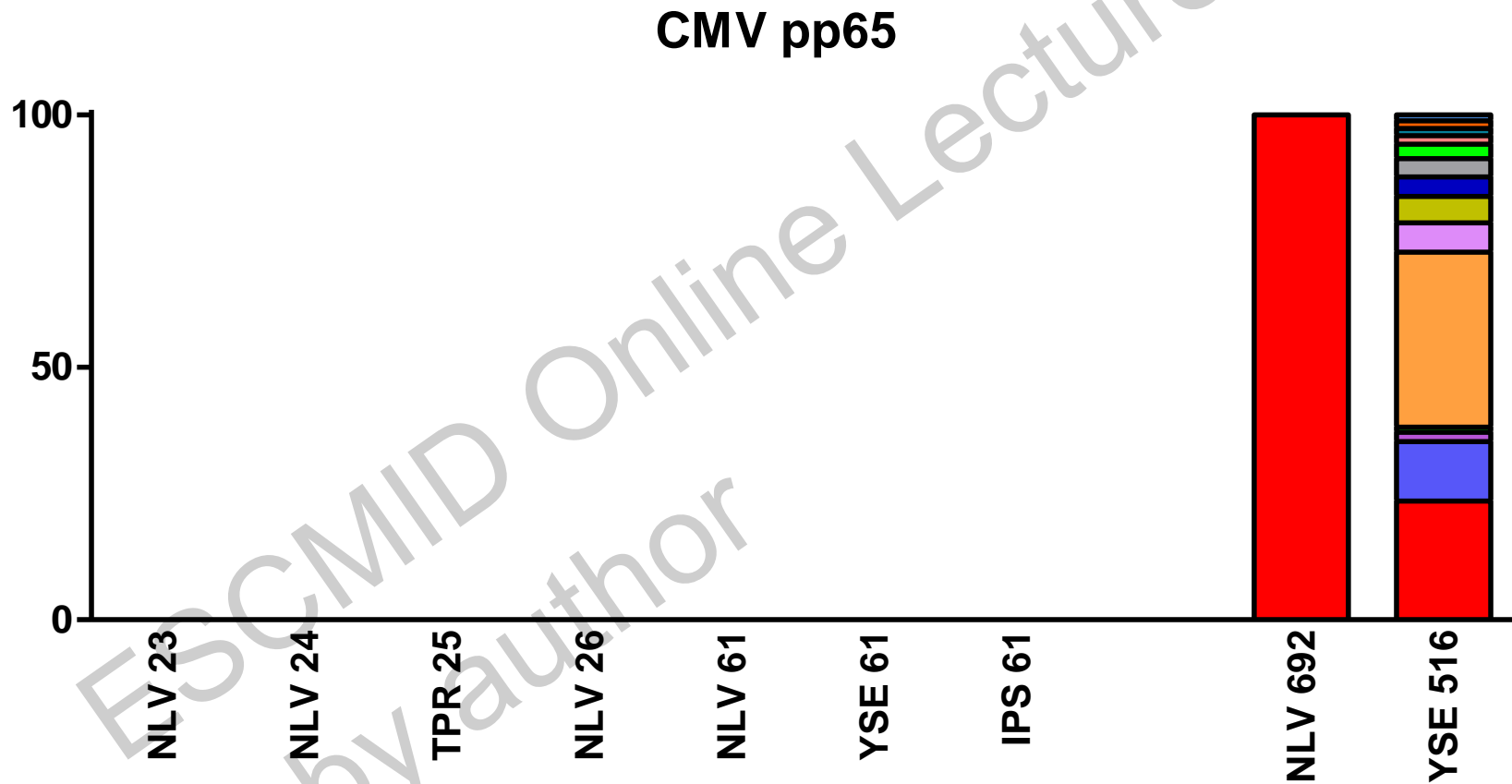
Activation of naive CD8⁺ T cells by MHC/peptide results in multiple activated clones of different affinity



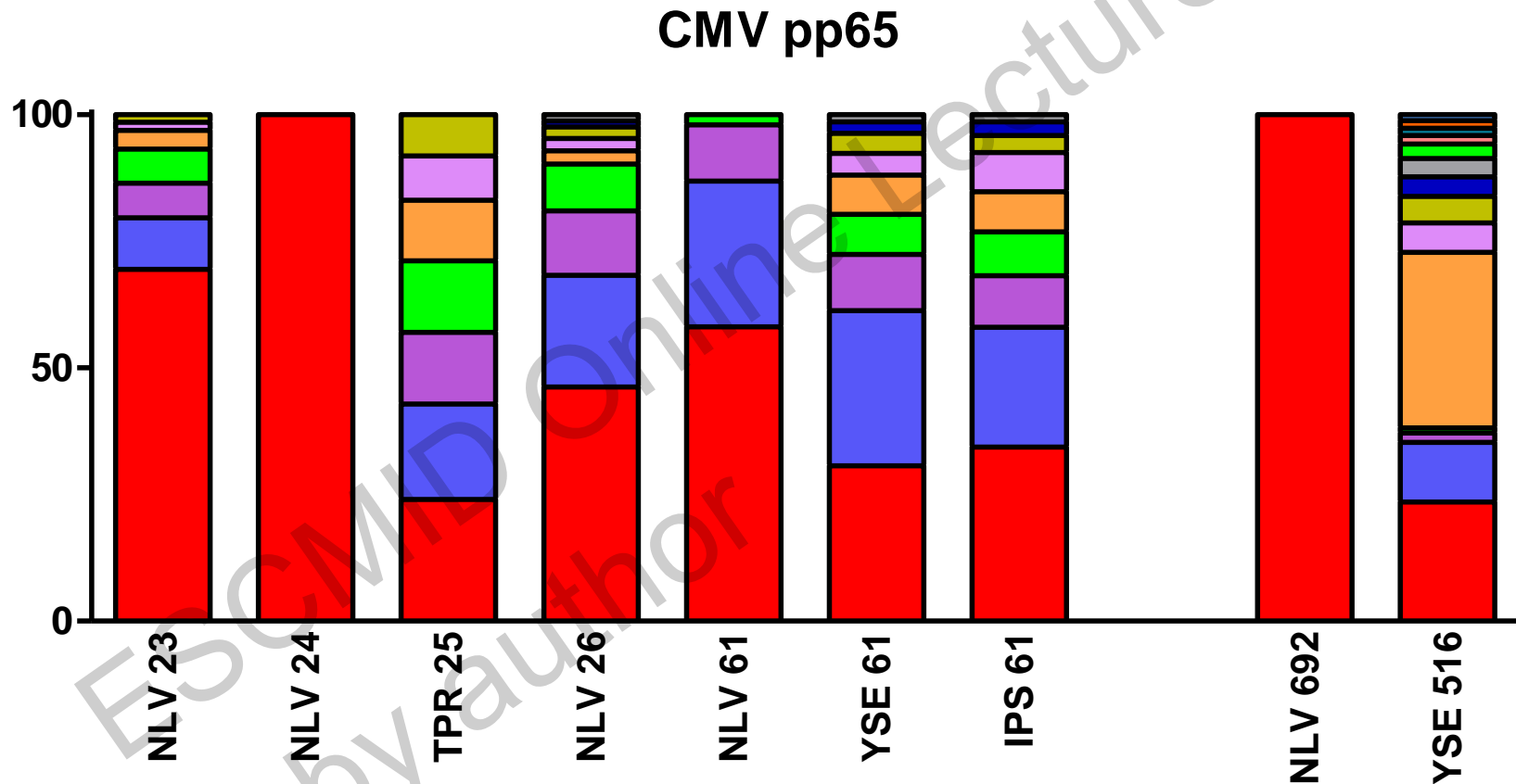
Identification and quantification of CMV specific clones within the CD8⁺ T cell compartment



Diversity of PB CMV specific T cell clones in renal Tx recipients (latency)



Diversity of PB CMV specific T cell clones in healthy CMV-carriers and in renal Tx recipients



Mean = 7 clones / tetramer⁺ T cell population

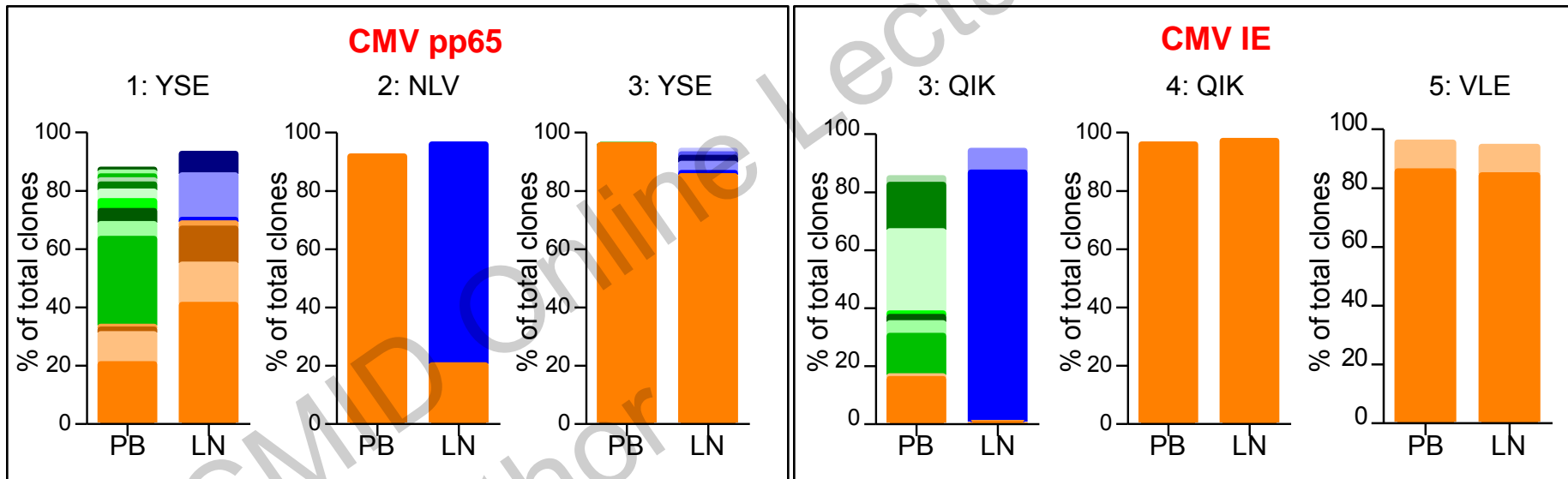
Both peripheral blood and lymph nodes may contain unique CMV specific CD8 clones

Pre-Tx

overlapping PB and LN clones

unique PB clones

unique LN clones

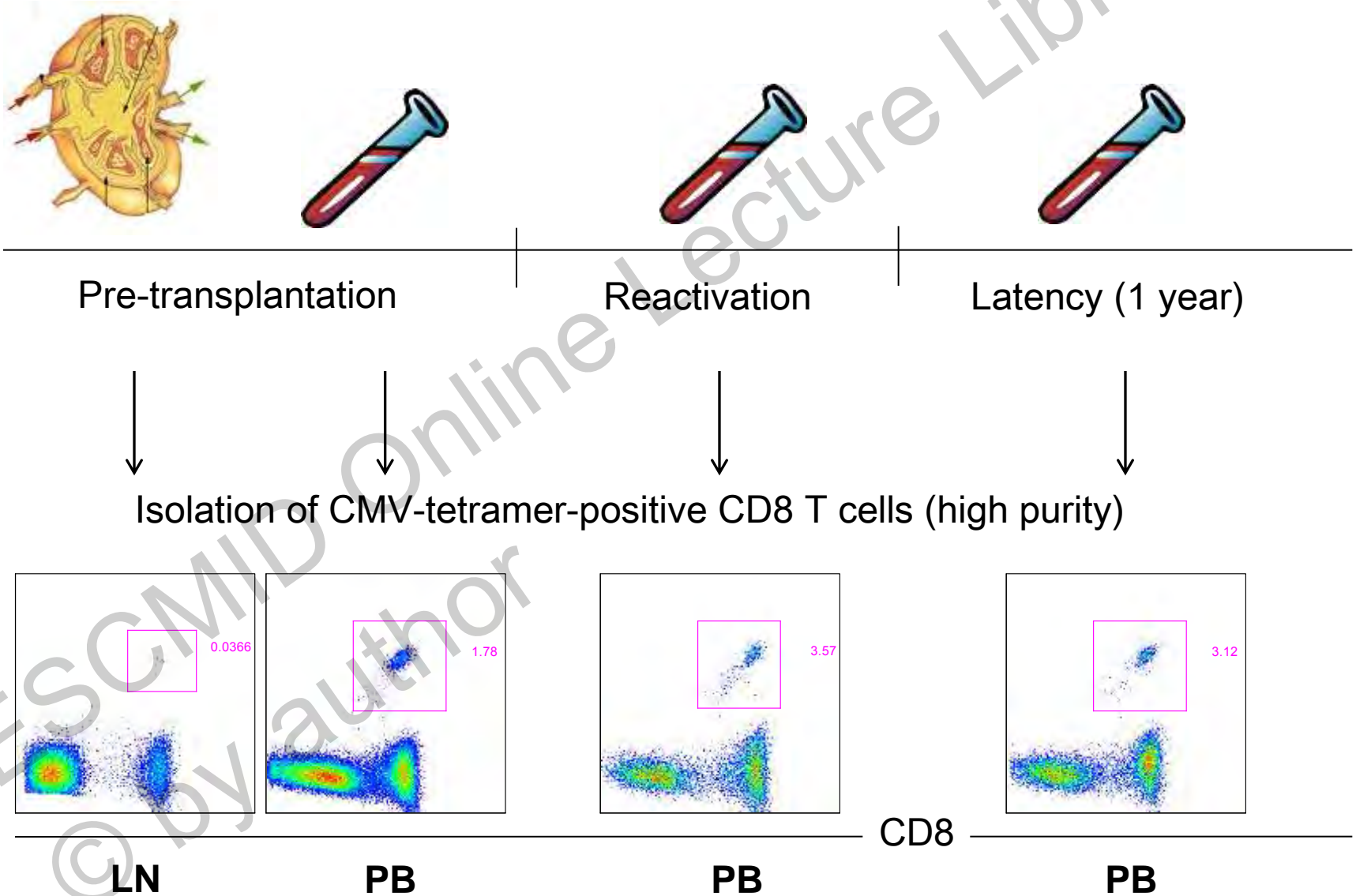


PB/LN derived clones

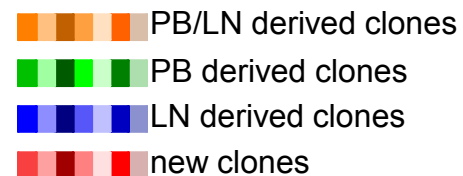
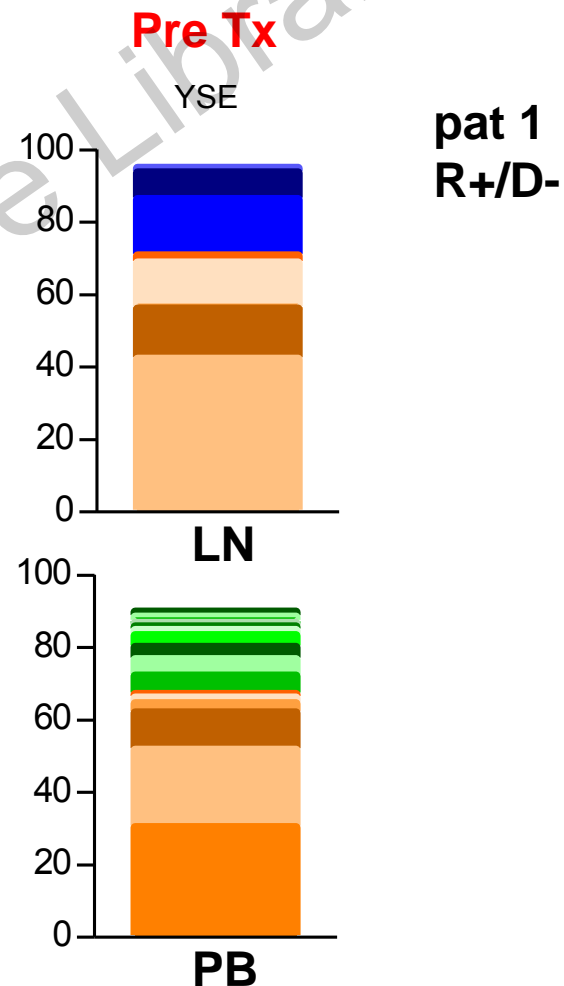
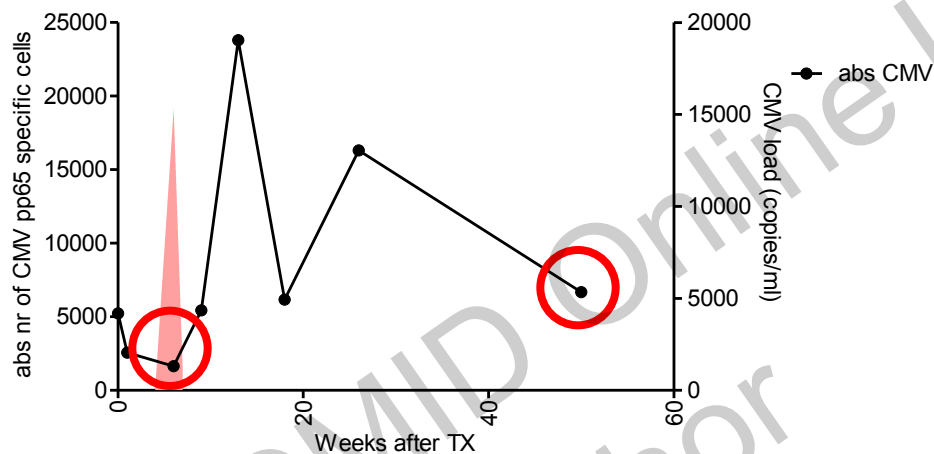
PB derived clones

LN derived clones

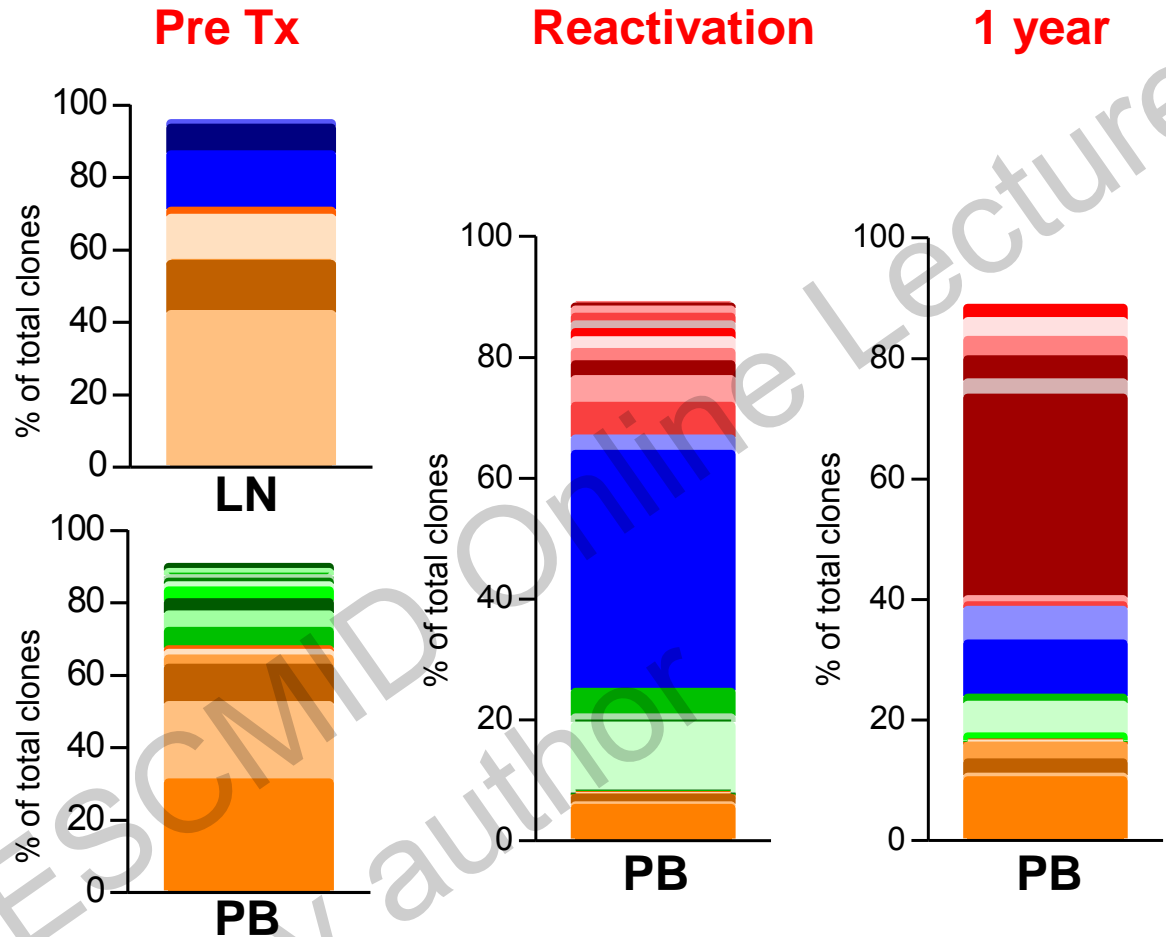
Are lymph node CMV-specific cells a source for CMV effector cells during reactivation?



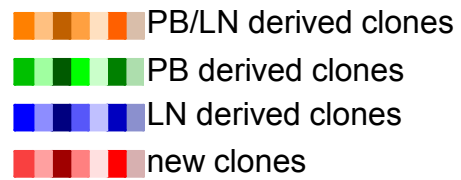
Longitudinal analysis of CMV-pp65 specific cells during endogenous reactivation



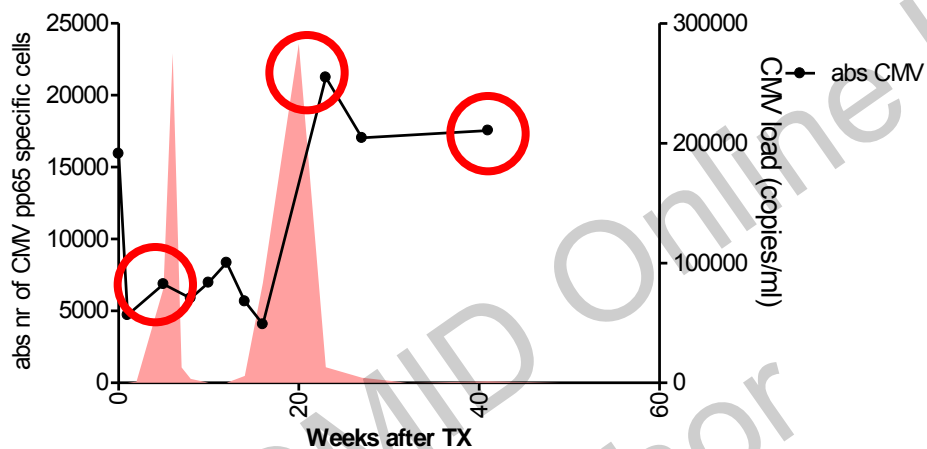
Some CMV-pp65 specific CD8 T cells are recruited from LN to PB



pat 1
R+/D-



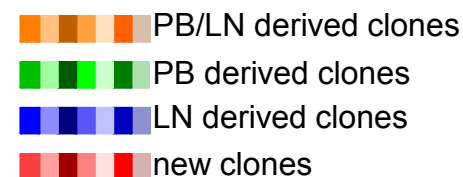
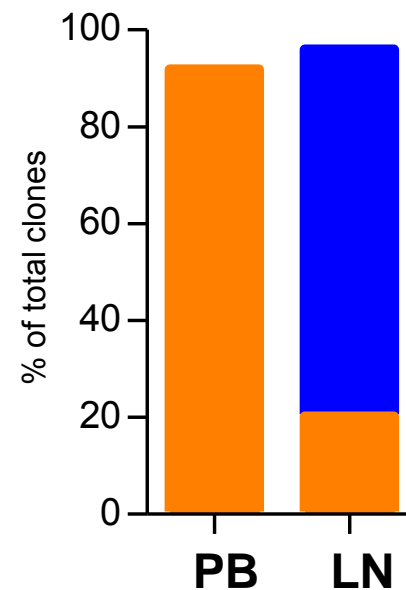
Longitudinal analysis of CMV-pp65 specific cells during reactivation / reinfection



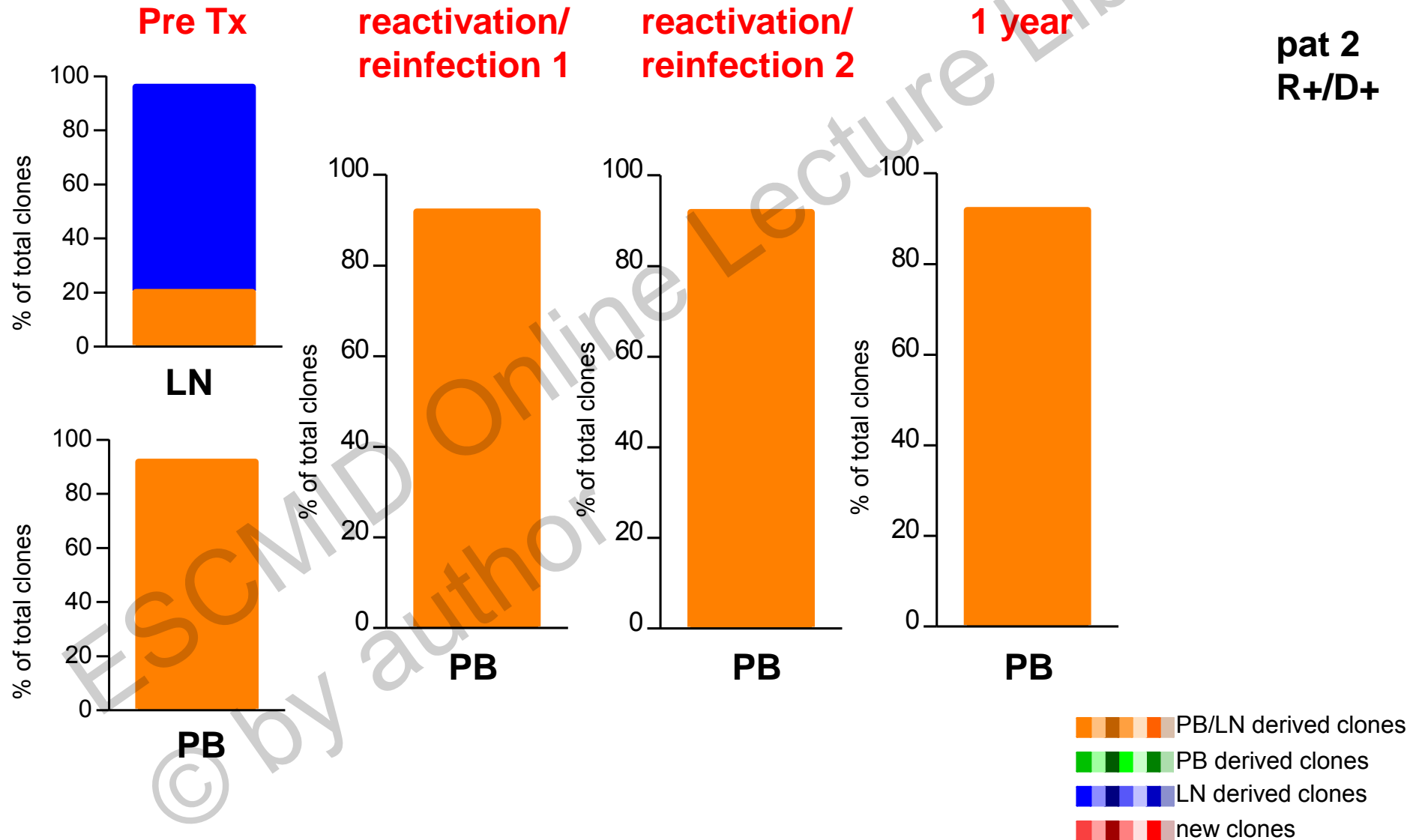
pat 2
R+/D+

Pre Tx

NLV



Unique LN CMV-pp65 specific CD8 T cell clone is not recruited to PB

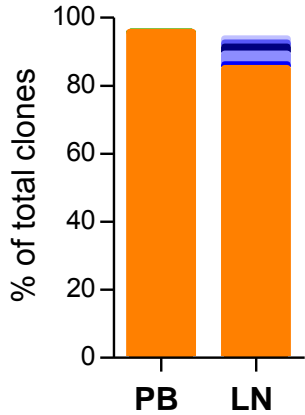


Longitudinal analysis of CMV-pp65 and -IE specific cells during reactivation / reinfection

pat 3
R+/D+

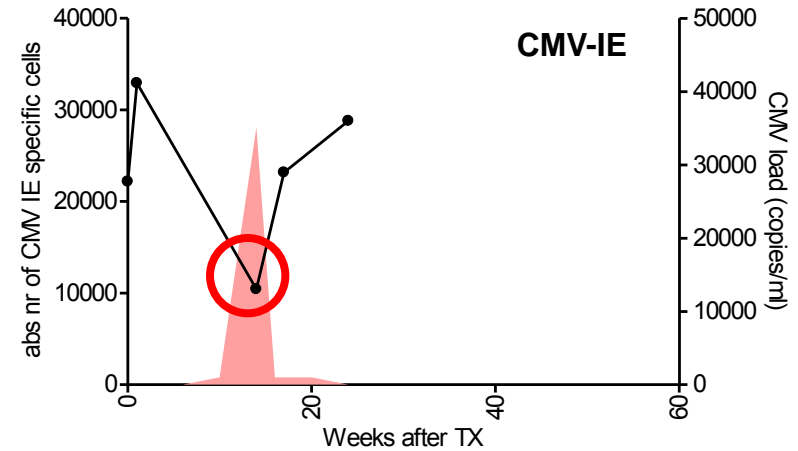
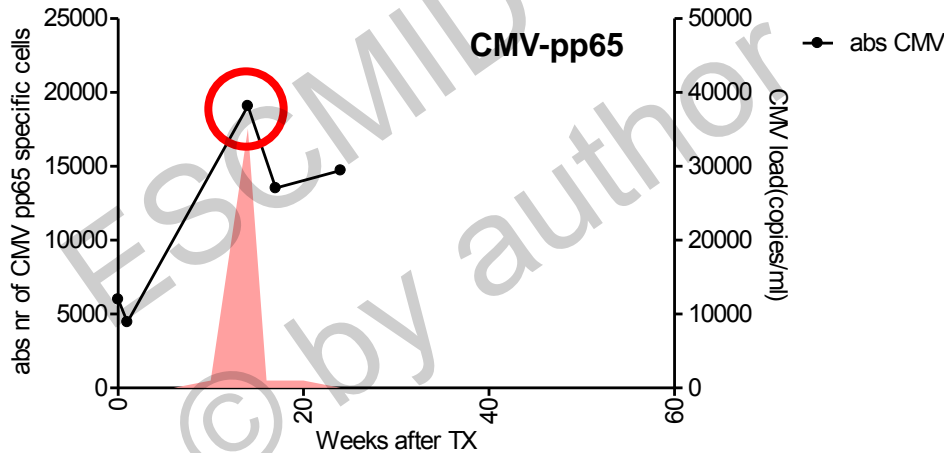
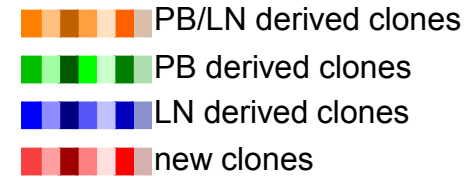
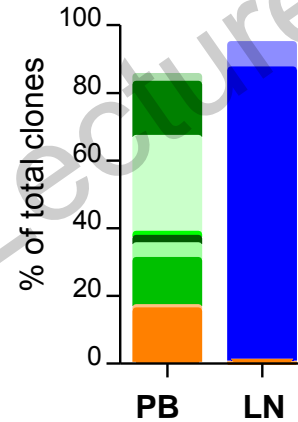
Pre Tx

YSE



Pre Tx

QIK



Unique LN CMV-specific CD8 T cell clones are not recruited to PB

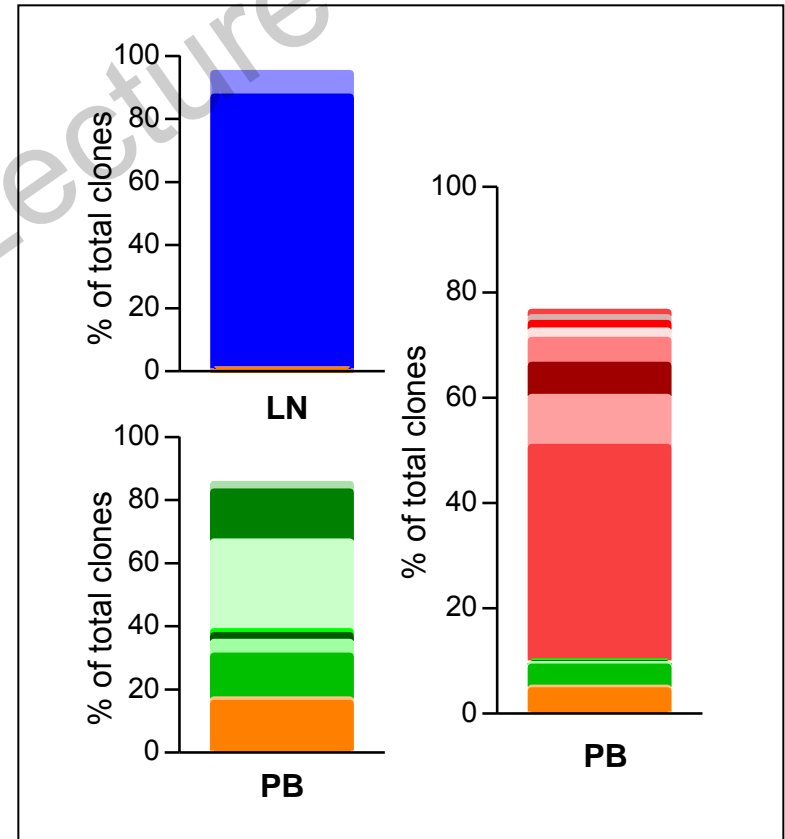
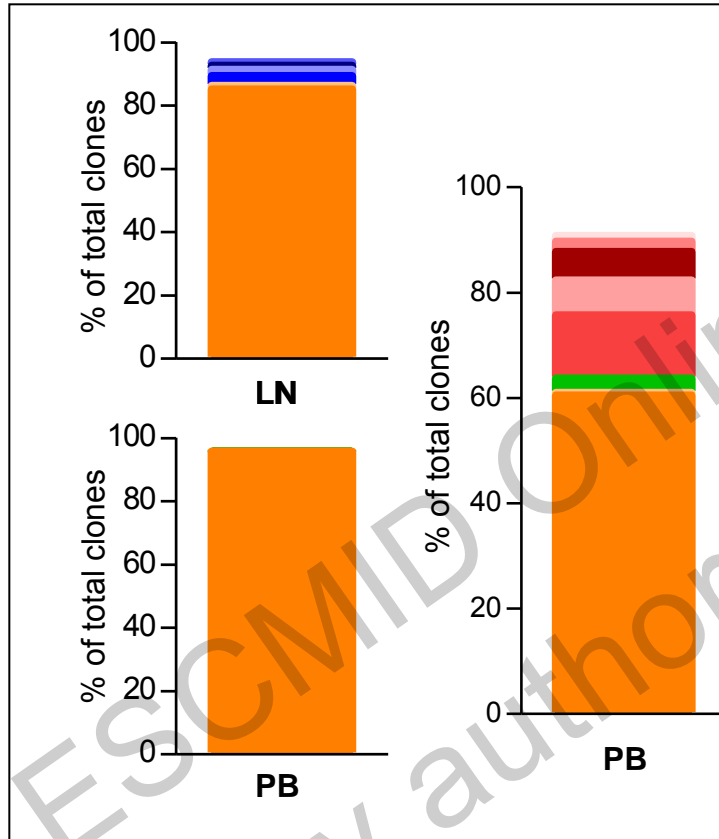
pat 3
R+/D+

Pre Tx

reactivation/
reinfection

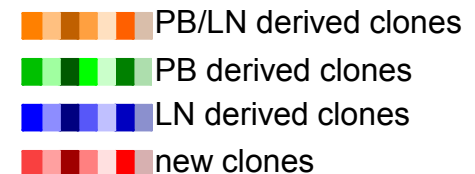
Pre Tx

reactivation/
reinfection



CMV-pp65, YSE

CMV-IE, QIK



Summary

- High Throughput Sequencing shows that the clonal distribution of tetramer-binding, CMV specific CD8⁺ T cells is highly diverse.
- CMV (pp-65 and IE) specific CD8 T cells in lymph nodes may be different from those in peripheral blood.
- Recruitment of LN derived CMV-specific cells towards the PB compartment upon reactivation appears to be a rare event.

overall conclusions

- CMV infection leaves a fingerprint in the T cell compartment
 - lifelong protection against of the virus
 - Immunopathology
- Changes in the transcriptomes of CMV specific T cells
- CMV specific T cells do not limit immunological space in lymph nodes
- Differences in phenotype and clonal distribution of CMV specific T cells between peripheral blood and lymph nodes

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