What is the most efficient immunotherapy to Epstein-Barr virus infection?

Ingemar Ernberg
Riga
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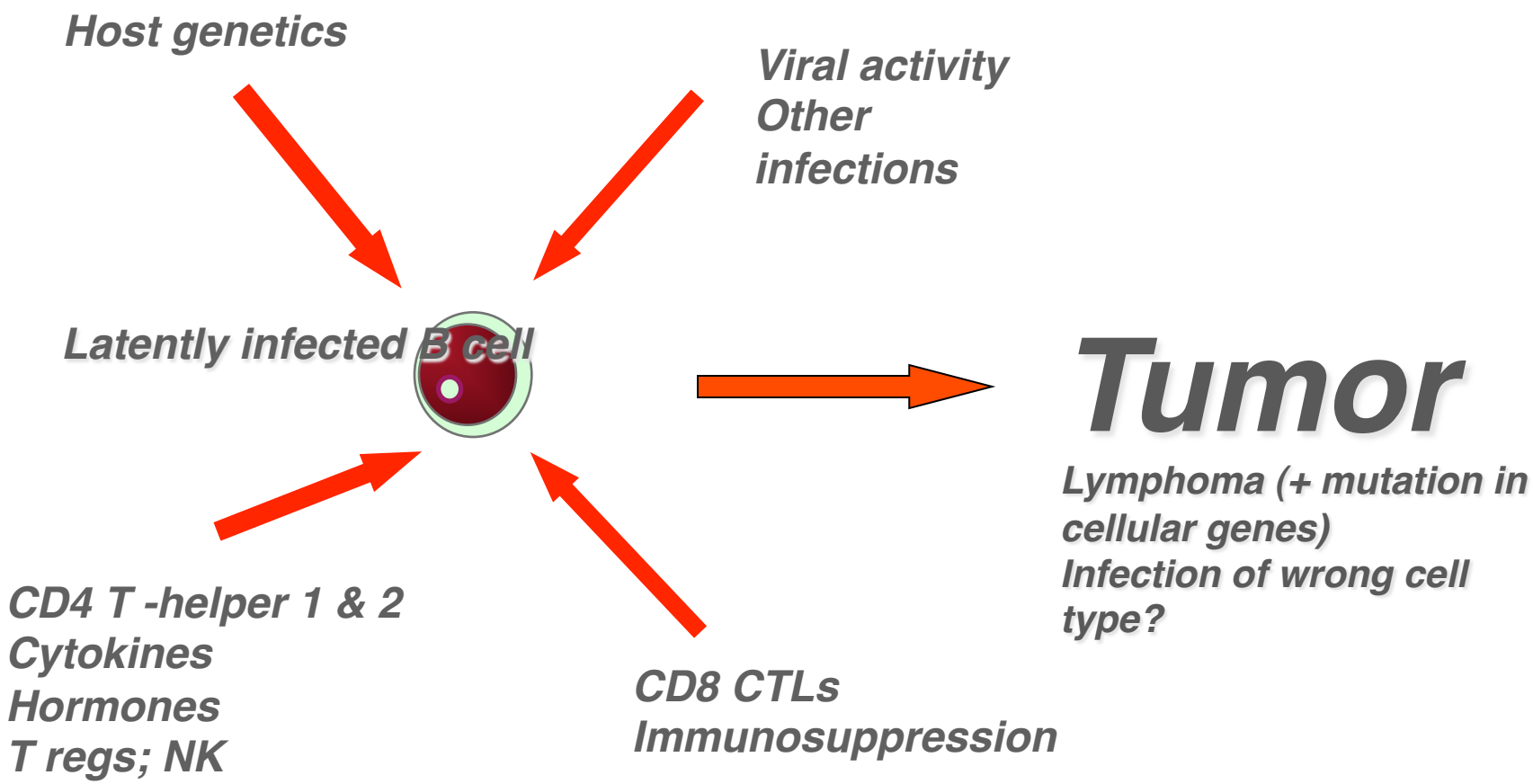
Dept of Microbiology, Tumor and Cell Biology (MTC)

Control and disruption of EBV latent infection

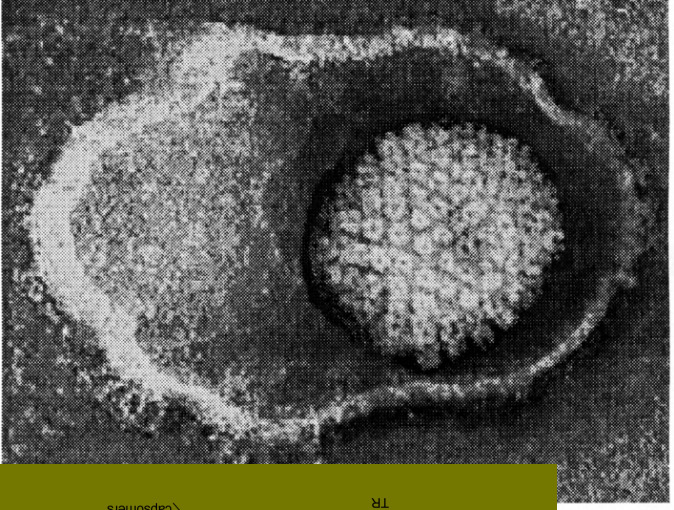


Prevention/vaccination or Immunotherapy

Why? Who? When? How?

My points

- EBV & infection in vivo
- Vaccine?
- Tumors
- High risk: transplant pat:s (& HIV-carriers)
- T-cell therapy



Epstein-Barr virus

The paradoxes

EveryBodies Virus

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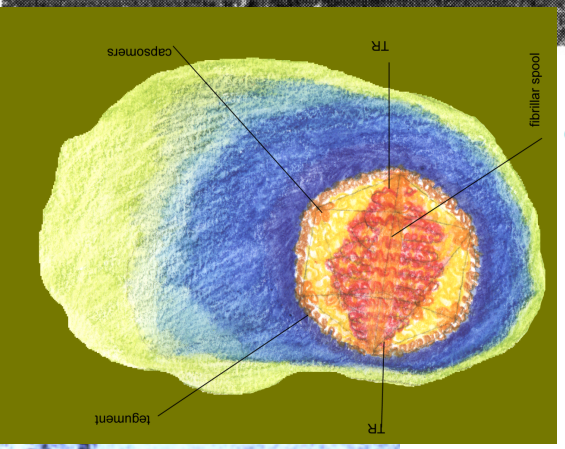
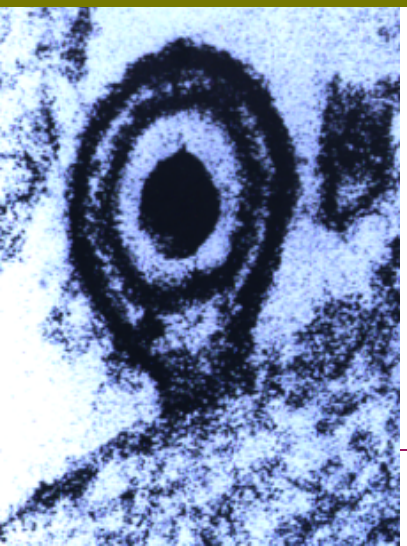
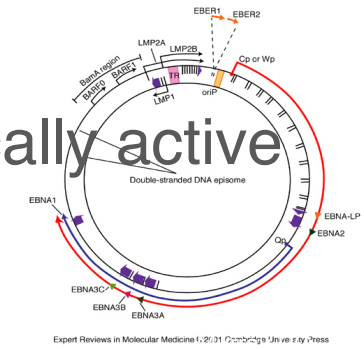


- Most common virus in the human population (>90% of all adults, life long persistence)
- Tumor-associated virus
- 80% of carriers secrete biologically active virus (saliva)
- Very efficient in immortalizing B-cells





(2001)

Stability
A sutibable vaccine target

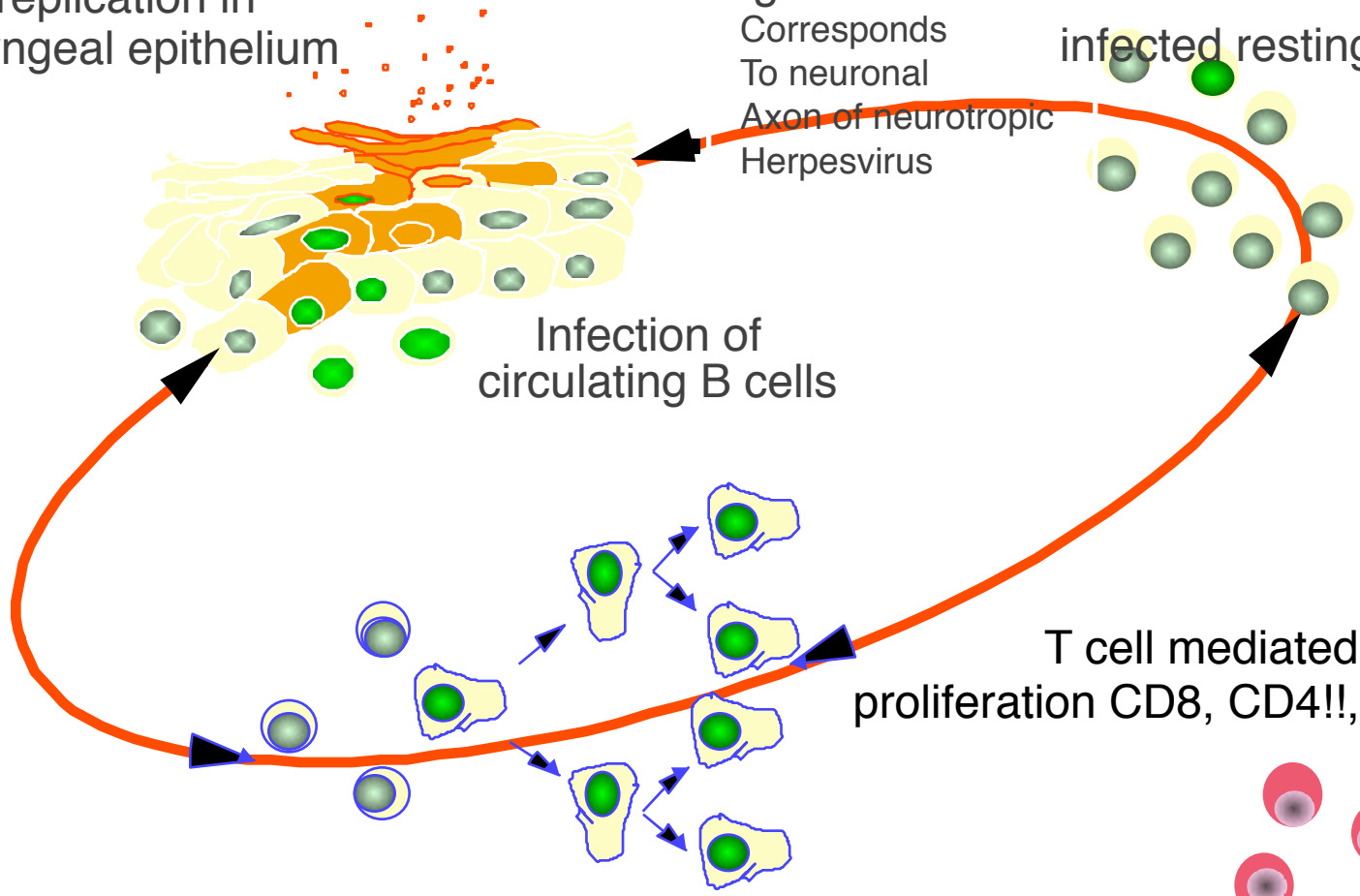
- 50+ complete DNA-sequences
- Two major types: type I=type A and type II=type B (98 % homology)
- Subtypes ill-defined: a chinese?, deletions in critical genes (LMP 1)
- Variants: appr = familiar isolates (recombination between large repeats in latency genes)

EBV(s) has(ve) adapted over millions of years to a very successfull life within the immune system.

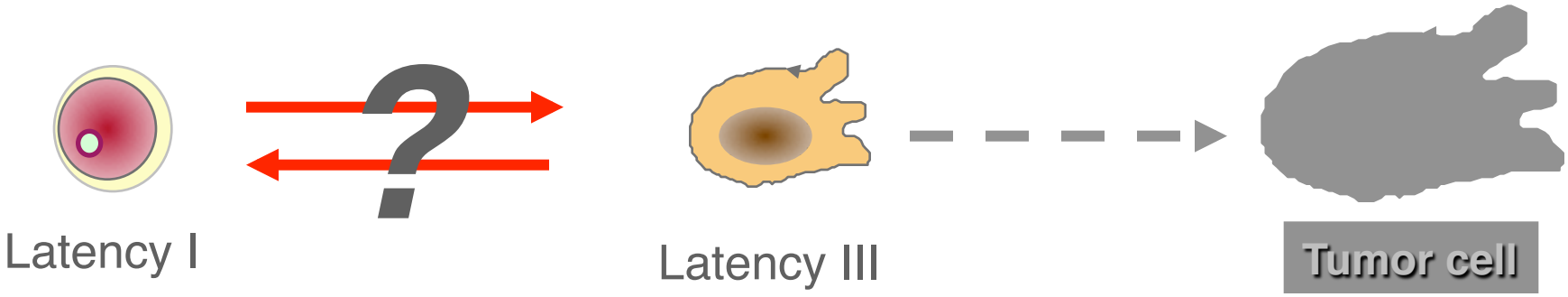
The viral strategy for survival, latency and spread in the population has evolved numerous mechanisms to master this challenging situation.

EBV-associated tumors are a rare trade off of this normally balanced virus-host interaction

EBV life cycle in vivo



Control of the latency-switch, latency I to III and reverse

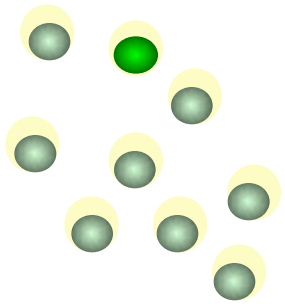


Model system for transformation in vitro

- HBV/HCV -
- HPV (+)
- EBV ++
- HHV 8/KSHV -
- HTLV +

- *H.Pylori* -
- Parasites (liver fluke, *Shistsomiasis*) -

Latency in vivo



One infected B-lymphocyte/ 100ul-10 ml of blood (0,1-10/100.000 B-cells)

5000 infected cells in blood in a healthy carrier (+ X in lymphoid system)

Increases 100-10.000-fold in high risk groups

Host-virus interaction & diseases

- Childhood infection *subclinical*
- Primary infection > 5 yrs 35% risk of *infectious mononucleosis* (“an acute autoimmune syndrome”)
- In immunosuppressed: (Fatal/Chronic) *lymphoproliferative syndrome*
- *Lymphomas, epithelial cancers* as a result of accidents of the host-virus interaction

To think about

Most biologically active EBV on earth is swallowed with the saliva.

300-500 ml/day, appr 500 infectious units entering the GI every day in 80% of humans.

“Part of the normal flora”

Role in infection, immune boosting, and pathogensis?

Infectious agents
Number of new tumors annually & globally

HBV/HCV	500,000
HPV	450.000
EBV	200.000
HHV 8/KSHV	45.000
HIV	10.000
HTLV	2.200
H.Pylori	460,000
Cl.psitacci, Borrelia burgdorfei, Campylobacter jejenum	500?
Parasites (liver fluke, *Shistsomiasis*)	11,000

EBV-associated tumors in man

Lymphoid tissues

Burkitt´s lymphoma, endemic	98%
Burkitt´s lymphoma, sporadic	30%
AIDS-immunoblastic lymphoma	30%
-in CNS	100%
Post-transplant lymphoma	100%
Hodgkin´s lymphoma	50%
T-cell lymphomas	10-30%
--lethal midline granuloma	>90%
Primary effusion lymphomas	>90%

Epithelial tissues

Oral hairy leukoplakia	100%
Gastric adenocarcinoma	5-10%
Nasopharyngeal carcinoma, undifferentiated	100%
Salivary gland carcinomas	<100%

Smooth muscle tissue

Leiomyosarcoma in immunosuppressed	100%

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Geographic distribution of EB virus-associated tumors

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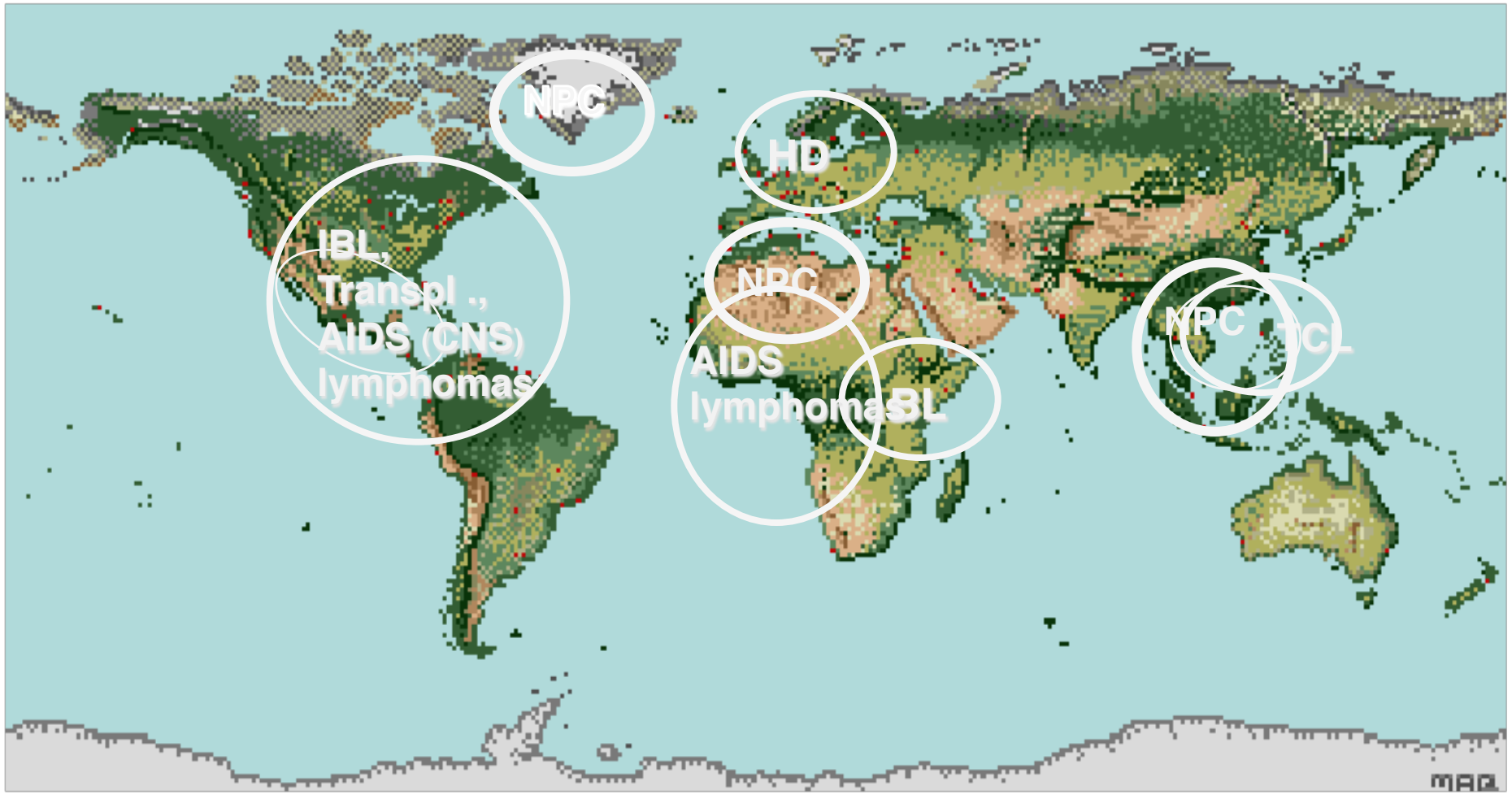
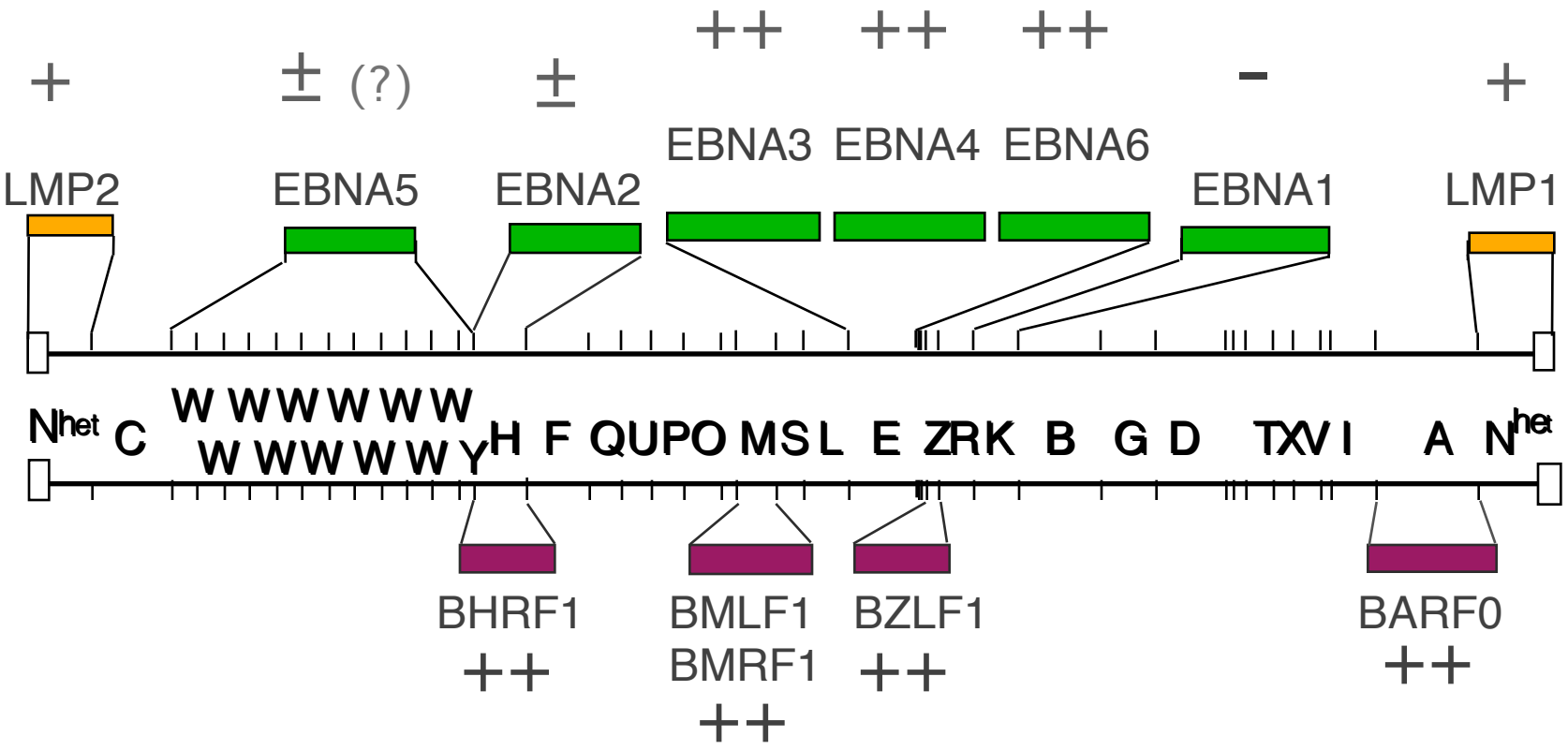


EBV - therapeutic strategies

- Conventional cancertherapy
- Anti-viral drugs: spec DNA-polymerase inhibitors (acyclovir etc.) clears replication but not latent infection. Effect on lymphoproliferative syndrome
- Bone marrow transplantation can clear out the whole EBV-infection including latency
- Therapeutic vaccines
- Adoptive transfer of EBV-specific cytotoxic T-lymphocytes (CTL) reeducated in vitro

(Check point-blockers PD1/CTLA4)

EBV specific cytotoxic T-cell (CTL) responses



- polyclonal responses preferentially directed against EBNA3,4,6 and antigens of the lytic cycle
- strongly focused on certain MHC:peptide combinations
- endogenously expressed EBNA1 is not recognised

Components of immunity to EBV

- Antibodies are efficiently neutralizing infection (anti-gp 350; anti-MA)
- Antibodies + complement/LAK-cells eliminate virus replicating cells
- Earlier dogma: Cytotoxic T-lymphocytes are instrumental in controlling the life-long latency (Masucci, Rickinson, Moss)
- Recent discovery: CD4 cells producíng specific cytokines (IL21) control the latent infection (Nagy, Kis, Klein; KI)

Receptors

B-cells: C3d (CR2)-receptor, CD 21

MHC Class II (co-receptor)

Epithelial cells: MHC Class II

Unorthodox mechanisms

Virus envelope receptor: gp 350/220

EBV vaccine

Primary preventive

- Recombinant gp350, major envelope glycoprotein, receptor binding, neutralizing epitope (prevents lymphomasin monkey models)
- To be used when, where and how? Risk groups for NPC, EBV-negative adolescents (IM)

EBV vaccines…

Therapeutic

- "Polytope", several MHC Class I epitopes combined in a vaccinia-vector based vaccine (D.Moss et al)
- Peptide-loaded dendritic cells (NPC; A.Rickinson et al)
- Adoptive cytotoxic T-cell transfer (C.Rooney; M.Masucci et al)
- Recombinant gp350, major envelope glycoprotein, receptor binding, neutralizing epitope (therapeutic in post-transplants?; A.Morgan et al +CRC, UK)
- CTL modulation combined with demethylation (Rich Ambinder et al.)?

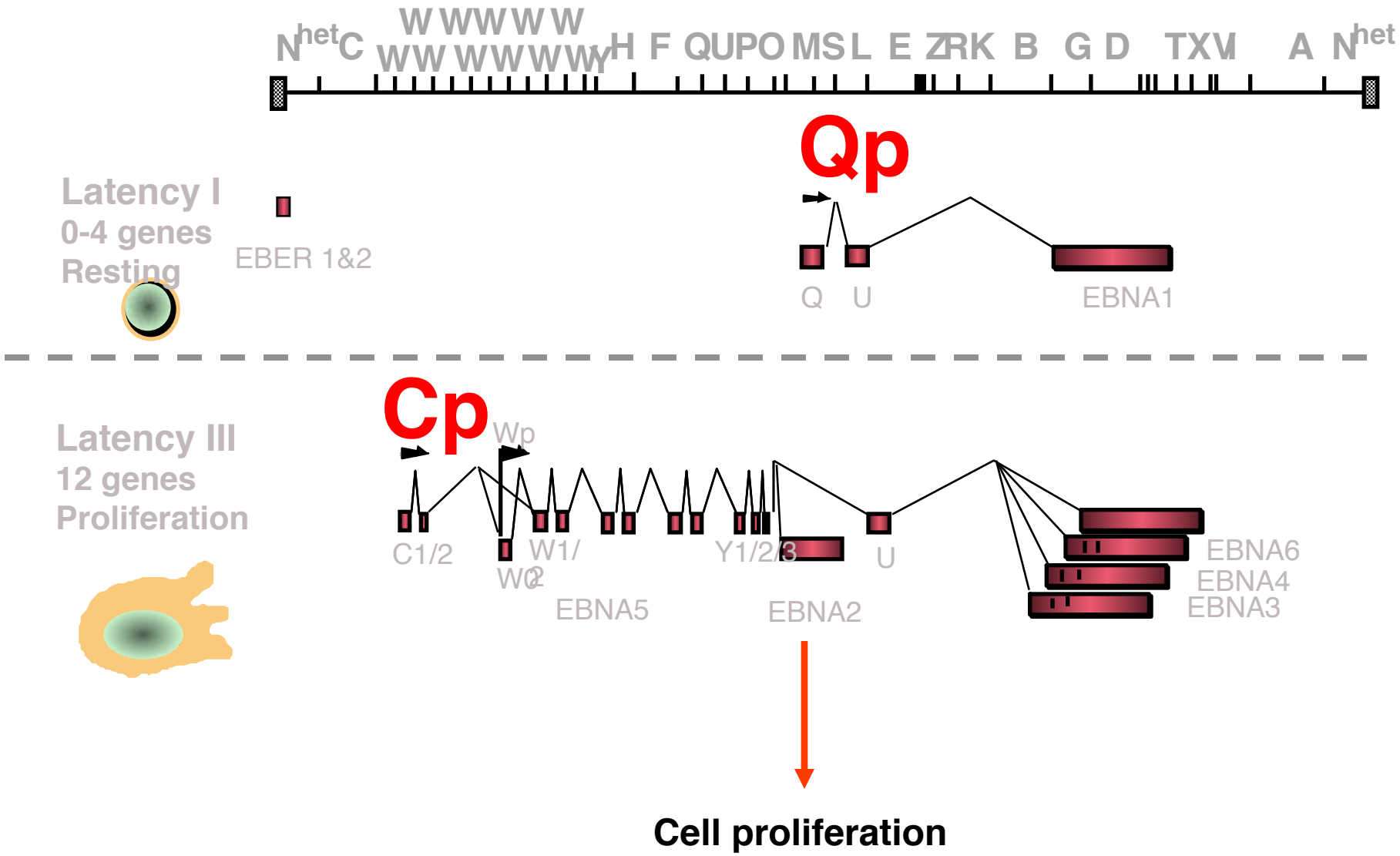
Groups with high risk for EBV positive lymphomas

- Bone-marrow/HSC transplanted pat:s (with minor mismatch)
- HIV-carriers (before HAART; 15-20 % CNS lymphomas)

- Children with chronic malaria

- Immunodisregulation+Chronic antigen stimulation

Proliferation-rest is regulated by viral expression programs in latency



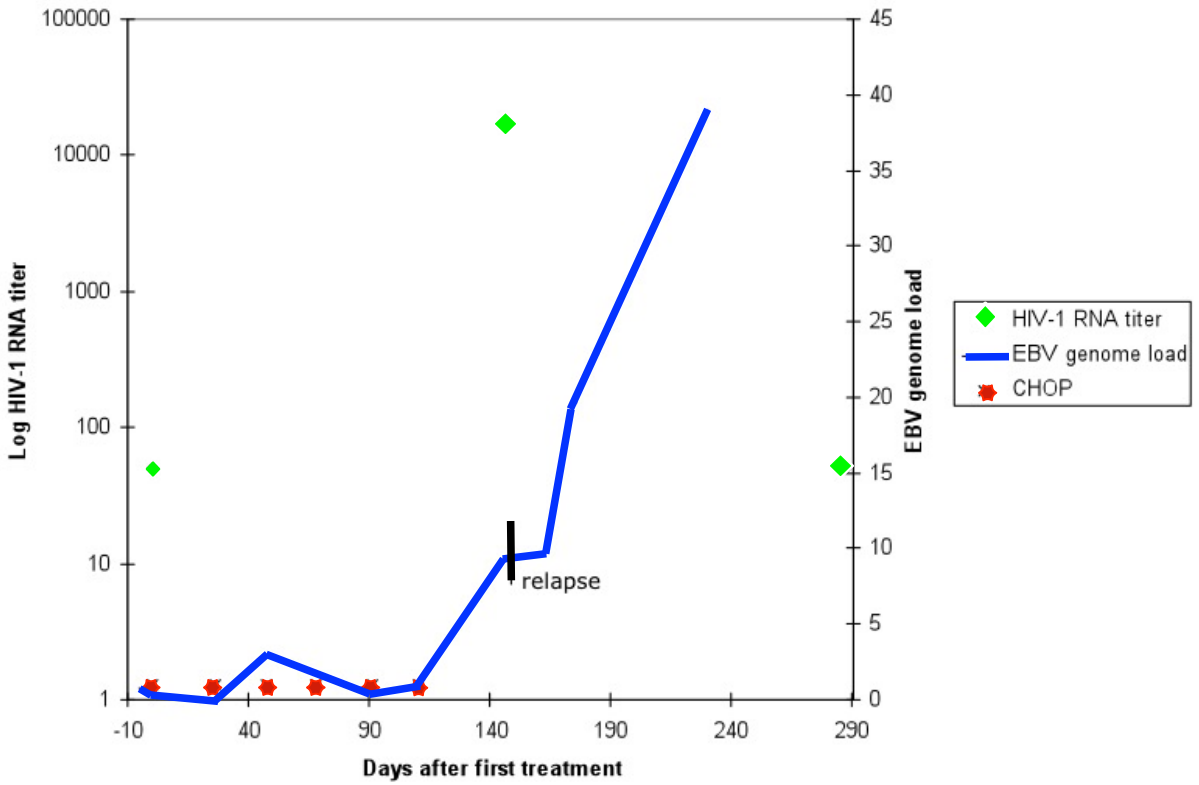
Incidence of post-transplant lymphoproliferative disease (PTLD) depending on type of transplant

- Kidney 1 %
- Liver 2 %
- Heart 3 %
- Lung 1 – 8 %
- Heart and lung 9 %
- Intestine 7 – 11 %
- Multivisceral 13 – 33 %
- BMT 1 % (up to 30 %)

Risk factors for post-transplant lymphoproliferative disease (PTLD)

- Mismatched, unrelated stem cell grafts
- Primary immune deficiency
- T-cell depletion techniques which do not concomitantly reduce the B-cell content
- Use of anti-T-cell antibodies (ATG, OKT-3)
- Heavy immune suppression
- High levels of circulating EBV-infected cells *
- EBV-seronegative recipient
- CMV infection / Young age ?/ GVHD treatment

EBV-DNA load and relapse in EBV-associated lymphoma (HIV-infected patient)

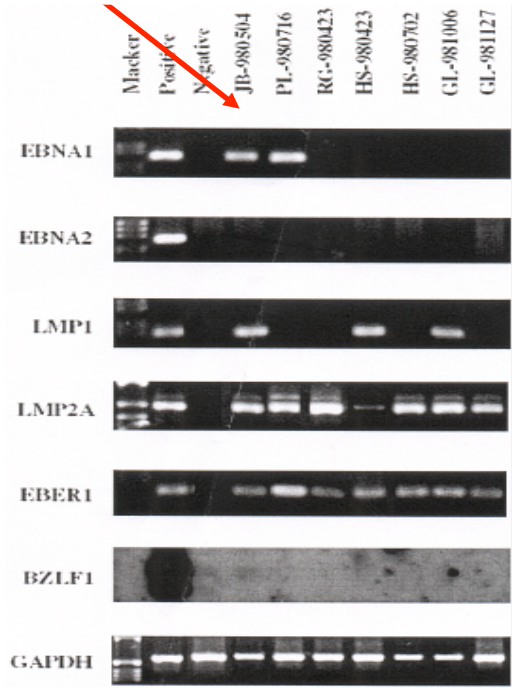


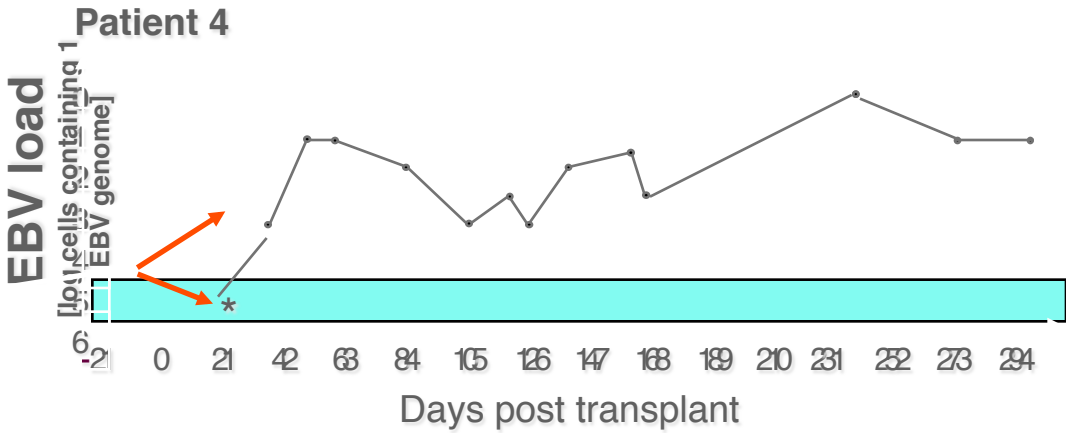
Increased EBV load in patients receiving T-cell depleted BMT

Several log-fold increase of EBV-load post-BMT…

…. related to T-cell depletion of graft

….only latency 1 & 2 (restricted, non-proliferating in blood)

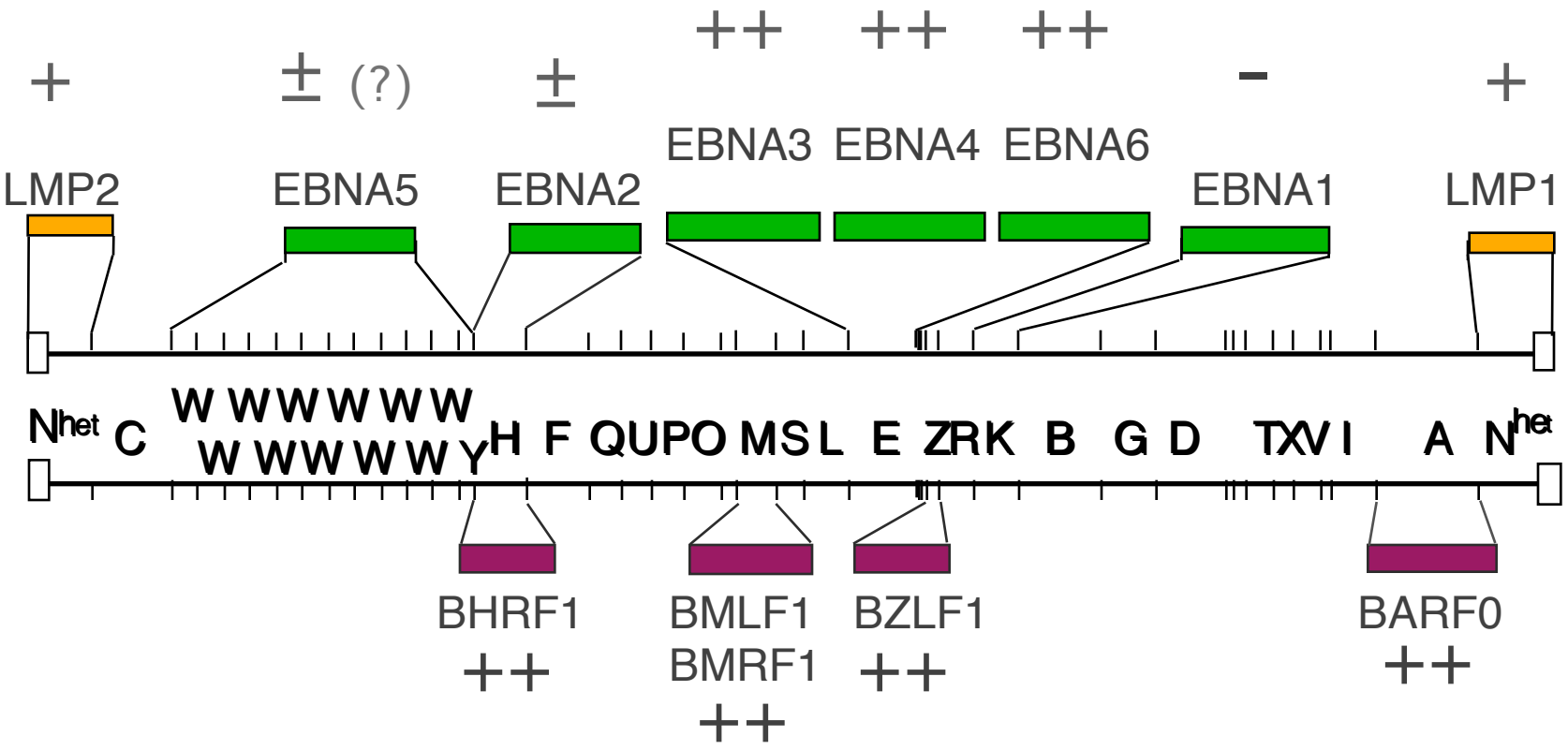




Maximal EBV load recorded in BMT recipients with high or low risk of PTLD

Donor	Type of BM graft	Maximal EBV load (genomes/10^6 cells)	Days post BMT
High risk patients			
JB[a]	TCD	1,250	31
UH	TCD	6,250	129, 149, 366
PL	TCD	31,250	53, 63
OL	TCD	31,250	49, 125
RG	TCD	156,250	42, 52
GL	TCD	31,250	80
HS[a]	U	156,250	80
mean±SE		59107±25963	
Median		31250	
Low risk patients			
MU	U	50	34
NJ	U	250	51, 62
SG	U	1250	62, 79, 93
RT	U	50	95
EE	U	1250	55
PZ	U	1250	335
LS	U	250	13, 27, 53, 62, 73, 81
GS	U	1250	67
mean±SE		700±269	
Median		750	

EBV specific cytotoxic T-cell (CTL) responses



- polyclonal responses preferentially directed against EBNA3,4,6 and antigens of the lytic cycle
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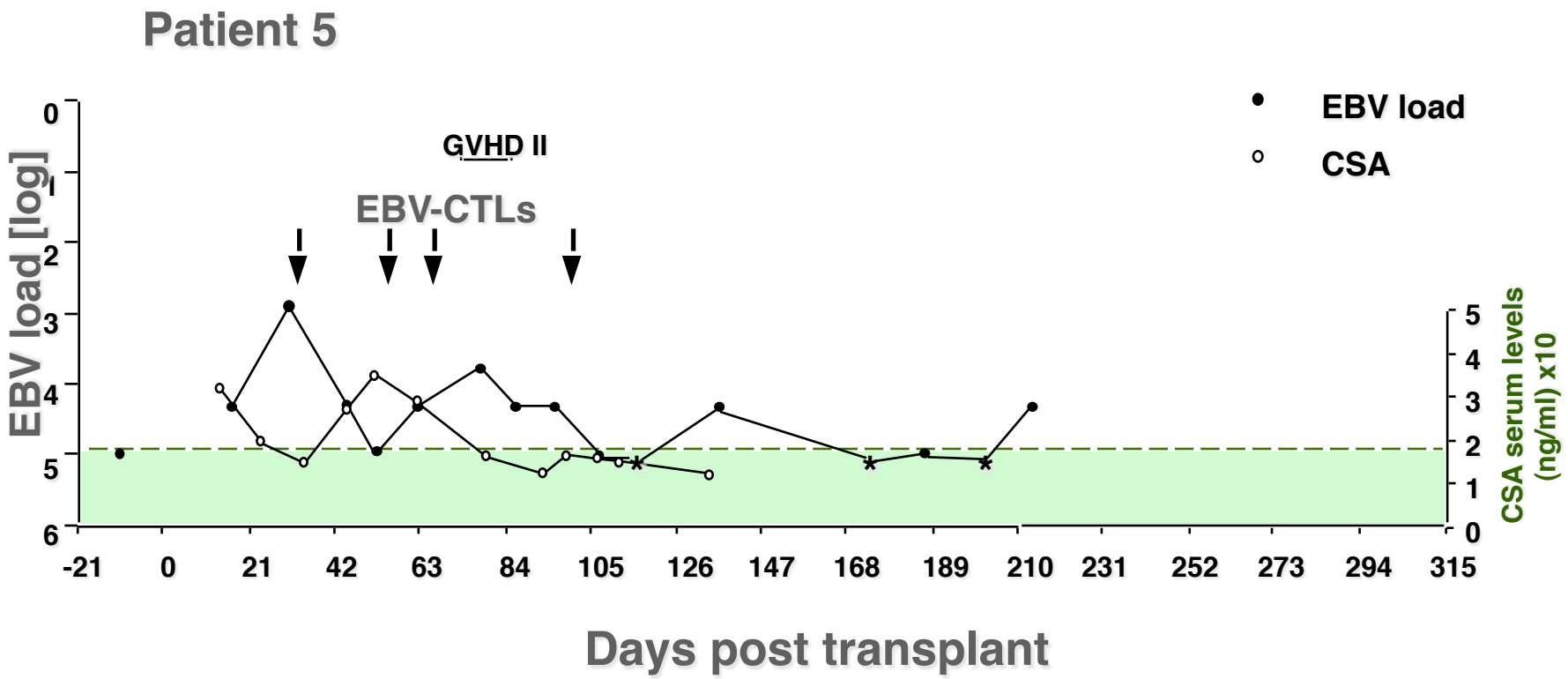
Early administration of EBV-CTLs prevents the increase of EBV load



Study of ”EBV” at transplant unit of Karolinska University Hospital South (Huddinge)

Question

Can we identify better tools for PTLD risk prediction at individual patients level?

T cell phenotyping on EBV-related immune reconstitution (tetramers, FCM)

Cohort

Entered so far: 350 adults & 50 children entered (open)

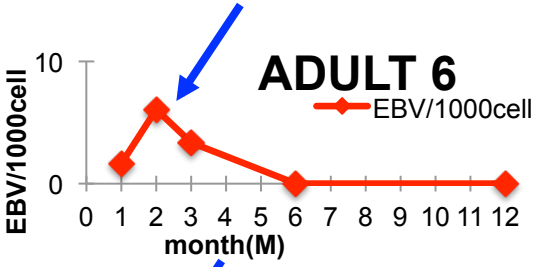
Sampled 1,2,3,6, 12 and 24 months > transplantation

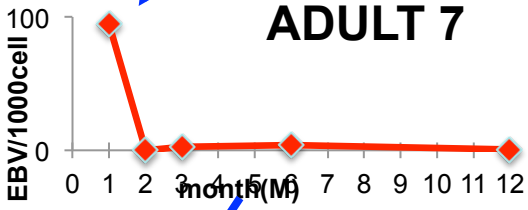
Colaborators

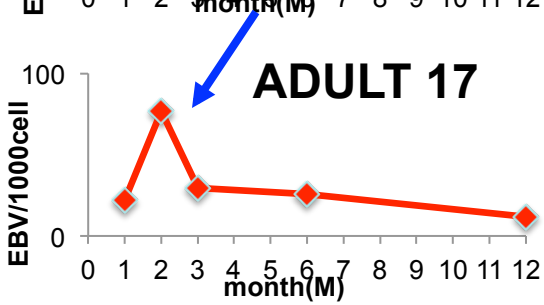
Markus Maeurer, Olle Ringdén, Johan Matsson, Jacek Winiarski,

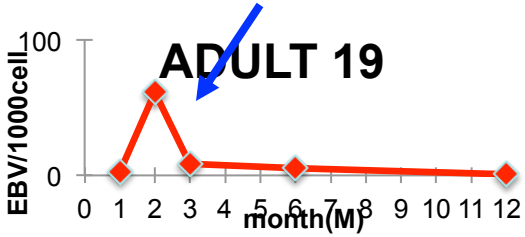
EBV DNA load over time: TWO patterns

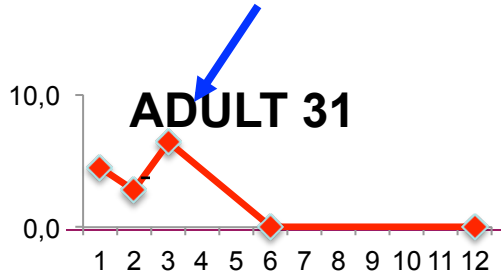
Type I
Typell

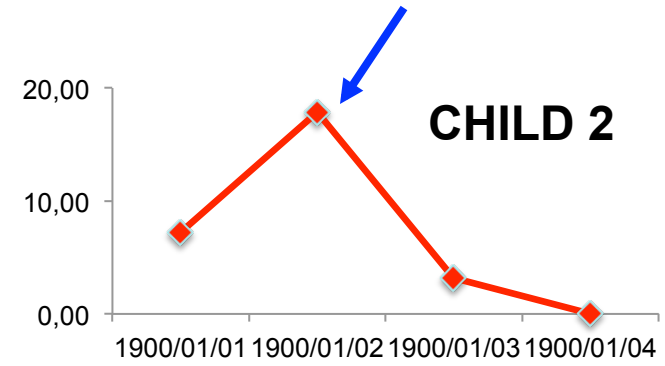


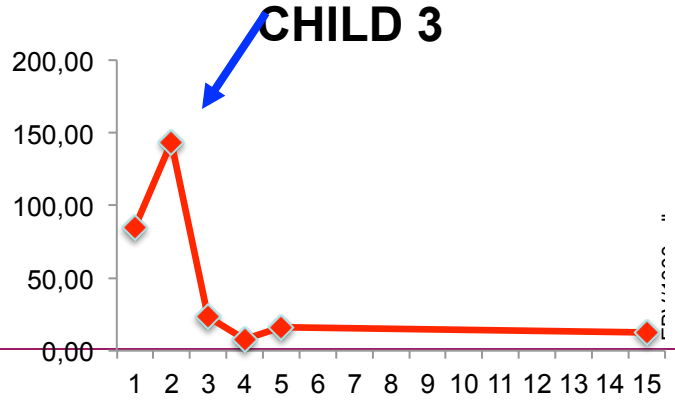


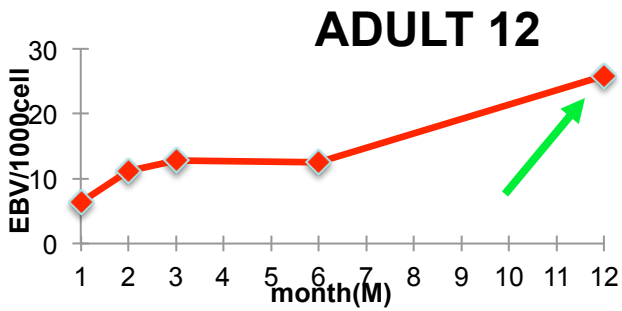


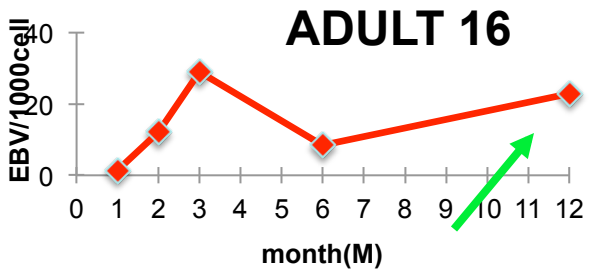


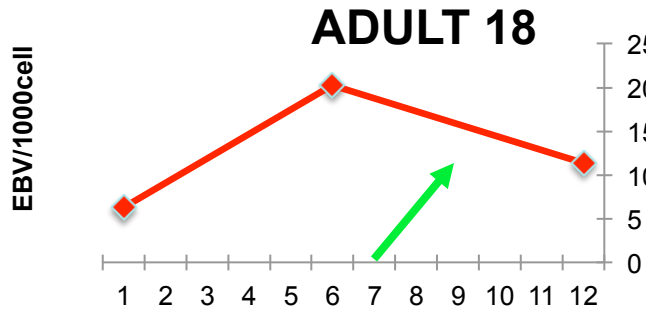


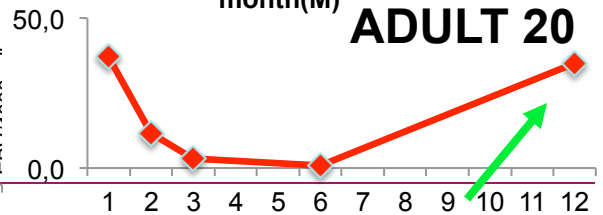












Fraction CD3+ cells

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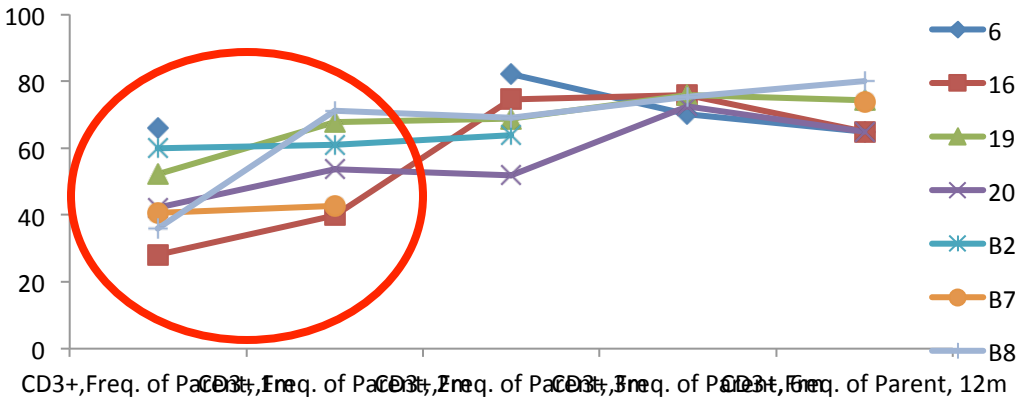
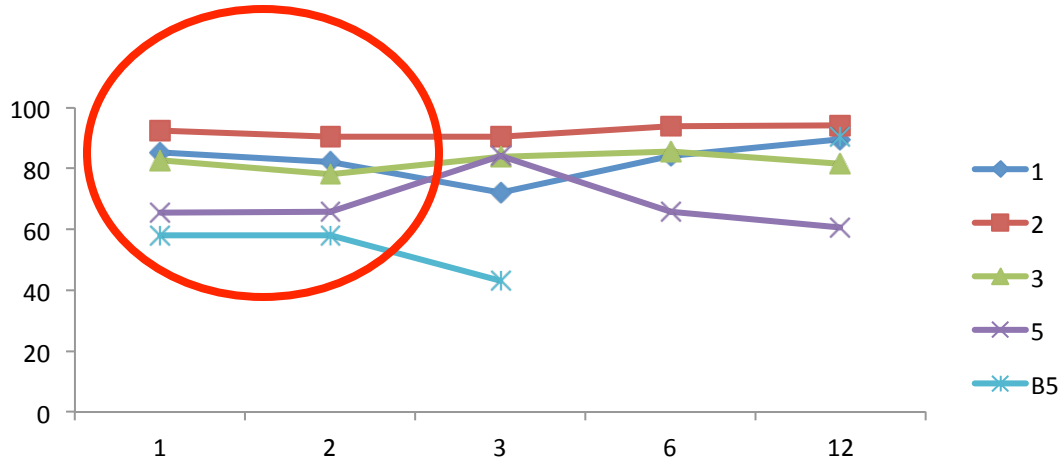
Intermediate



Low



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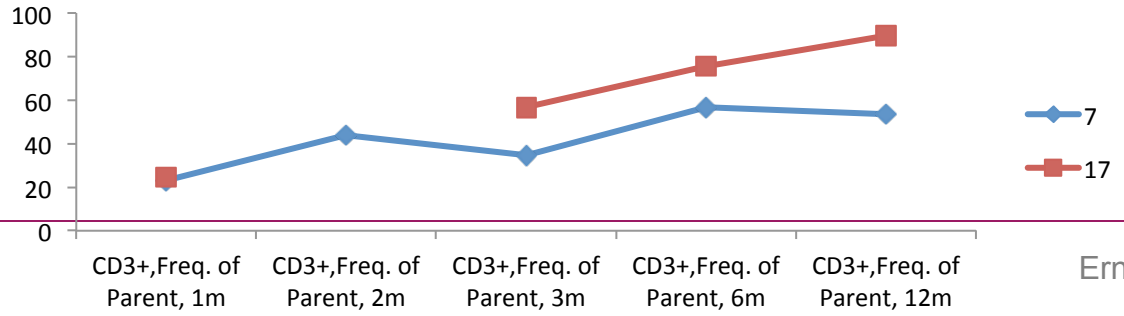
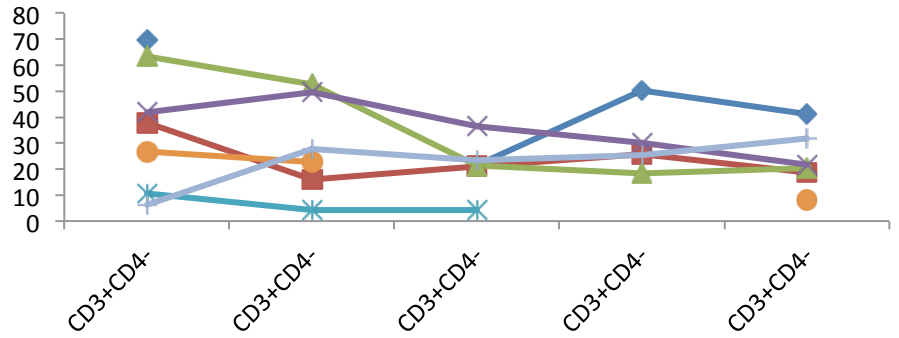


CD4-CD8- T cells

Intermediate



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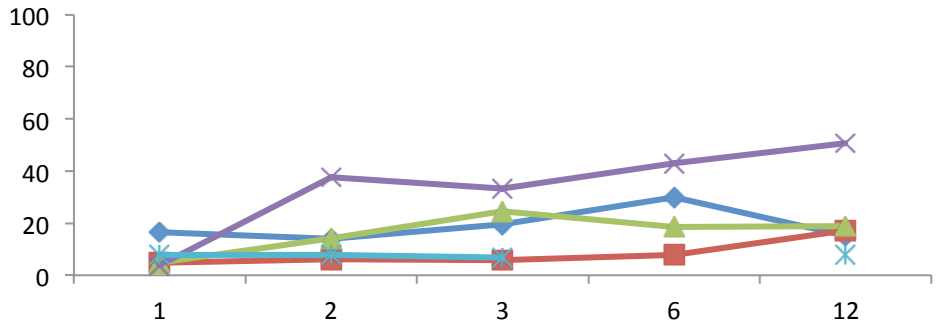

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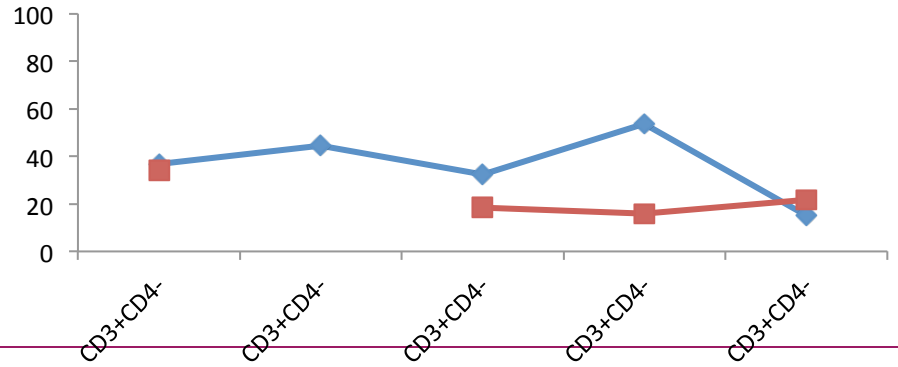

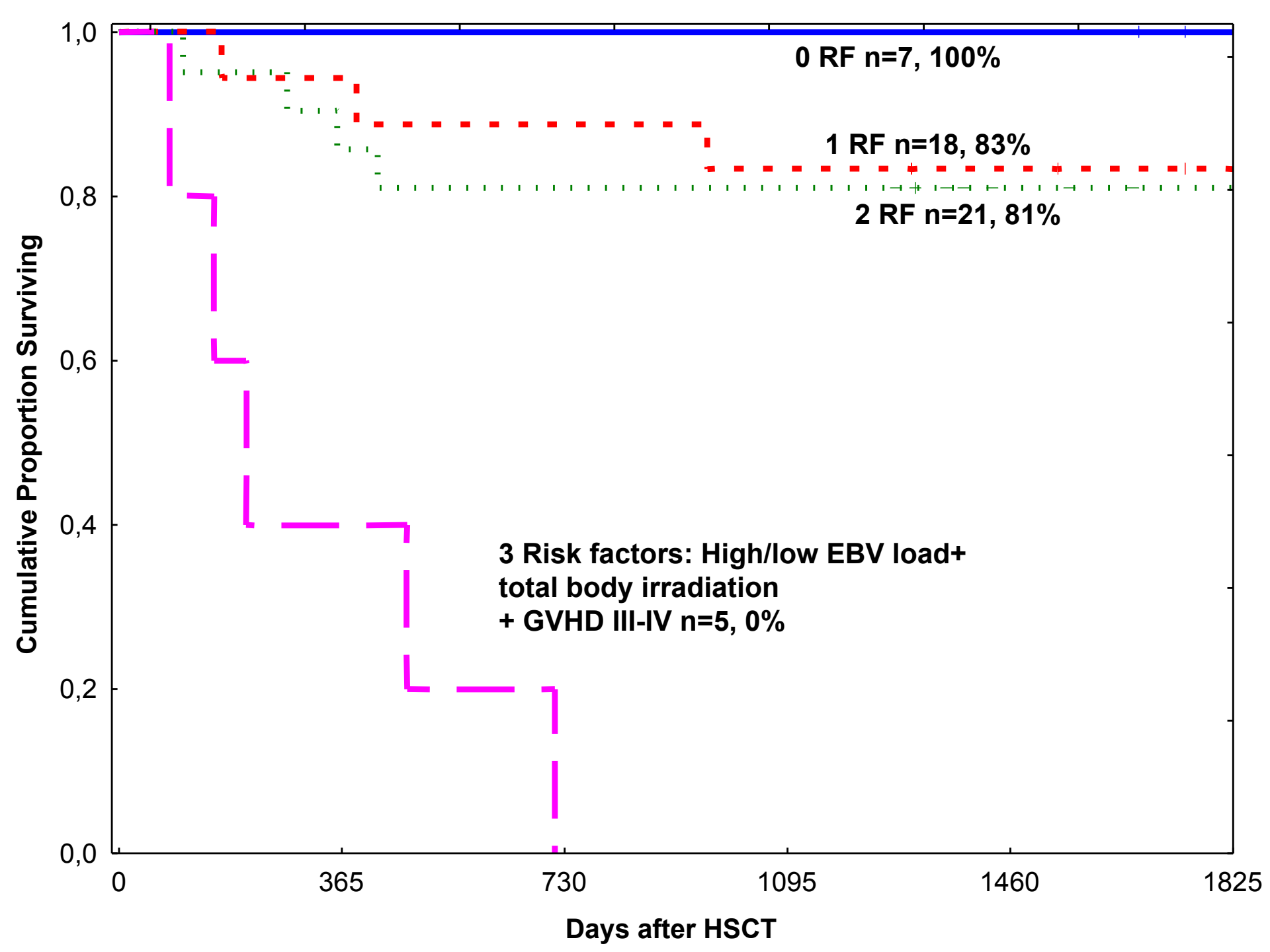
1,0
0,8
0,6
0,4
0,2
0,0
Cumulative Proportion Surviving
0 RF n=7, 100%
1 RF n=18, 83%
2 RF n=21, 81%
3 Risk factors: High/low EBV load+
total body irradiation
+ GVHD III-IV n=5, 0%
0
365
730
1095
1460
1825
Days after HSCT

Concluding remarks

Target population for vaccination against EBV not identified; efficient vaccine exist (rec gp350); duration of protection not known

Normalization of EBV load in risk groups depends on functional immunereconstitution (cART; post-BMT)

Adoptive (EBV-specific) T-cell therapy prevents PTLD and cures some lymphomas (post HSCT)

New possibilities: check point inhibitors, CD4, interleukins (IL21?)

Extreme low or high EBV load in blood (B-cell bound) post-BMT/HSCT poor prognosis. With aGCVD & TBI predicts (100%) failure

Collaborators

Huddinge Transplantation &
Pediatric clinic
Jacek Winiarski
Olle Ringdén

CTL work
Maria Masucci, CMB
Teresa Frisan, CMB
Viktor Levitsky, CMB

EBV
Jie-Zhi Zou, MTC

HIV
Anna Friis
Börje Åkerlund

T-cell phenoptyping
Nalini Vudattu, SMI &MTC
Markus Maeurer, SMI & MTC

Members; now and recent

Andrey Alexeyenko
Rozina Caridhe
Sun Di
Nguyen van Do
Ziming Du
Anna Friis
Elvira Grigorieva
Sven Grüzmier
LiFu Hu
Qin Li
Liudmila Matskova
Tore Midtvedt
Imran Nawaz
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Jie-Zhi Zou
Xiaoying Zou