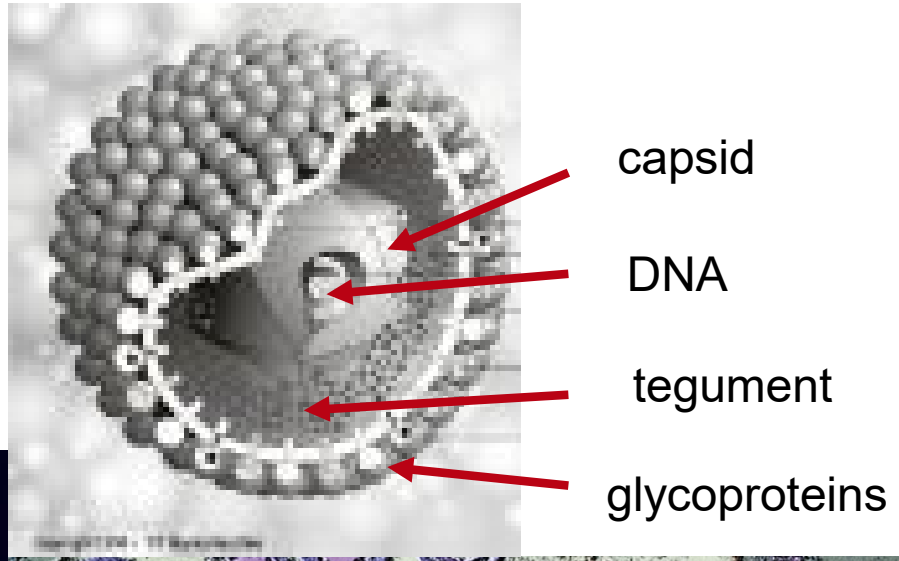
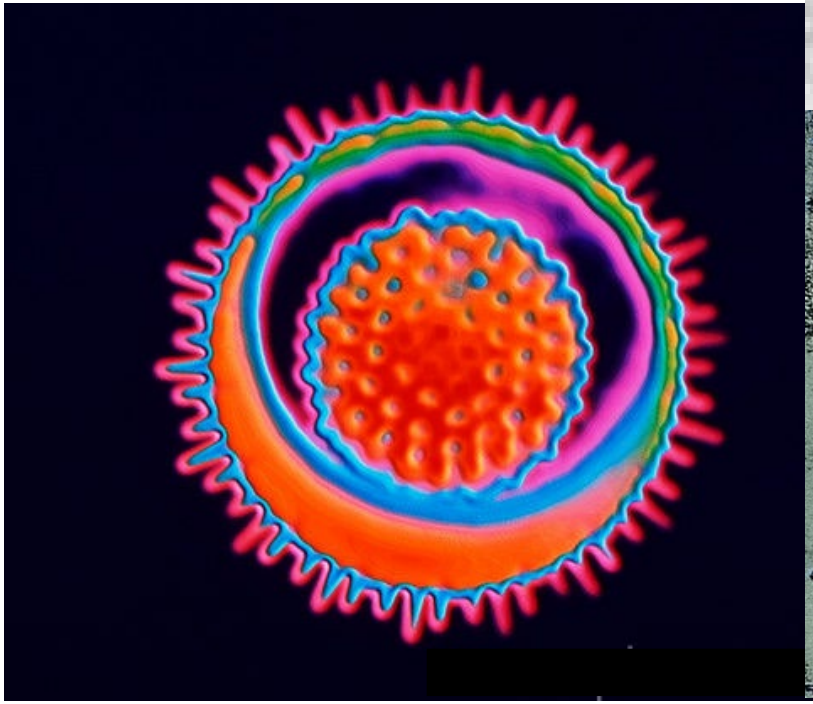
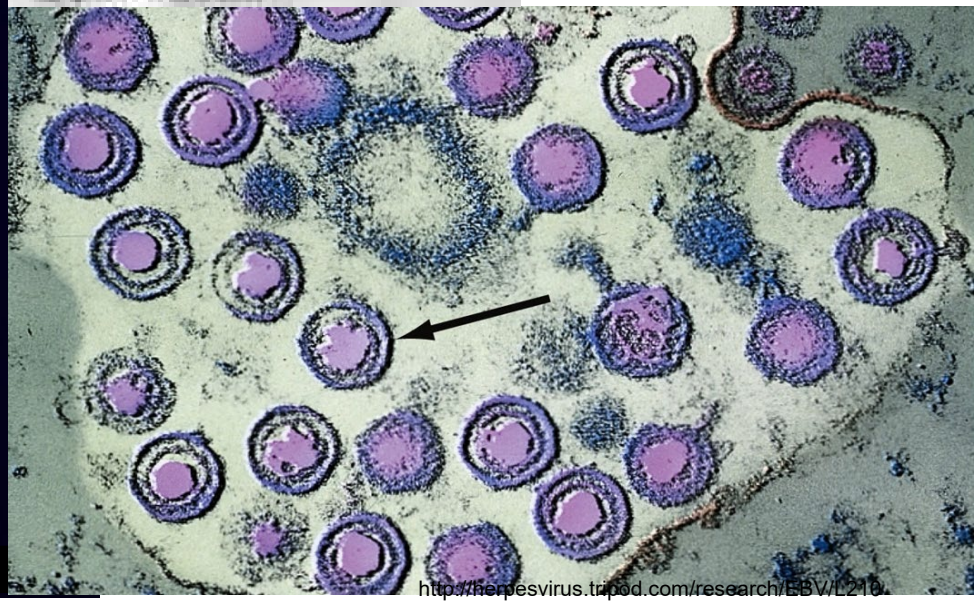
Herpesviruses

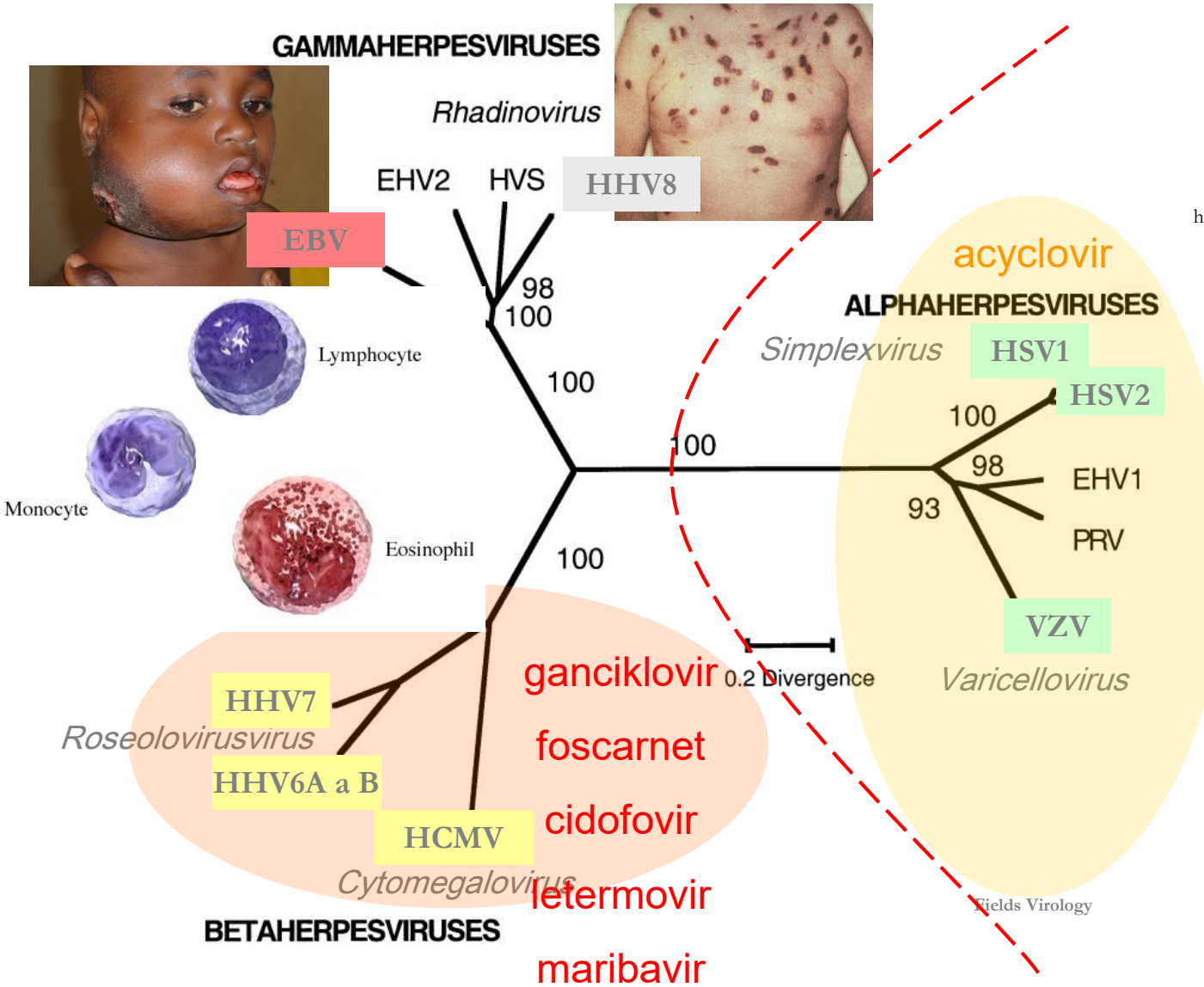
- Enveloped ds DNA viruses
- Genome of length 125-240 kb
- Icosahedral capsid
- Diametre of capsid of approx. 100 nm

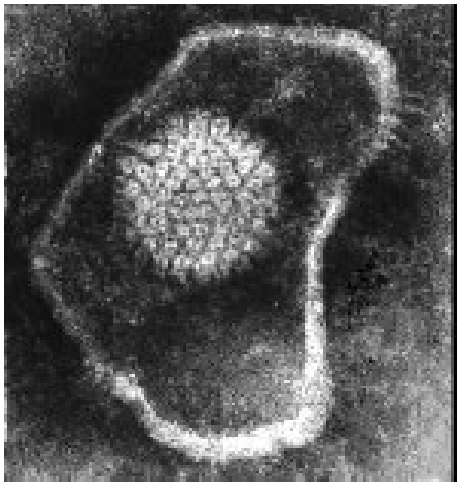




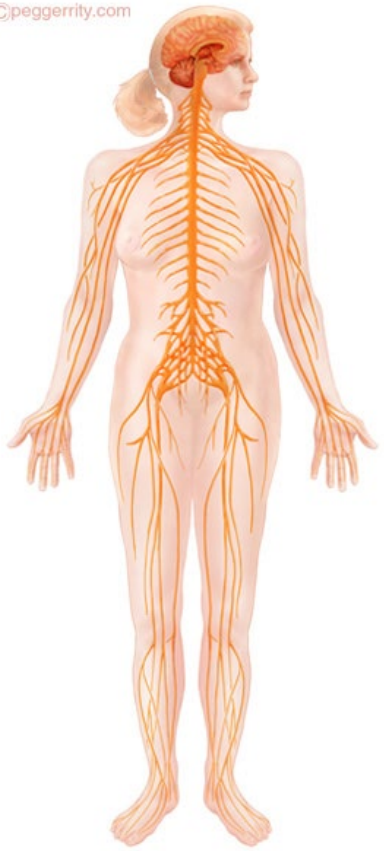


Taxonomy

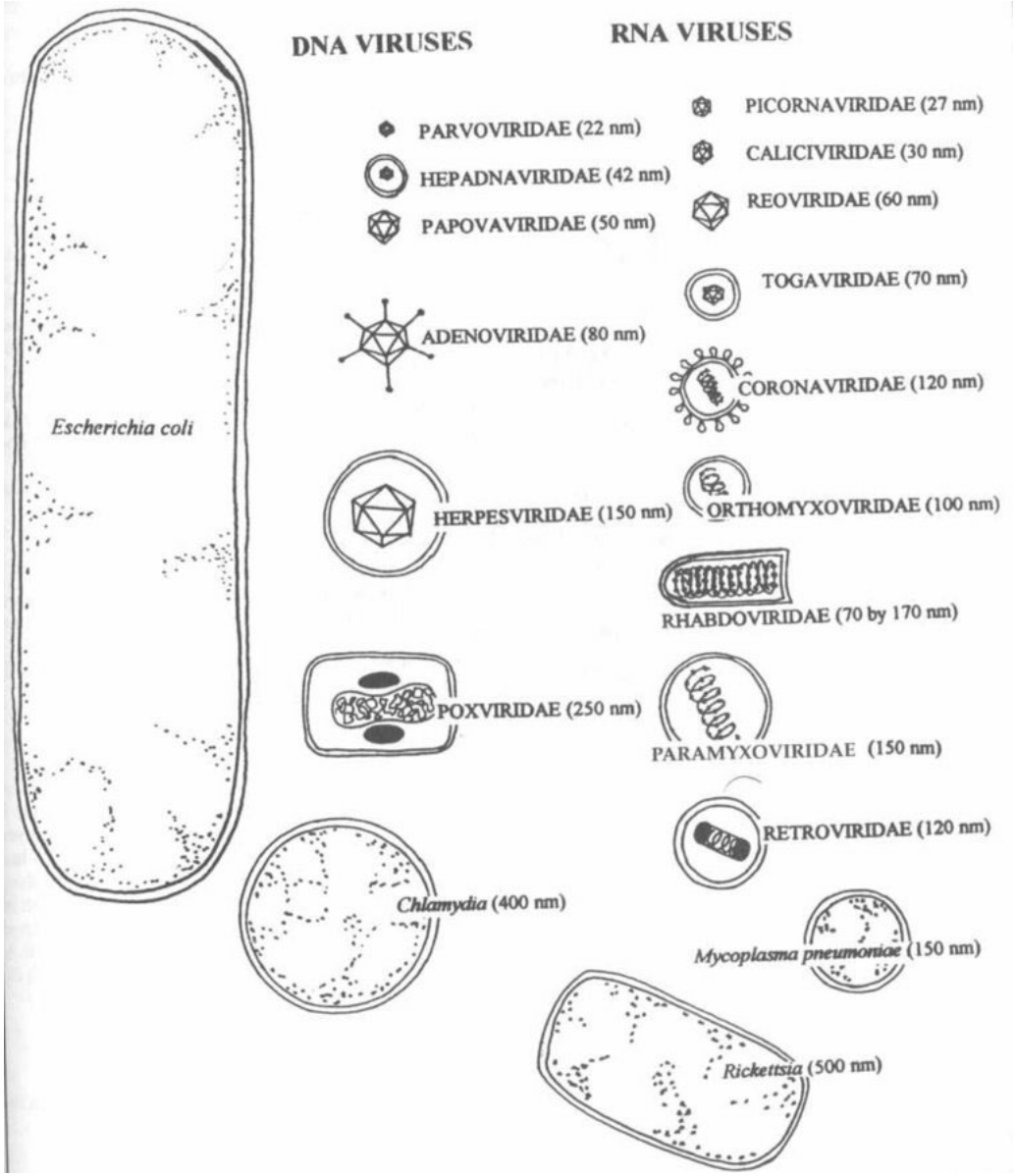




http://www.tulane.edu/~dmsander/garryfavweb.html



How they looks like?

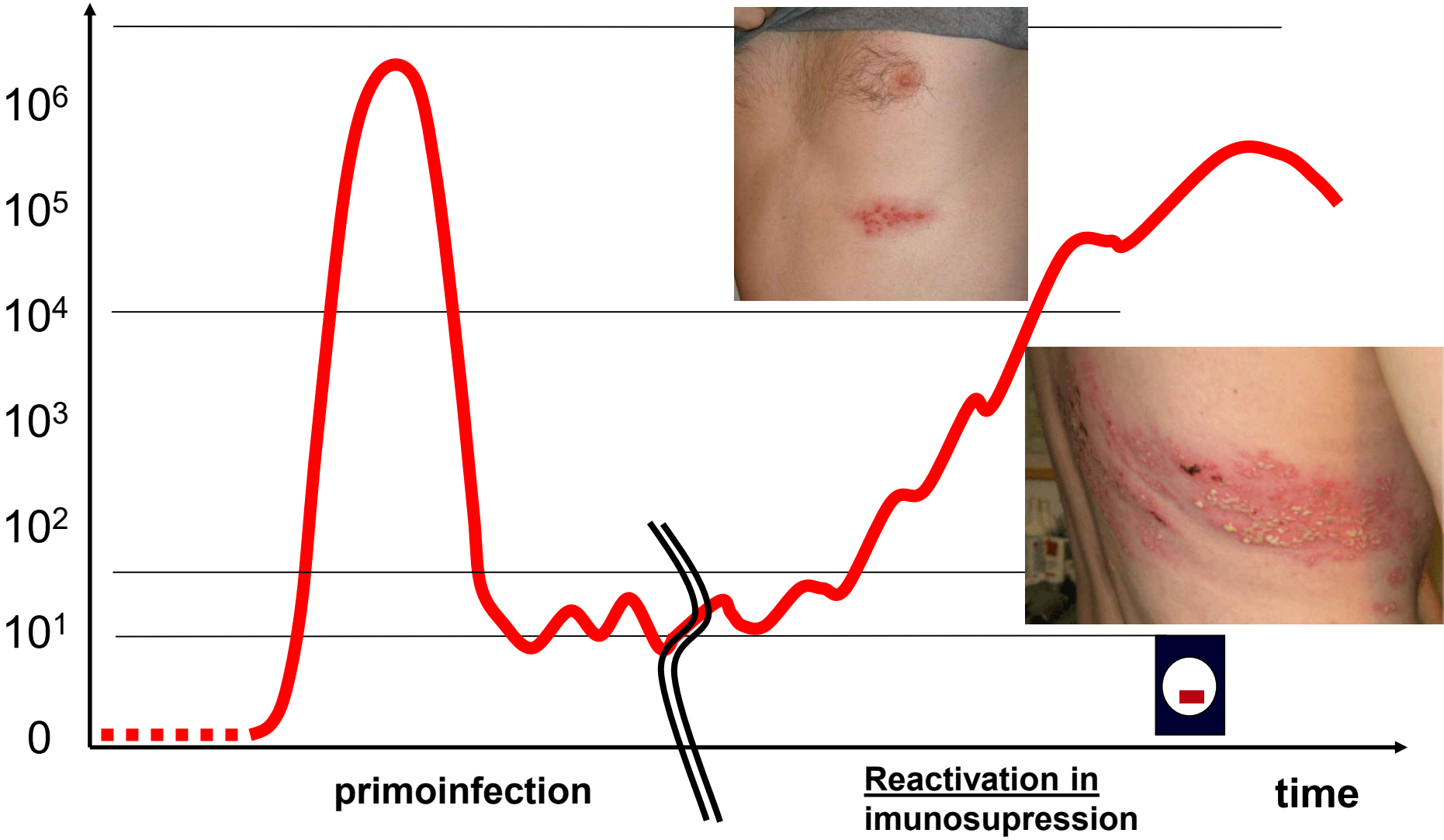


- Coding nucleic acid can be both ss or ds and RNA and DNA
- Size of the genome is approx. between 3 kB and ≈ 200 kB
- For infection are important molecules on the viral surface which determines the cell receptors for virus binding and so specificity of viral infection for different cell types.

http://www.epubbud.com/read.php?g=2RBLFKRP&tocp=5

Latency with possibility of reactivation

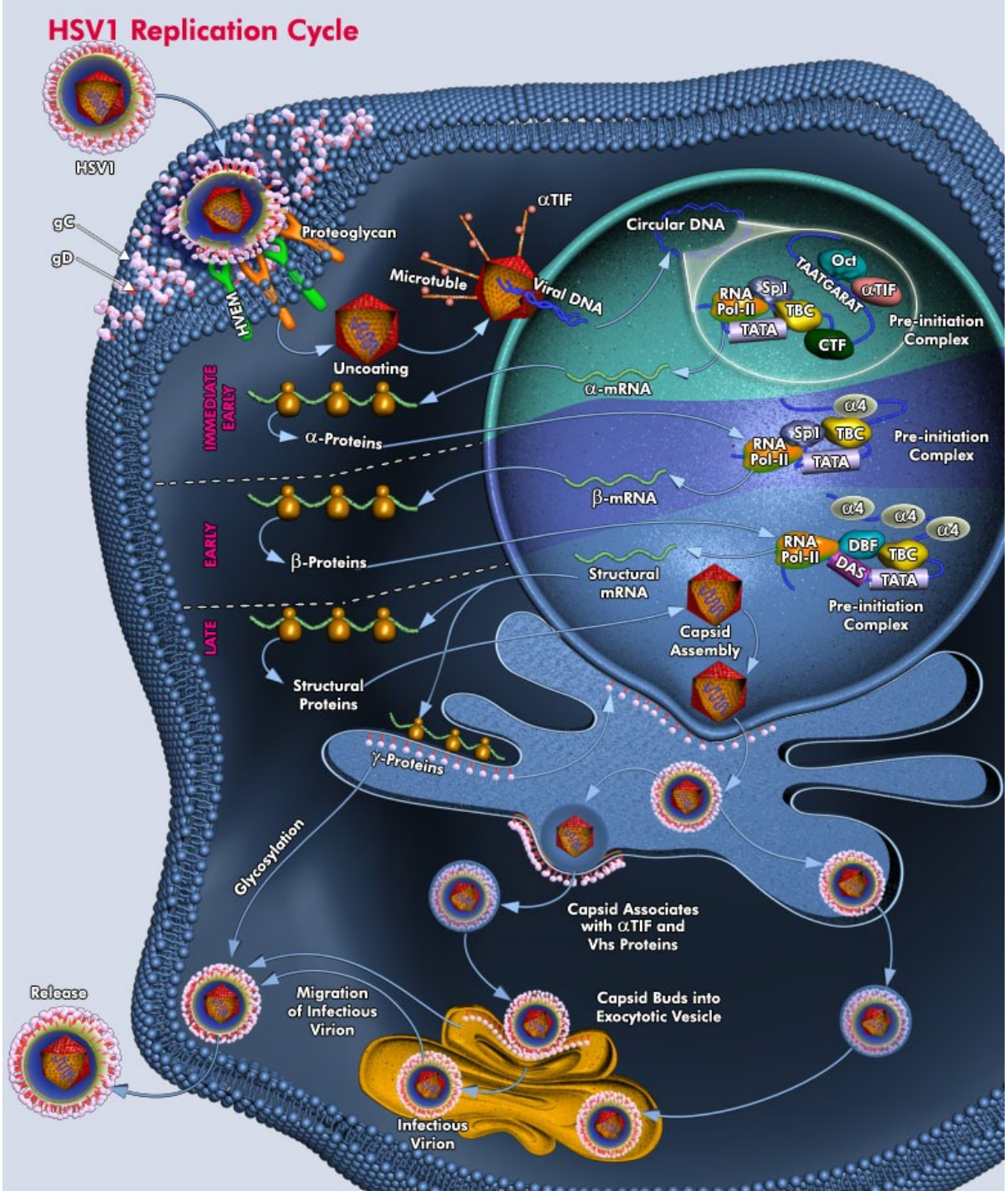
Transmission – by body fluids (saliva, urine, breast milk, blood, …)



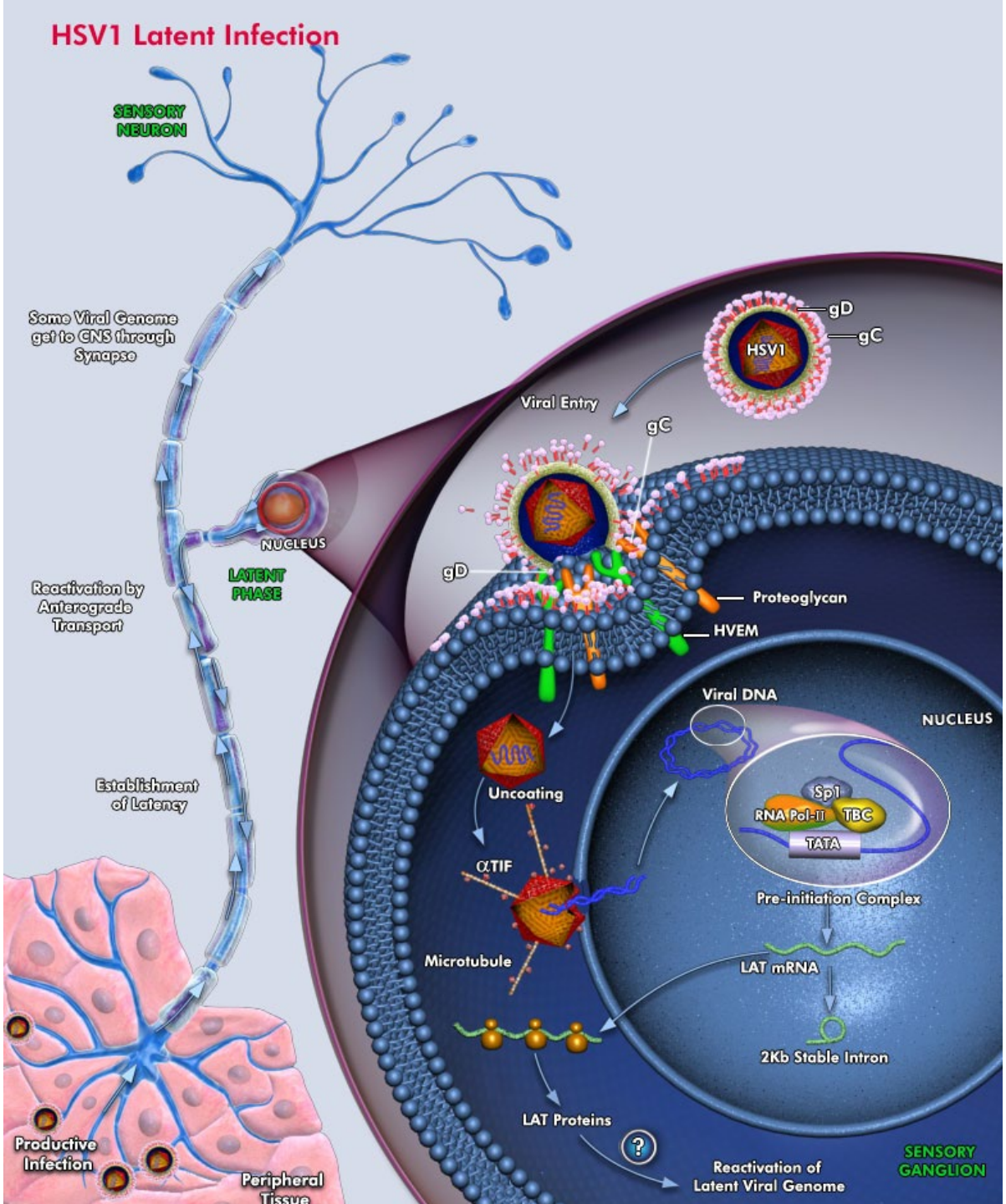
Why herpesviruses?

Indicative disease for HIV re-classification to AIDS stage (WHO criteria):

1. pneumocystis pneumonia
2. toxoplasma encefalitis
3. esofageal, tracheal, bronchial or lung candidiasis
4. **Chronic anal herpes simplex or herpetic bronchitis, pneumonia or esofagitis**
5. **CMV retinitis**
6. **generalized CMV infevtion (excluding liver and spleen)**
7. **progresive multifocal leukoencefalopatia**
8. repeating salmonela bacteriemia
9. repeating pneumonia within 1 year
10. chronic intestinal cryptosporidiosis
11. chronic intestinal isosporosa
12. extrapulmonary cryptococcus infection
13. Disseminated or extrapulmonary histoplasmosis
14. disseminated coccidioidomycosis
15. tuberkulosis
16. disseminated or extrapulmonary atypic mycobacteriosis
17. **Kaposhi sarkoma**
18. **malignant lymfoma (Burkitt‘s lymfoma, imunoblastic and primary cerebelar lymfoma)**
19. Invasi carcinoma of cervix
20. HIV encefalopatia
21. wasting syndrom


HSV1 Replication Cycle
HSV1
gC
gD
Proteoglycan
HVEM
Uncoating
αTIF
Microtuble
Viral DNA
Circular DNA
Oct
TAATGARAT
αTIF
RNA Pol-II
Sp1
TBC
TATA
CTF
Pre-initiation Complex
α-mRNA
IMMEDIATE EARLY
α-Proteins
α4
Sp1
TBC
RNA Pol-II
TATA
Pre-initiation Complex
β-mRNA
EARLY
β-Proteins
α4
α4
α4
RNA Pol-II
DBF
TBC
DAS
TATA
Pre-initiation Complex
Structural mRNA
Capsid Assembly
LATE
Structural Proteins
γ-Proteins
Glycosylation
Capsid Associates with αTIF and Vhs Proteins
Release
Migration of Infectious Virion
Capsid Buds into Exocytotic Vesicle
Infectious Virion

QIAGEN®


HSV1 Latent Infection
SENSORY NEURON
Some Viral Genome get to CNS through Synapse
NUCLEUS
LATENT PHASE
Reactivation by Anterograde Transport
Establishment of Latency
Productive Infection
Peripheral Tissue
gD
gC
HSV1
Viral Entry
gC
gD
Proteoglycan
HVEM
Uncoating
αTIF
Microtubule
Viral DNA
NUCLEUS
Sp1
RNA Pol-II
TBC
TATA
Pre-initiation Complex
LAT mRNA
2Kb Stable Intron
LAT Proteins
?
Reactivation of Latent Viral Genome
SENSORY GANGLION

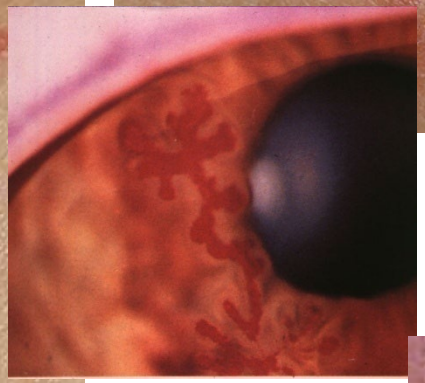
Pathological impact of HSV and VZV

HSV – herpes simplex, benign crbl. ataxia, gingivostomatitis, faryngotonsititis, **encefalitis, pneumonie, hepatitis**

VZV – varicella, herpes zoster, encefalitis, pneumonie, hepatitis

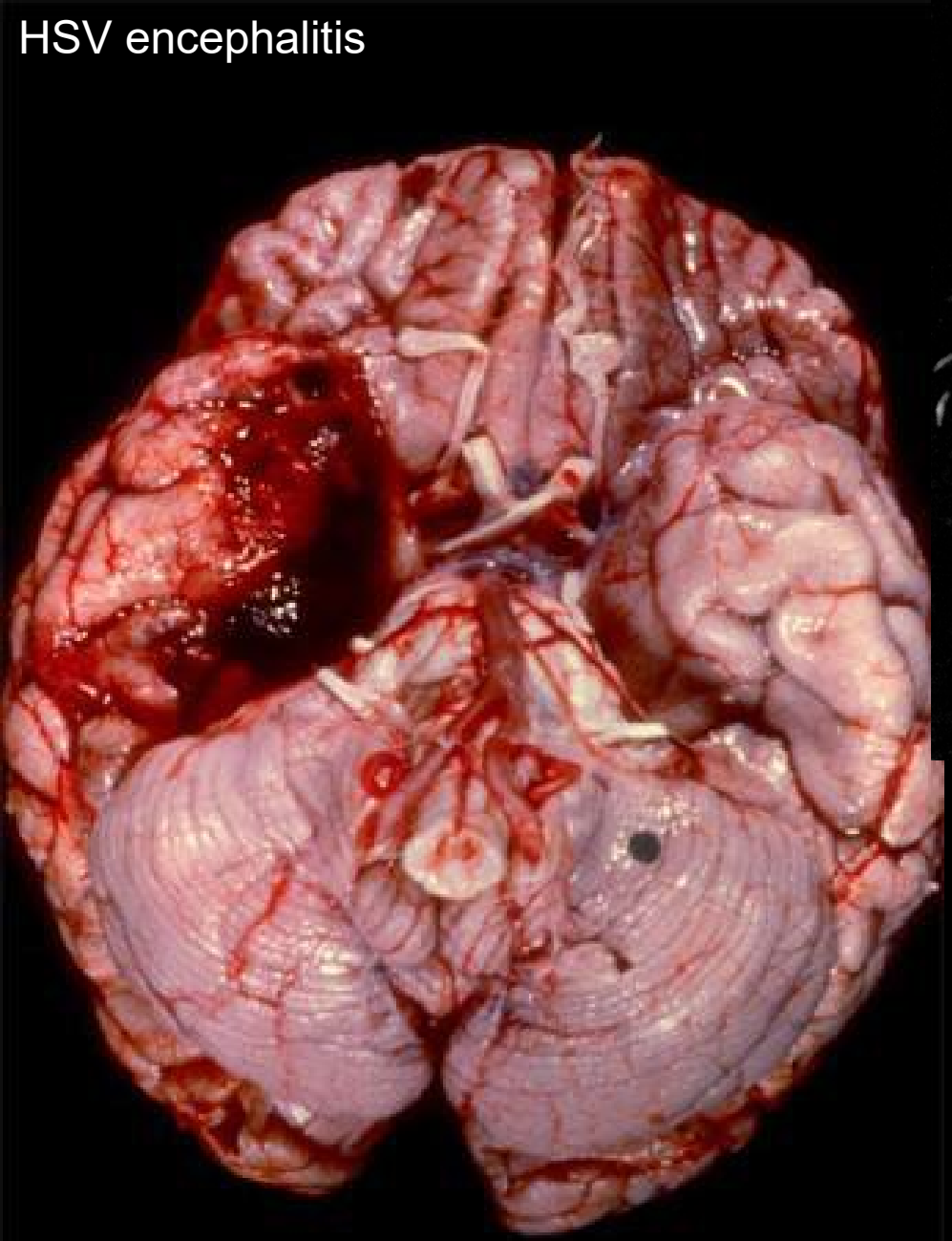
– *In allogeneic HSCT setting less frequently in case of acyclovir prophylaxis; reactiovation of HSV without ACV prophylaxis in 80% of patients*

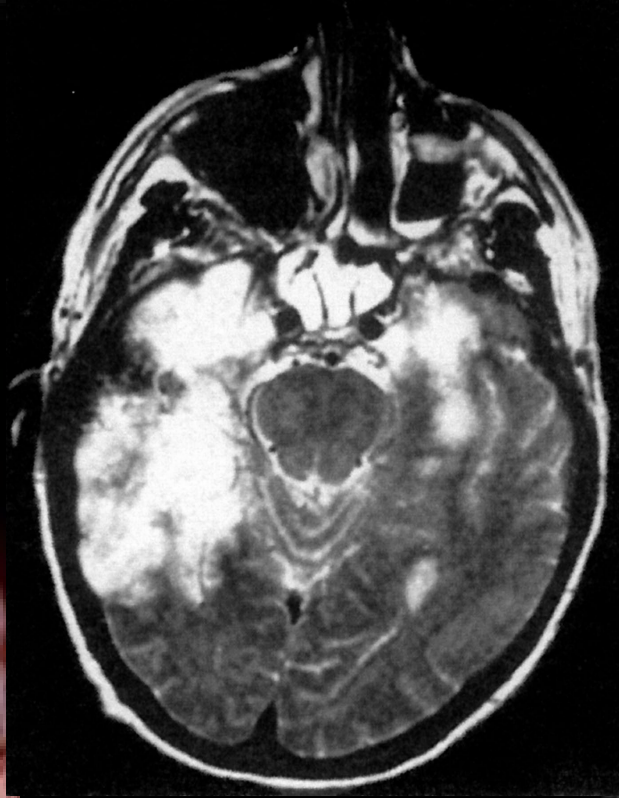
Varicella – chicken pox

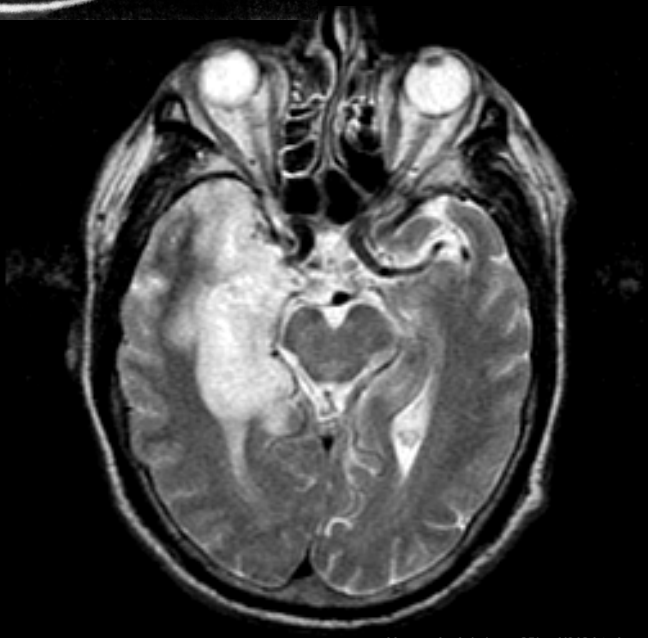


www.hiv-aids.cz

HSV encephalitis



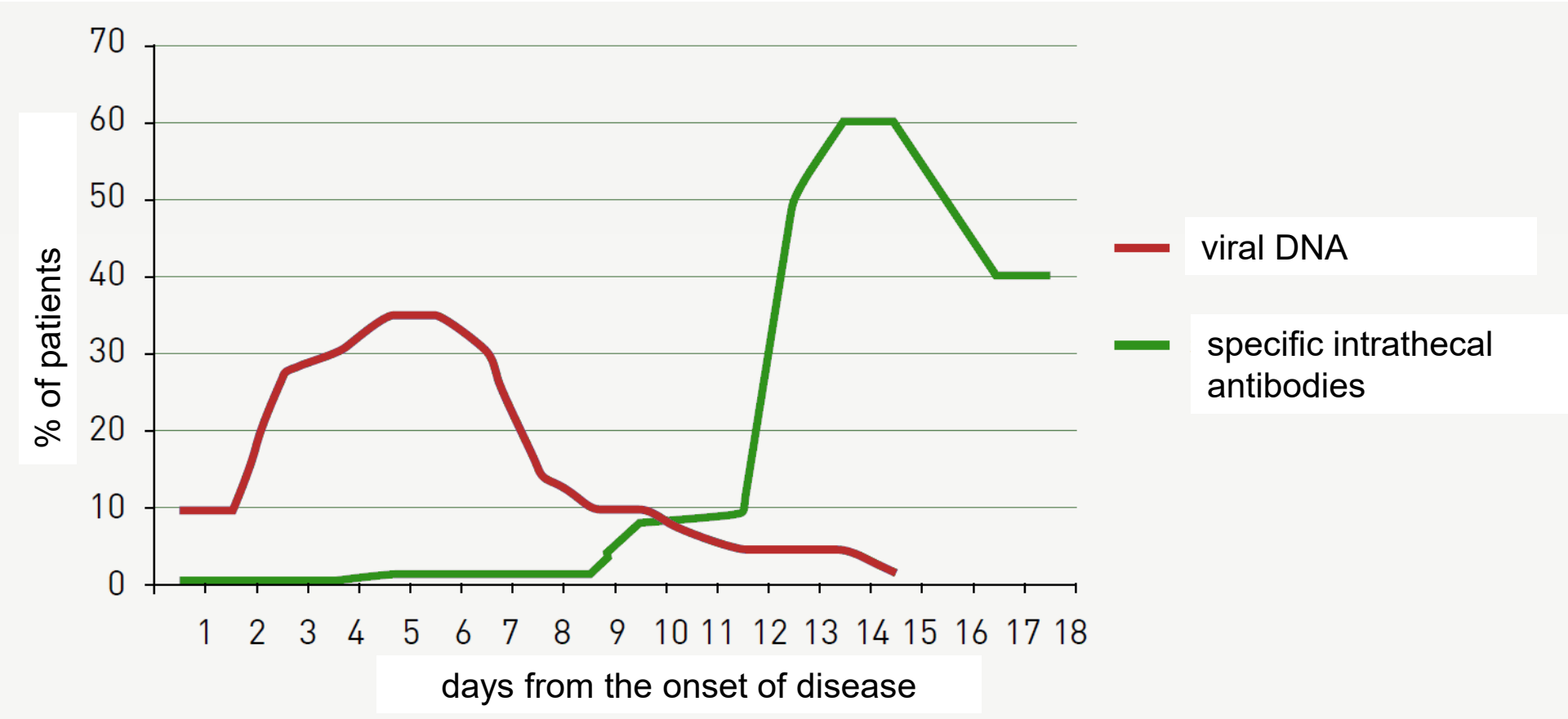


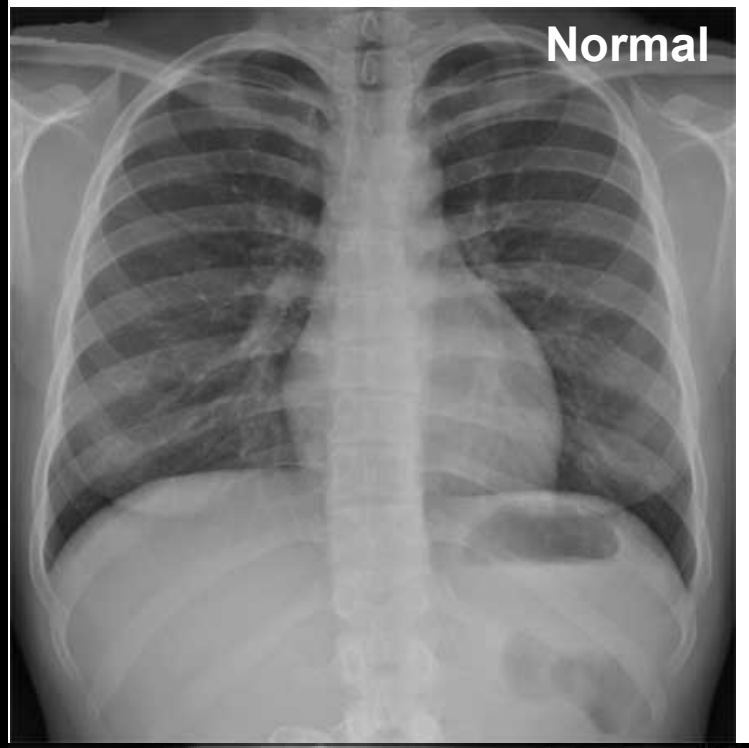


Incidence : 1990-2001: 2,2/1 000 000 citizens

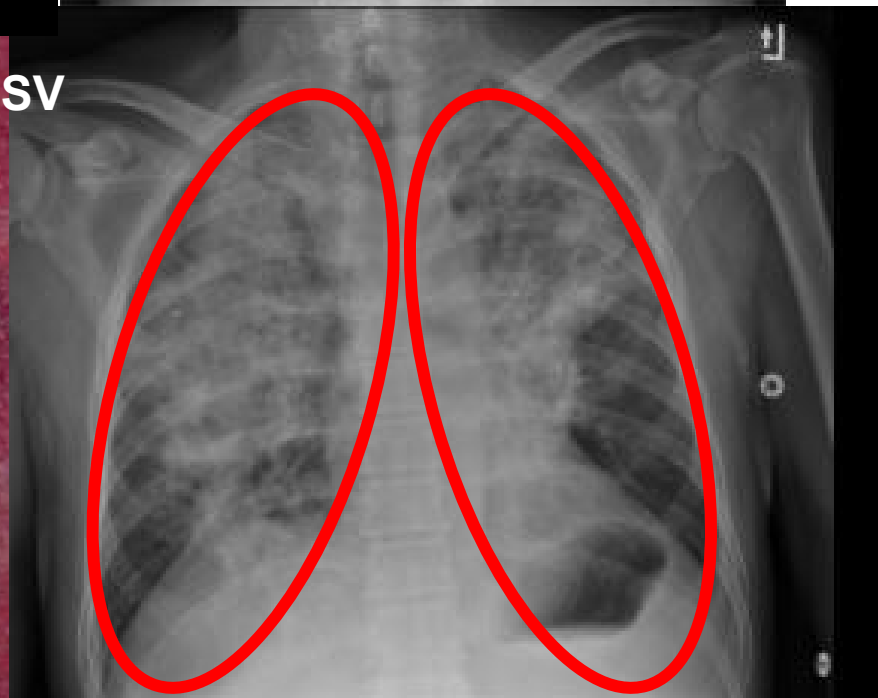
www.med.harvard.edu/.../ case25/mr1/012.html

Antibody response to viral infection and detection of virus in CSF



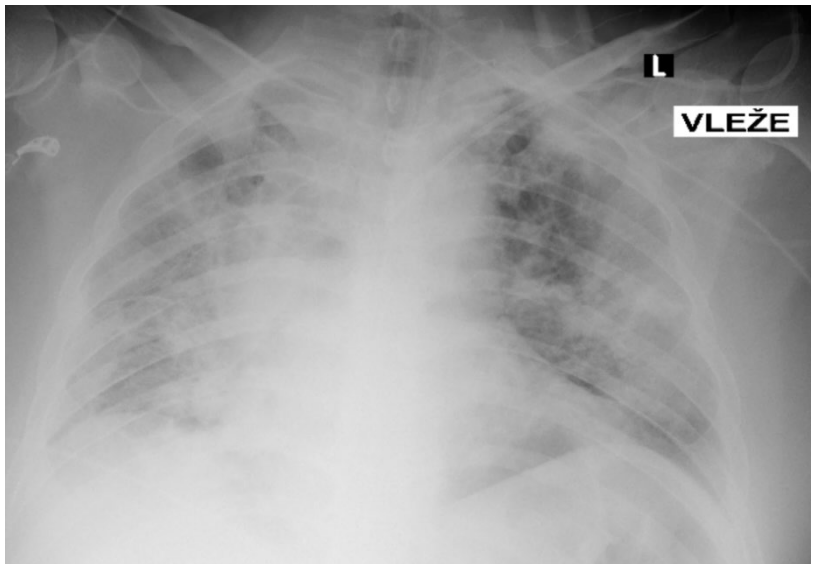


HSV

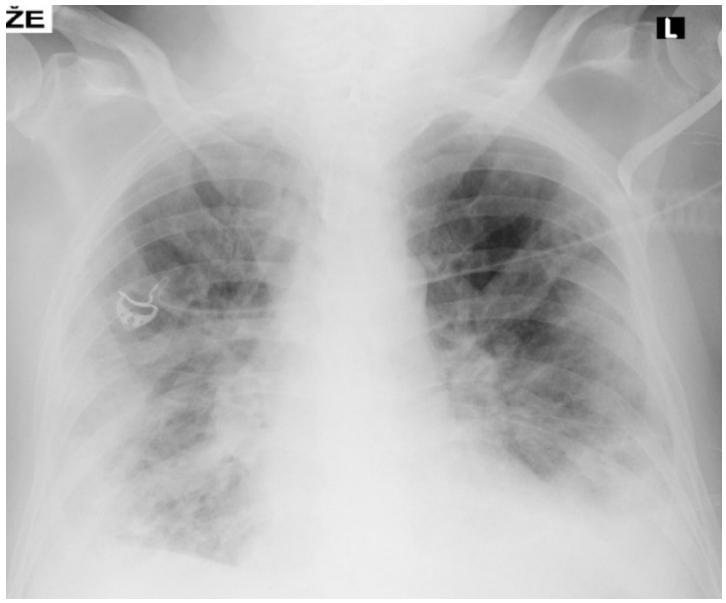


HSV in critically ill patients – X-ray

COVID-19

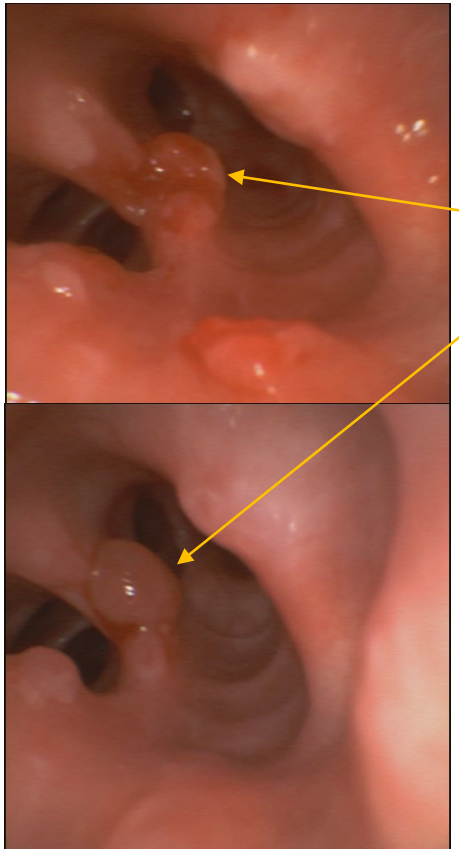


HSV



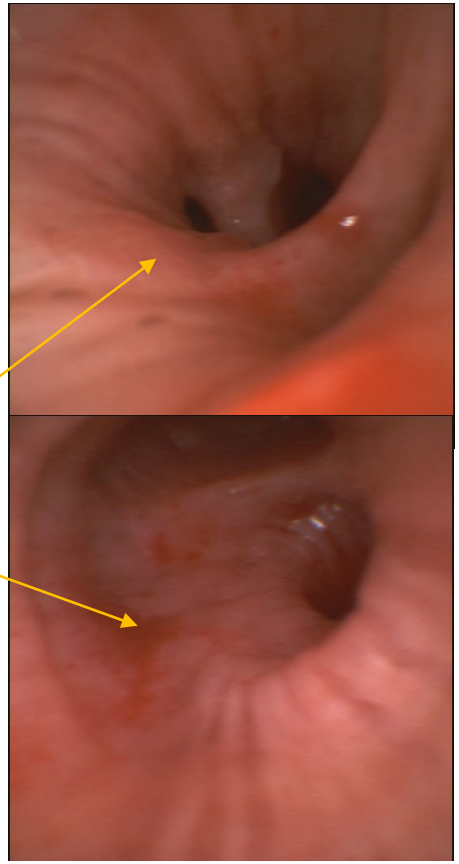
CAVE Herpes sine herpes.

HSV in critically ill patients -
Bronchoscopic picture



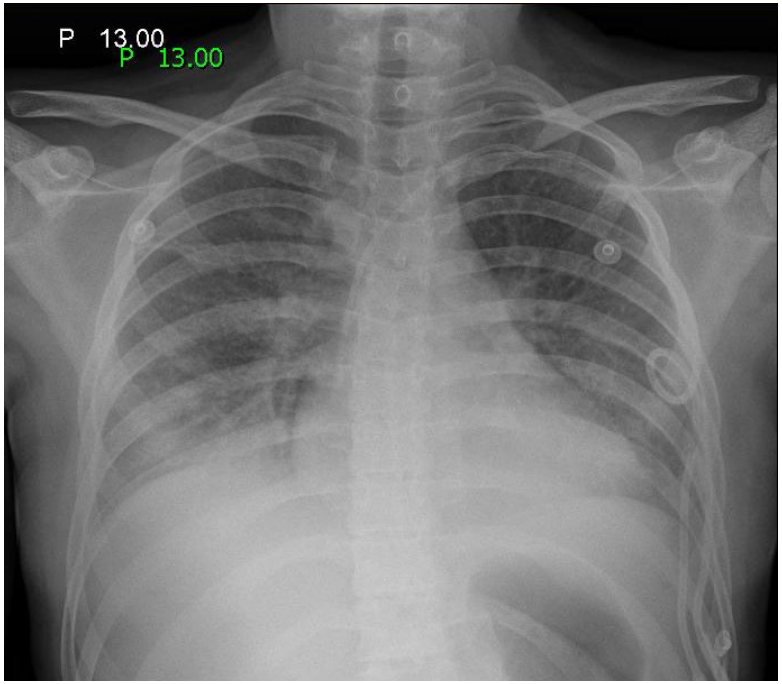
Sometimes herpetiformic vesicles

Frequently oedematic, fragile, swollen mucous with tendency to bleeding.

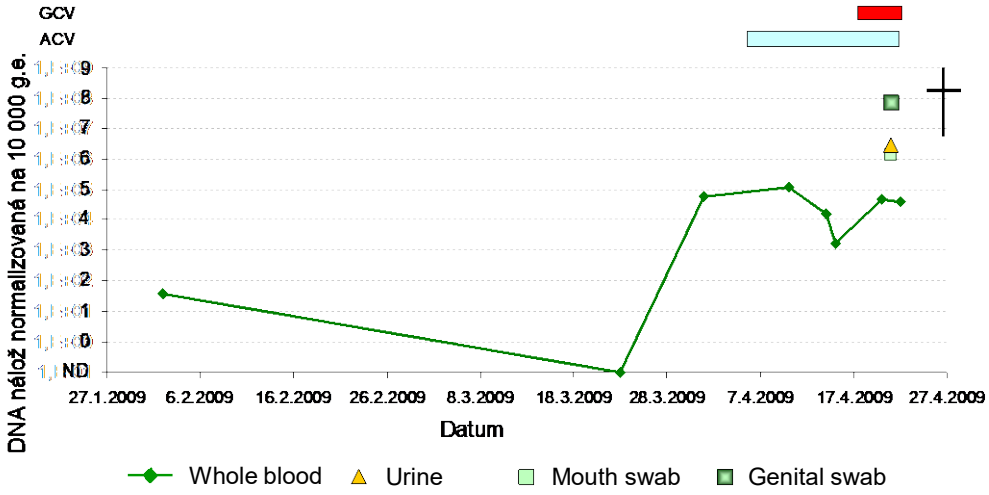


Different impact and destruction in different organs

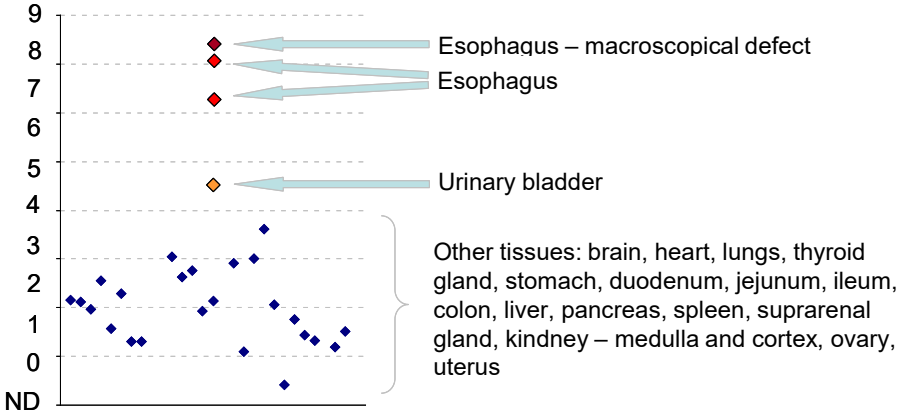
HSV (girl treated for ALL)



HSV pneumonia

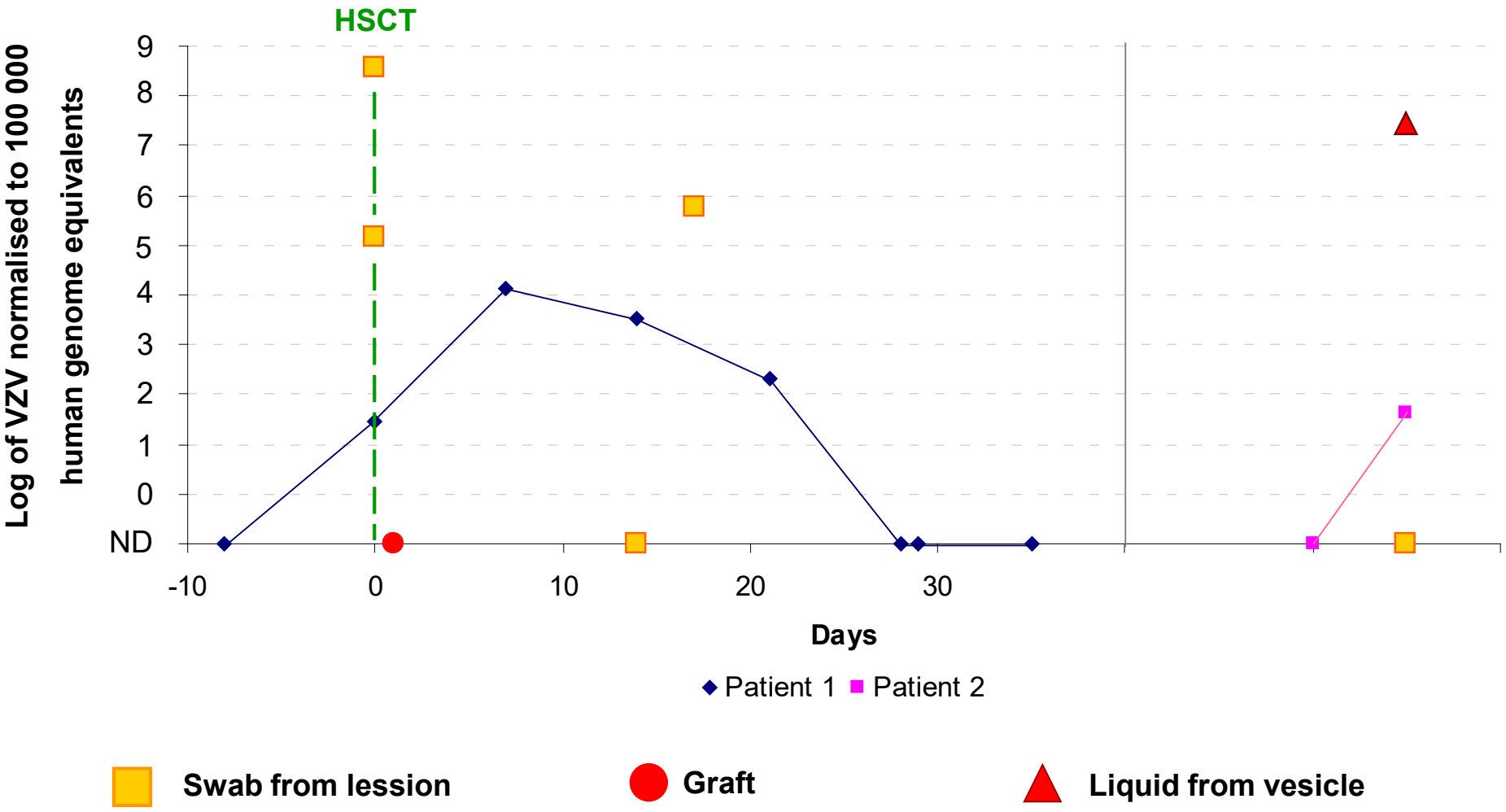


HSV quantity detected in the tissue at the authopsy

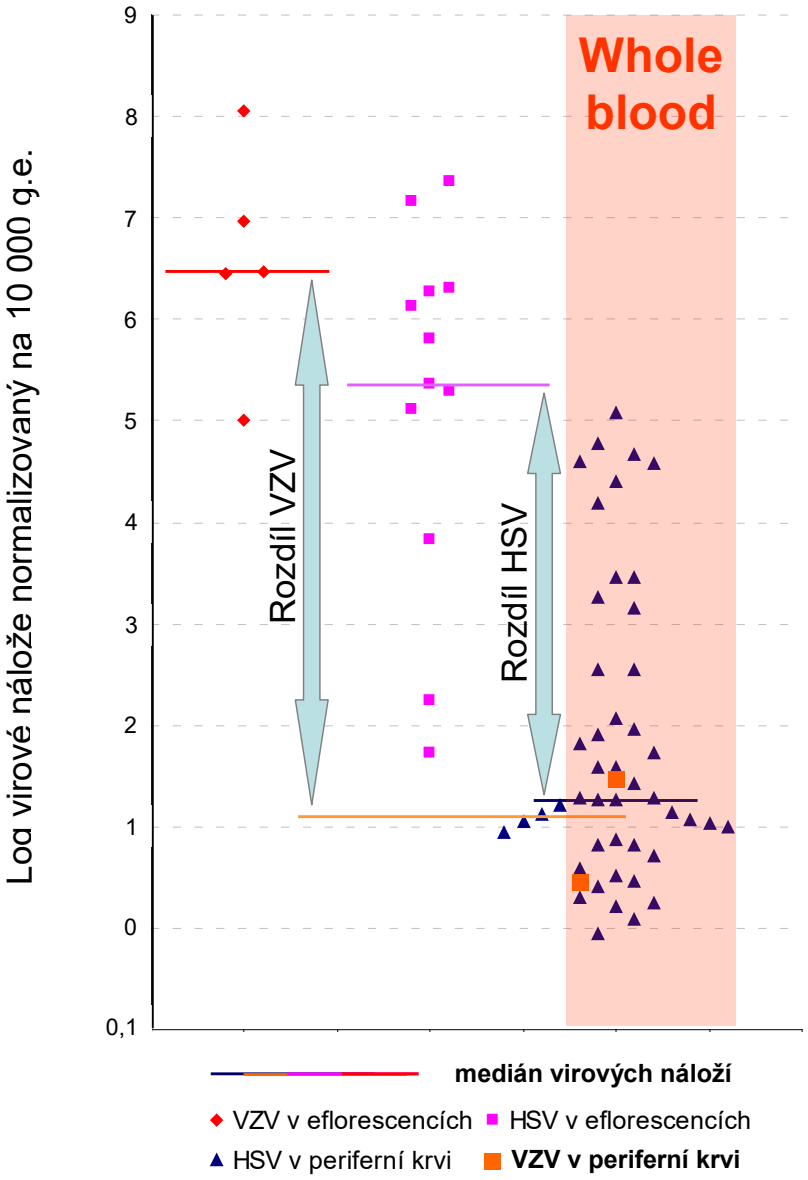


Difference in materials

VZV – chicken pox at D+0



Source for viral detection



January 2004 to August 2011

- **HSV in 735 samples from 266 patients**
  VZV in 587 samples from 148 patients
- **569 whole blood samples**
- **43 swab samples from skin, mucousal tissue and aspirates from vesicles (from 15 p.)**
- **227 samples from other biological materials (stool, urine, CSF, tissues)**

HSV detected

- **in 12 samples from eflorescence from 9 pts; medián of quantity 439,465 NVC**
  (range 53-23,380,000 NVC)
- **6 pts in whole blood samples; median of viral load 18.7 NVC**
  (range 0.88 – 1,216,650 NVC)
- **4 in stool with median 53,662 NVC**
  (range 1,248-900,000 NVC)

VZV detected

- **in 8 samples from skin eruption from 5 pts; median of quantity 2,856,124 NVC**
  (range 13,939-114,464,380 NVC)
- **in 2 pts. In whole blood (quantity 30 and 2.9 NVC)**

viruses

VZV and organ involvement

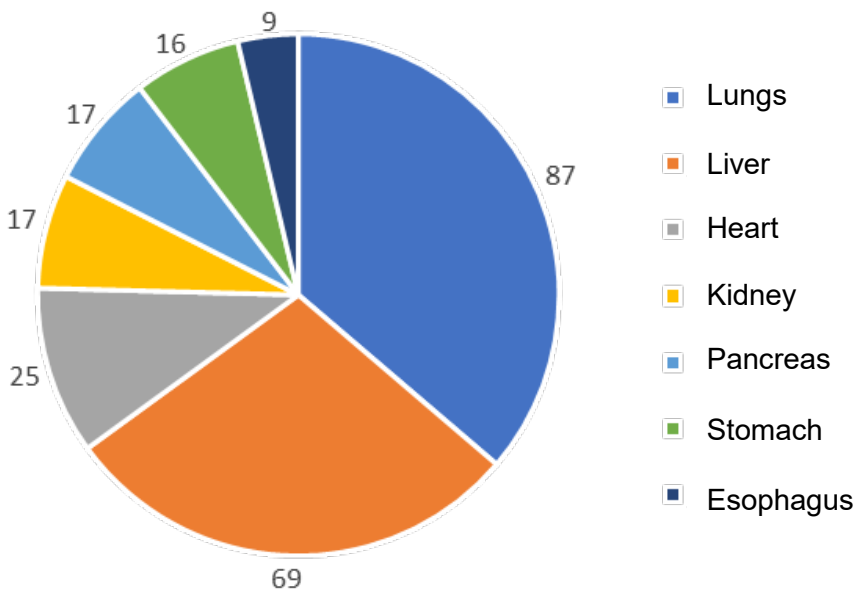
MDPI

Article

Disseminated Varicella-Zoster Virus Infection with Internal Organ Involvement: A Scoping Review of 156 Cases

Aleksandar Timotijevic [1,2], Pratyusha Kodela [1], Vladislav Glušac [3], Sara Bokonjic [4], Bojan Joksimovic [5,6], Juan Vera Gomez [1,7], David Ladin [1,7] and Igor Dumic [1,7,*]

- review 594 articles - 156 cases
- diagnóza often late due to unspecific signs of infection
- Vesikular signs on skin are missing in about 5% of cases
- mostly men (53.8%), median age 42.3 yrs
- overall mortality 25.0%
- multiple organ involvement in 46.1% of cases
- early diagnosis in 65.4% of patients; associated with survival (p = 0.043).
- mortality was associated with comorbidity (p = 0.004), and autoimune disease with immunosupressive therapy (p = 0.048).



Pathological impact of CMV

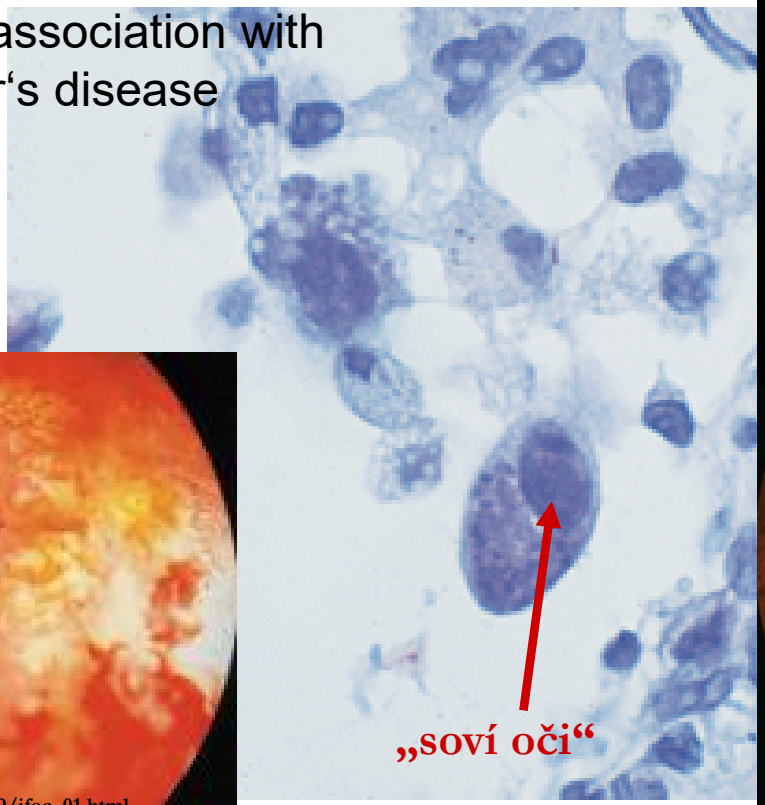
In immunocompetent

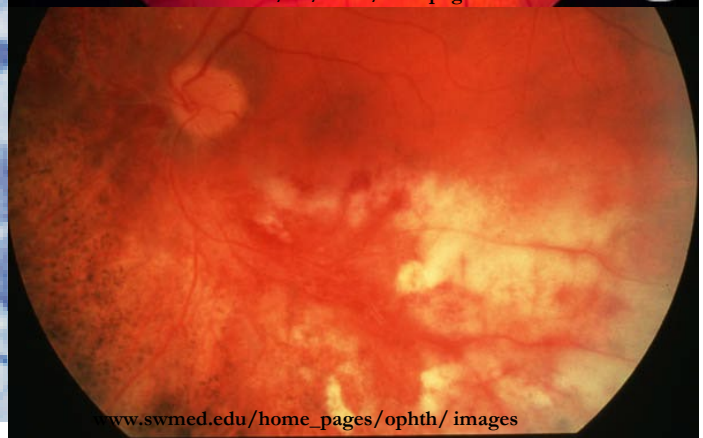
- Asymptomatic in 95% of children
- mononukleosis like sy.
- In pregnant woman teratogenic
- Associations with malignant glioma, ca. of breasts
- Possible association with Alzheimer‘s disease

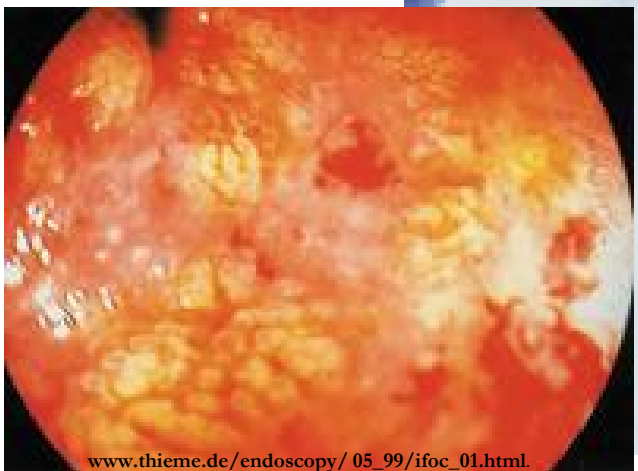
In immunocompromissed

mainly

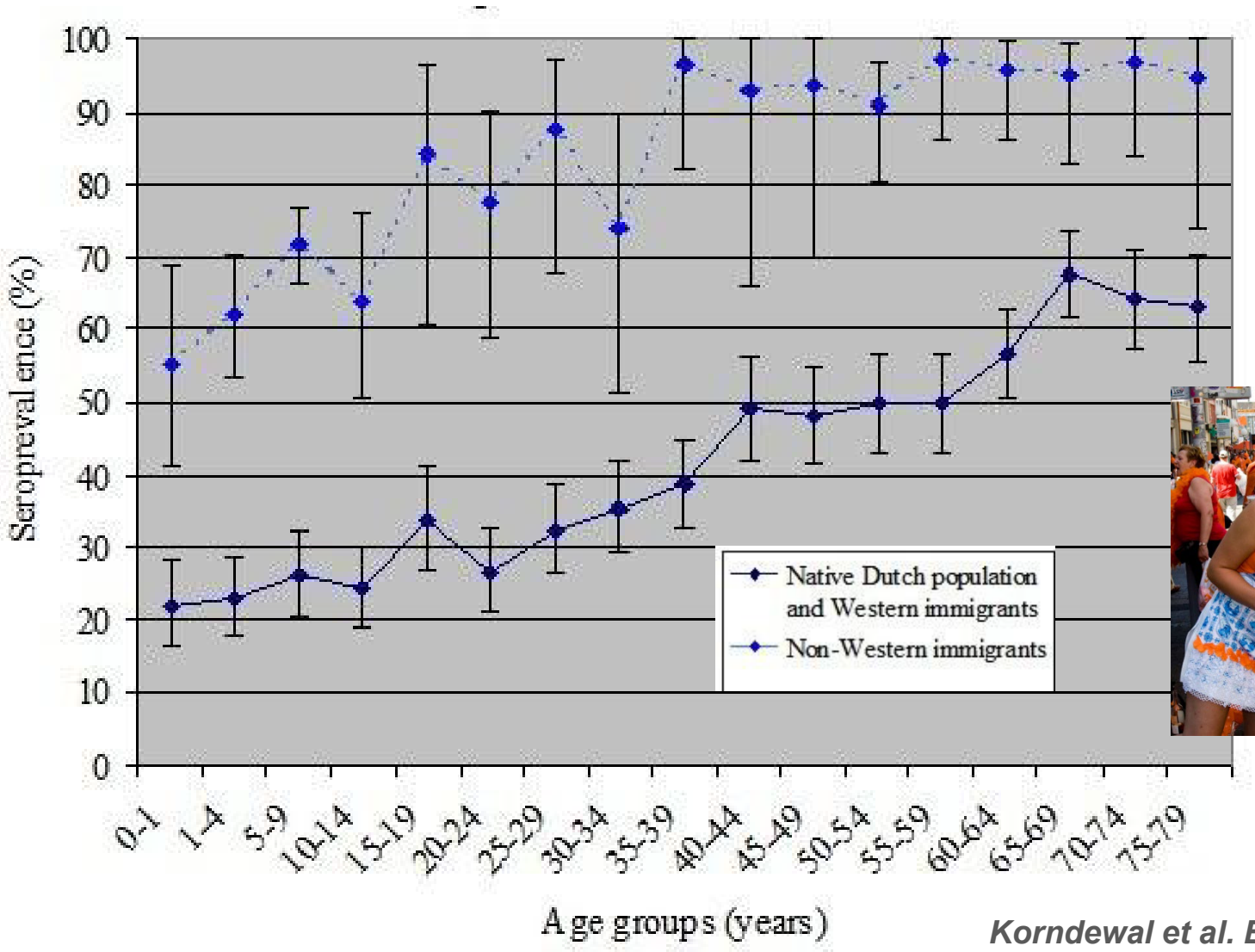
trombocytopenia, pneumonitis, hepatitis, encefalitis, retinitis, colitis, esofagitis, pankreatitis, vasculitis, malaise, vomitting, artralgia, myalgia







CMV seroprevalence

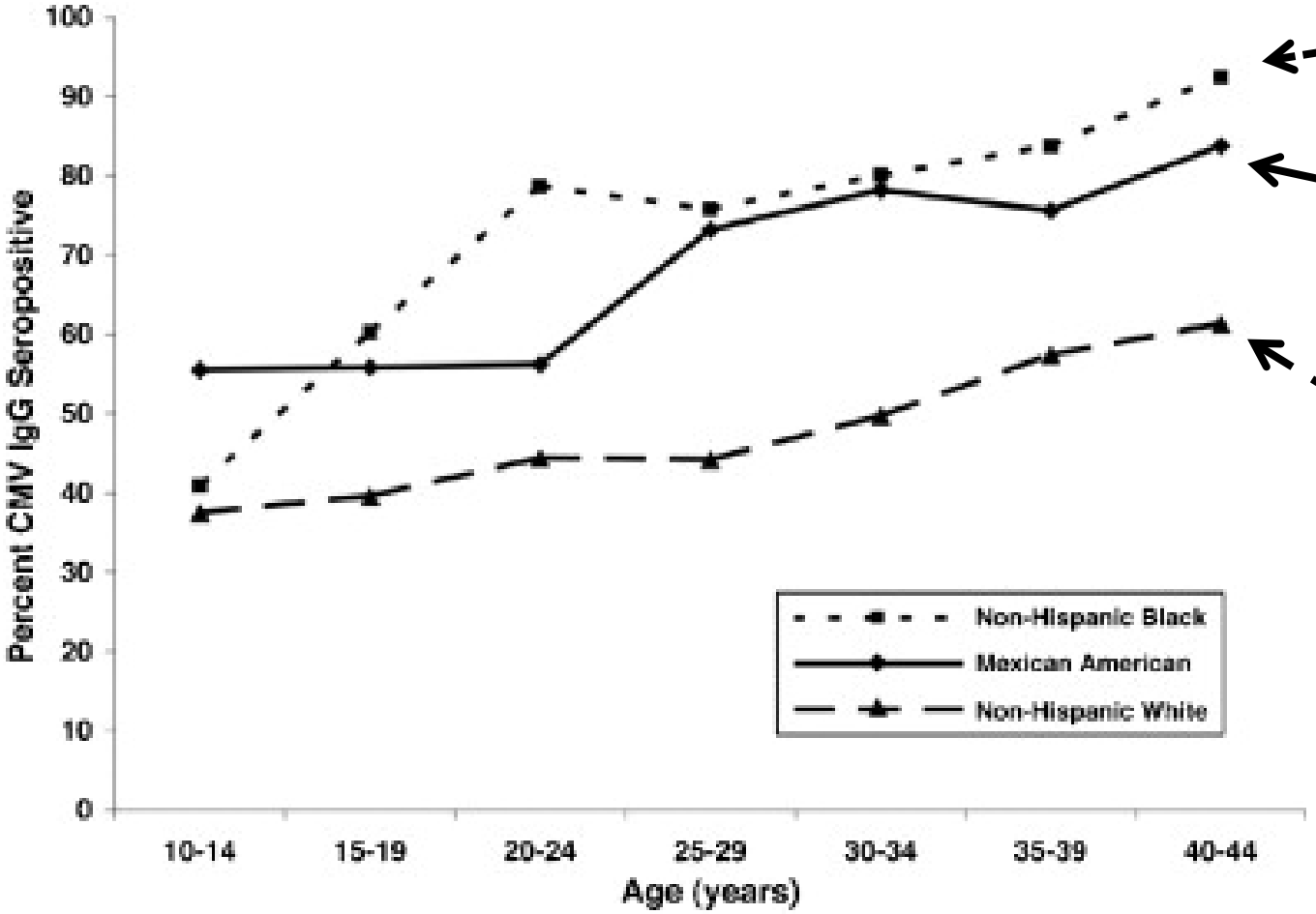


Korndewal et al. European Society for Paediatric Infectious Disease 2012

https://www.abstractserver.com/ESPID2012/pictures/p_435_00079.jpg

CMV seroprevalence

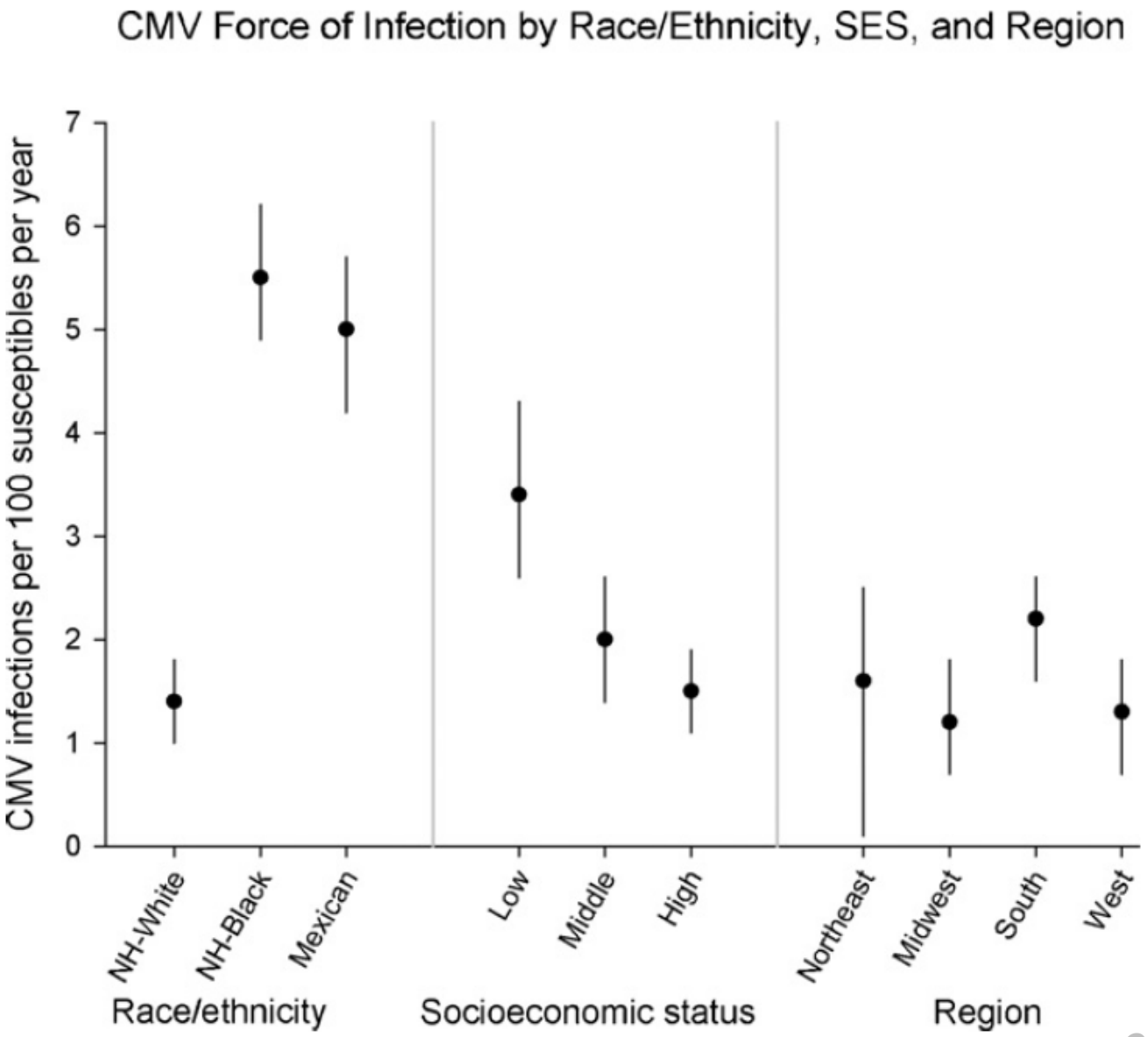
- 60-90% of healthy adult population http://www.tulane.edu/~dmsander/garryfavweb.html
- increases with age and decrease in developed countries



Cannon JCV 2009

https://encrypted-tbn3.gstatic.com/images?q=tbn:ANd9GcRpA_jZXafN6tARnBRXk6Mh32MDm7OAdNLwYoi2X8kgQF7gLtHzgg

Incidence of CMV primoinfection

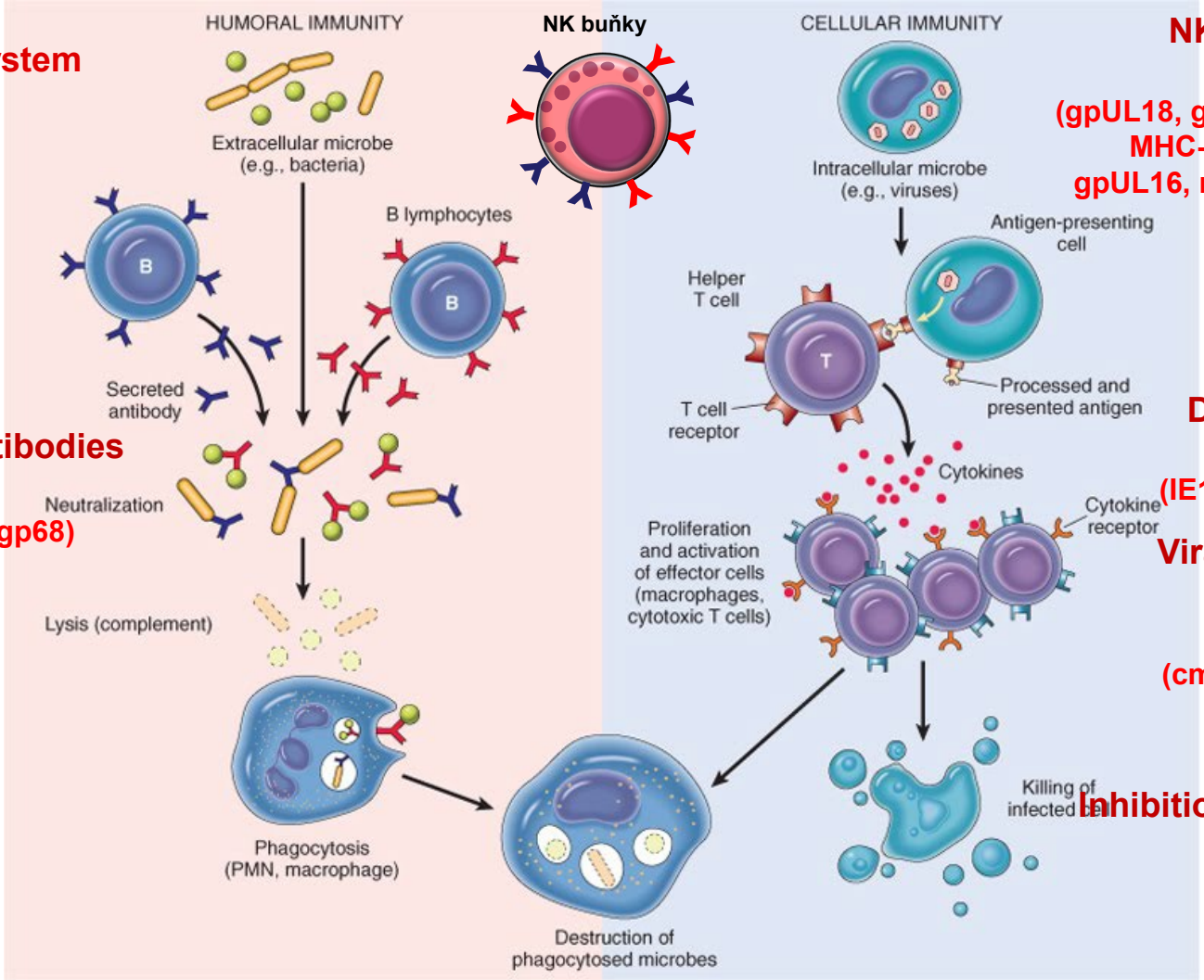


Cannon JCV 2009

How CMV manipulates with immunity?

Complement system inhibition (?)

Inhibition of antibodies by FcR homolog (gp34, gp68)



© Elsevier. Kumar et al: Robbins Basic Pathology 8e - www.studentconsult.com

NK cells function inhibition (gpUL18, gpUL142-homolog MHC-I, pp65, gpUL141, gpUL16, miR-UL112, UL40)

Decrease of INF production (IE1-p72, IE86, pp65)

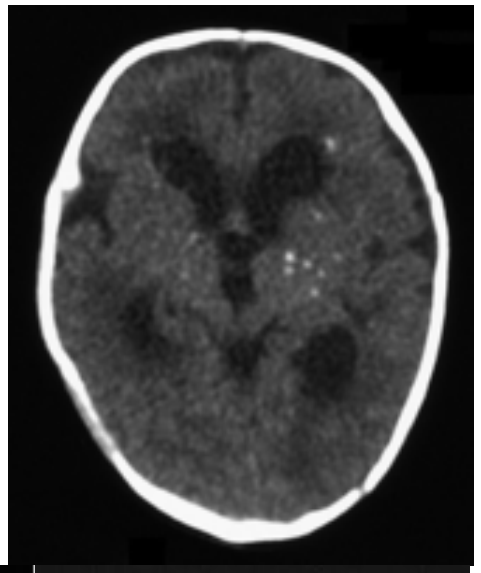
Viral homologs of cytokines and chemokines (cmvIL-10, vCXCL-1, pUL147, pUL128-131)

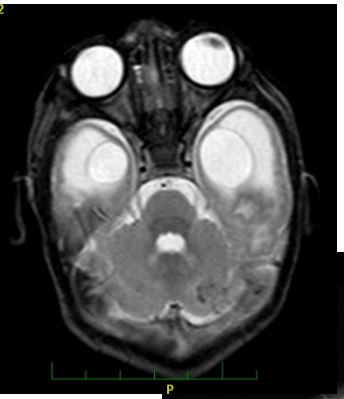
Inhibition of apoptosis (vICA, vMIA)

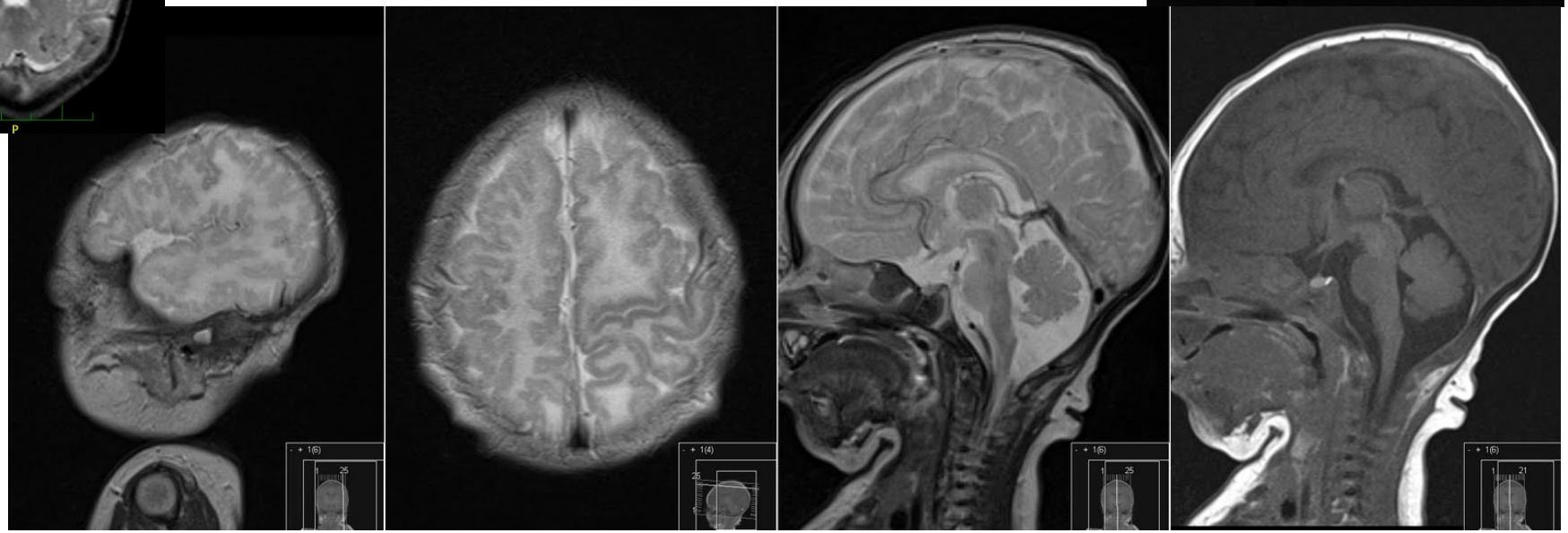
Inhibition of expression and function of MHC-I and II (gpUS2, gpUS3, gpUS6, gpUS8, gpUS10, gpUS11, gp34, gp40,gp48, pp65)

Teratogenic impact of CMV

- In primoinfection in pregnancy or reactivation
  - TORCH (Toxoplasmosis, O – Other infections, Rubella, CMV, HSV-2)
  - Brain destruction, hepatopathy, problems in blood count
  - Cause of sensoneural hearing loss in about 30-50% clinically symptomatic children and 8-12% of asymptomatic children.







Symptoms and impact of cCMV

Placental infection

– swalling of the placenta – worse difussion characteristics

- smaller cotymedon development – smaller placental surface

IUGR

Fetal infection

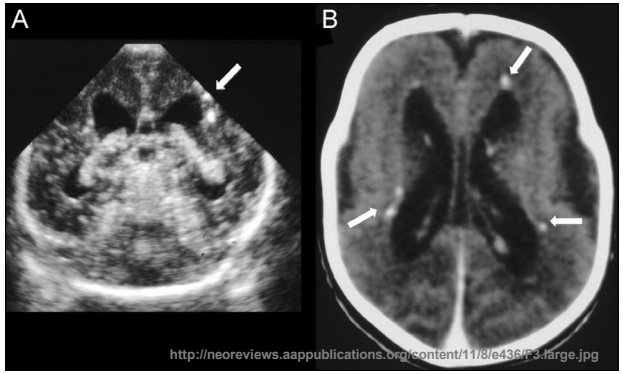
- bone marrow supression **petechia, „blueberry muffin baby“**
- CMV end-organ infection
- vasculitis – especially eyes and a CNS

Neurologic problems/seisures

Brain calcification/ cavity

CMV excretion to urine

Premature delivery



Symptoms and impact of cCMV

Asymptomatic

90% of children with cCMV

Symptomatic

http://medicotrivia.files.wordpress.com/2010/07/blueberry-baby1.jpg

Congenital CMV infection (cCMV)

According to CDC

Symptoms of congenital CMV at delivery

Premature birth
Hepatopathy
Pulmonary signs
Splenomegaly
IUGR
Neurological seizures

Long term effects of cCMV

Sensoneural hearing loss (SNHL)
Visual loss
Mental disorder
Mikrocephaly
Motorical problems (coordination) ce)
Neurological seizures (epilepsy)
Rarely death

Blueberry muffin baby characterized by purpura as a sign of extramedullary hematopoesis.

Symptoms and impact of cCMV

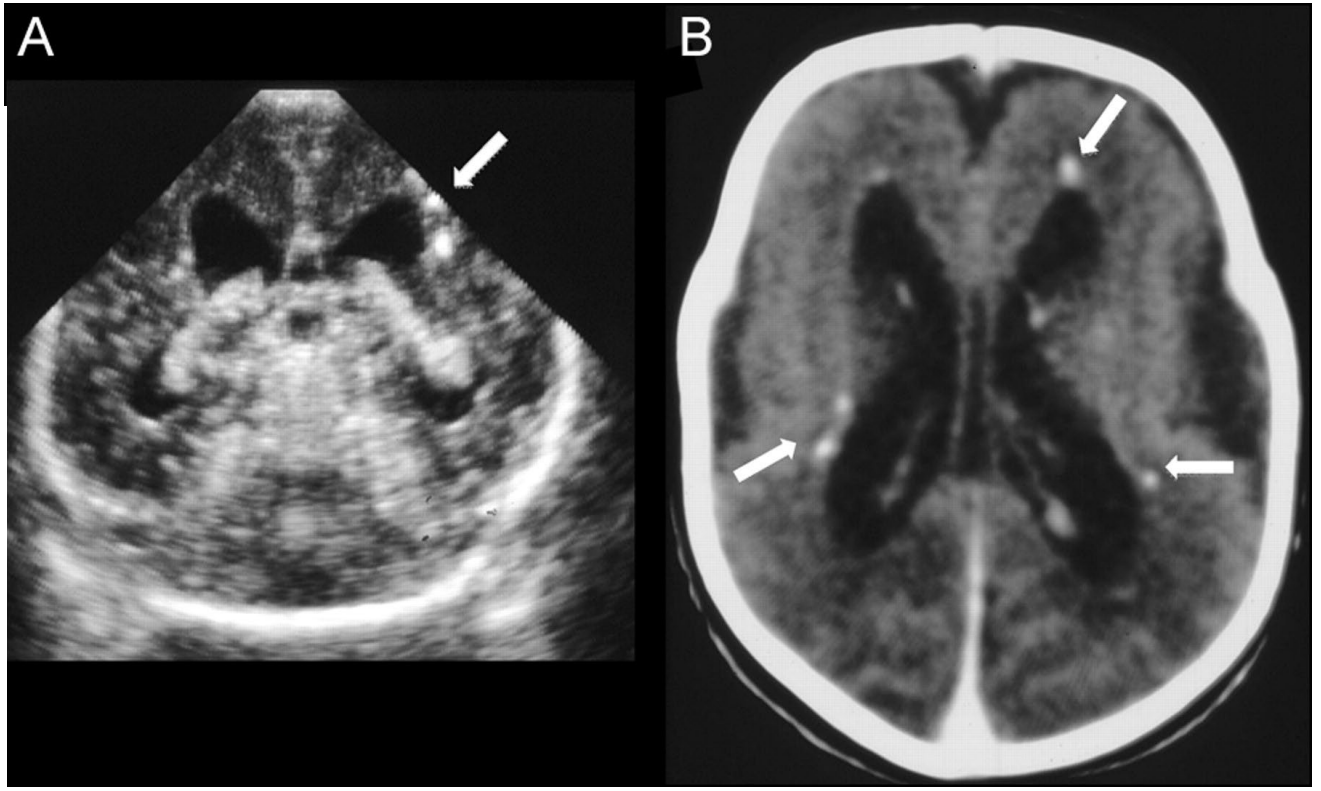
Transient Outcomes

- Hepatomegaly
- Splenomegaly
- Jaundice
- Petechia and purpura
- Pneumonitis
- Fetal growth retardation
- Seizures

Permanent Outcomes

- Microcephaly
- Vision loss
- Hearing loss
- Mental retardation
- Motor disabilities
- Seizures
- Death

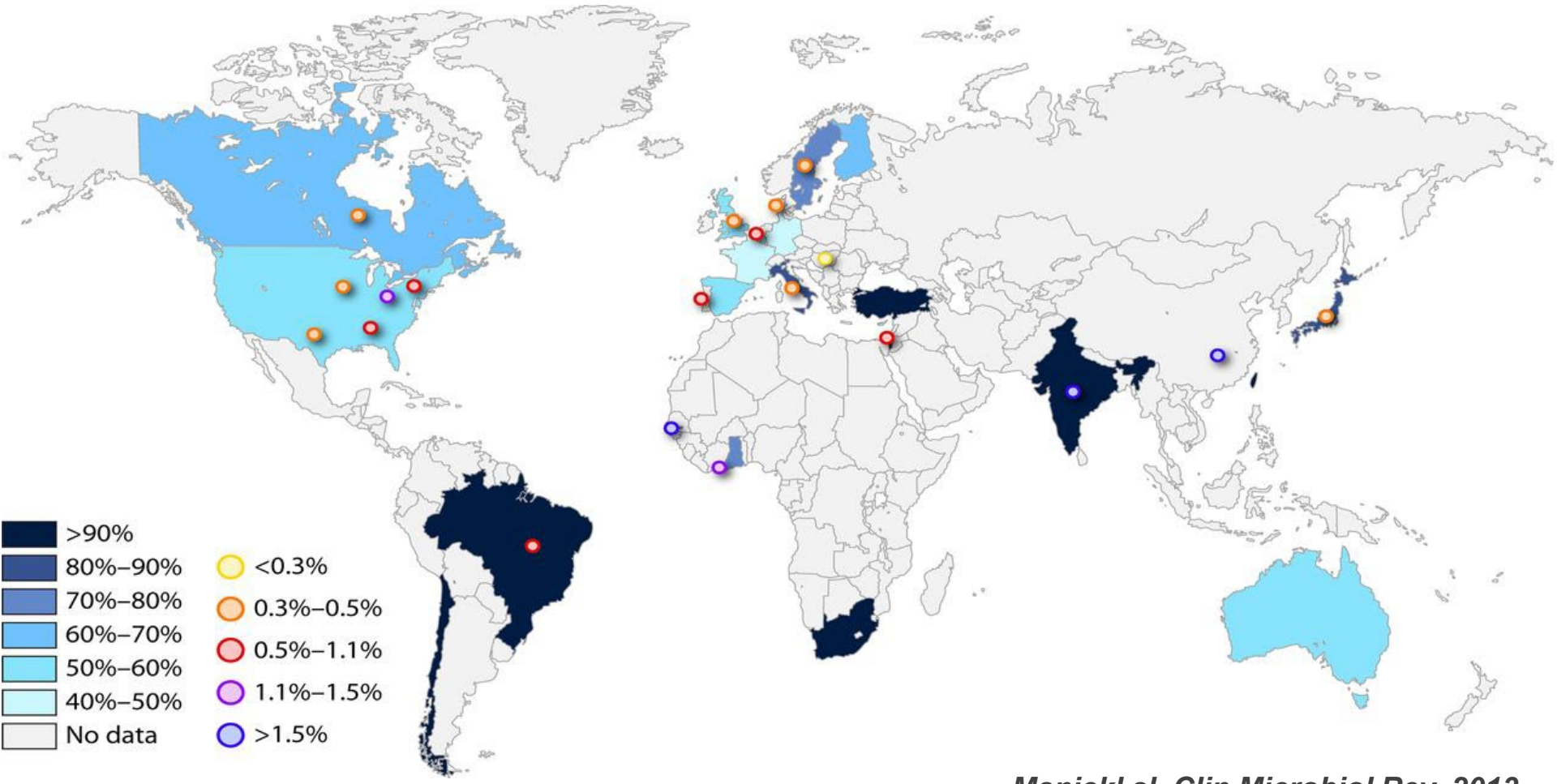
Brain calcification



http://neoreviews.aappublications.org/content/11/8/e436/F3.large.jpg

What is the frequency of cCMV?

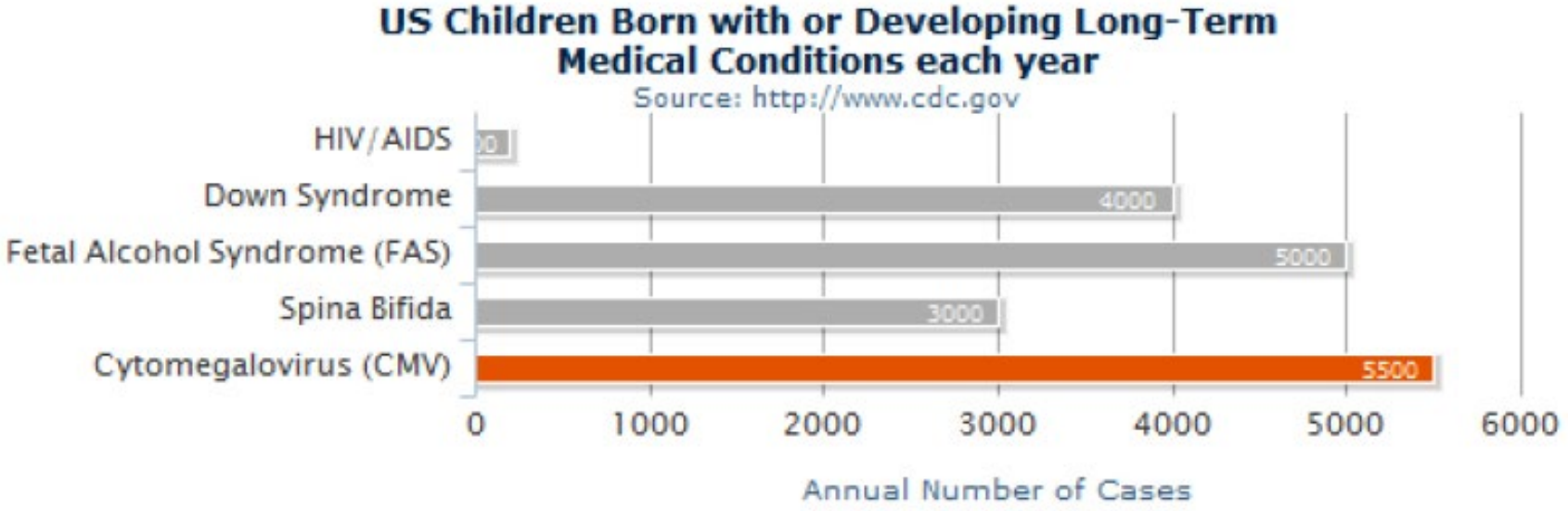
There is 370,000 children born every day in the world, representing 134 millions/year.
Average frequency approx. 1,5% of living birth – 2.01 millions of children with cCMV/year.
In Europe and Czech Republic is estimated frequence 0.5-1% cCMV of living neoborns.

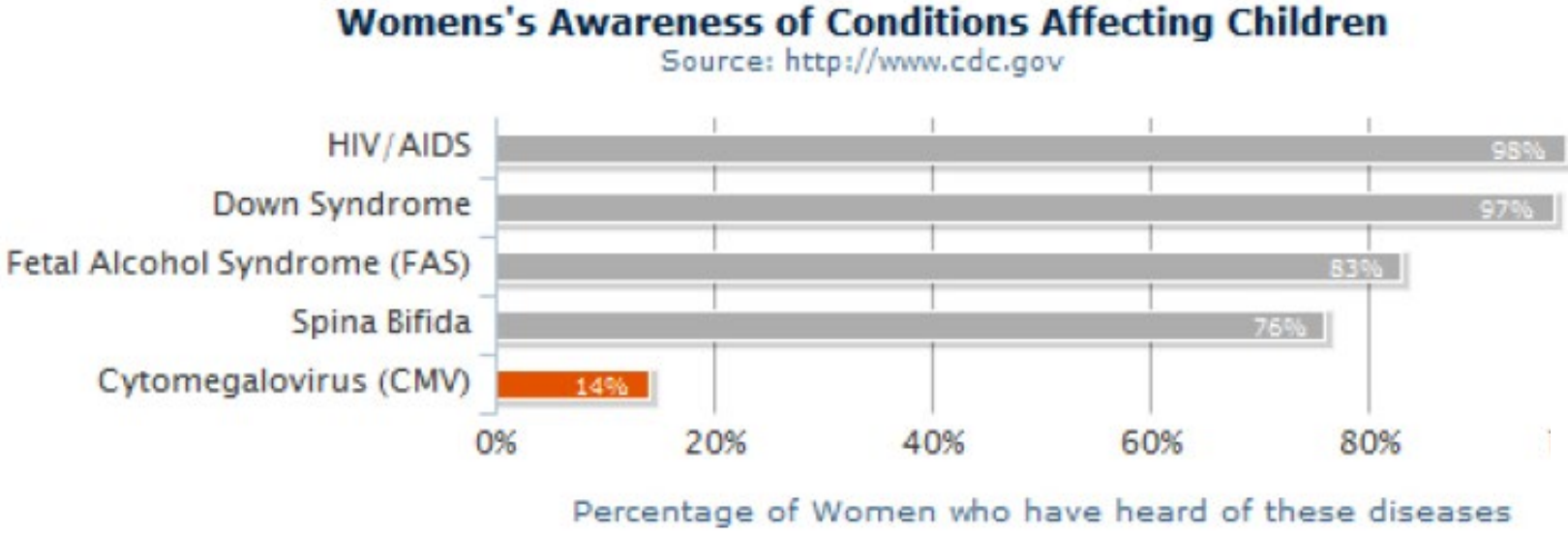


Manickl al. Clin Microbiol Rev. 2013

http://cmr.asm.org/content/26/1/86/F7.large.jpg

What is a knowlegde about cCMV and its impacts?





STOP CMV
The CMV Action Network
www.stopcmv.org

What to do with cCMV? Review

Consensus recommendation for prenatal, neonatal and postnatal management of congenital cytomegalovirus infection from the European congenital infection initiative (ECCI)

Marianne Leruez-Ville,[a,b,u,*] Christos Chatzakis,[c,d,u] Daniele Lilleri,[e,v] Daniel Blazquez-Gamero,[f,v] Ana Alarcon,[g] Nicolas Bourgon,[c] Ina Foulon,[h] Jacques Fourgeaud,[a,b] Anna Gonce,[i] Christine E. Jones,[j] Paul Klapper,[k] André Krom,[l] Tiziana Lazzarotto,[m,n] Hermione Lyall,[o] Paulo Paixao,[p] Vassiliki Papaevangelou,[q] Elisabeth Puchhammer,[r] George Sourvinos,[s] Pamela Vallely,[k] Yves Ville,[a,c,w] and Ann Vossen[t,w]

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[b]Virology Laboratory, Reference Laboratory for Cytomegalovirus Infections, Hôpital Necker Enfants Malades, GHU Paris Centre, AP-HP, Paris, France
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[k]Microbiology and Virology Unit (EIGen), School of Biological Sciences, University of Manchester, Manchester, M139PT, UK
[l]Department of Medical Ethics and Health Law, Leiden University Medical Center, Leiden, the Netherlands
[m]Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy
[n]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy
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[p]CHRC, NOVA Medical School/Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, 1169-056, Lisbon, Portugal
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[s]Laboratory of Clinical Virology, Medical School, University of Crete, Heraklion, Crete, 71003, Greece
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Patient 1

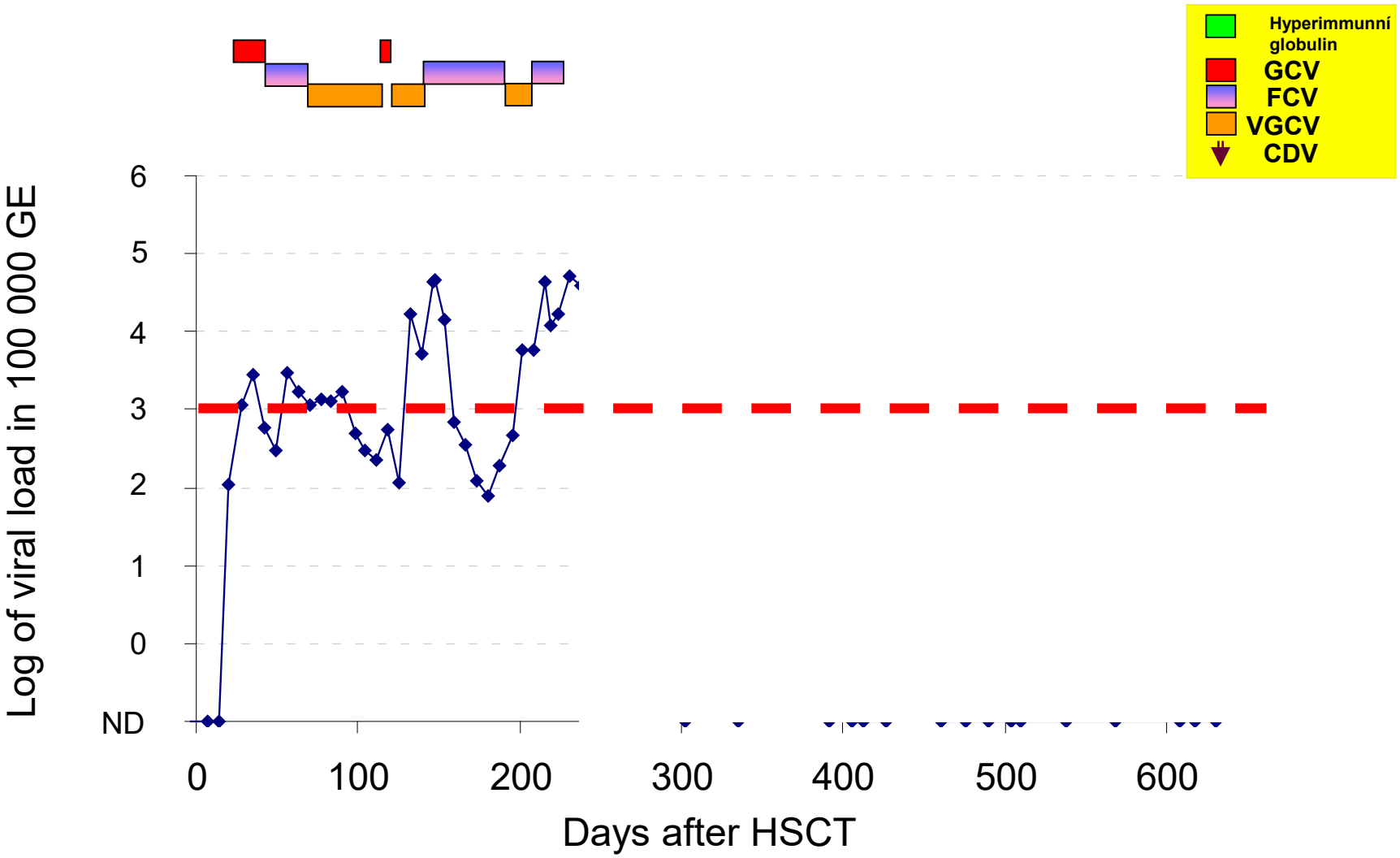
Girl, 16.5 yrs of age at HSCT
Allogeneic HSCT for AML M2 (AML1/ETO+) in 2nd CR
MMUD – 7/10
Conditioning: Busulphan, Cyclofosfamid, Melphalan, ATG
Graft: Periferal stem cells
CD34+: 11,12 x 10^6 /kg; CD3+: 302,1 x 10^6 /kg; NC: 12,09x10^8 /kg
GvHD profylaxis: MTX and CsA
CMV status donor/recipient: D-/R+

Non-CMV complications:

D+16 haemorrhagic cystitis –hyperhydration
D+61 –GvHD grade II (skin and GIT)
therapy : steroids 1 mg/kg
D+377 – Herpes zoster – acyclovir treatment
D+440 – Laser coagulation of retinal bleeding
(not proven, suspected, active CMV retinitis)

Patient 1

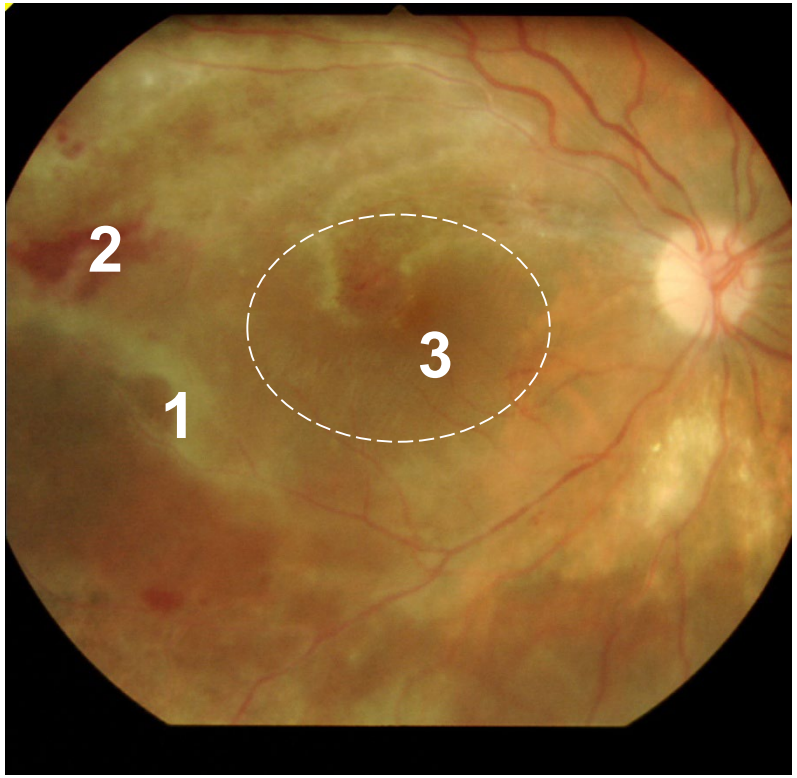
D+ 29 – first CMV treatment



D+230 – during foscarnet treatment patient presented diplopy, headache, vomitting and sleepness.

CMV detected in CSF (approx. 2 600 000 copies / ml) and increase of viral load in peripheral blood.

Results confirmed **encephalitis and** bilateral **chorioretinitis**.

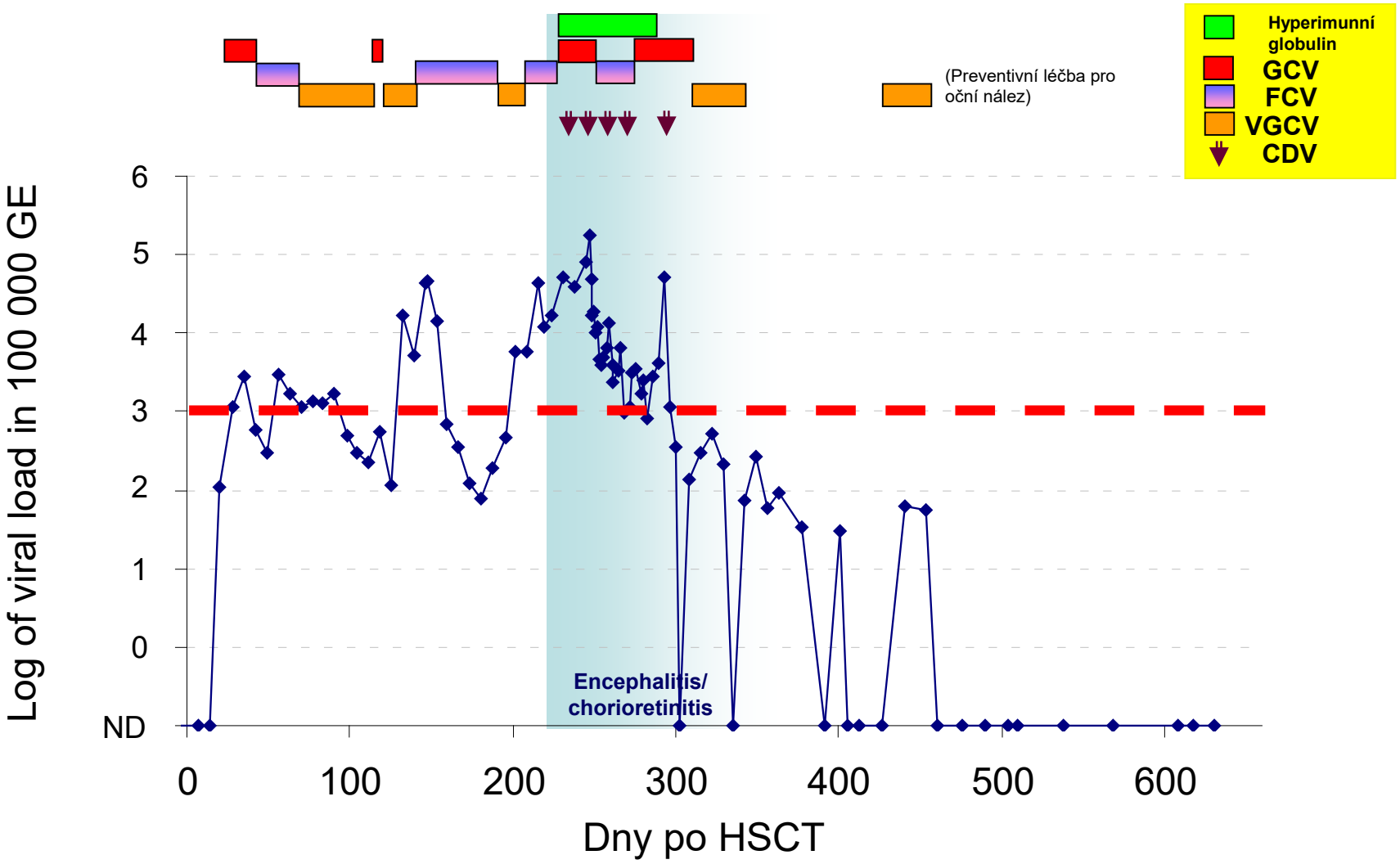


1 – retinal fibrotisation

2 – intraretinal bleeding

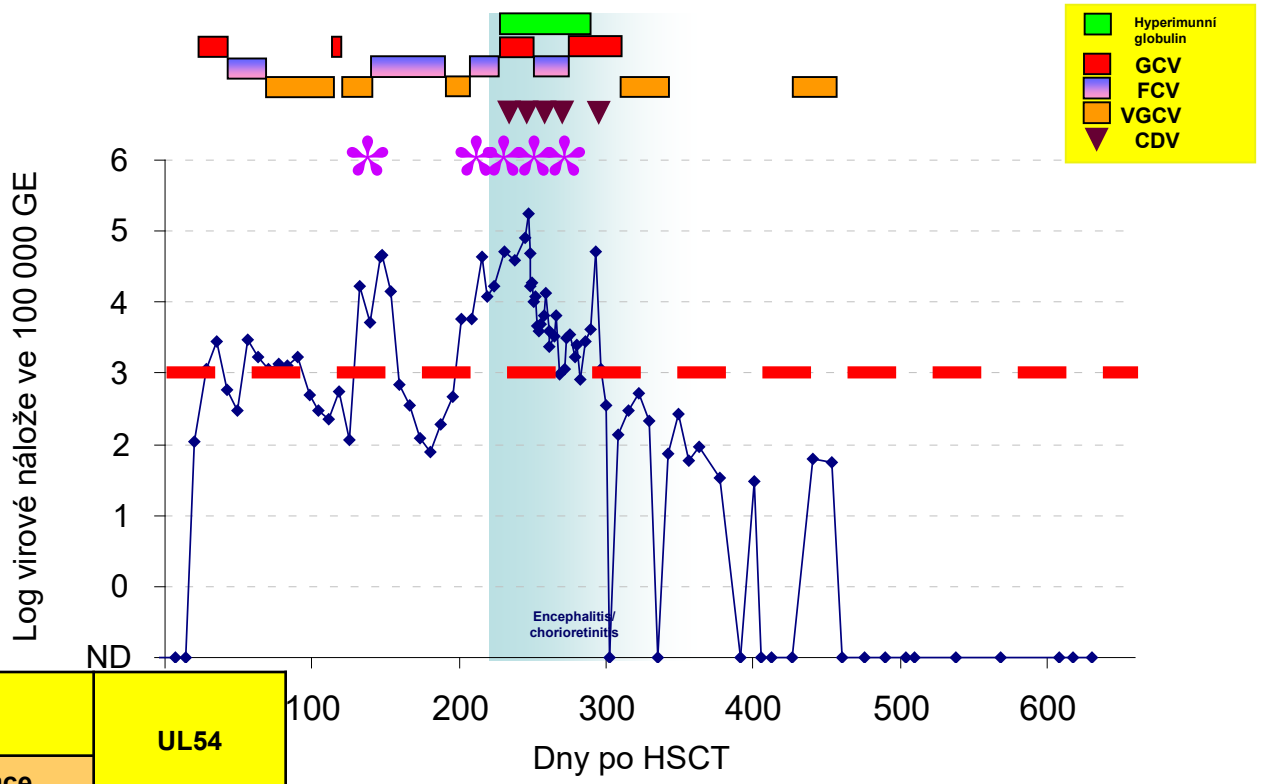
3- epiretinal pseudomembrane

Patient 1


Hyperimunní globulin
GCV
FCV
VGCV
CDV
(Preventivní léčba pro oční nález)
Log of viral load in 100 000 GE
6
5
4
3
2
1
0
ND
Encephalitis/ chorioretinitis
0
100
200
300
400
500
600
Dny po HSCT

Patient 1

Ganciclovir resistance

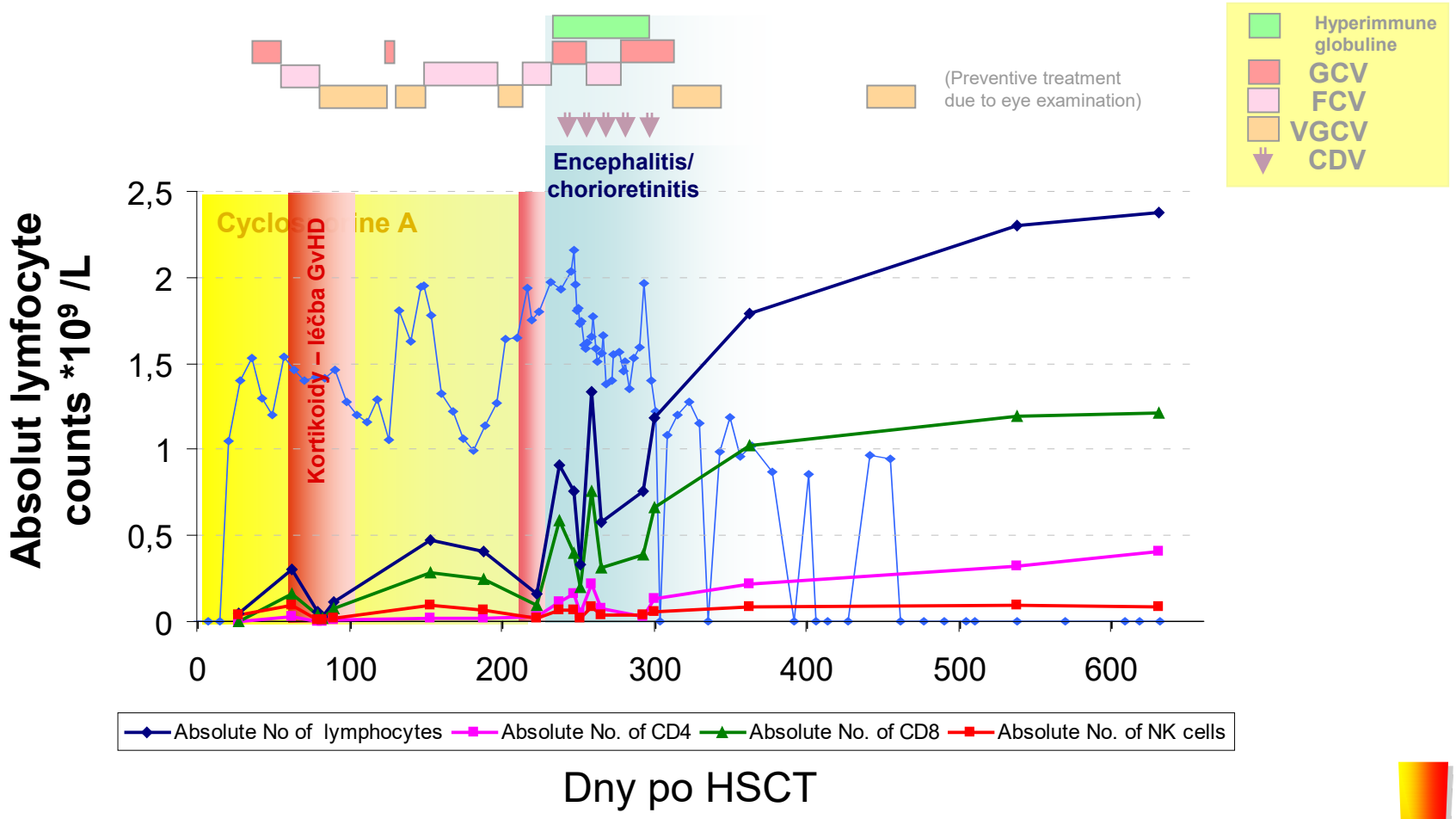


Den	Materiál	Gen UL97		UL54
		Mutace A594V	Mutace L595S	
146	*Krev*	WT+mutation	WT+mutation	ND
209	*Krev*	WT	Mutatiom	ND
231	*Krev*	WT	Mutatiom	ND
234	*CSF*	WT+mutation	Mutatiom	ND
238	*Krev*	ND	ND	Mutatiom
247	*Krev*	ND	ND	Mutatiom
248	*Krev*	WT+mutation	Mutatiom	ND
276	*Plazma*	WT	Mutatiom	ND

Possibility of resistance mutant detection is in case of long-lasted treatment usefull.

Patient 1

Lymfocyte counts



Even a short term steroid treatment leads to decrease of the lymfocyte count neccessary for infection control

Outcome

- Recently the patient is regularly controlled by ophtalmologests. ´Visus in one eye is very limited, however the second eye is healthy. In boths eyes there is limitation of peripheral visus.
- There are no signs of relaps of the primary disease, GvHD and other infections including CMV.

1 – retinal fibrotization

Patological activities of HHV-6

HHV-6

Recently 2 distinct viral species

HHV-6 A

Unknown

„Orphan virus“

Rash during sixth disease

Fitzpatrick´s Dermatology

HHV-6 B

Immunocompetent host

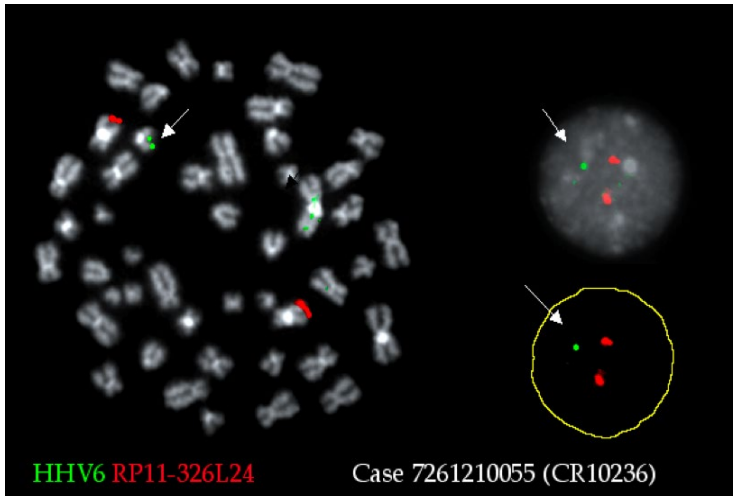
- **Sixth disease**
- **Febrile seizures**
- **Encephalitis**

Immunocompromised host

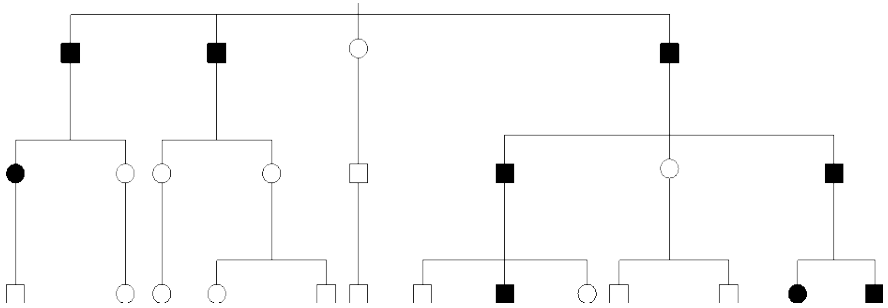
- **Encephalitis**
- Hepatitis
- Pneumonitis
- Pericarditis
- **Delayed engraftment after HSCT**

Chromosomally integrated HHV-6 (CI-HHV-6)

- Viral DNA integrated into human chromosomes
  - Inherited from parents to child
  - Viral DNA is present in every body cell
    (e.g.hair roots, nails)
  - Ratio of viral DNA : human DNA = 1:1
- Described frequency in population between 0.2-2.9% (Tanaka-Taya 2004, Ward 2007)
- Both variants (A or B) integrates
- No clear observed reactivation CI-HHV-6 to active infection in vivo
- In vitro reactivations are doubtful

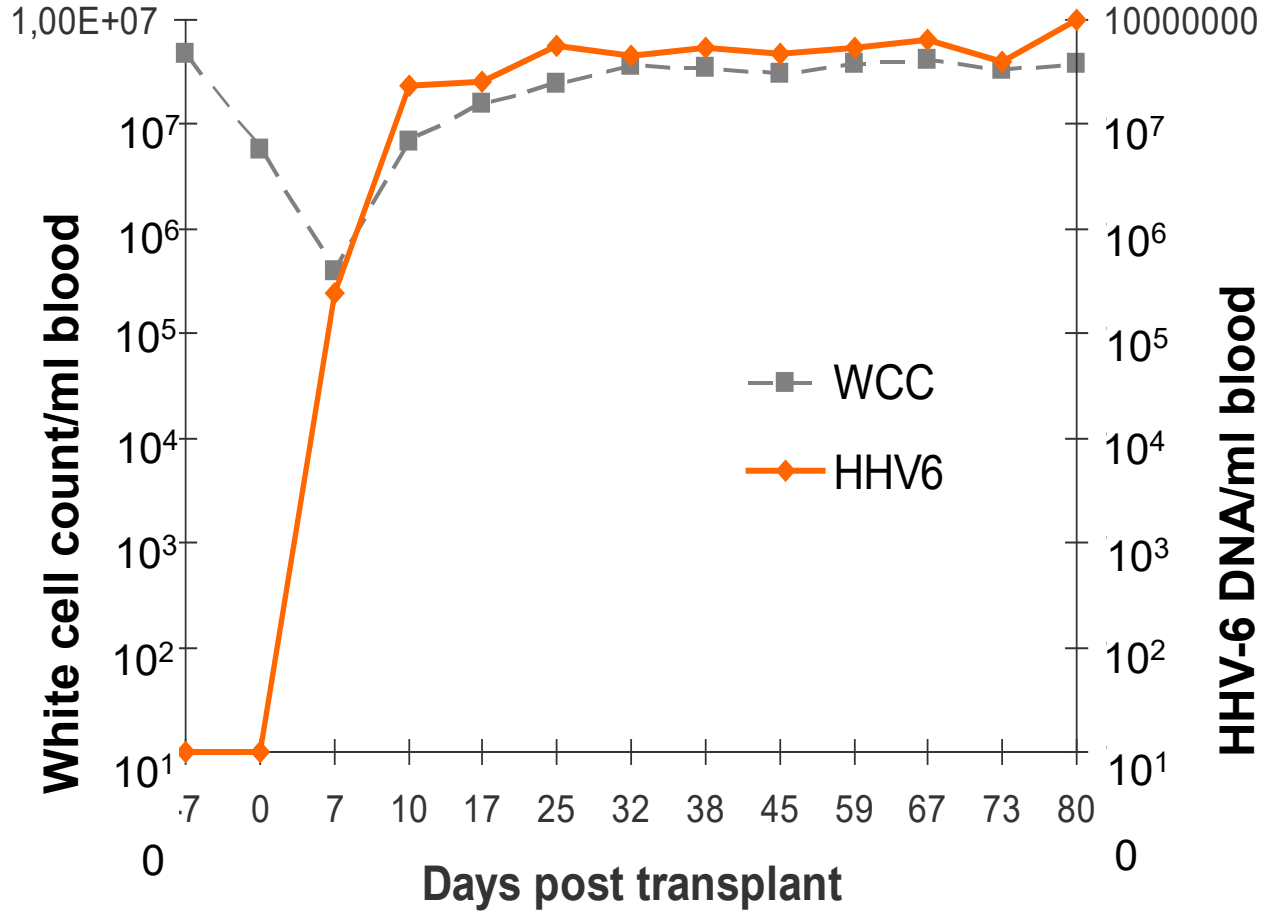


HHV-6 integration at 22q13.3 control probe on 9q34.4



HHV6 DNA in blood after HSCT

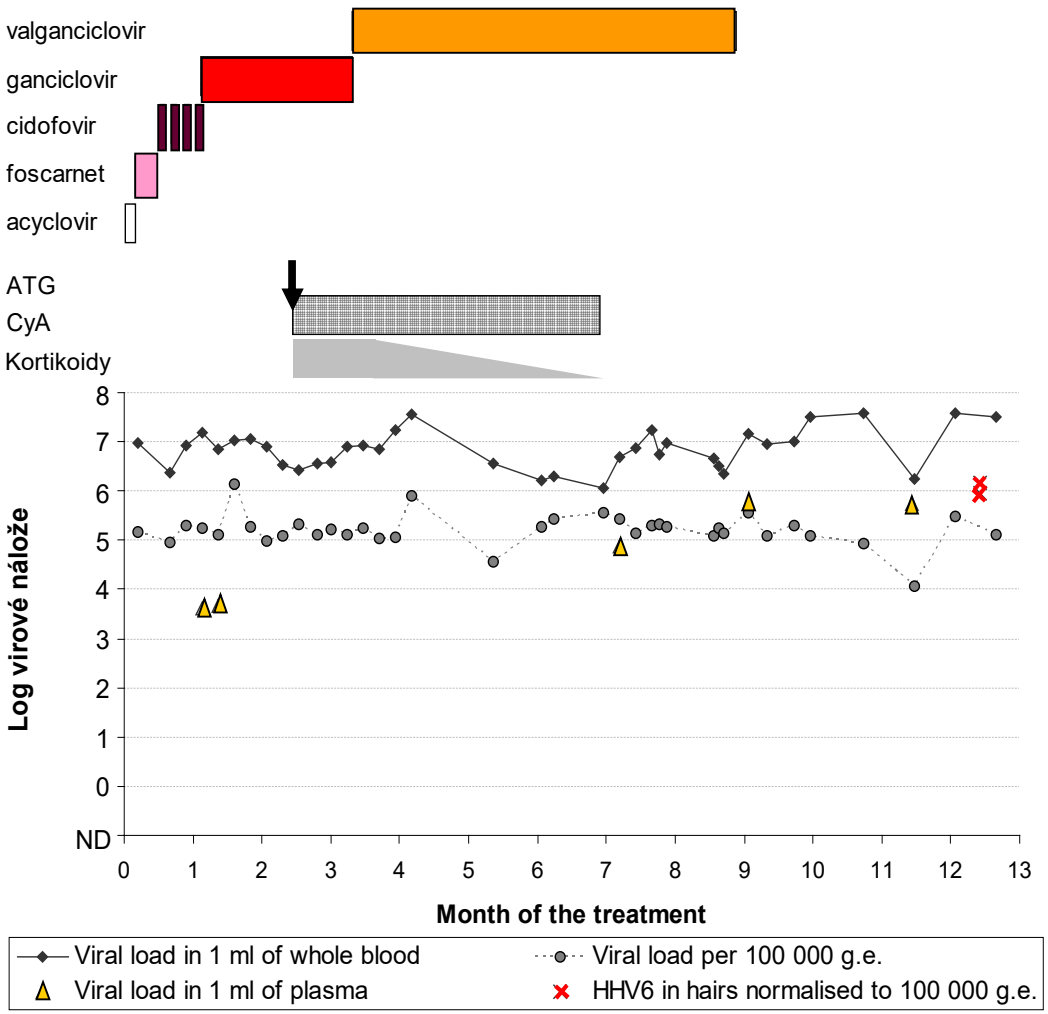
donor with Ci-HHV-6



Clark et al., JID 2006

Patient 2

Chromosomally integrated HHV-6 (Ci-HHV-6)



ND – not detected

Patient with SAA

50 years

After start of the IS therapy– partial response only

Dependent of thrombocyte infusion

G-CSF therapy

Died due to peracute sepsis of *St. aureus*.

Detection of high HHV-6 DNA quantity is NOT NECESSARY an active infection.

Detection in hair, or nails detects Ci-HHV-6 safely.

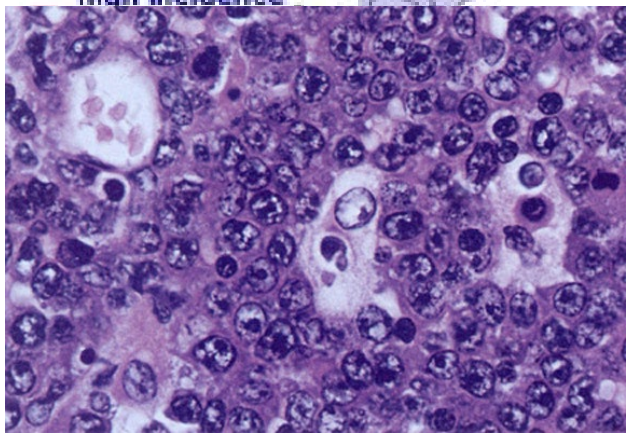
EBV discovery

surgeon

1958

„A sarcoma involving the jaws of African children.“British Journal of Surgery

1st described human oncogenic virus



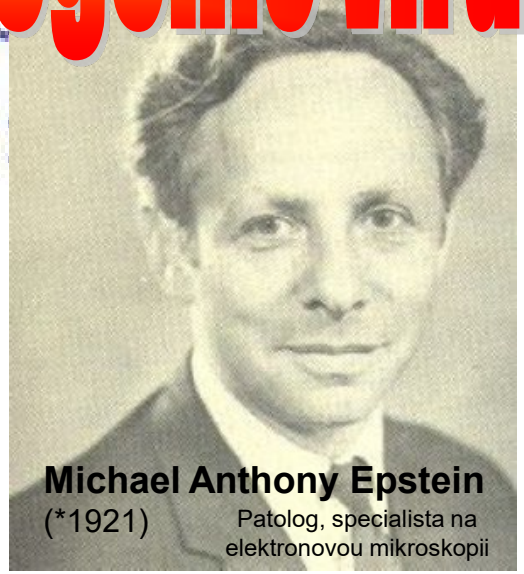
1961

„The Commonest Children's Cancer in Tropical Africa — A Hitherto Unrecognised Syndrome.“

1963 - 1. kultivace viru

1964 – Publikováno v Lancet:

„Cultivation in vitro of human lymphoblasts from Burkitt's malignant Lymphoma“



Michael Anthony Epstein
(*1921)
Patolog, specialista na elektronovou mikroskopii

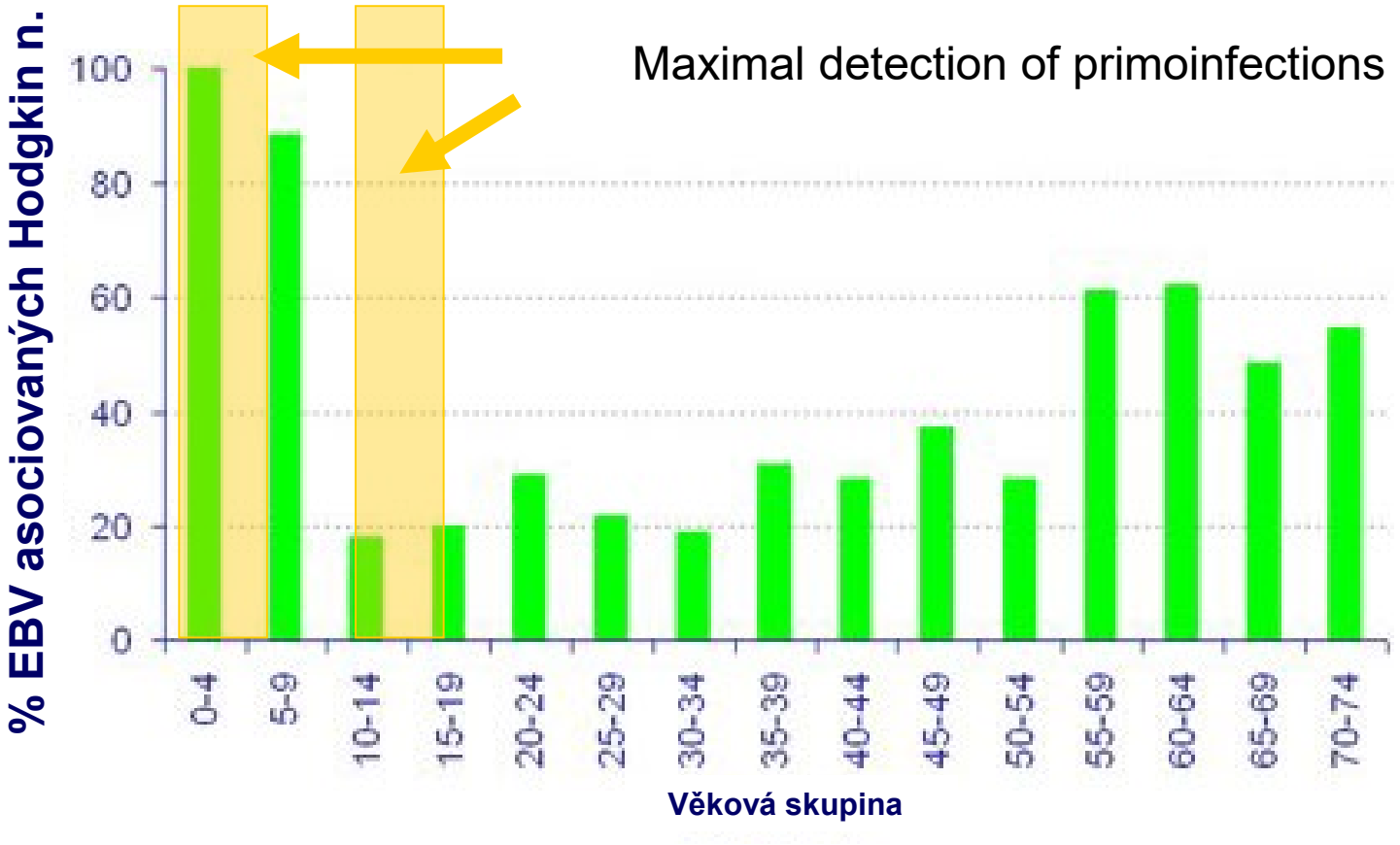
Yvonne M. Barr
(*1932)

Bert Geoffrey Achong
(1928-1996)

Transmission and epidemiology

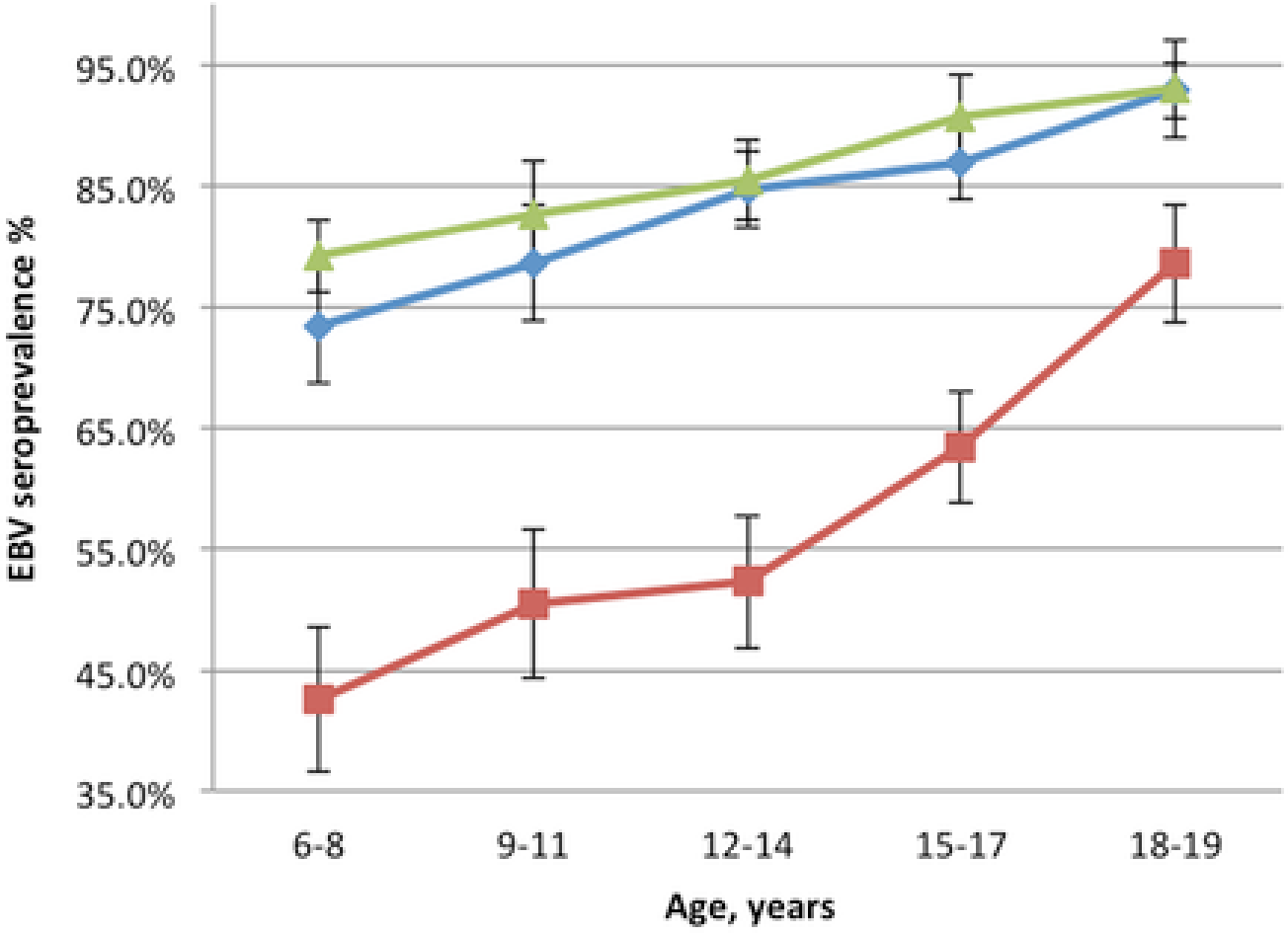
- **Transmission through saliva by oral route**
- **80 - 90% adult population is seropositive**

(in developing countries, it is 90% of children older 2 yrs)



Transmission and epidemiology

- **Transmission through saliva and oral route**
- (permissive cells: B lymfocytes and epithelial cells)
- **80 - 90% of adults population is seropositive**



Median of EBV seroprevalenceaccording to age, race/ethnicity, National Health and Nutrition Examination Survey, 2003–2010.

Non-Hispanic Black

Non-Hispanic White

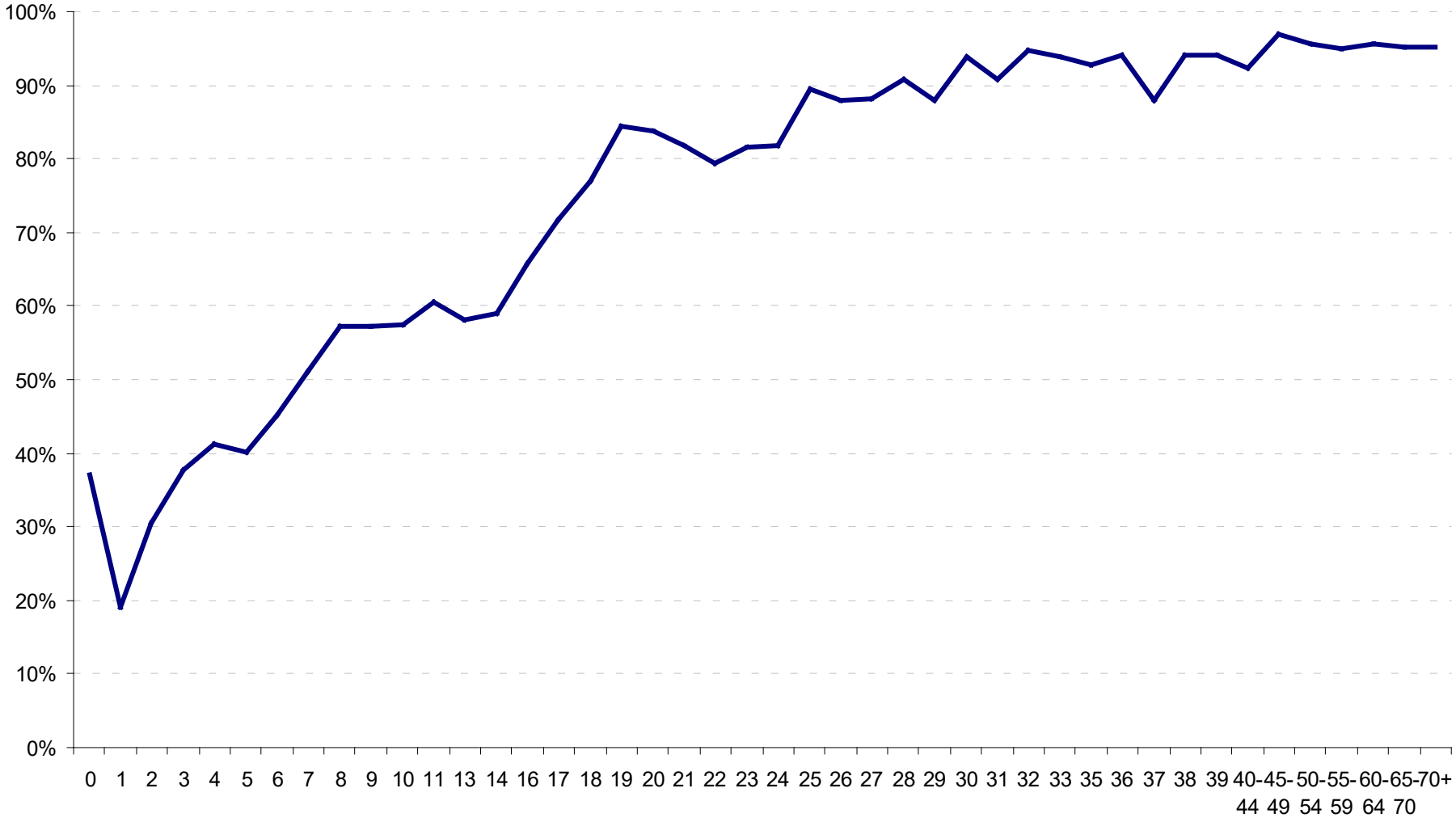
Mexican-American

PLOS ONE

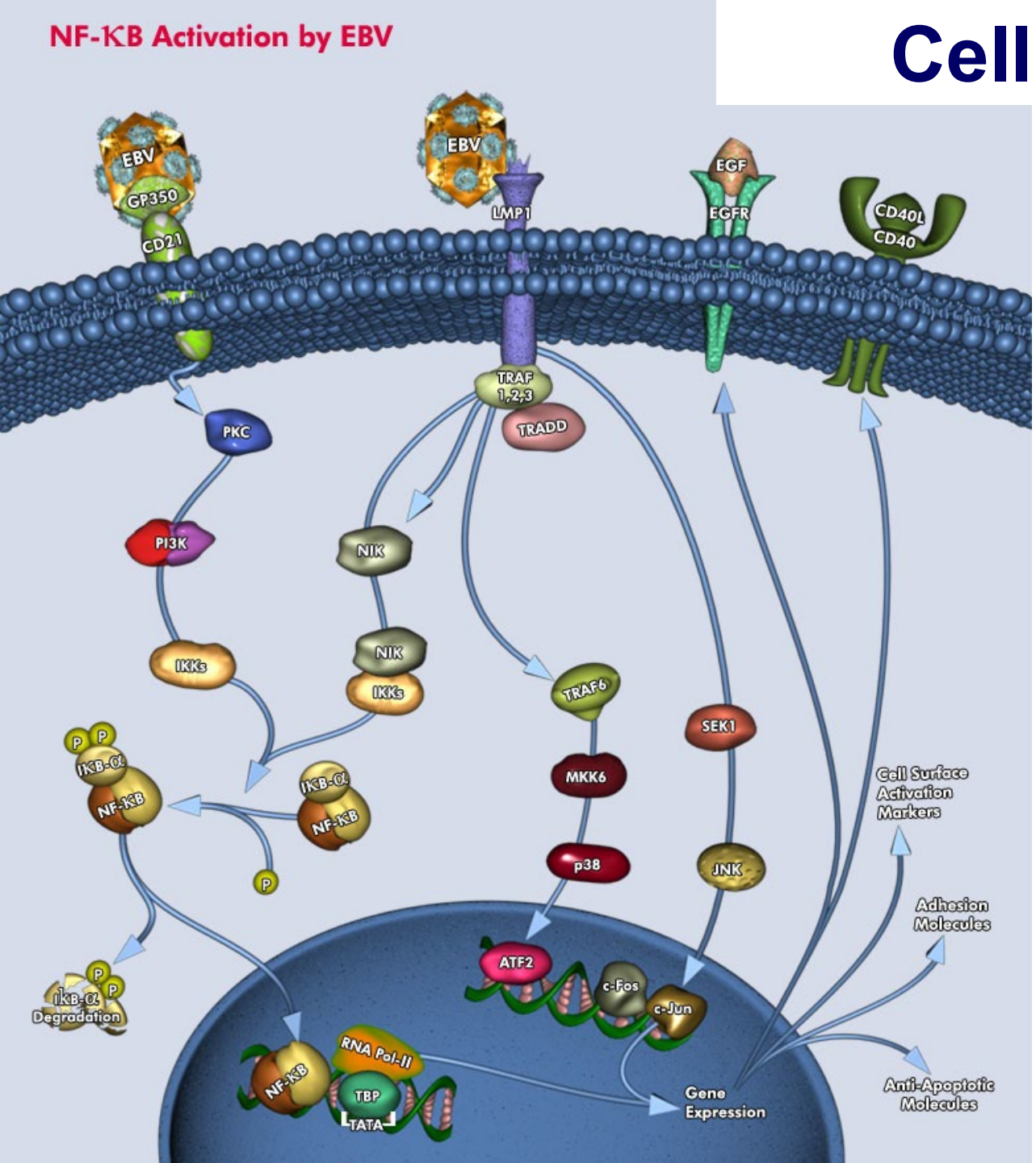
Dowd JB, Palermo T, Brite J, McDade TW, et al. (2013) Seroprevalence of Epstein-Barr Virus Infection in U.S. Children Ages 6-19, 2003-2010. PLoS ONE 8(5): e64921. doi:10.1371/journal.pone.0064921http://www.plosone.org/article/info:doi/10.1371/journal.pone.0064921

Transmission and epidemiology of EBV in Motol UH

FN MOTOL



Cell entrance



gp350/220

Binds to:

CD21(CR-2)

HLA II

Permissive cells

B lymfocytes

Epithelial cells

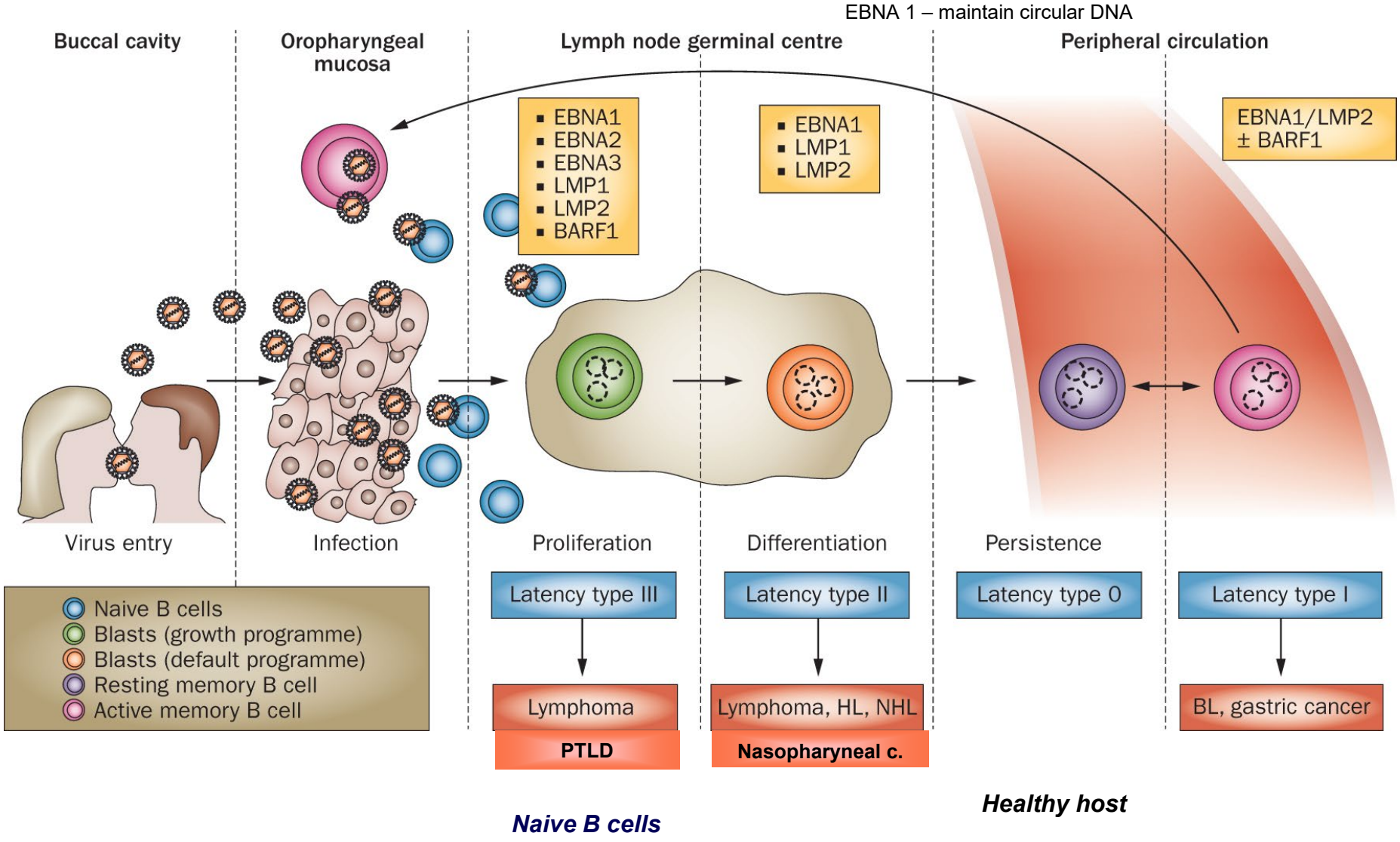
How EBV manipulates the immunity /prolipheration?

EBNA-1	Sequence-specific DNA-binding protein to EBV element; sequence-nonspecific chromosome association protein; transactivator of viral latent genes and host genes; responsible for episome replication, segregation and persistence of viral genome; involved in p53 degradation and oncogenesis
EBNA-LP	Transcriptional coactivator of EBNA-2-dependent viral and cellular gene transcription; primarily indirectly associates with host DNA sites located at or near the transcriptional start; associates with cellular transcriptional (co)factors and EBNA-2; dismisses repressor complex from promoter or enhancer sites; is essential for EBV-mediated B-cell transformation
EBNA-2	Together with EBNA-LP cooperatively activates viral and cellular gene transcription for transformation; primarily indirectly associates with host DNA sites located at the enhancer or intergenic region; associates with cellular transcriptional (co)factors and EBNA-LP; is critical for EBV-mediated B-cell transformation
EBNA-3A	A coactivator of EBNA-2, EBNA-3A and EBNA-3C associations with RBPJ inhibit RBPJ recruitments to DNA; downregulate cMyc transcription and block EBNA-2 activation effects; and induce CDKN2 and chemokines. Induces G1 arrests, which is essential for EBV-mediated B-cell transformation
EBNA-3B	A coactivator of EBNA-2; dispensable for B-cell transformation; viral tumor suppressor; and upregulates CXCL10. EBNA-3B-knockout induces DLBCL-like tumors
EBNA-3C	Coactivates with EBNA-2 host *CXCR4* and *CXCL12* genes; induces CDKN2, chemokines and aurora kinase B; mediates RB degradation; attenuates H2AX expression and overcomes EBV-infection-mediated DNA damage response; promotes cell proliferation; induces G1 arrests; essential for EBV-mediated B-cell transformation
LMP-1	Mimics the constitutively active form of CD40, a major EBV-encoded oncogene; activates NF-κB, JNK and p38 pathways; is critical for EBV-mediated B-cell transformation, a major EBV-encoded oncogene; activates NF-κB, JNK and p38 pathways; and induces EMT of NPC and acquisition of CSC-like properties
LMP-2A	Mimics constitutively active, antigen-independent BCR signaling through constitutive activation of the ERK/MAPK pathway224; blocks antigen-dependent BCR signaling; induces B-cell lymphoma in transgenic condition; is important but not essential for *in vitro* primary B-lymphocyte growth transformation; rescues the LMP-1-generated impairment in germinal center in the response to antigen in animals; confers resting B cells sensitive to NF-κB inhibition and apoptosis; suppresses differentiation and promotes epithelial cell spreading and motility in epithelial cells; and enriches cancer stem cell-like population
EBER	Most abundant EBV-encoded noncoding RNAs; augments colony formation and induces growth; confers cells resistance to PKR-dependent apoptosis; induces cytokines and modulates innate immune response; binds to La, PKR, L22, PRR and RIG-I; and EBER-mediated RIG-I activation likely contributes to EBV oncogenesis. EBER blockades of PKR-mediated phosphorylation of eIF2α results in blockage of eIF2α-mediated inhibition of protein synthesis and resistance to IFNα-induced apoptosis
miRNAs	Transcribed from BART and BHRF1; validated targets include Bim, BRUCE, CXCL11, DICER1, PUMA; has a role in sustaining latently infected cells. BHRF1 miRNA and BART miRNAs interfere with apoptosis. The miR-BART15-3p promoted apoptosis 331

Experimental & Molecular Medicine (2015) 47,

How EBV manipulates the immunity

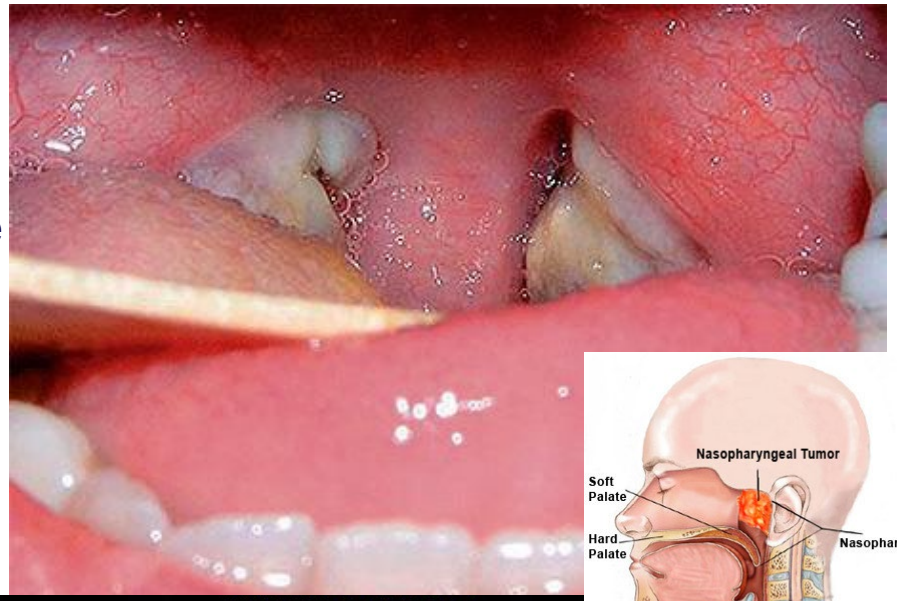
(antigens and latency)

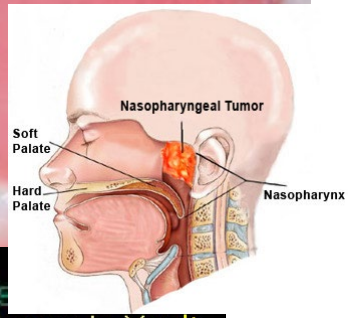


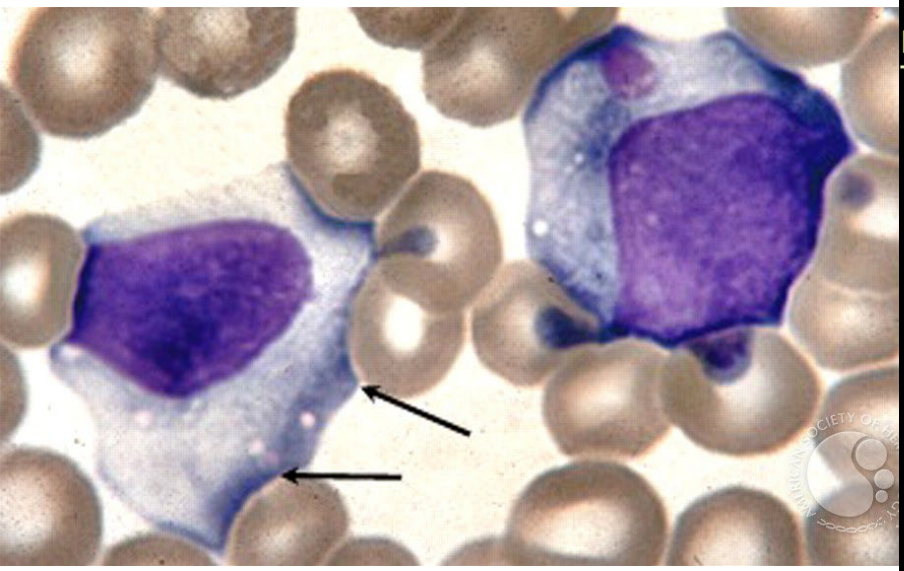
Bollard, C. M. et al. (2012) T-cell therapy in the treatment of post-transplant lymphoproliferative disease
Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2012.111
nature REVIEWS CLINICAL ONCOLOGY

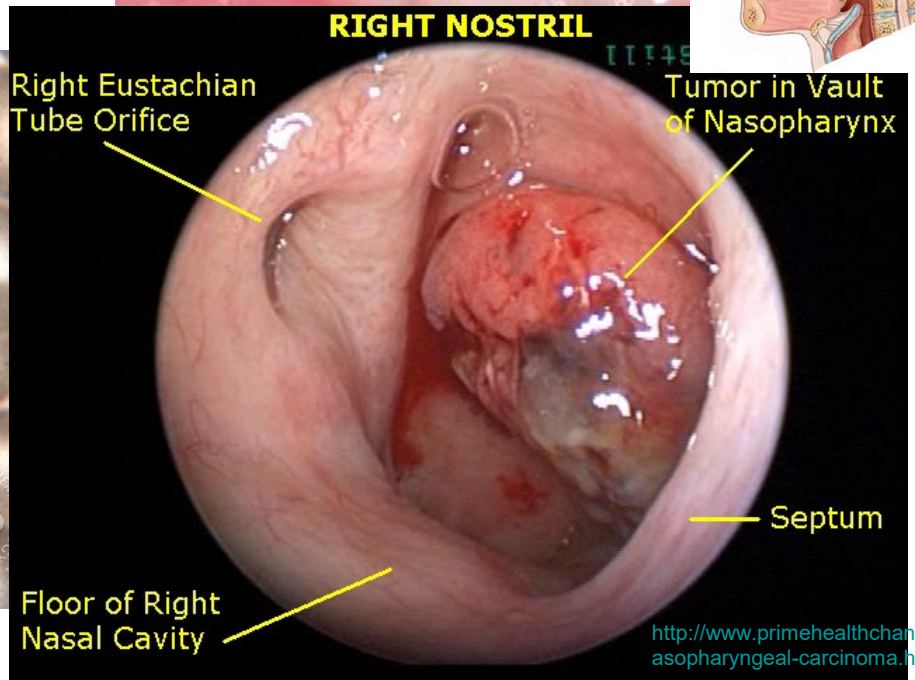
Patological activities of EBV

- **Immunocompetent host**
  - Infectious mononucleosis
  - Chronic active EBV infection
  - X-binded lymfoprolipherative disease
  - Malignant diseases
    - Hodgkin disease
    - Burkitt‘s lymphoma
    - non-Hodgkin T/NK lymphoma
    - Nasopharyngeal carcinoma
    - Gastric carcinoma
    - Angioblastic T lymphoma





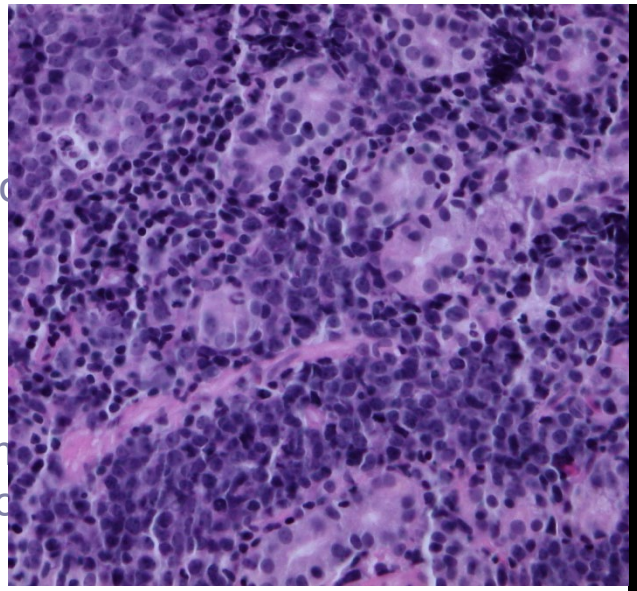


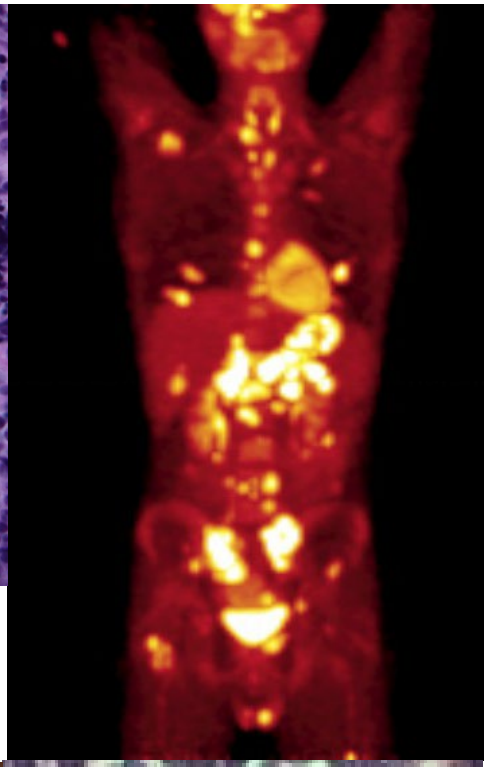


http://www.primehealthchannel.com/nasopharyngeal-carcinoma.html

Patological activities of EBV

- **Imunokompetentní hostitel**
  - Infekční mononukleóza
  - Chronická aktivní EBV infekc
  - X-vázaná lymfoproliferativní
  - Maligní onemocnění
    - Hodgkinova nemoc
    - Burkittův lymfom
    - non-Hodgkinský T/NK lym
    - Nasopharyngeální karcinc
    - Karcinom žaludku
    - Angioblastický T lymfom
- **Immunocompromised host**
  - Hairy leukoplaky
  - Above listed malignant diseases
  - Post-transplant lymfoproliferative disease (EBV-LPD)
  - Encefalitis/myelitis
  - Pneumonie
  - Hepatopathy/hepatitis





http://www.kcom.edu/faculty/chamberlain/Website/lectures/lecture/aids.htm

www.med-ed.virginia.edu/courses/path/innes/wcd/hodgkin

Infectious mononucleosis

- „Kissing disease“
- Proliferation affects spleen, liver and lymph nodes
- Tiredness lasting for weeks, increased temperature and fevers (often approx. 39 °C), pharyngitis and swelling of the lymph nodes (submandibular and cervical); hepatosplenomegaly, hepatopathy, swelling of the eye lashes and face, malaise
- Inkubation period 4-6 weeks
- At the beginning seems like „tonsilitis“
- Transmission by saliva
- Treatment approx. 6 months

  Relax and diet (2-3 months);

  Subsequently it is neccesary to have some relax in physical activity

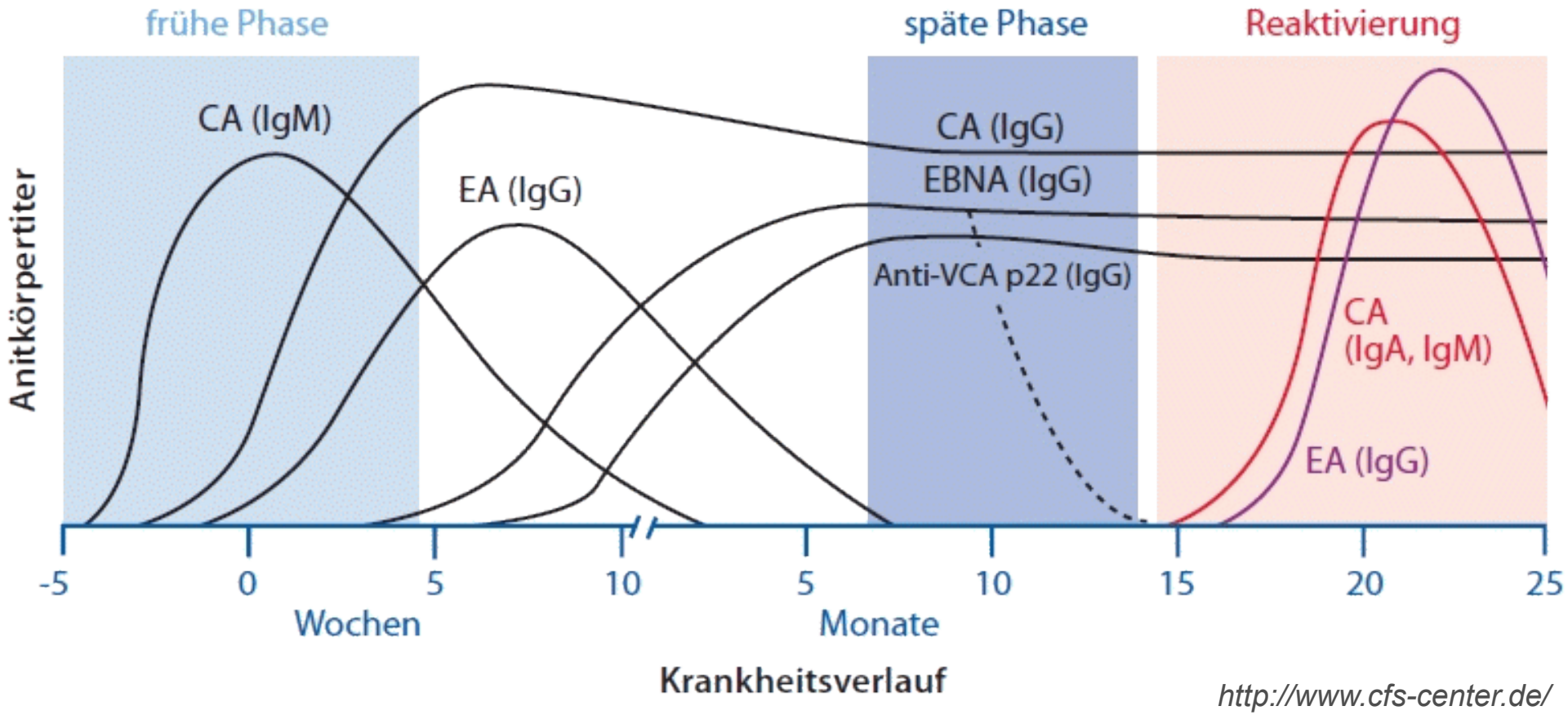
http://home.teleport.com/~bobh/InfectiousMononucleosis.htm

Diagnostics

Basic diagnosis of EBV is indirect – serological.

Early phase | Post-acute phase | Late phase | Reactivation

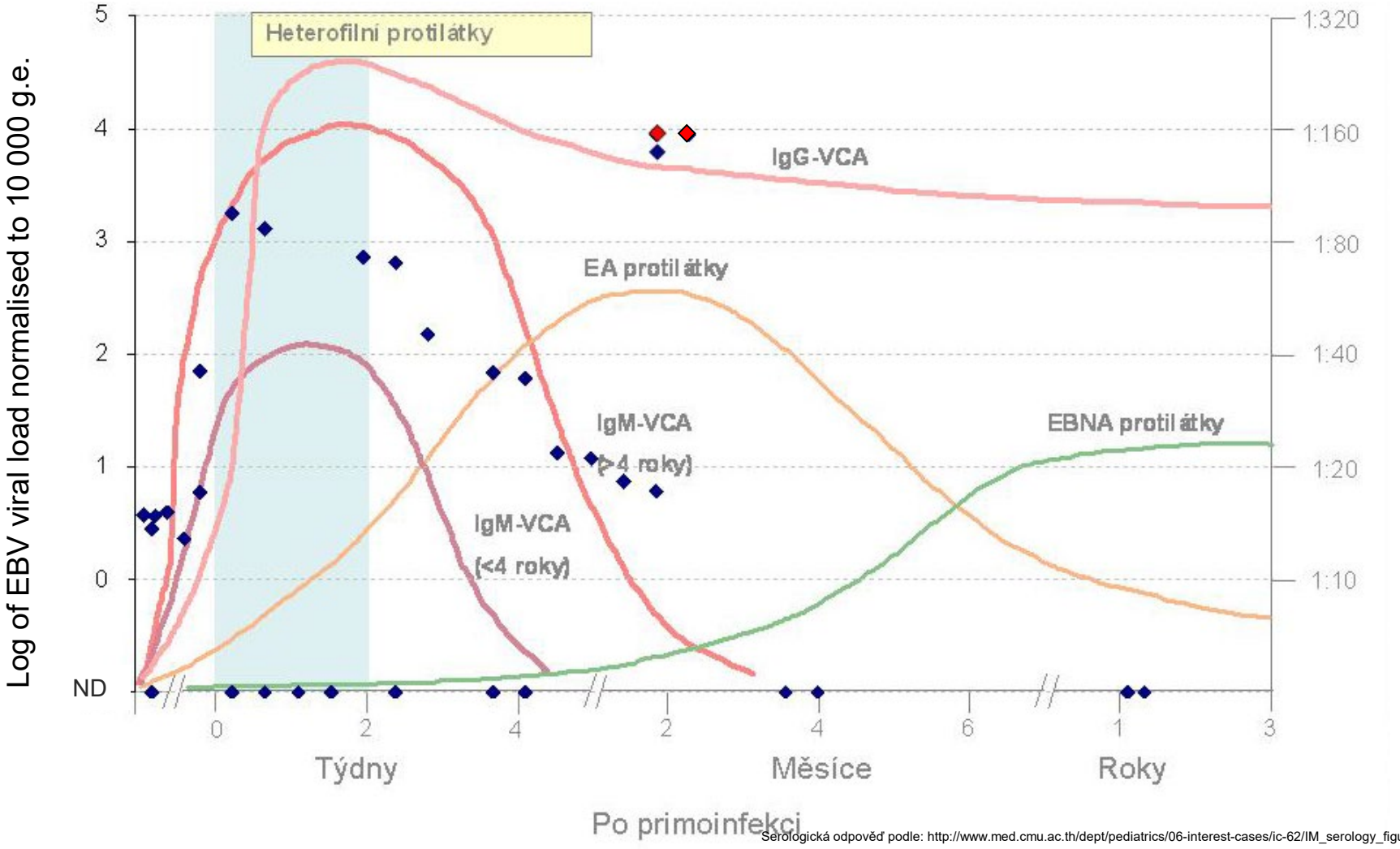
Heterofile antibodies Paul-Bunnelova reaction



http://www.cfs-center.de/

Viral load in patients with dg. B27 - IM

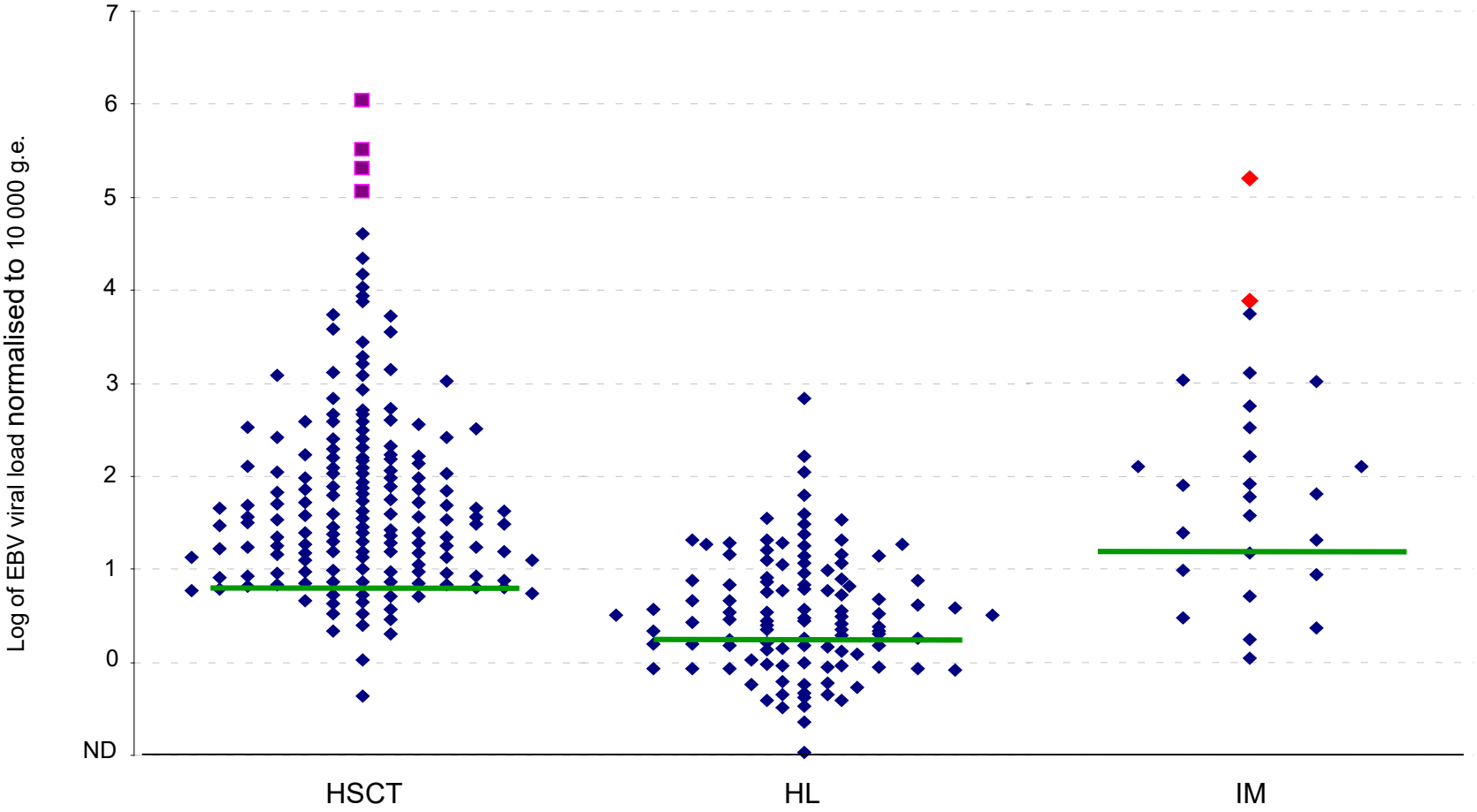
- Positive – 26 patients (62%)
  - 50 samples positive (65%); median of positivity 110 (range 11-157,670) in 100 000 g.e.



Serologická odpověď podle: http://www.med.cmu.ac.th/dept/pediatrics/06-interest-cases/ic-62/IM_serology_figure2.1.jpg

Direct detection - PCR

- Detection in peripheral blood (plasma, whole blood), possibly in the tissue
- In HL and IM, EBV is detected in peripheral blood in low quantity.
- Median of detected quantity in whole blood increased from HL→ HSCT → IM



Chronic active EBV infection

Infected T lymfocytes and NK cells

Signs often connected with prolonged presence of interferons in the organism.

Diagnostic criteria of a case definition for SCAEBV [15]

Category	Criteria
Clinical	Intermittent fever, lymphadenopathy, and hepatosplenomegaly.
Hematologic	Anemia, thrombocytopenia, lymphocytopenia or lymphocytosis, neutropenia, and polyclonal gammopathy.
Virological	Elevated antibody titers and positivity for antibodies to EBV-related antigens (VCA IgG, $\geq$5120; VCA IgA, positive; EA [D] IgG, $\geq$640; EA [D] IgA, positive; and EA [D] and EA [R] IgG, $\geq$640) and/or detection of EBV genomes in affected tissues.
Other	Chronic illness that cannot be explained by other known disease processes.

F. Sánchez et al. / Annals of Diagnostic Pathology 12 (2008) 368–371

Chronic active EBV infection

	T-cell type (n = 16)	NK-cell type (n = 12)	P
Symptoms			
Fever, > 1 d/wk (%)	67	25	**.04**
HMB (%)	13	**75**	**.002**
Splenomegaly (%)	73	100	.08
Large granular lymphocytosis (%)	13	83	**.0004**
Calcification in basal ganglia (%)	7	33	.10
Laboratory data			
IgG (mg/dL, mean ± SD)	2213 ± 1104	1682 ± 464	.11
IgE (IU/mL, mean ± SD)	282 ± 298	**2774 ± 3774**	**.04**
VCA IgG (geometric mean titer)	**2405**	446	**.01**
EA IgG (geometric mean titer)	831	119	**.02**
EBNA (geometric mean titer)	30	45	.24
Viral load			
PBMC (copies/μg DNA, mean ± SD)	$10^{4.1\pm0.5}$	$10^{4.4\pm0.4}$	.09
Plasma (copies/mL, mean ± SD)	$10^{2.9\pm1.1}$	$10^{2.4\pm2.1}$	.49

HMB indicates hypersensitivity to mosquito bites; VCA, viral capsid antigens; EA, early antigens; EBNA, EB nuclear antigens; PBMC, peripheral blood mononuclear cells. Fisher exact test was used to compare symptoms between groups. Student *t* test was used to compare the mean copy numbers of EBV-DNA or laboratory data. Bold letters indicate statistically significant results.

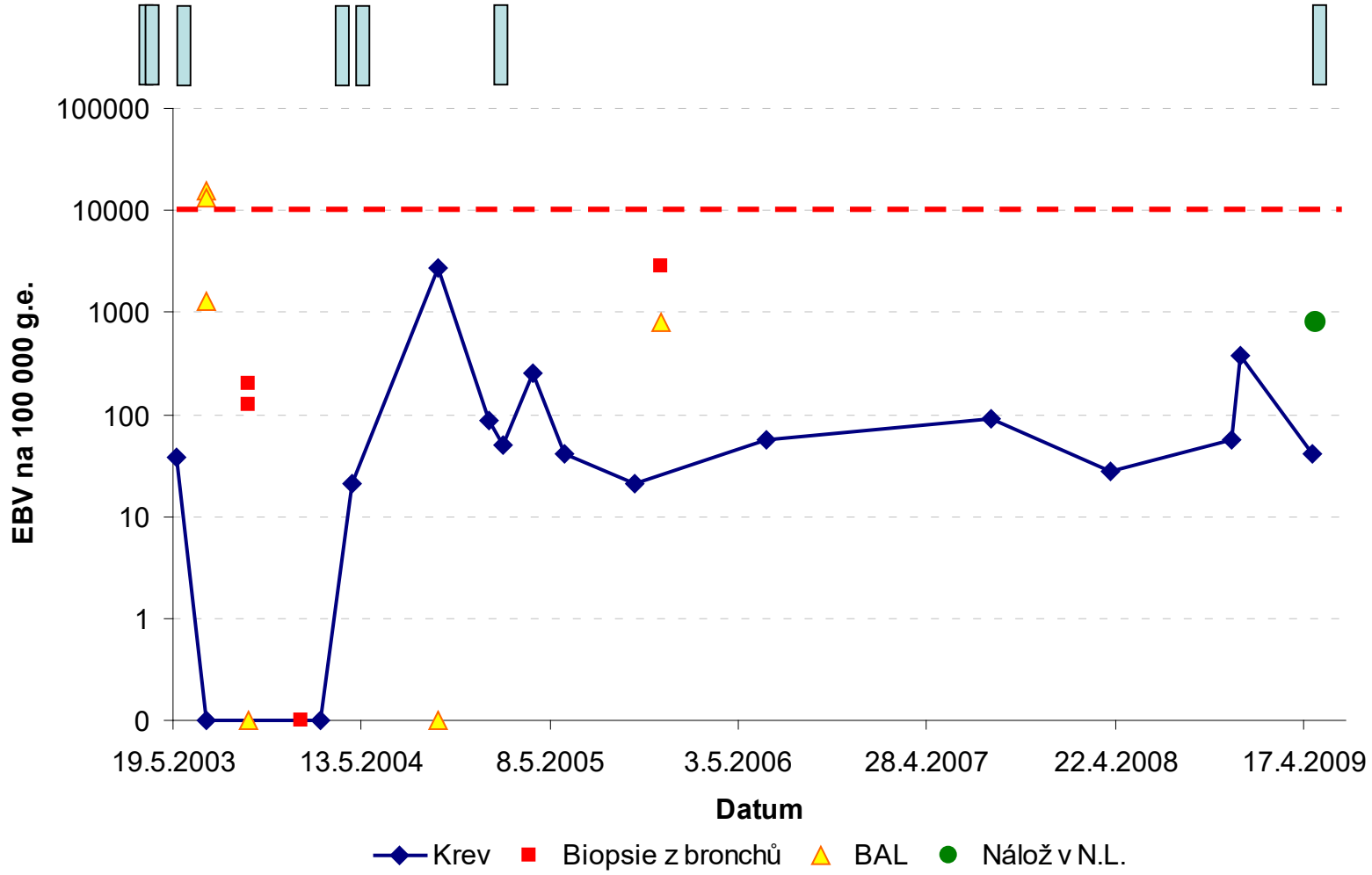
Table 2. Clinical features of 30 patients with chronic active Epstein-Barr virus infection

Symptoms and signs	(%)	Life-threatening complications	(%)
Fever	100	Hemophagocytic syndrome	21
Liver dysfunction	90	Coronary artery aneurysm	21
Splenomegaly	90	Hepatic failure	18
Lymphadenopathy	50	Malignant lymphoma	16
Thrombocytopenia	50	Interstitial pneumonia	12
Anemia	48	Central nervous system involvement	7
HMB	43	Sepsis	7
Skin rash	28	Pulmonary hypertention	4
Calcification in basal ganglia	18	Intestinal perforation	4
Oral ulcer	18	Myocarditis	4
Hydroa vacciniforme	14		

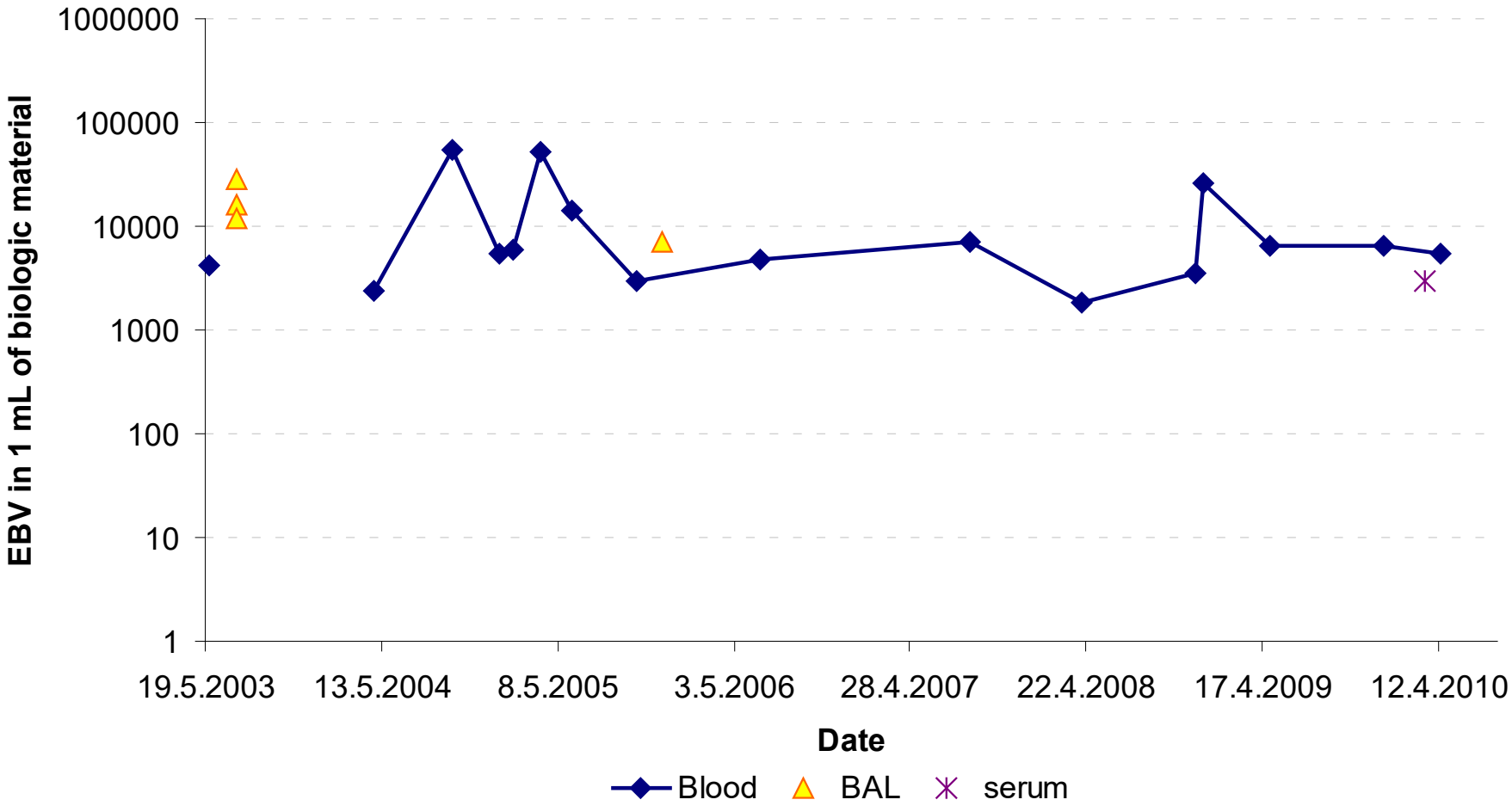
HMB indicates hypersensitivity to mosquito bites.

Kimura et al. Blood 15 July 2001, Vol. 98, No.2

Datum	6.2.2003	17.2.2003	27.5.2003	23.2.2004	26.3.2004	4.4.2005	4.5.2009
VCA IgG	+	+++		+	++	147 U/ml	119 U/ml
VCA IgM	+	+	-	+	+	72,5 U/ml	45,5 U/ml
EA-D	++	++	+++ (vysoká exprese)	++	++	90 U/ml	<150 U/ml
EBNA 1 IgG	-	-	-	+	+	52,5 U/ml	14,3 U/ml



EBV load in 1 ml of biological material



Malignant impact of EBV

NHL - Burkitt lymphoma

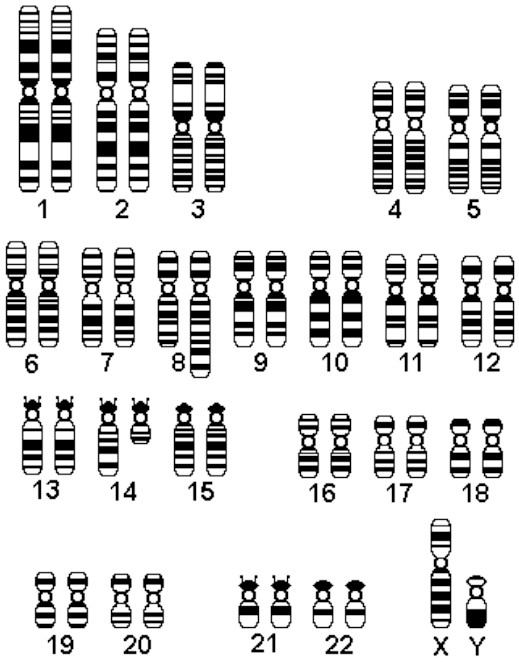
Very agressive

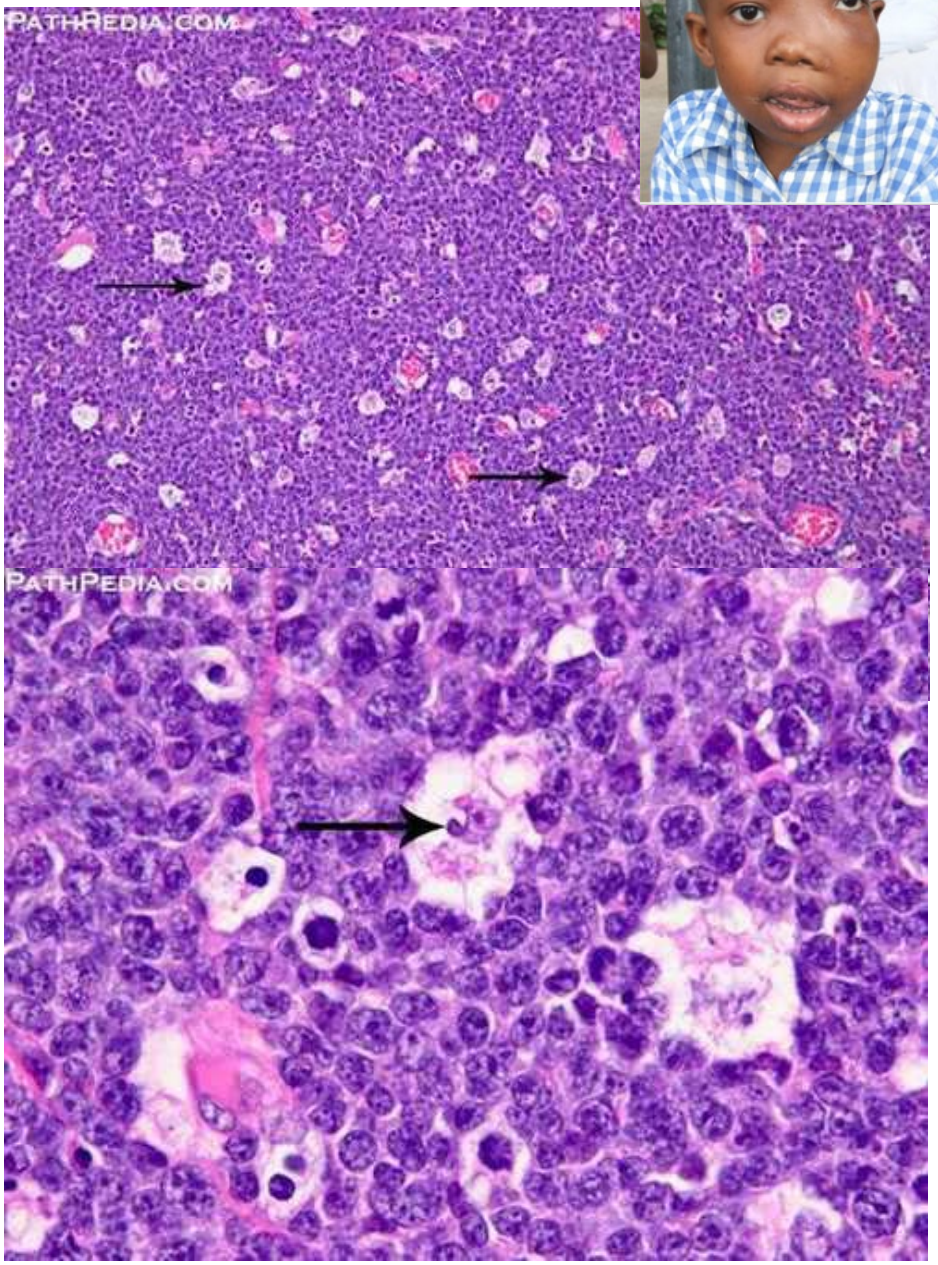
Picture of the **„Sky of stars "** – „stars" are apoptotic tumor cells which are fagocyte by macrophages; „sky" – represent tumor lymfocytes

Typical fusion t(8:14) chromosome 8 with c-myc oncogen

In the equatorial Africa incidence 5-15/100,000 of children

In Europe and USA 0,2-0,3/100,000 citizens





http://mynotes4usmle.tumblr.com/post/33262736354/burkitts-lymphoma#.VPgrFSx5vU4

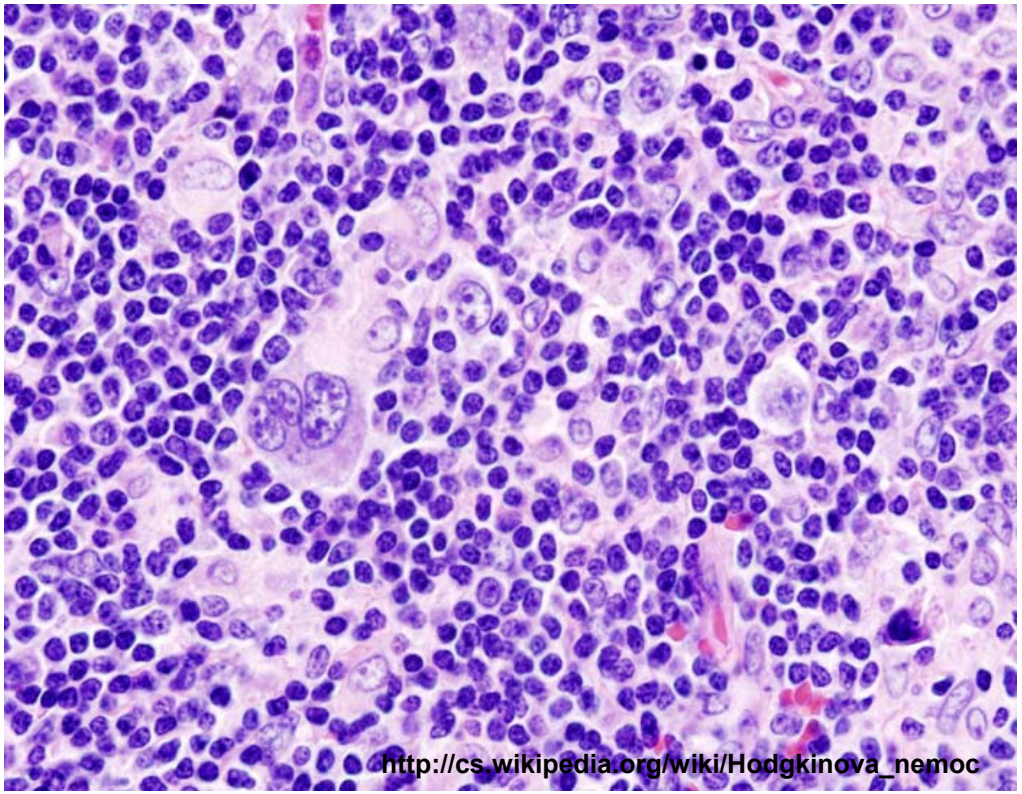
Malignant impact of EBV

Hodgkin lymfoma

High number of patients in long lasting remission.
Higher frequency in younger patients (approx. 20 yrs. of age) and in patients older 50-60 yrs. (median of age at dg. 35 yrs.)

Ratio of malignant and non-malignant cells approx. ~ 1:100

Incidence 2.4/100000 in ♀ and 3.1/100 000 ♂.

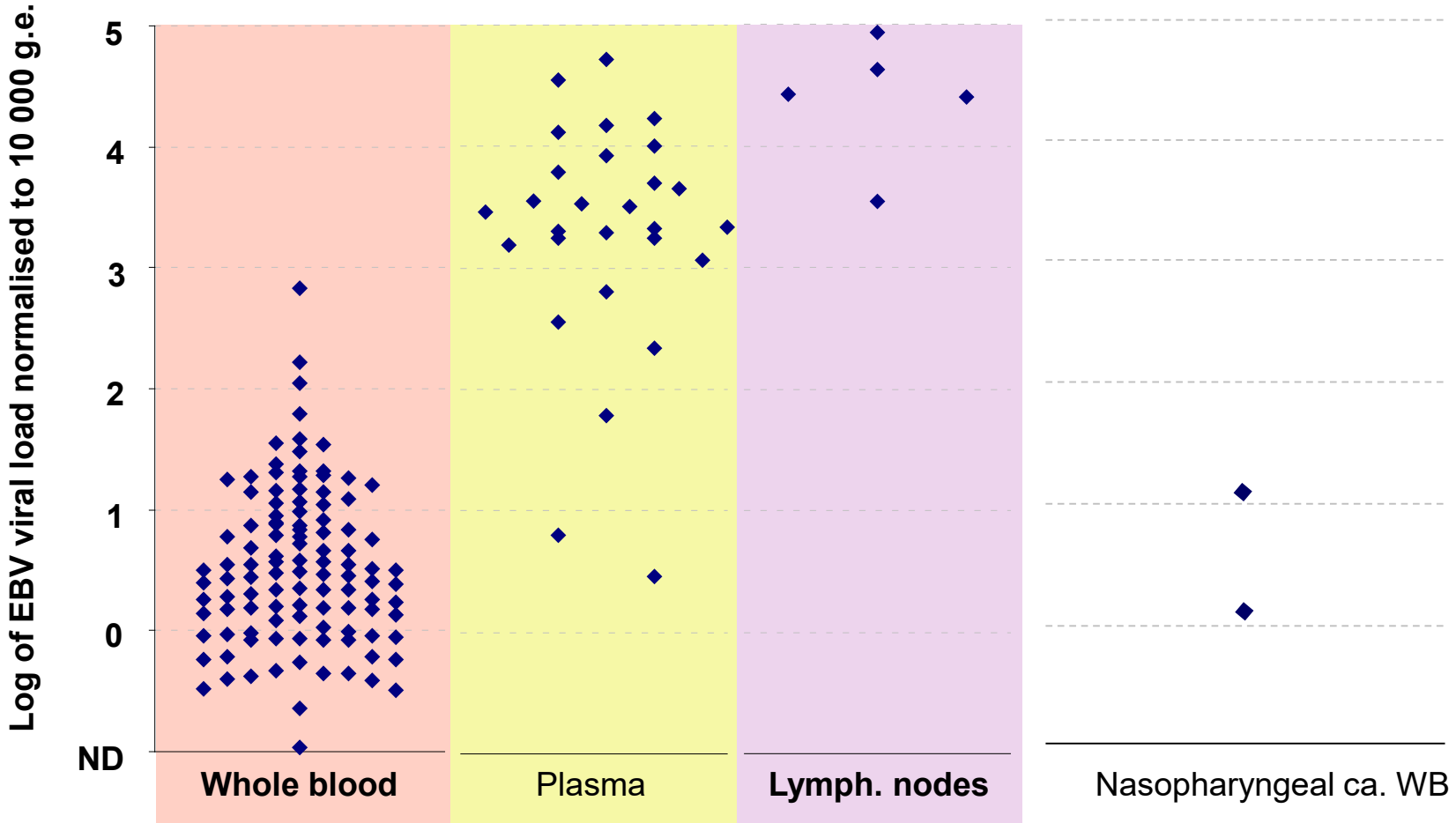


Histologically divided according to no. of **Reed-Sternberg‘s cells** (cells developed by mutation from B-cells) and according to the cellular frections:

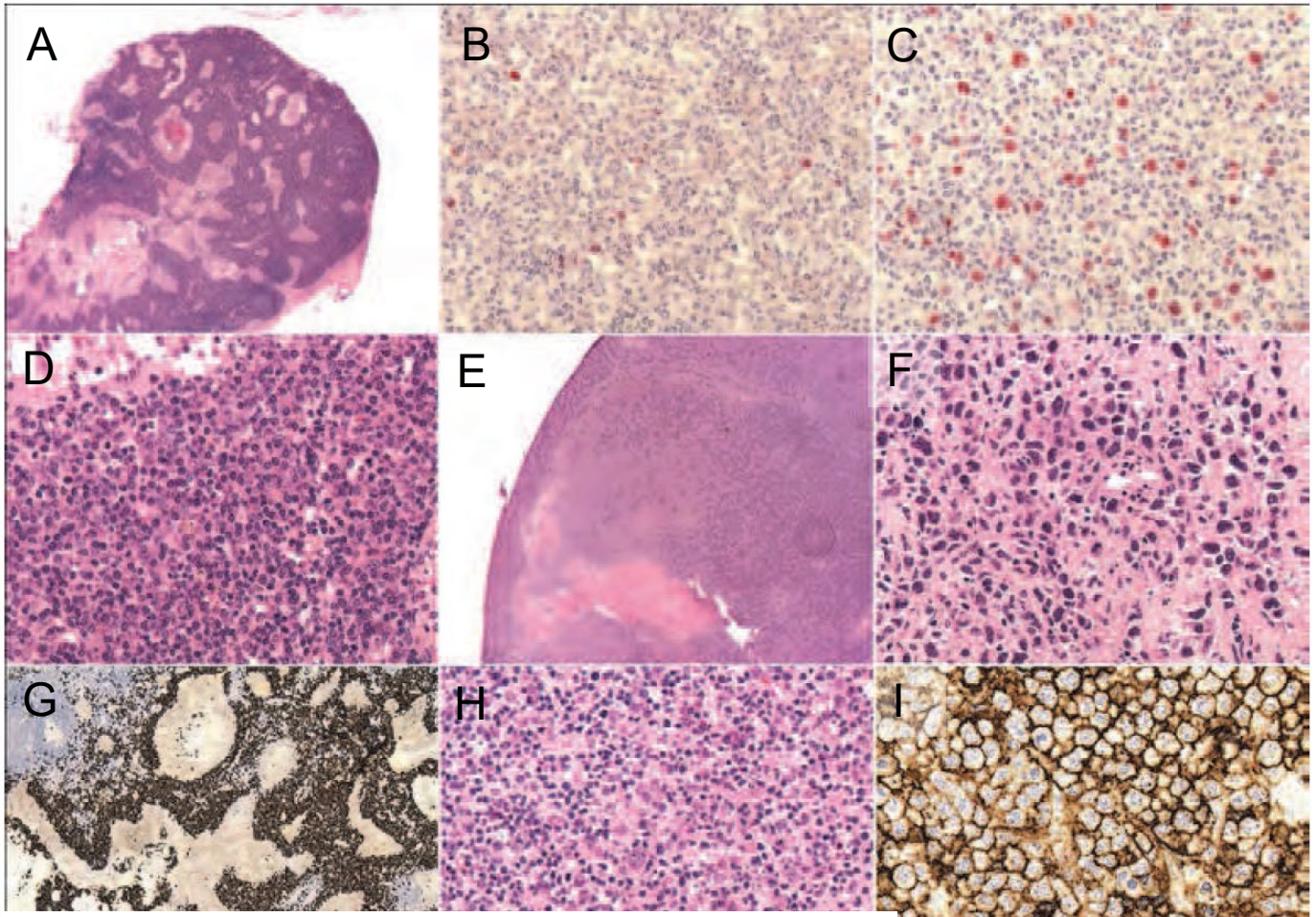
typ I with dominance of lymfocytes (few R-S cells, dominance of lymfocytes; best prognosis) (5 %);
typ II nodular-sclerotic (nodular centres, cells (reticular, lymphocytes, histiocytes) in colagen fibres) (70 %);
typ III mixed (20–25 %);
typ IV classical, few lymphocytes (No. of Sternberg‘s cells increased; worse prognosis) (1 %).

Patients with Hodgkin L. and NF ca.

- Positive HL – 69 patients (38%)
  - positive 110 whole blood samples (17%) and 30 plasma samples (4.8%)
  - median of positivity in whole blood 3.45 copy (range 0.11 - 721)
  - median plasma positivity 5,400 copies/ml (range 600 – 126,600);
    after normalisation to 10 000 g.e median 2,500 (range 3 - 52 162)

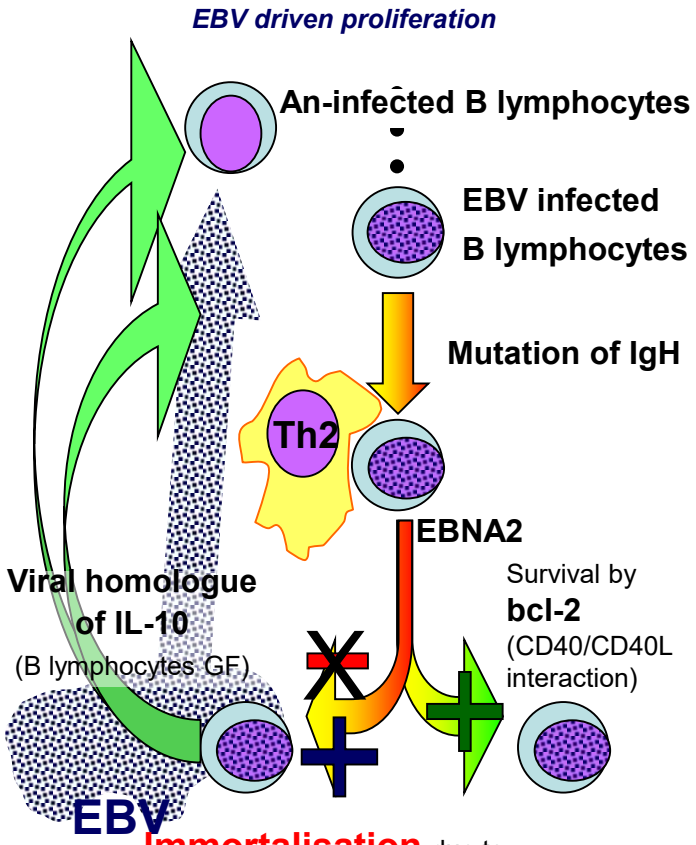


EBV associated posttransplant proliferative disease (EBV-LPD)



A-D. Early lesion, plasmacytic hyperplasia. A. H&E low power view. Preserved lymph node architecture. B. High power view shows numerous plasma cells. C. Plasma cells stain with CD138/syndecan. D. EBER *in situ* hybridisation shows small amount of positive cells.
E-G. Polymorphic PTLD. E. Low power view shows disturbed lymph node architecture. F. Higher power shows a polymorphic infiltrate composed of plasma cells, lymphocytes (small, medium-sized, large and Reed-Sternberg-like). G. EBER ISH shows numerous positive cells.
H-I. Monomorphic PTLD. H. Diffuse proliferation of large atypical cells. I. CD20 staining shows their B-cell origin (Courtesy to Prof Thomas Tousseyn).

Etiopathogenesis and classification EBV-LPD



Immortalisation due to

viral BHRF1 *(homologue of bcl-2)*

LMP-1 *(homologue of bcl-2)*

- *activation of NFκB pathway*
- *binding of TRADD from TRAF pro-apoptotic pathway*

World Health Organization Classification of Post-transplant Lymphoproliferative Disorder (PTLD)

Category	Subtype
Early lesions	Plasmacytic hyperplasia Infectious mononucleosis–like lesion
Polymorphic PTLD Monomorphic PTLD (classify according to lymphoma they resemble)	B-cell neoplasms – Diffuse large B-cell lymphoma – Burkitt lymphoma – Plasma cell myeloma – Plasmacytoma-like lesion – Other[a] T-cell neoplasms – Peripheral T-cell lymphoma NOS – Hepatosplenic T-cell lymphoma – Other
Classical Hodgkin lymphoma-type PTLD	cT1-2 gr 3 cT3-4

http://www.cancernetwork.com/oncology-journal/lymphoma-risk-and-response-after-solid-organ-transplant

Different symptoms of poly-, oligo- and monoclonal proliferation.

Mononucleosis-like syndrome

(fever, sore throat, myalgia, tonsillar hypertrophy and cervical lymphadenopathy, hepatopathy (bilirubinemia)

Tumorous form

(Symptoms secondary to the presence of lymphoid tumors: pain, obstruction, perforation, GI bleeding, respiratory distress, etc.)

Disseminated disease

(Proliferating B cells in blood and bone marrow, high fever and/or multi-organ failure)

EBV-LPD incidence and risk factors

Risk Factor	Degree of Risk	Study Reference(s)
EBV seronegativity pretransplant	24 × average risk	11–13
Younger age at transplantation	4–8 × adult risk	1,11
Type of immune suppression		
– Tacrolimus	2–5 × risk with cyclosporine	1,16,17
– OKT3 and/or ATG	3–4 × risk without these drugs	1
Type of organ transplant		9
Kidney	1%–3% of all transplant patients	
Liver	1%–3% of all transplant patients	
Hear	1%–6% of all transplant patients	
Heart-lung	2%–6% of all transplant patients	
Lung	4%–10% of all transplant patients	
Small bowel	20% of all transplant patients	
Time from transplant < 1 year	5–10 × risk at > 1 year	1
De novo CMV infection: CMV-positive recipient of a CMV-positive organ	4–6 × risk of CMV-negative recipient	21

ATG = anti-thymocyte globulin; CMV = cytomegalovirus; EBV = Epstein-Barr virus; OKT3 = muromonab-CD3 (Orthoclone OKT3); PTLD = post-transplant lymphoproliferative disorder.

In allogeneic HSCT incidence 2-25%.

- Cumulative intensity of immunosupresive treatment
- Use of anti-T lymfocytic antibodies in conditioning and/or posttransplant treatment
- T-cell depleted graft
- Intensive GvHD treatment
- Activation about 60 days after HSCT

EBV-LPD diagnosis

Diagnosis of <u>neoplastic</u> EBV-LPD should fulfill at least 2 of the following criteria:

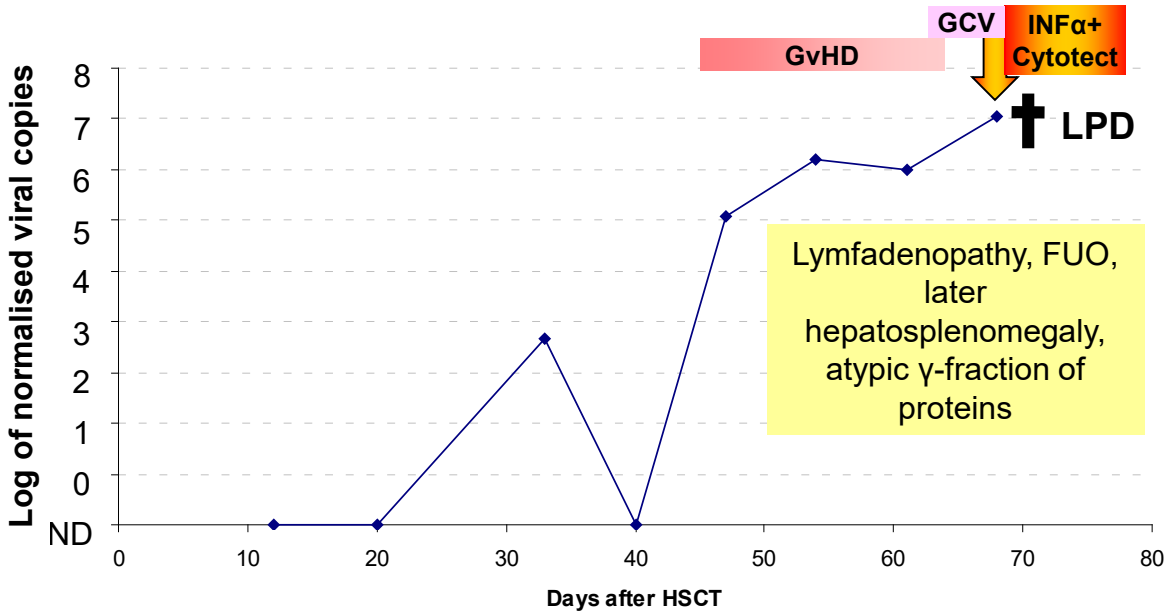
- **Change and/or destroy of the cell tissue culture by lymphoproliferative process**
- **Presence of monoclonal, or oligoclonal proliferation proven with cell and/or viral markers**
- **Evidence of EBV infection in many cells (e.g.. DNA, RNA, protein…**

EBV DNA detection in whole blood is not enough.

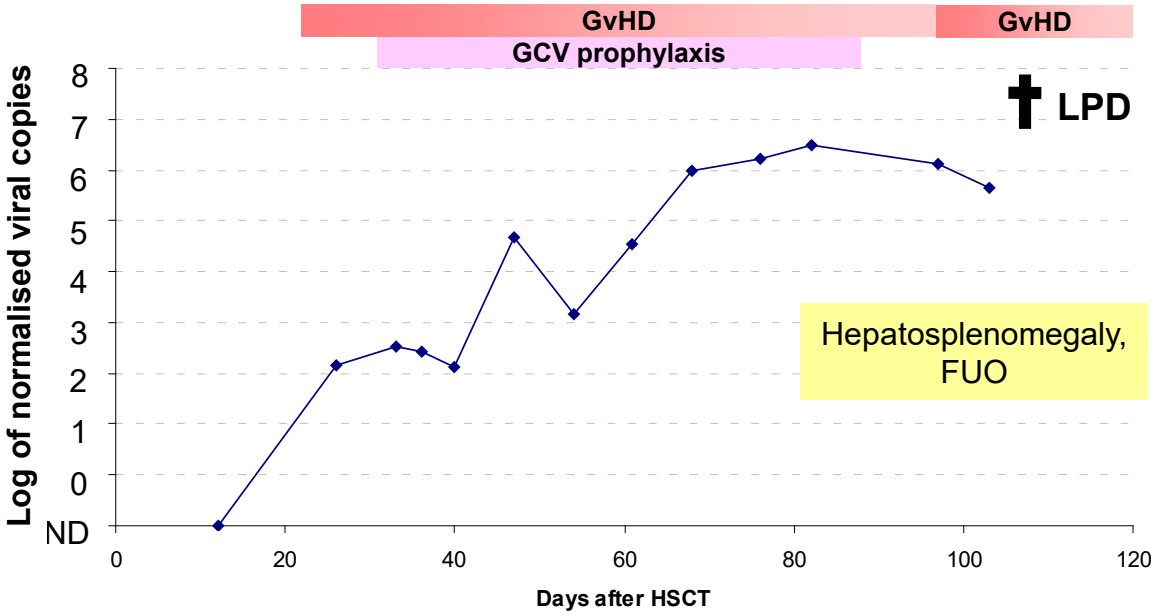
Dle definice EBMT IDWP, 2007

- Clinical symptoms
- Imagine methods
- Immunology (Flow cytometry, Ig levels, clonality)
- Histology N.L. (detecting the presence of EBV)
- Direct detection of virus
  - EBV load (based mainly on NA detection)
    - Sample type: plasma, whole blood, MNC
    - Different methods of PCR – most frequently quantitative real-time PCR

Retrospectively tested patients

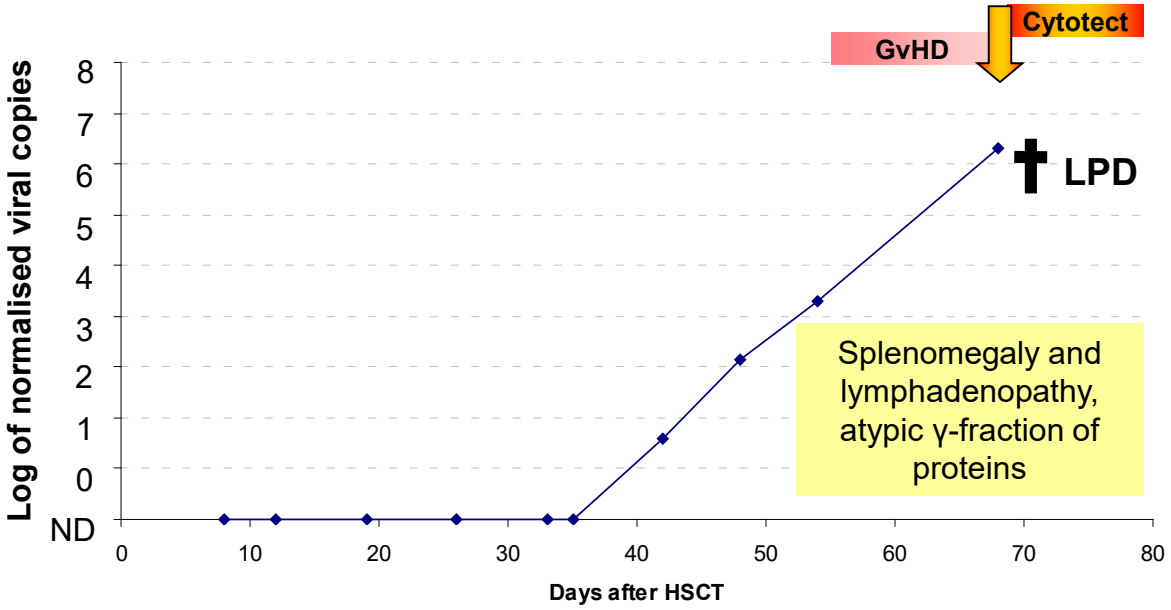


Girl, 5.5 yrs., HSCT for ALL CR2.
Donor: MMUD (8/10)
Graft: BM
Conditioning: TBI (12 Gy)
cyclophosphamide (120 mg/kg)
ATG (16 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade III (D+ 49; GIT)
Deceased on D+74 due to MOF.

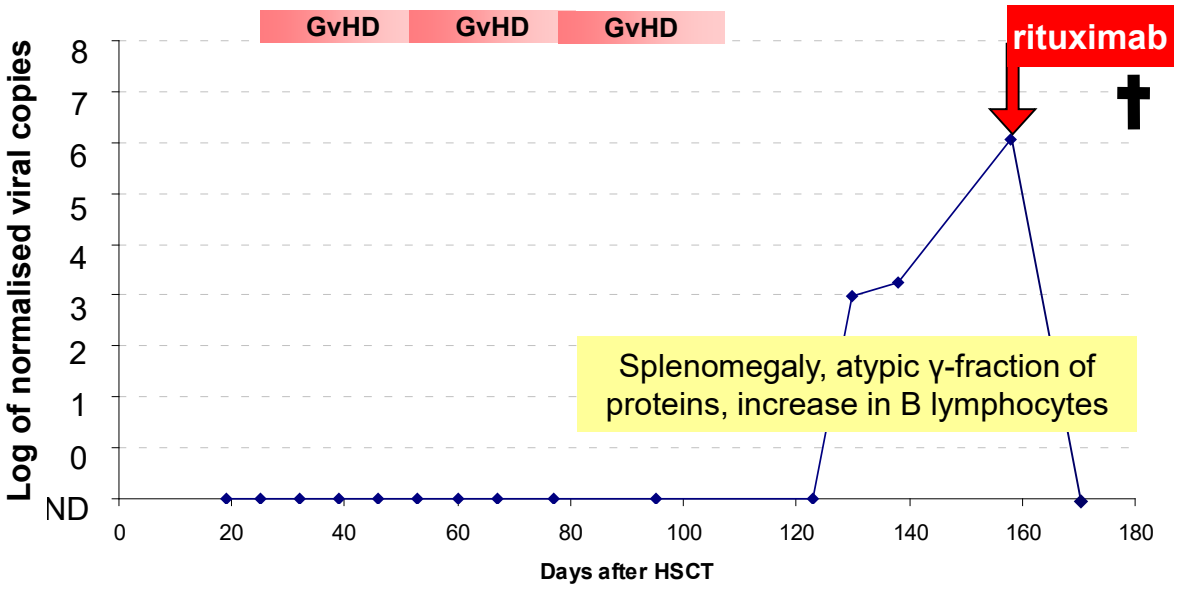


Girl, 4 yrs., HSCT for AML PR2
Donor: MMUD (9/10)
Graft: BM
Conditioning:busulphan(16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m^2)
ATG (40 mg/kg)
GvHD: grade III (D+ 25; GIT)
Deceased on D+117 due to bleeding and respiratory failure.

Retrospectively tested patients

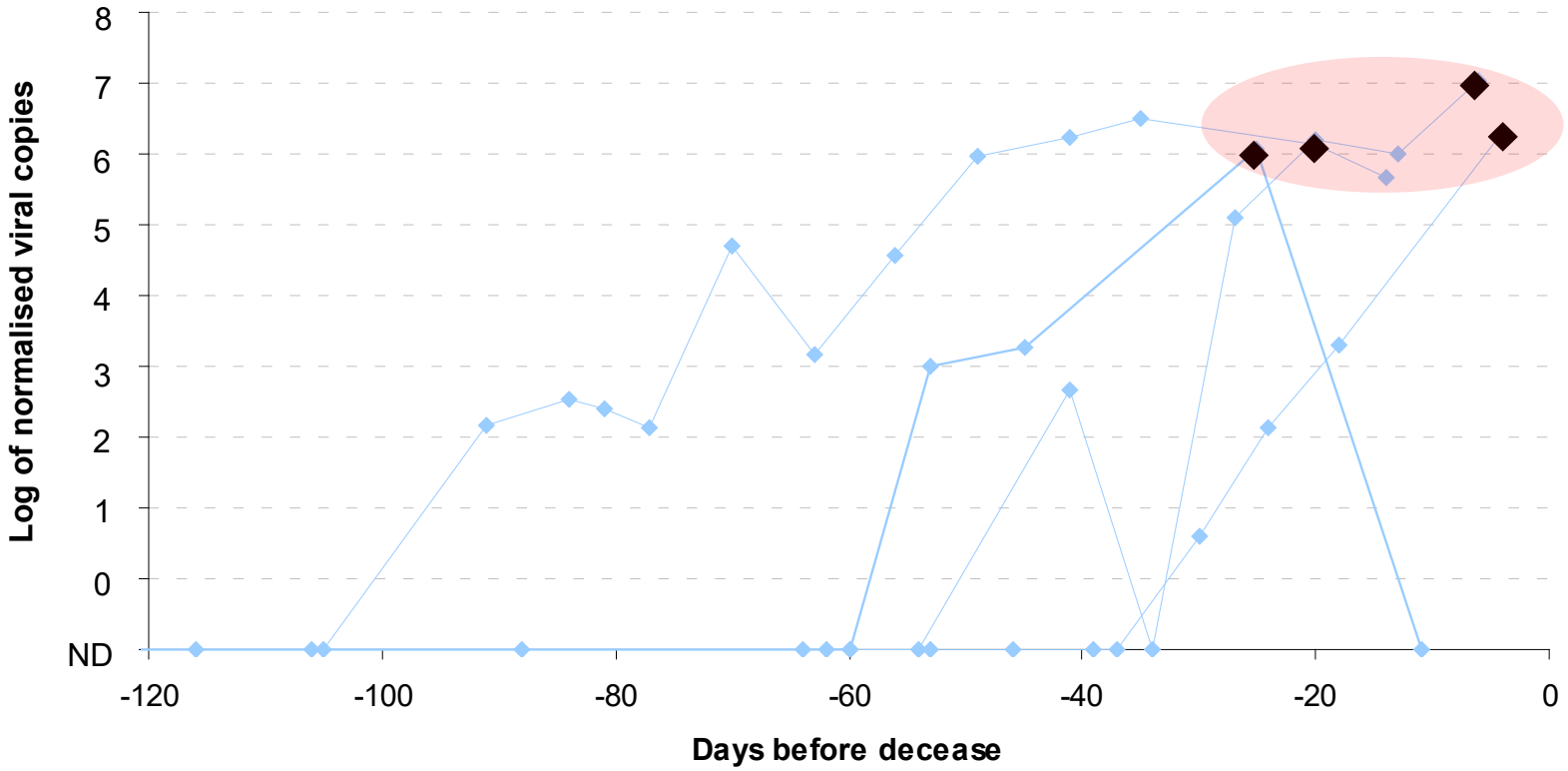


Boy, 5 yrs., HSCT for JMML
Donor: MUD
Graft: BM
Conditioning: busulphan (18 mg/kg)
cyclophosphamide (120 mg/kg)
ATG (16 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+ 56; GIT)
Deceased on D+72 due to MOF.



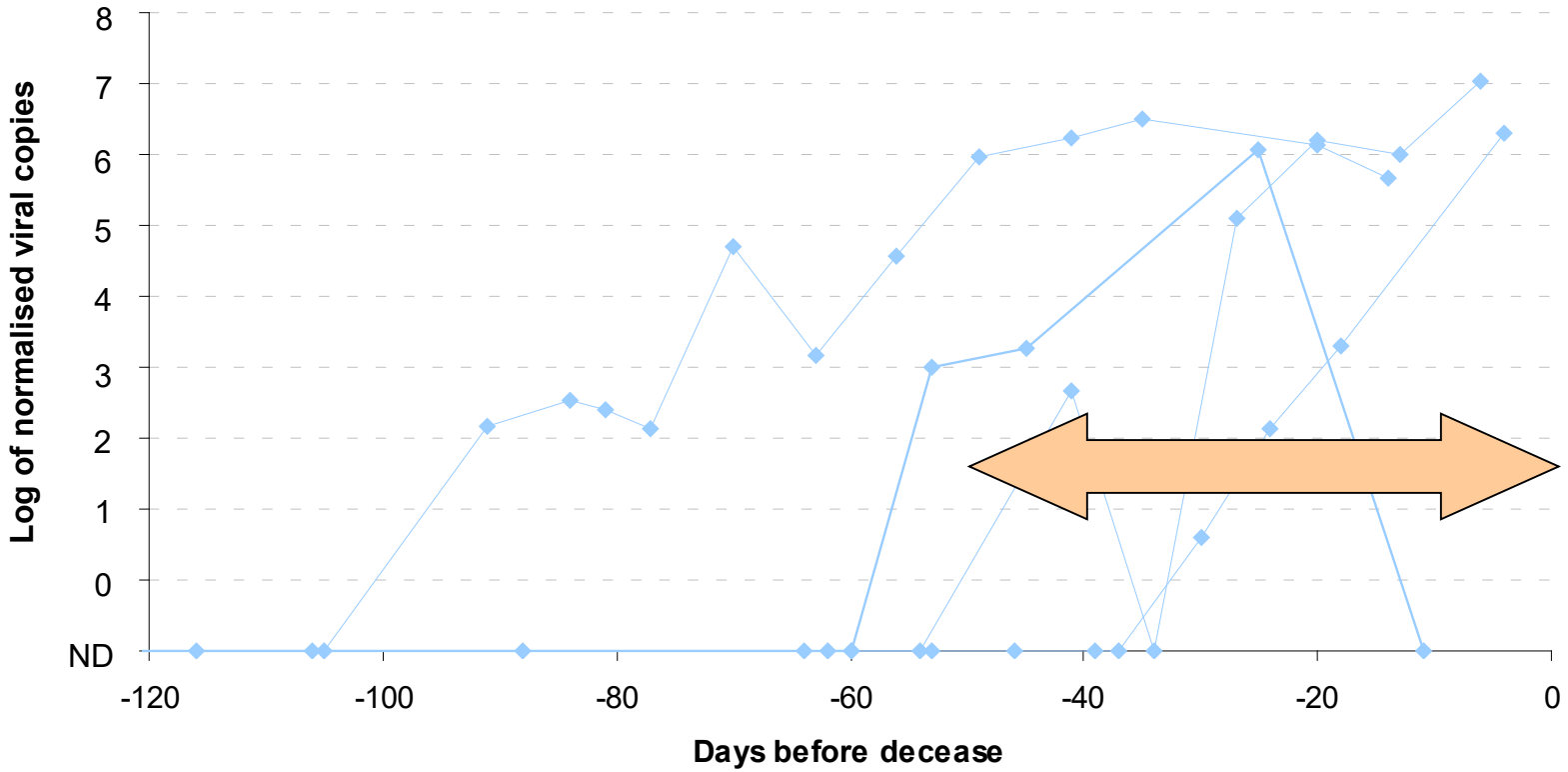
Boy, 11 yrs., HSCT for AML CR2.
Donor: MUD
Graft: BM
Conditioning: busulphan (16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m^2)
ATG (40 mg/kg)
GvHD: grade III-IV (D+ 23; GIT)
Deceased on D+182 due to MOF.

Retrospectively tested patients



Maximum detected quantity was between
1.16×10^6 and **1.17×10^7 NVCs**

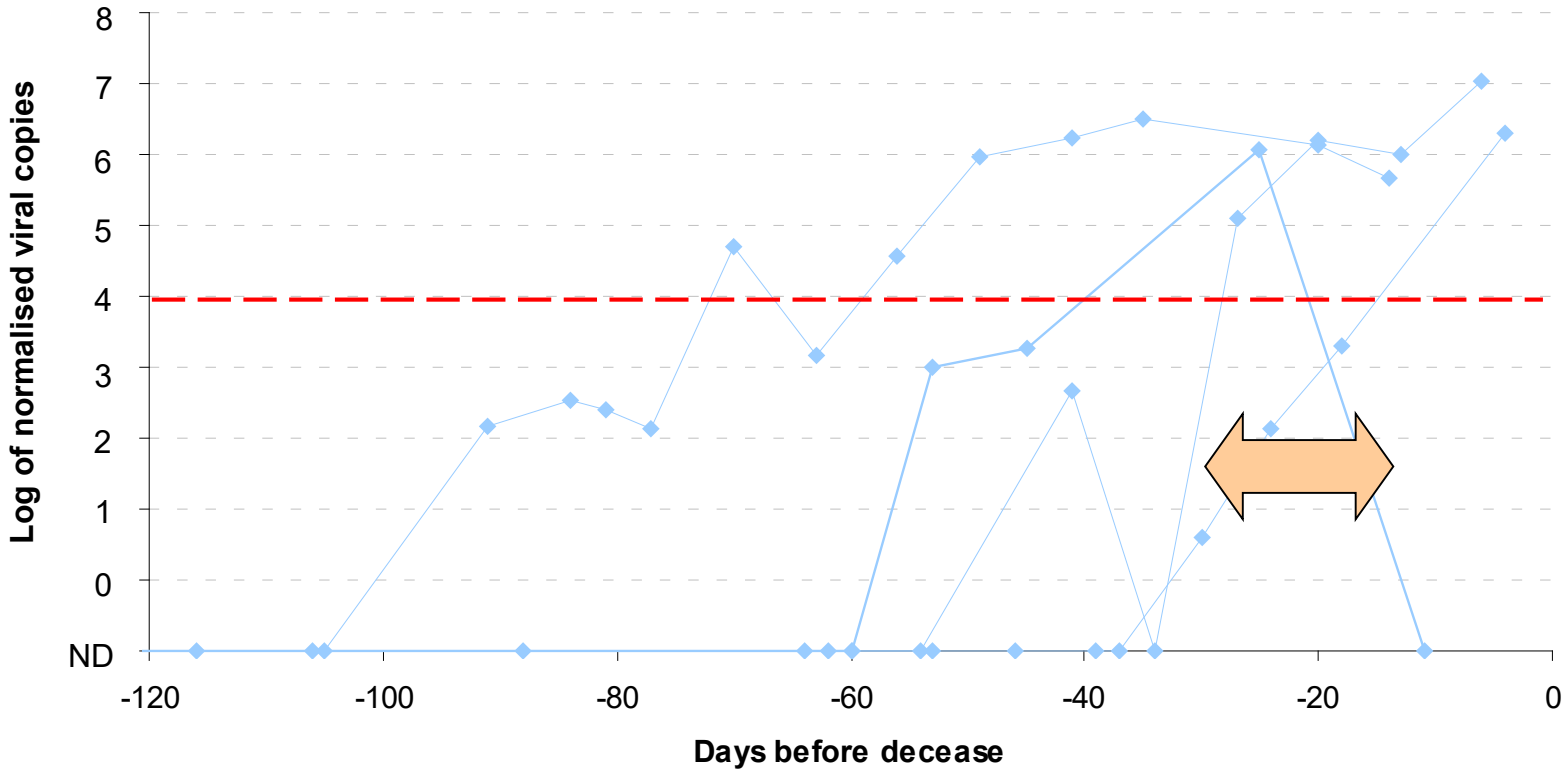
Retrospectively tested patients



Detection preceded decease with median of 47 days (-91 to -30)

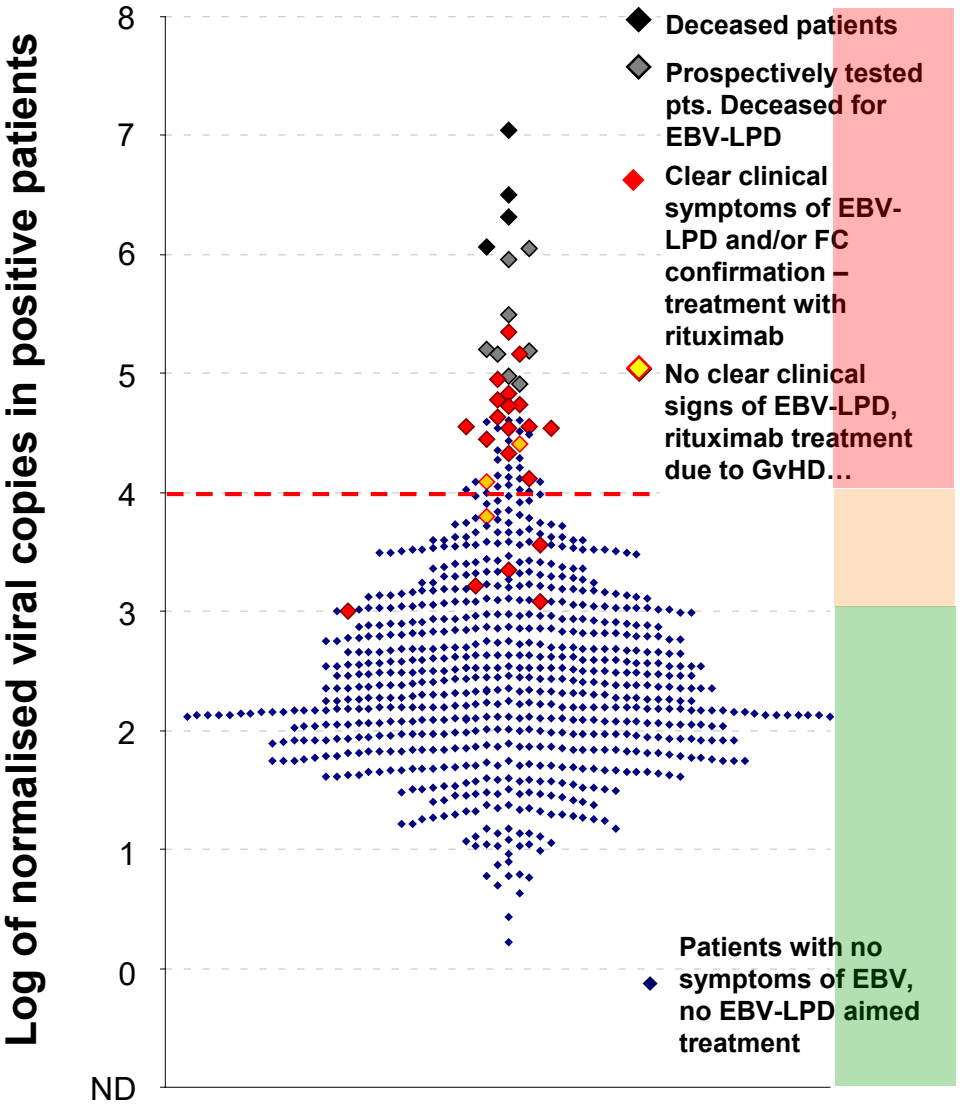
Detection preceded clinical signs of EBV-LPD with median of 35 days (-77 to -24)

Retrospectively tested patients



Quantity > 10^4 NVCs preceded clinical signs of EBV-LPD with median of 14 days (-56 to 2)

Prospective testing – maximal quantity

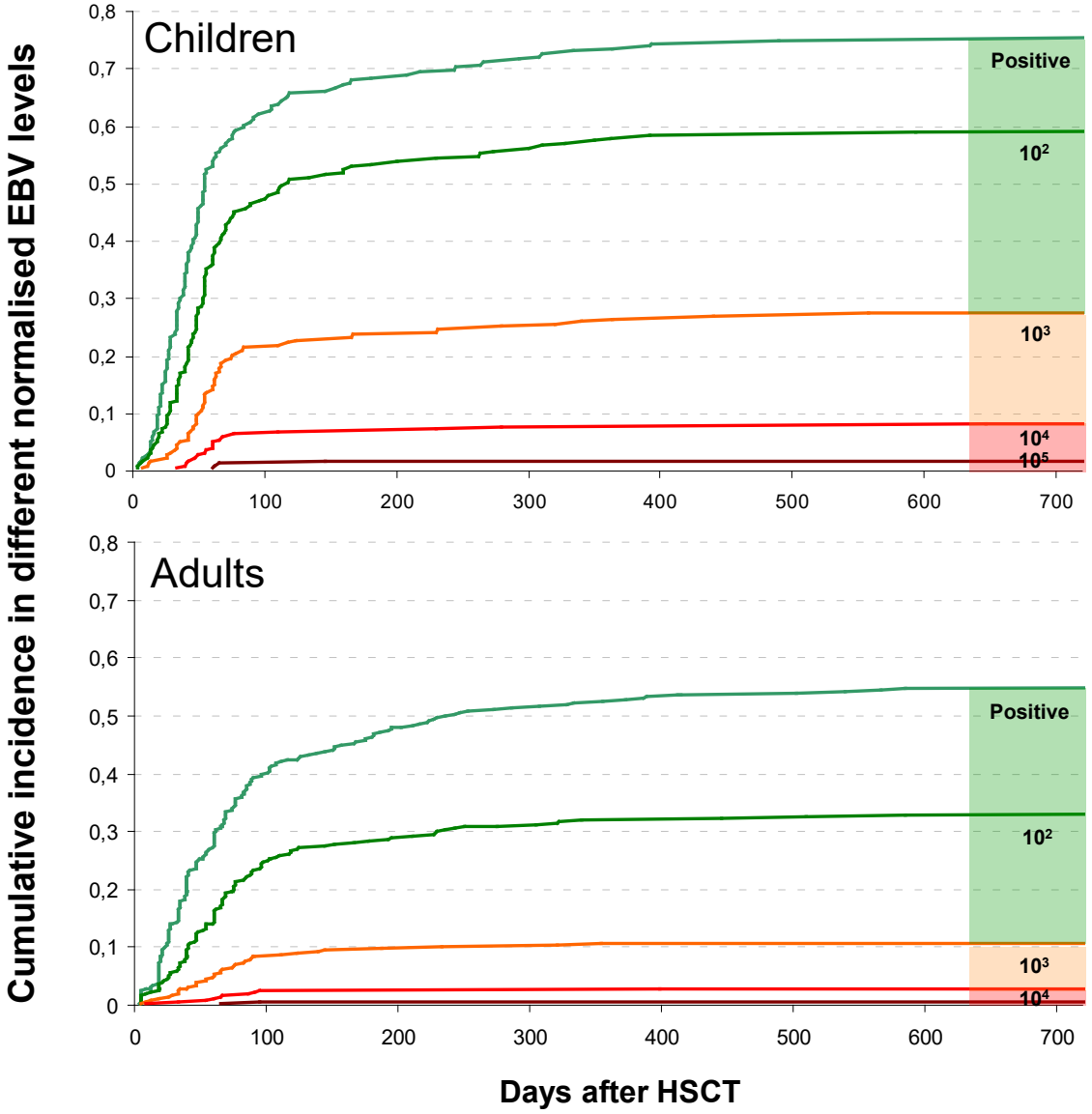


- **Very high risk level reactivation**
  > 10^4 NVCs – ≈ 50 IU/ng
  suspected and/or correlated with signs of EBV-LPD - 102 samples from 26 children (7.1%) and 22 adults (3.2%)
  EBV-LPD was confirmed in 22 (45%)

- **Risk level reactivation**
  > 10^3 and < 10^4 NVCs – ≈ 5-50 IU/ng
  687 samples from 95 (26%) children and 104 adults (15%)

- **Benign level reactivation**
  < 10^3 NVCs ≈ 5 < IU/ng
  low quantities with no clinical signs of EBV were detected in majority of samples from 275 children and 481 adults.

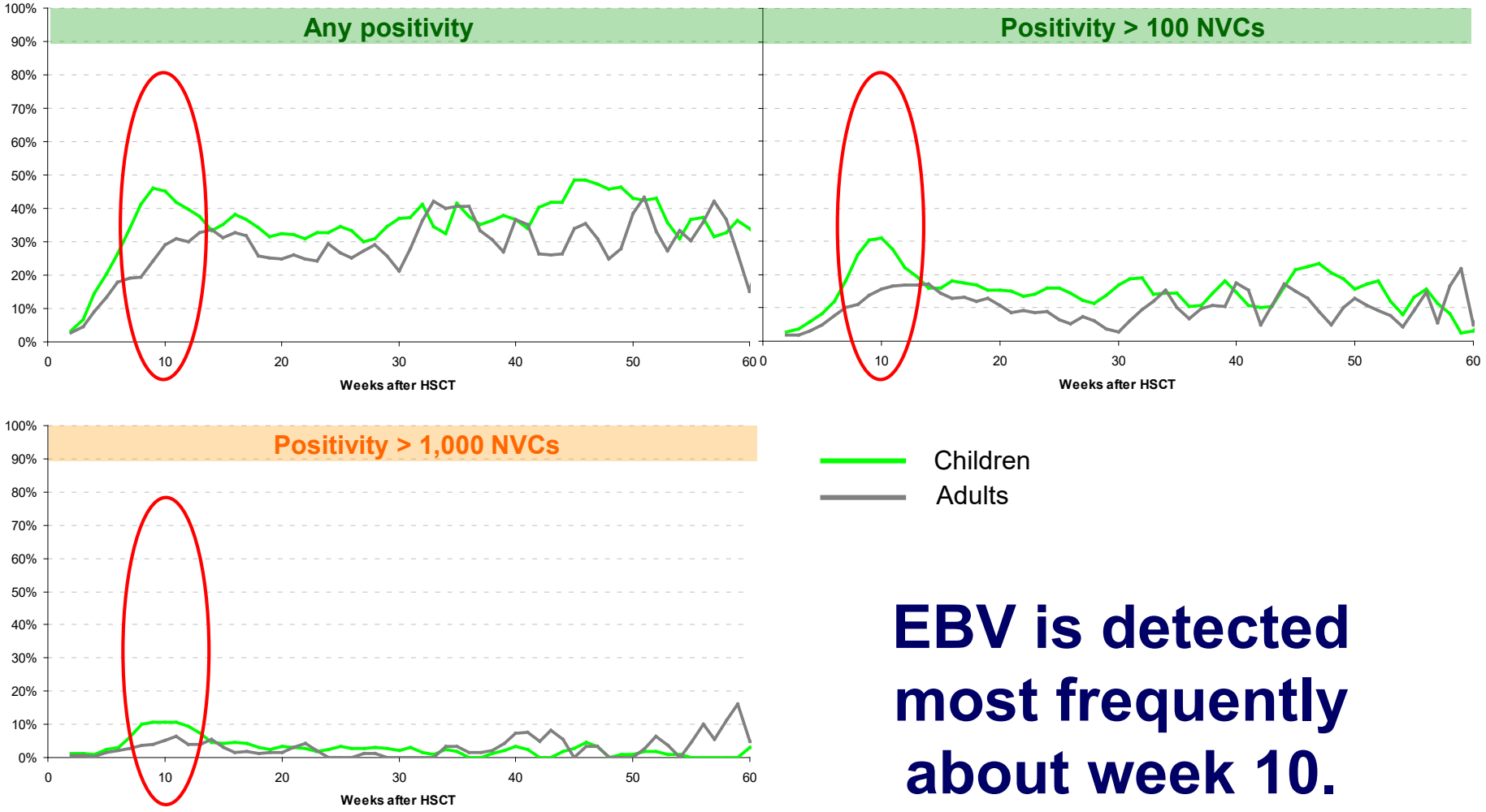
Prospective testing – incidence in time



Higher incidence in paediatric patients at every level

$p<0.007$

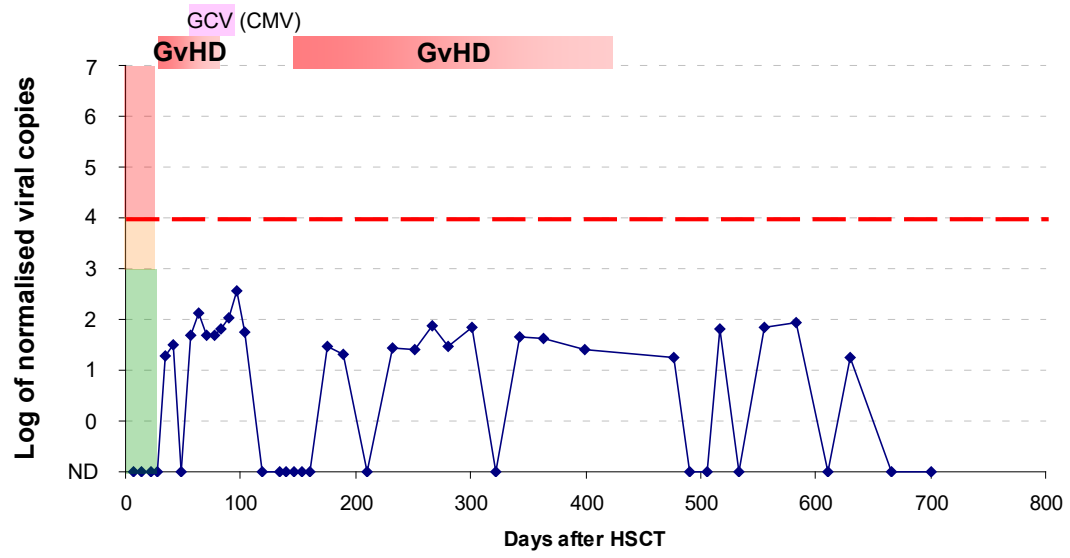
Proportion of positive patients by week and level



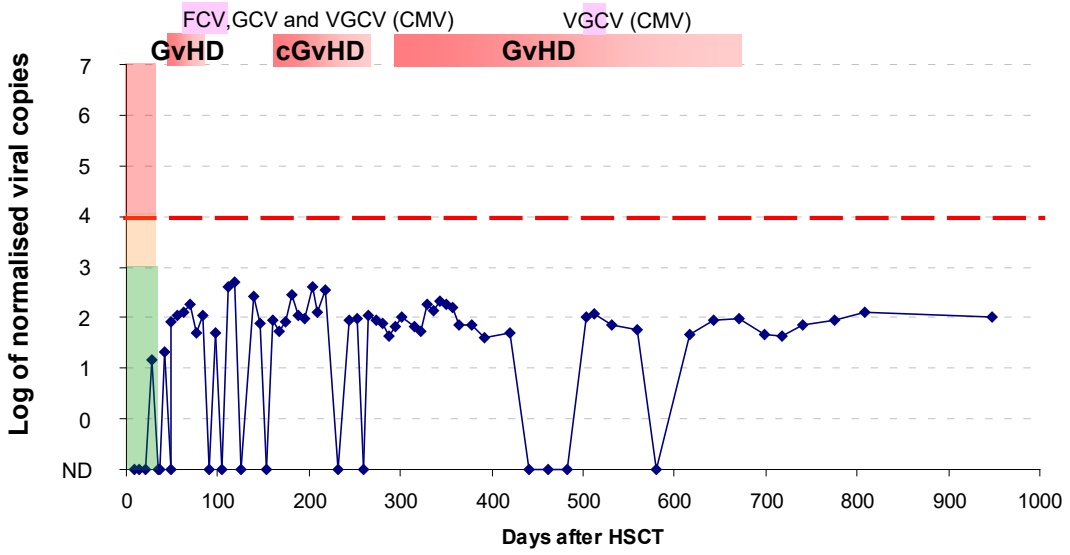
EBV is detected most frequently about week 10.

Benign level reactivation

< 10^3 NVCs ≈ 5 < IU/ng



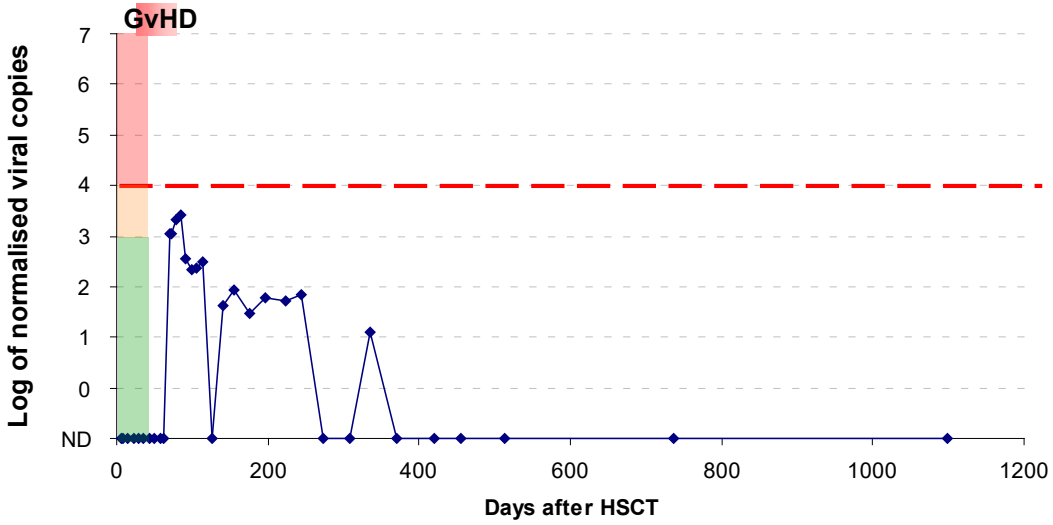
Boy, 13 yrs., HSCT for MDS-RAEB
Donor: MMUD (8/10)
Graft: PBSC
Conditioning: busulphan (16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m^2)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+28;GIT)
Other: BKV-HC(D+40), CMV(D+55)
Outcome: alive, no clin. problems



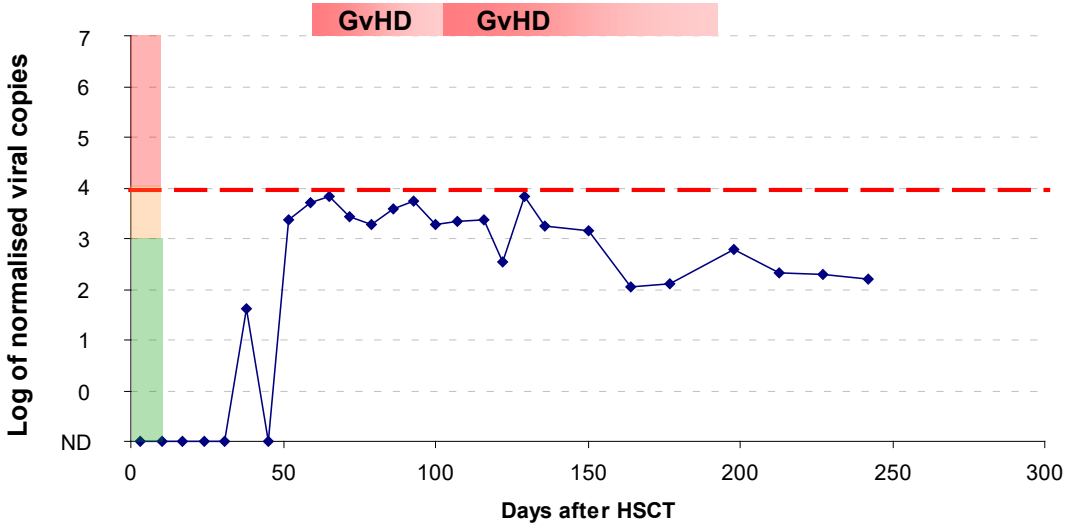
Girl, 13 yrs., HSCT for SAA
Donor: MMUD (9/10)
Graft: BM
Conditioning: TBI (5,4 Gy)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+40;GIT)
Other: CMV(D+46), lung affection of unknown ethiology, steroid DM
Outcome: alive, no clin. problems

Risk level reactivation

> 10^3 < 10^4 NVCs ≈ 5 - 50 IU/ng



Boy, 6.5 yrs., HSCT for ALL
Donor: RD (10/10)
Graft: BM
Conditioning: TBI (12 Gy)
etoposide (60 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+42;GIT+skin)
Outcome: alive, no clin. problems



Boy, 15 yrs., HSCT for BAL(ALL/AML)
Donor: MMUD (9/10)
Graft: PBSC
Conditioning: TBI (12 Gy)
etoposide (60 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade I (D+66)
Other: pulmonary mycosis
Outcome: alive, no clin. problems

EBV-LPD

- **Detected: 28 patiens (2.65% of tx, 3.3% of EBV positive)**
  15 adults (1.98%) and 13 children (3.5%)

- **Mononucleosis like syndrom:** 1 adult
  peak at 54 days after HSCT (1,198 NVCs)

- **Localised :** 12 patients (9 adults, 3 children)
  median peak level at 68 days after HSCT
  median peak level 32,400 NVCs

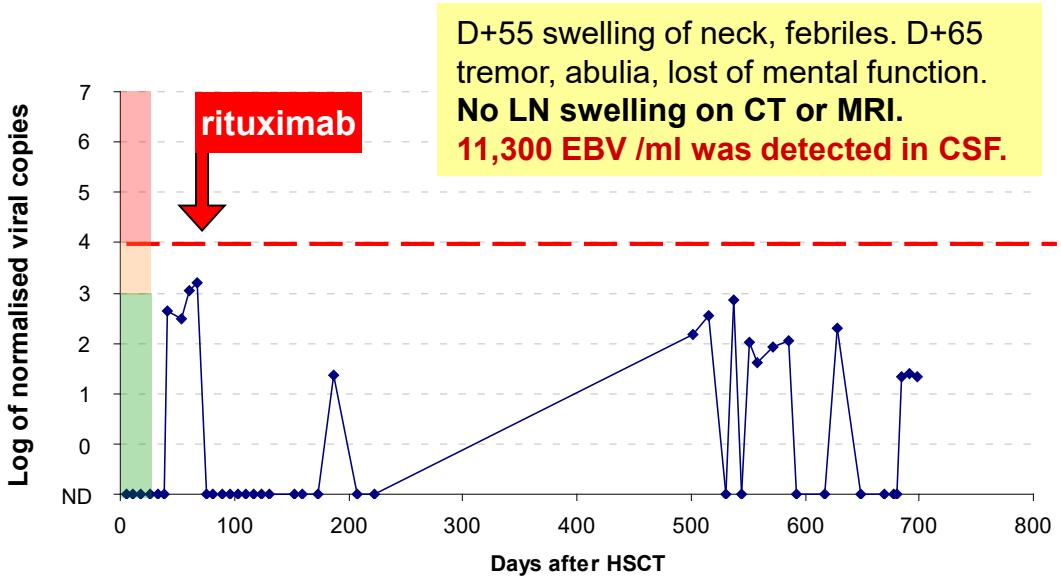
- **Generalized:** 15 patients (10 children, 5 adults)
  median of peak level at 71 days after HSCT (range 41-230)
  median peak level 56,600 NVCs (27,407-220,716)

Confirmed by Flow cytometry.

Rituximab therapy was successful in all but 1 patient.

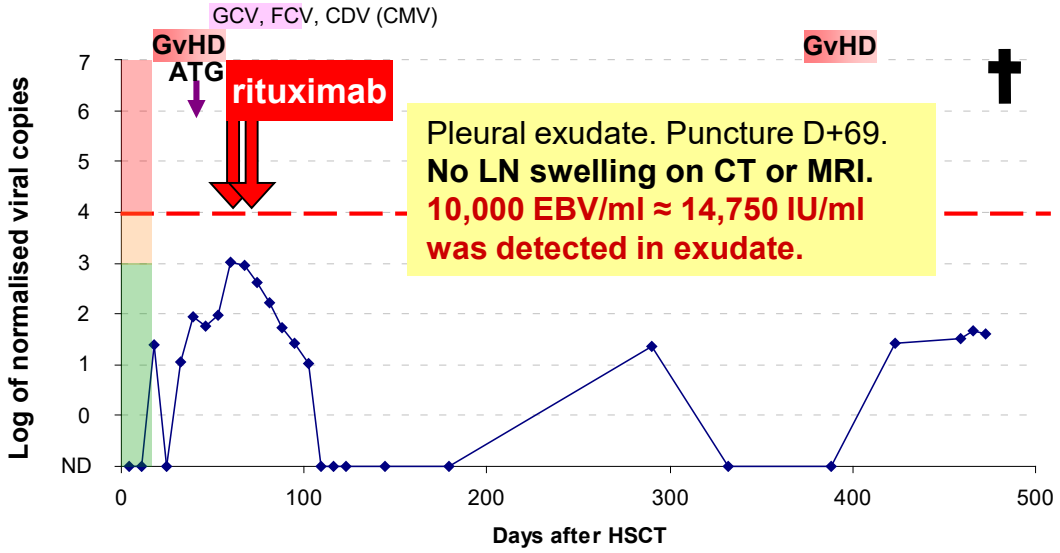
- **EBV 1 was detected in all but one patient with EBV 2.**

Localised EBV-LPD



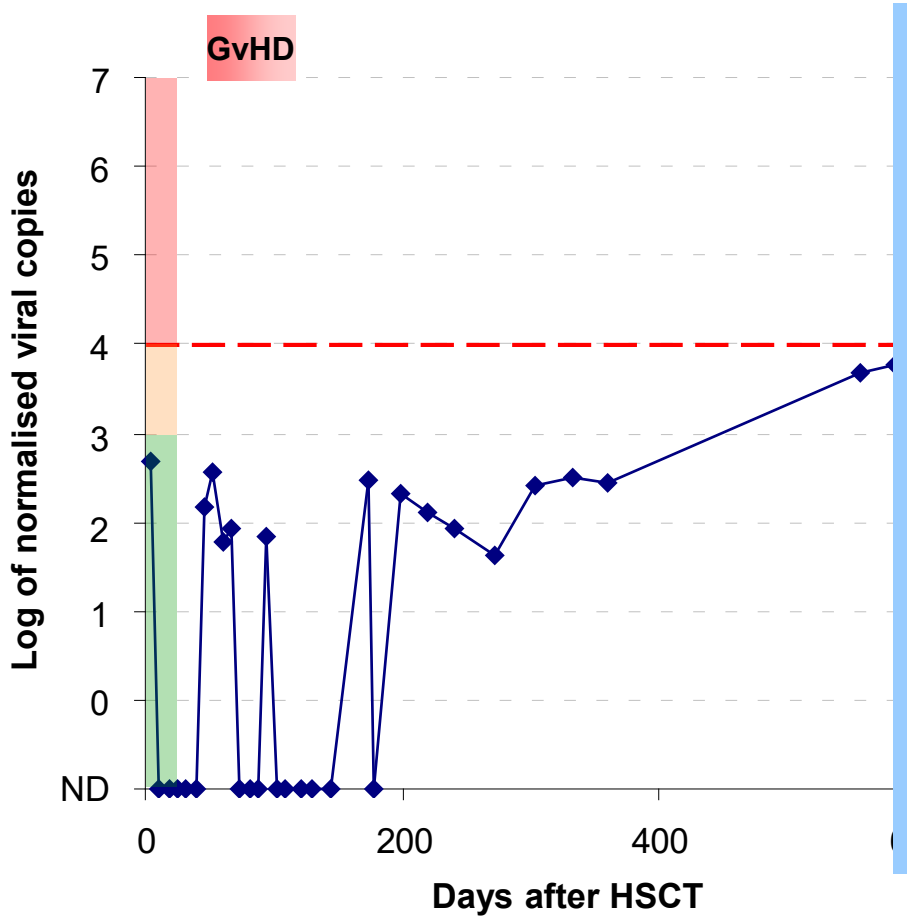
Woman,58 yrs.,HSCT for MDS/AML
Donor: SD (8/10)
Graft: PBSC
Conditioning: idarubicine (21 mg/m²)
fludarabine (25 mg/m²)
TBI (12 Gy) ATG (40 mg/kg)
GvHD prophylaxis: CyA, MMF
GvHD: grade II (D+22)

Outcome: rapid improvement,alive



Woman, 39 yrs., HSCT for AML
Donor: MMUD (9/10)
Graft: PBSC
Conditioning: idarubicine (21 mg/m²)
fludarabine (25 mg/m²)
TBI (12 Gy) ATG (40 mg/kg)
GvHD prophylaxis: CyA+MMF
GvHD: grade III-IV (D+15)
Other: pulmonary proces of unknown origin
Outcome: deceased on D+478 due to MOF

Localised EBV-LPD (NHL)



Boy, 15 yrs., HSCT for ALL in 1. CR
CMV, EBV, HSV seronegative

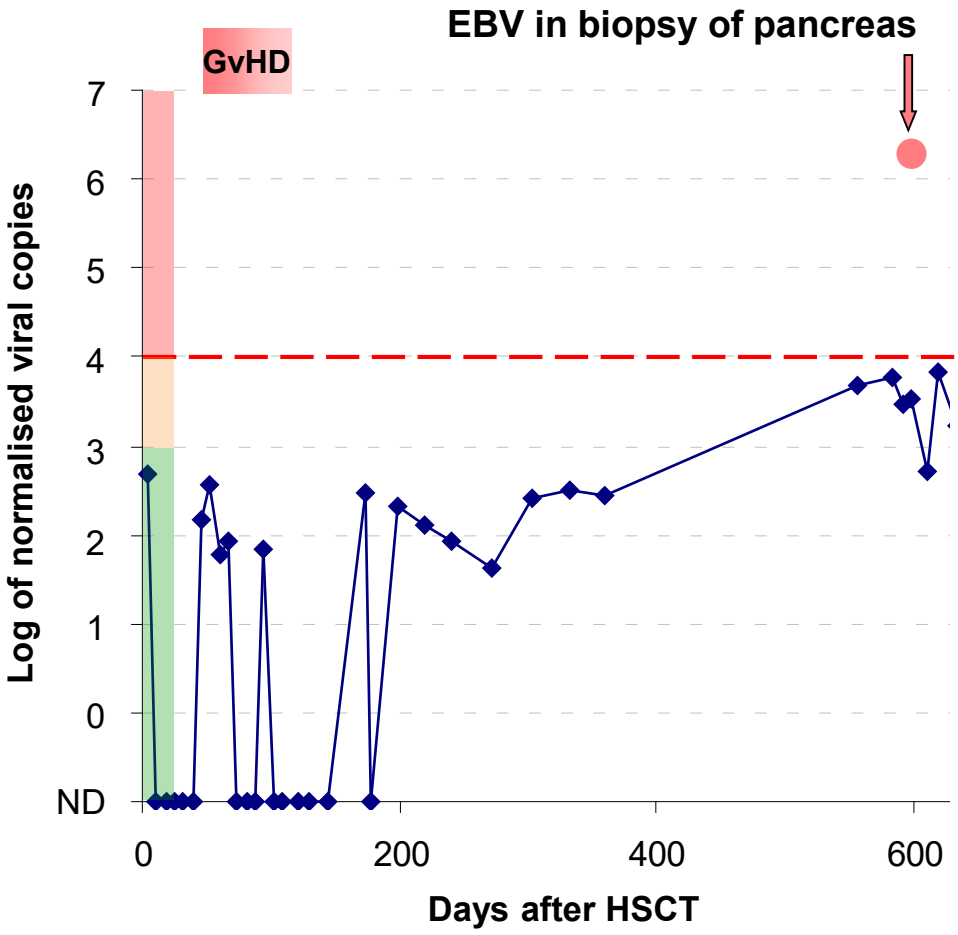
Donor: MSD (brother)
Graft: PBSC
Conditioning: TBI (12 Gy)
etoposid (60 mg/kg)
GvHD prophylaxis: CyA

Engraftment: D+14
Chimaerism: CC D+28
D+95 – 20% autologous (negative MRD)
D+130 – 1% autologous (negative MRD)

GvHD: grade II (D+28;GIT, skin)
Th: MP (1 mg/kg; until D+74) + CsA (until D+102)

D+280 – Herpes zoster (ACV)

Localised EBV-LPD (NHL)



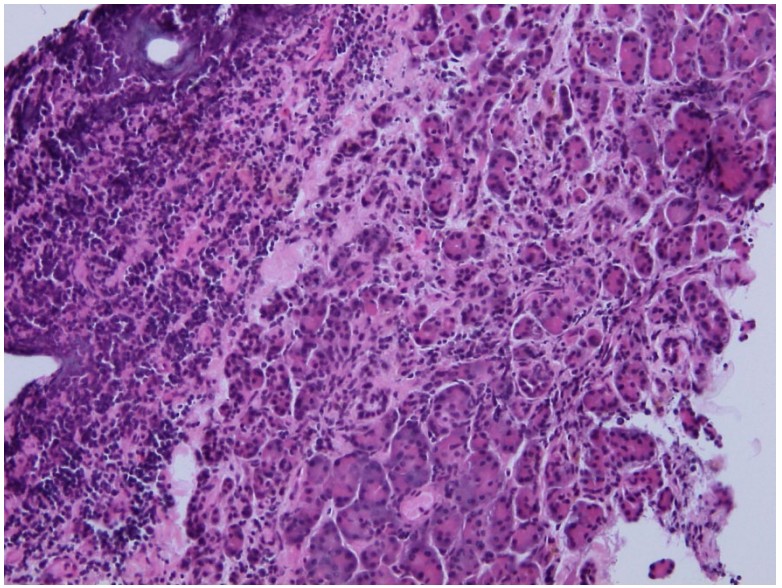
Pancreatopathy of unknown origin, confirmed by CT and US.
MRD negative.

↑CRP, ↑ IgG, no autoantibodies.

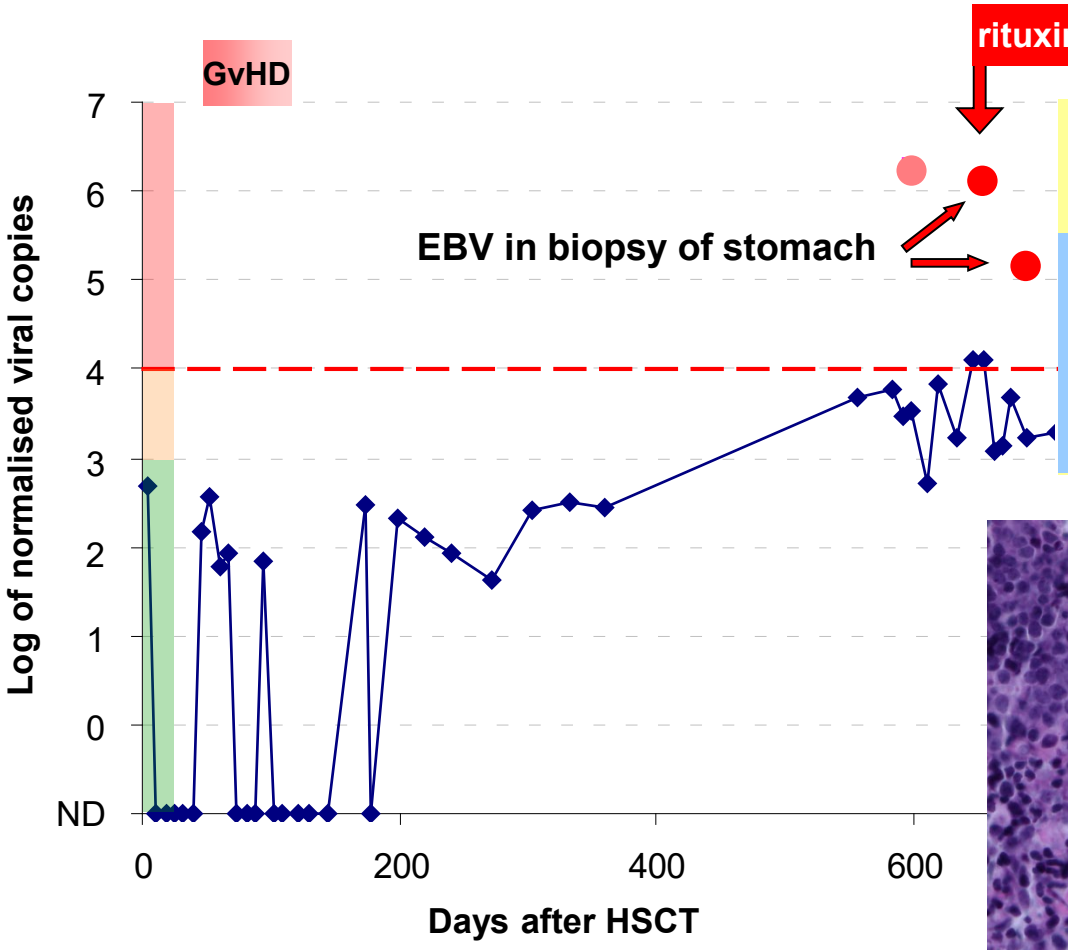
Laparotomy.

All types of lymphocytes detected in the biopsy. No malignant cells.

DR+ lymphocytes detected



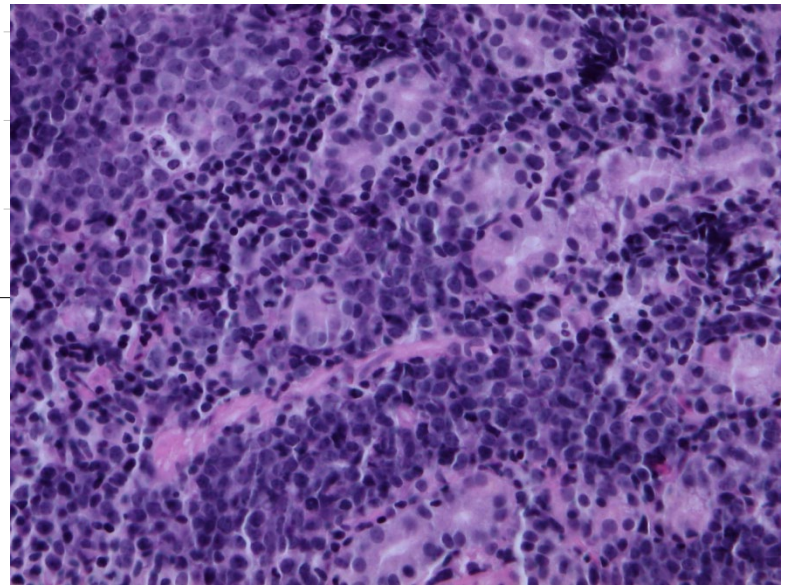
Localised EBV-LPD (NHL)



Anaemia. Gastric bleeding.
4 x rituximab 375 mg/m^2

Gastric biopsy proves EBV+ Non Hodgkin Lymphoma (DLBCL)

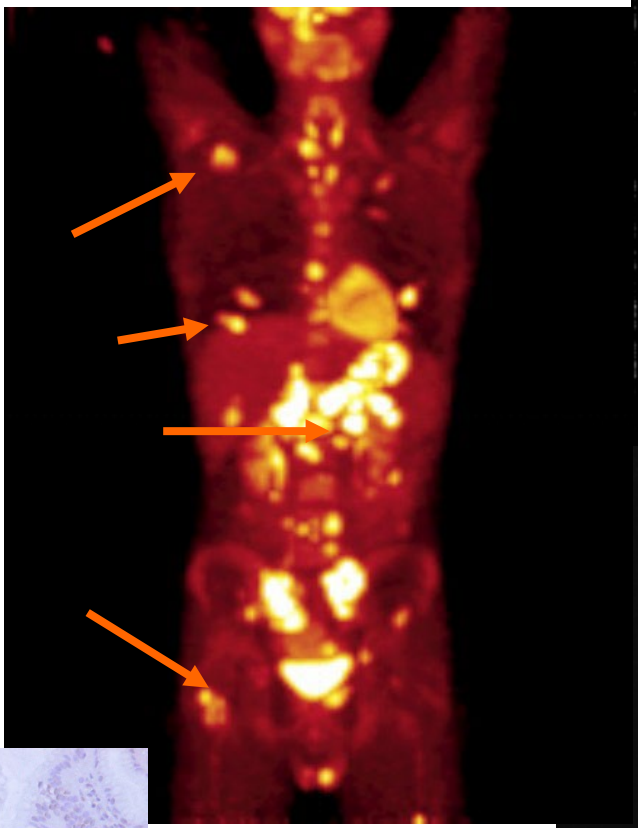
Malignant cells of donor origin.

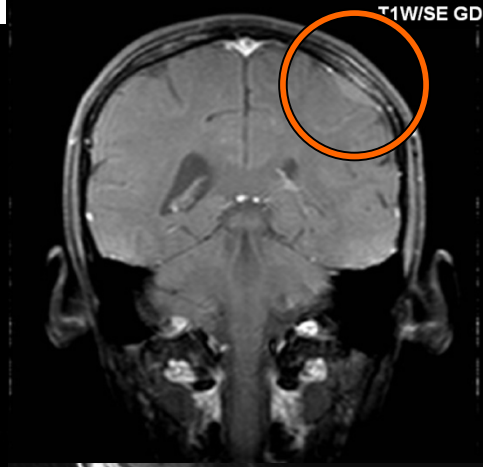


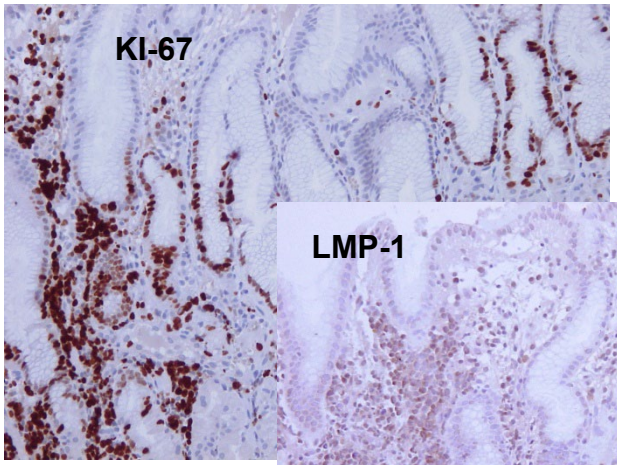
Treatment according to Protocol **BFM NHL 2004**

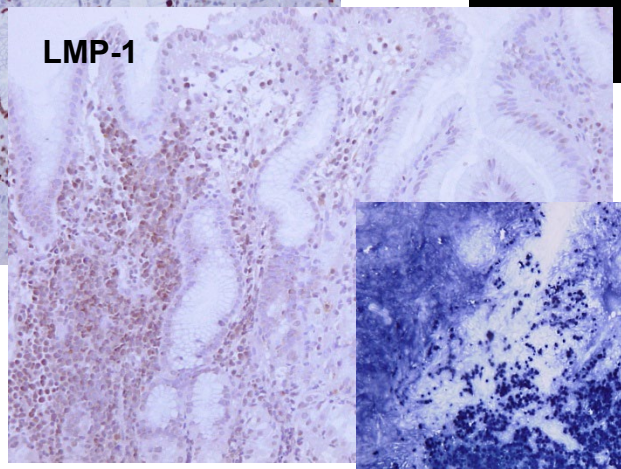
During last chemotherapy sepsis cased by Pseudomonas aeruginosa.

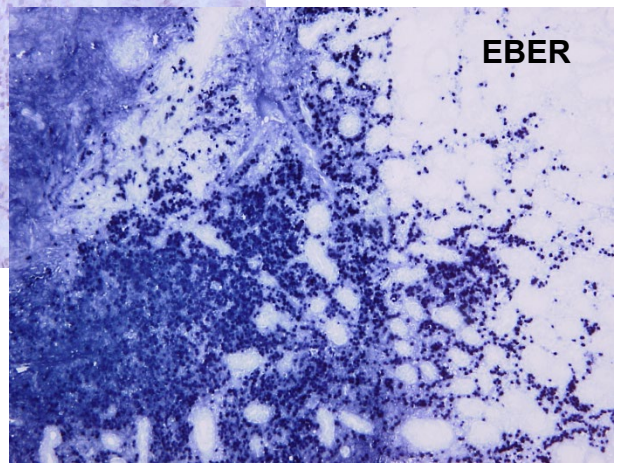
Last PET CT confirmed Remission of NHL.



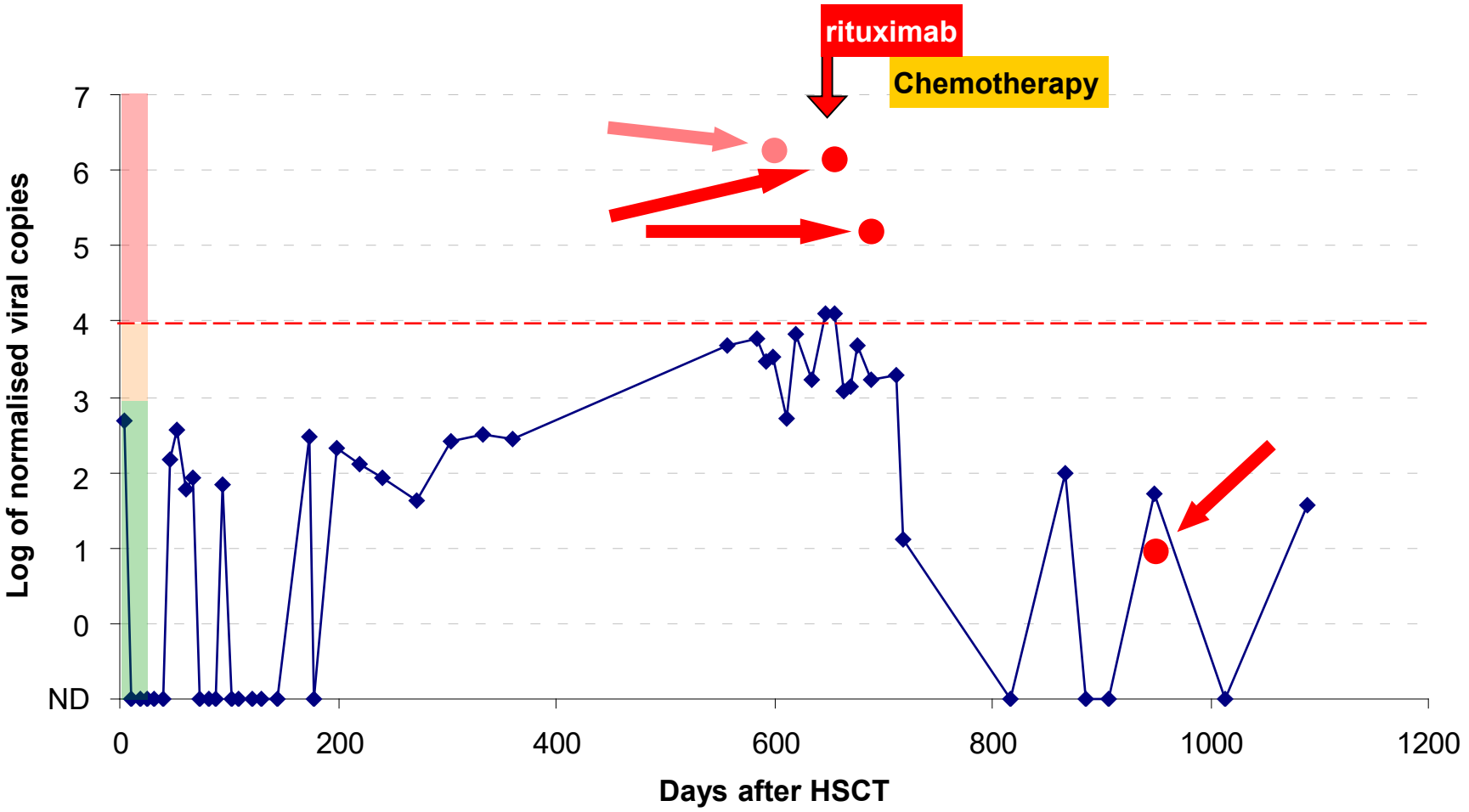






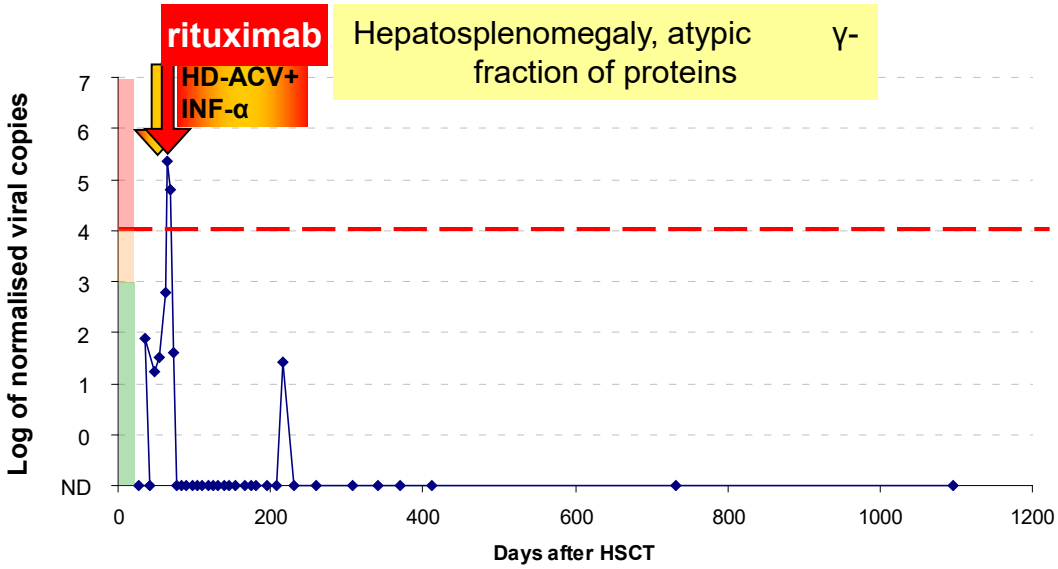


Localised EBV-LPD (NHL)

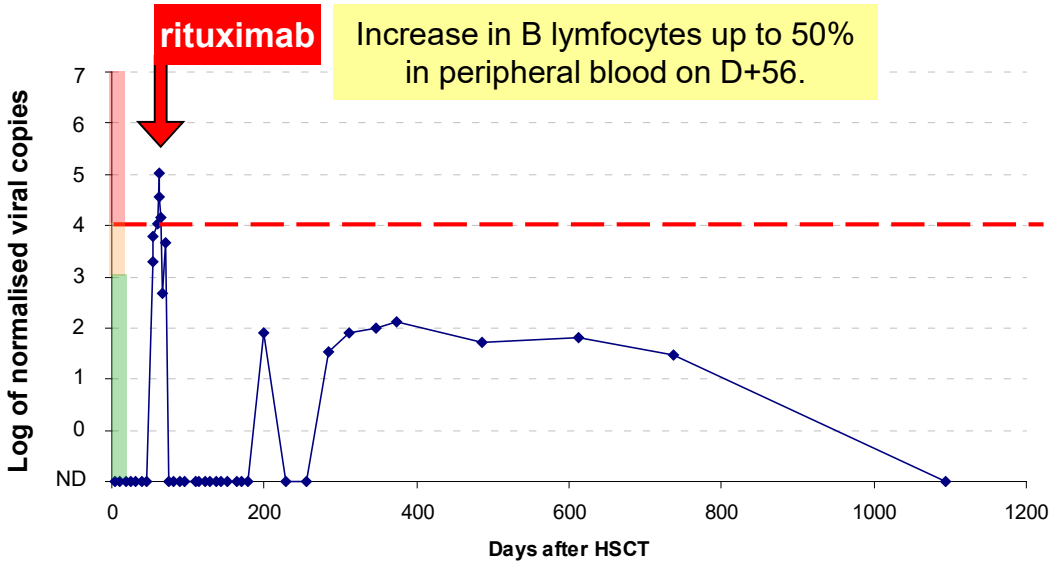


The patient remains in the remission of ALL and NHL.

Generalized EBV-LPD

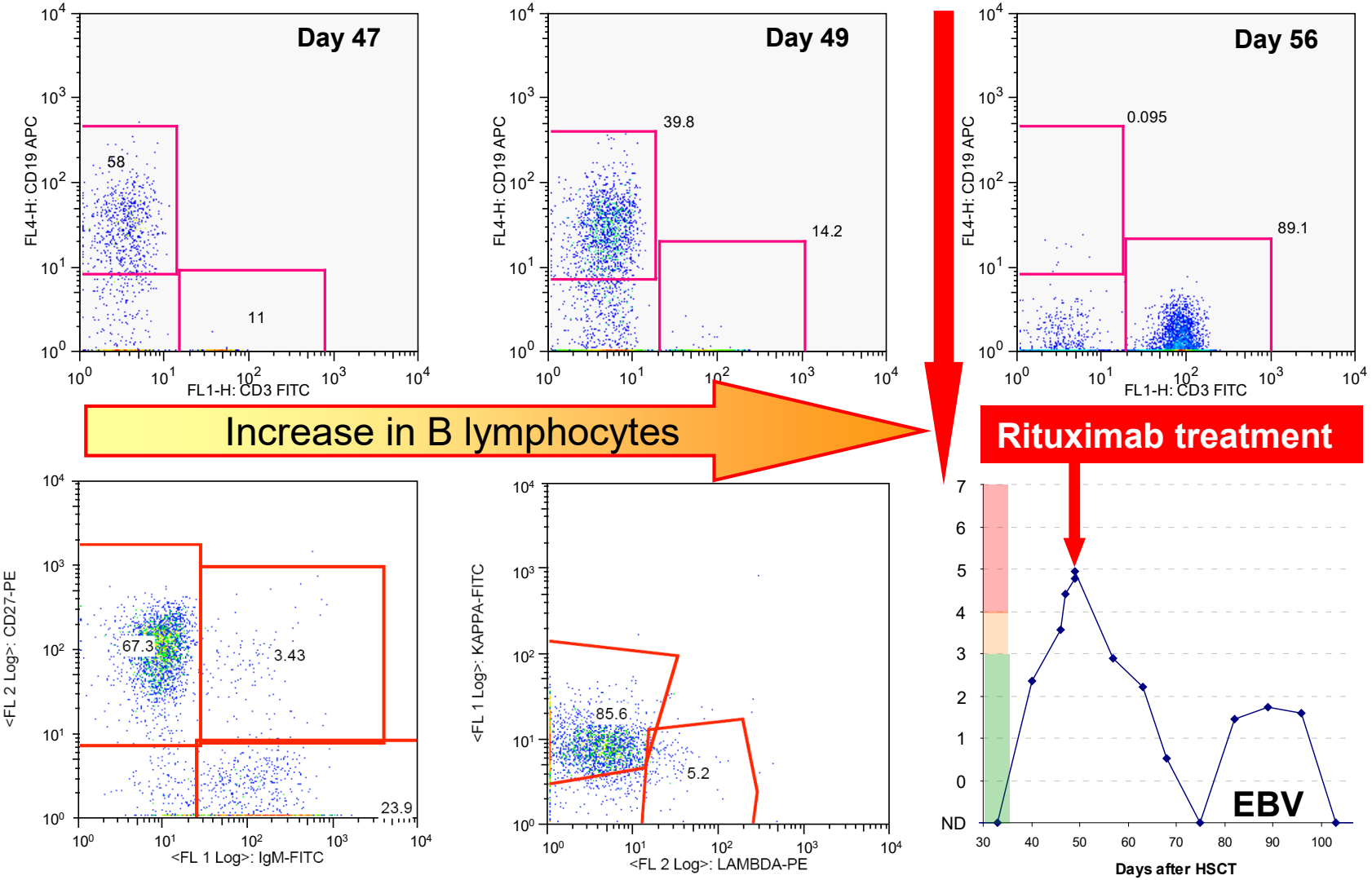


Boy, 3.3 yrs., HSCT for MPS type I
Donor: MMUD (5/6)
Graft: CB
Conditioning: busulphan (24.5 mg/kg)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA, MP
GvHD: grade II (D+49; GIT)
rituximab on D+69
Outcome: alive, no clin. problems



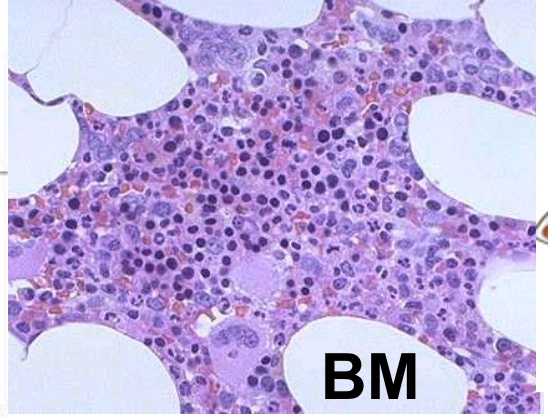
Boy, 4.4 yrs., HSCT for WAS
Donor: MUD
Graft: BM
Conditioning: busulfan (15.3 mg/kg)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA, MTX
GvHD: grade II (D+54; GIT)
rituximab on D+56.
Outcome: alive, no clin. problems

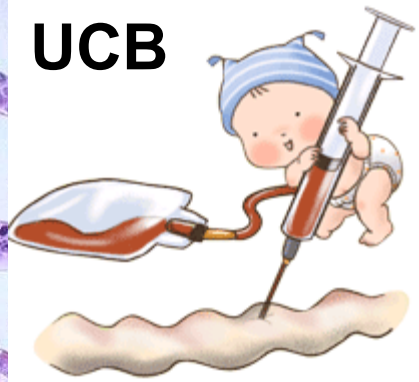
Flow cytometry EBV-LPD confirmation

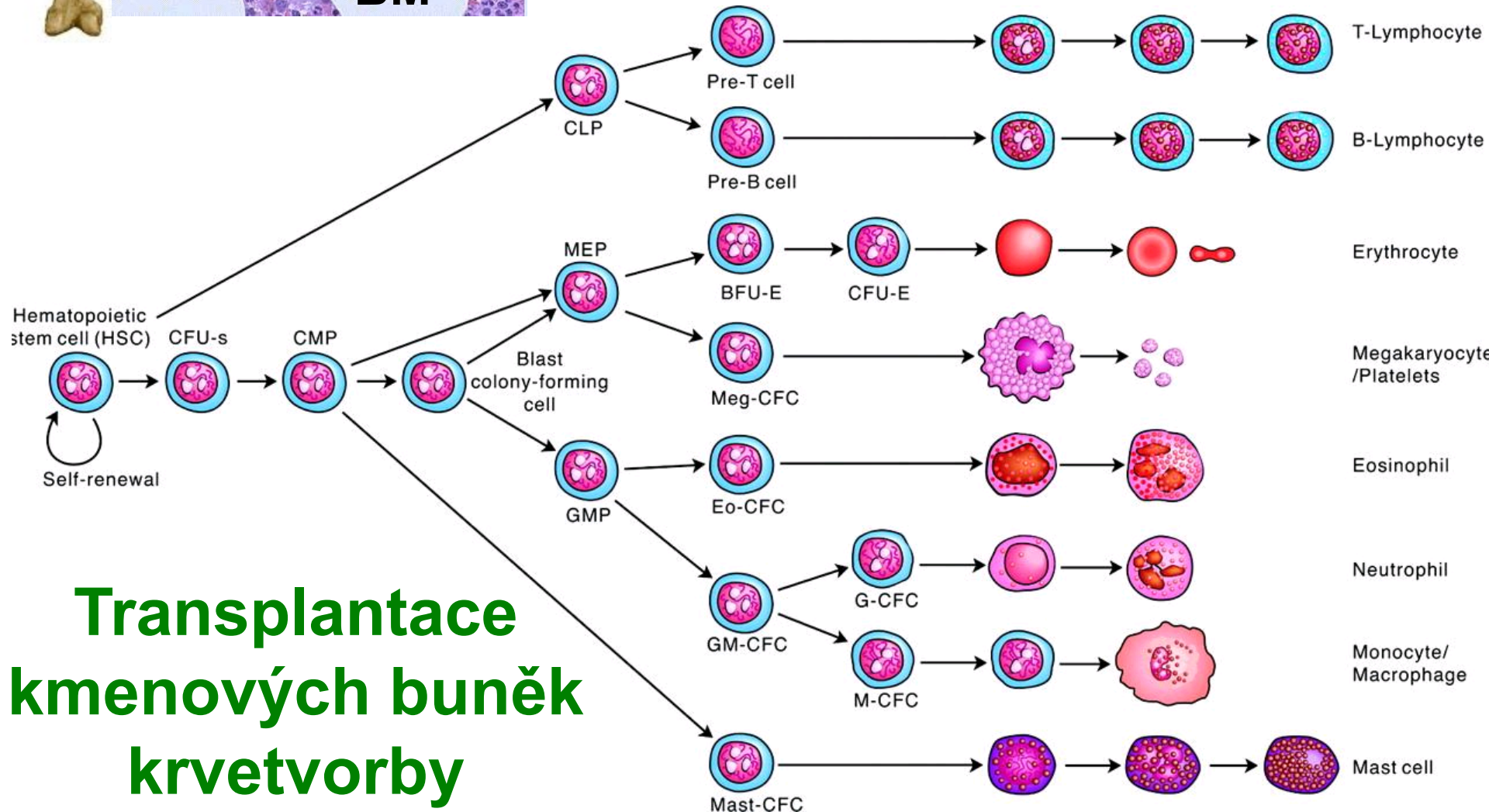


Proliferation of memory B cells

KAPPA IgL+ cells







Transplantace kmenových buněk krvetvorby

HHV-8 = KSHV (Kaposhi sarcoma associated herpesvirus)

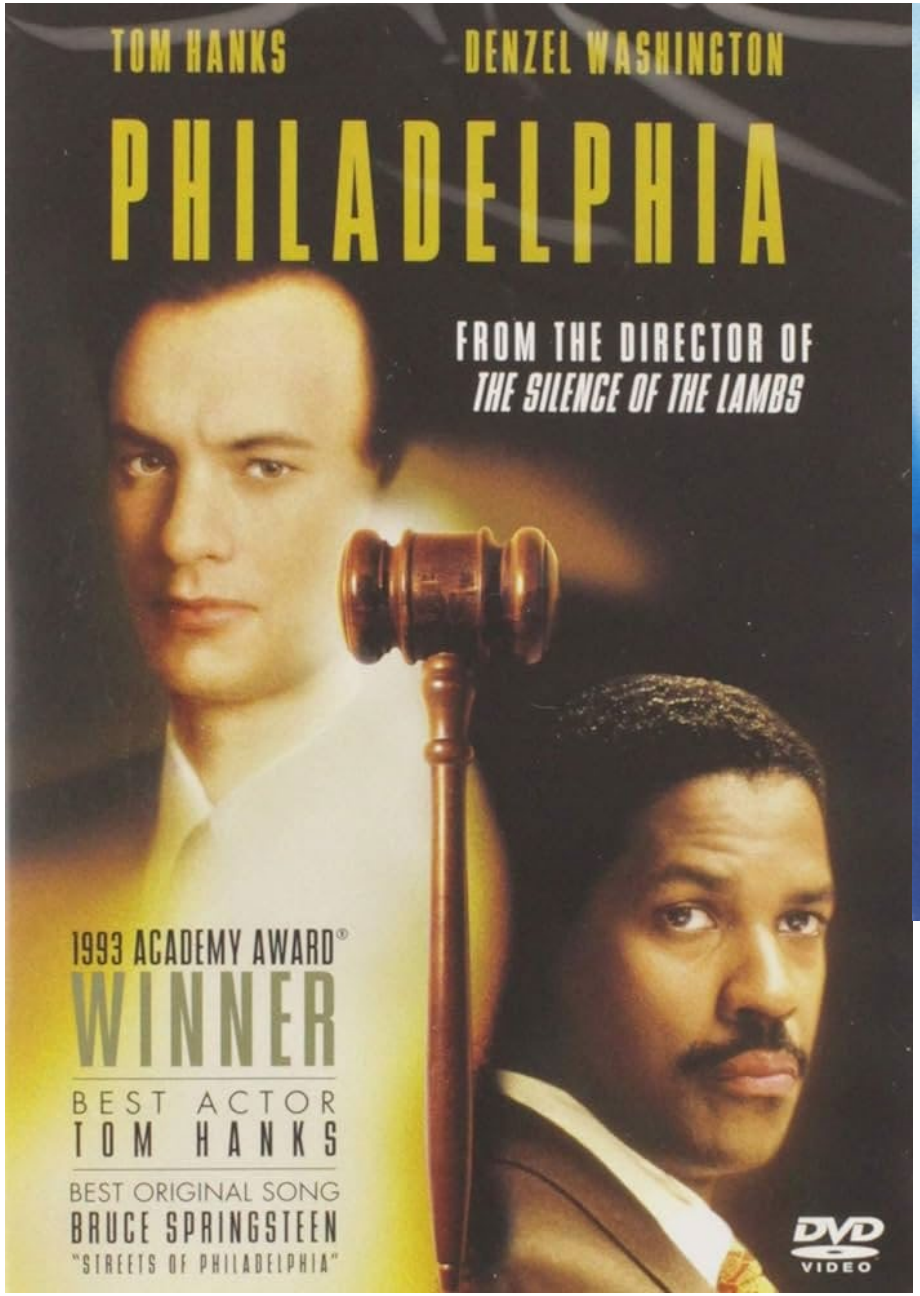
https://cdn.britannica.com/81/131881-004-7B1624F6/Kaposi-sarcoma-lesion.jpg?w=400&h=300&c=crop

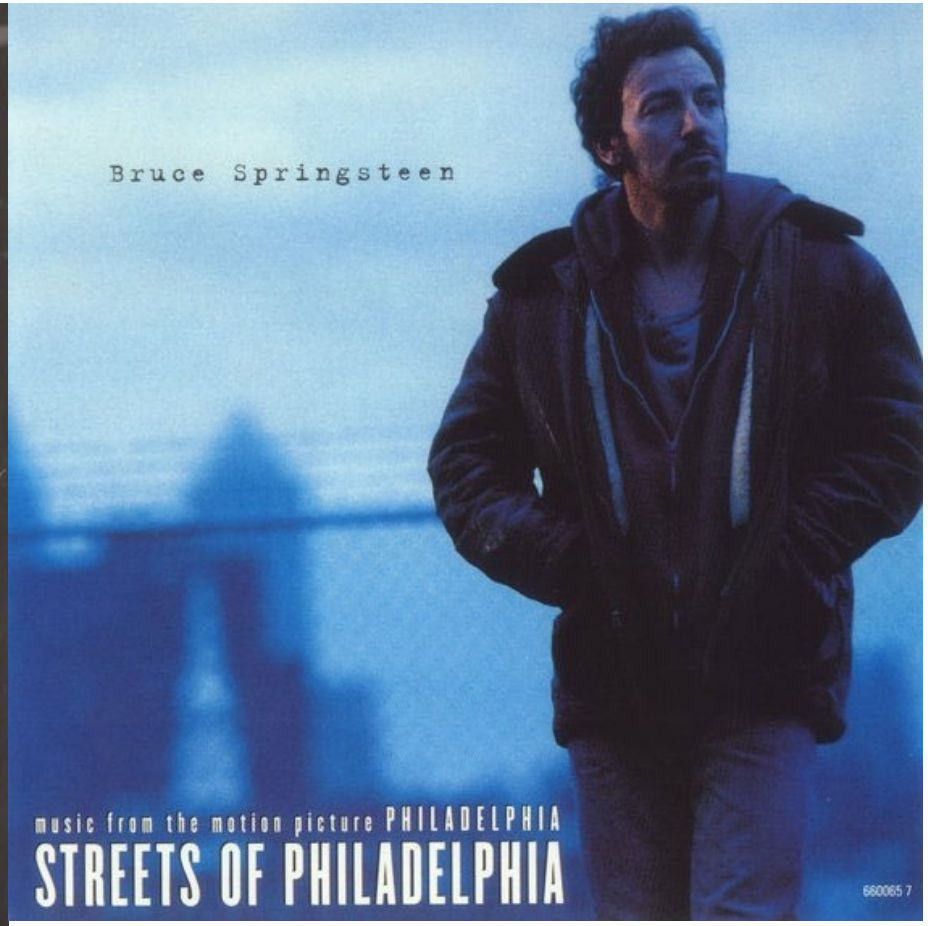
https://upload.wikimedia.org/wikipedia/commons/thumb/1/1c/Kaposis_sarcoma_01.jpg/220px-Kaposis_sarcoma_01.jpg

endothelial tumor of blood and lymphatic veins and lymph nodes

https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcR6dYPRe2lkion2MMu5ZfRaLmaNllksIoYo1Mt3DI62CLxmbzPEs5i2o885TVMdgU2Lkf8&usqp=CAU

https://media.springernature.com/lw685/springer-static/image/chp%3A10.1007%2F978-3-662-45139-7_46/MediaObjects/978-3-662-45139-7_46_Fig3_HTML.png





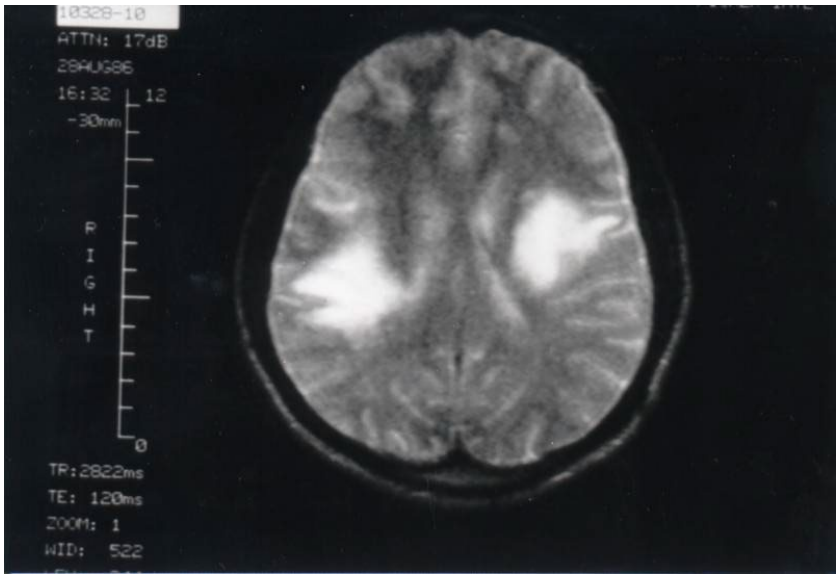
https://www.youtube.com/watch?v=4z2DtNW79sQ

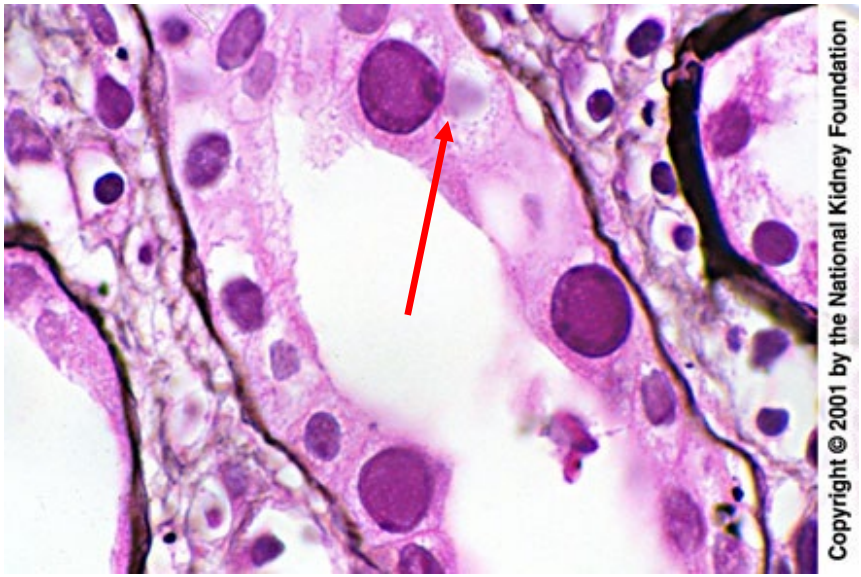
Polyomaviruses

- small ds DNA viruses with circular NA
- Capsid diameter 42-45 nm, genome: 5 kbp
- Transmission by fecal-oral route

- **JC virus** – progressive multifocal leukoencephalopaty PML
- **BK virus** – hemorrhagic cystitis, nephropathy (graft rejection in kidney transplant)
- **WUV and KIV** – respiratory infections
- **MCV** – Merkel cell carcinoma virus (rare skin carcinoma)
- **HPyV 7-12** (Human Polyomavirus) – mainly skin viruses

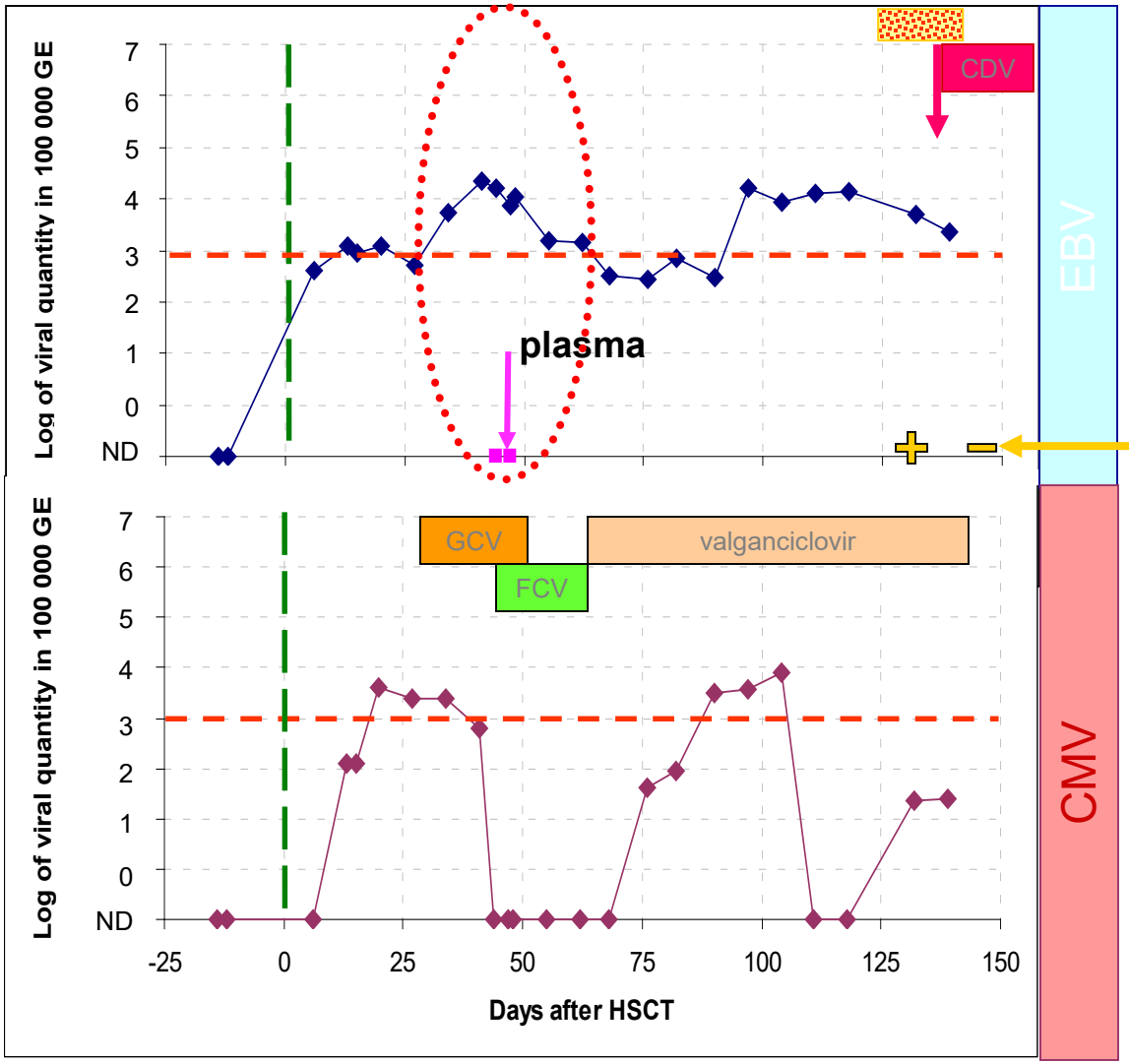
- Potencially treatable with **cidofovir**



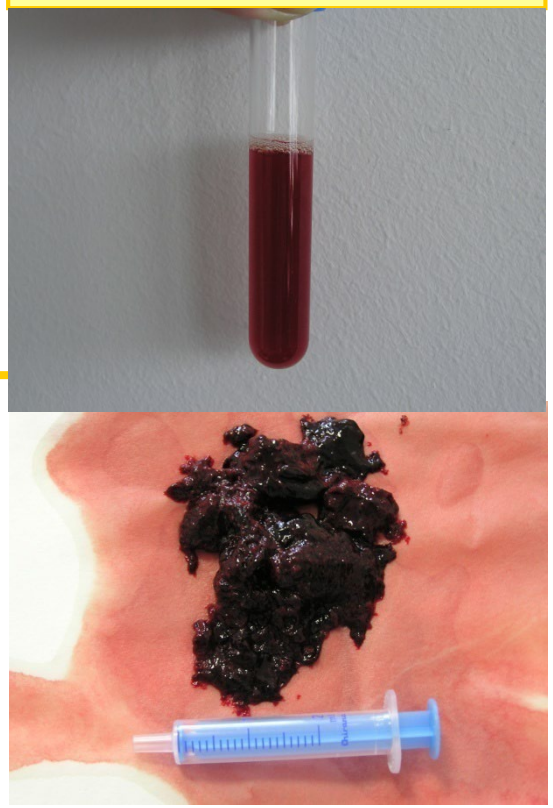


www3.us.elsevierhealth.com/ajkd/ atlas/37/1/atlasf02.htm.

BKV – haemorrhagic cystitis



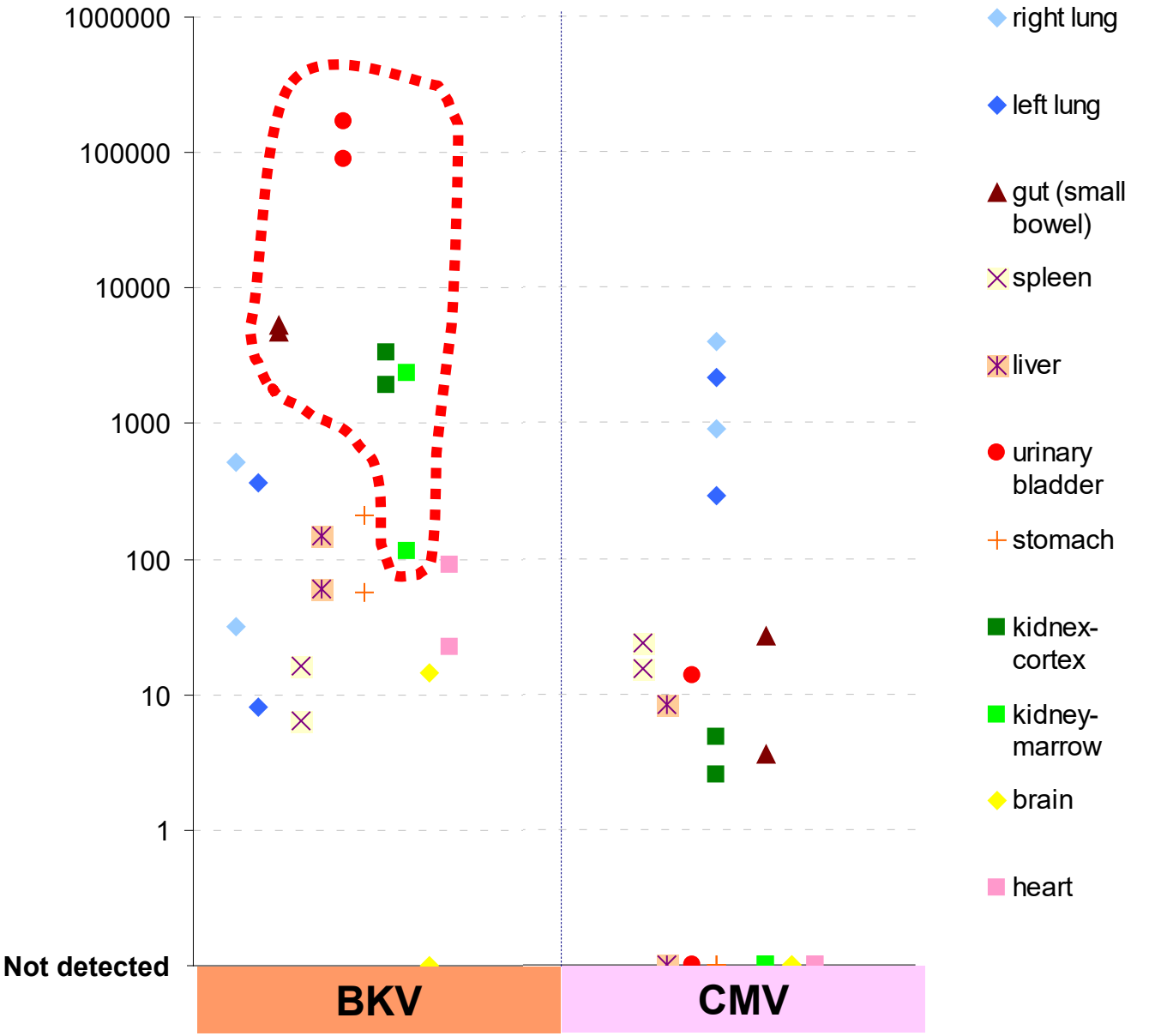
BKV - urine



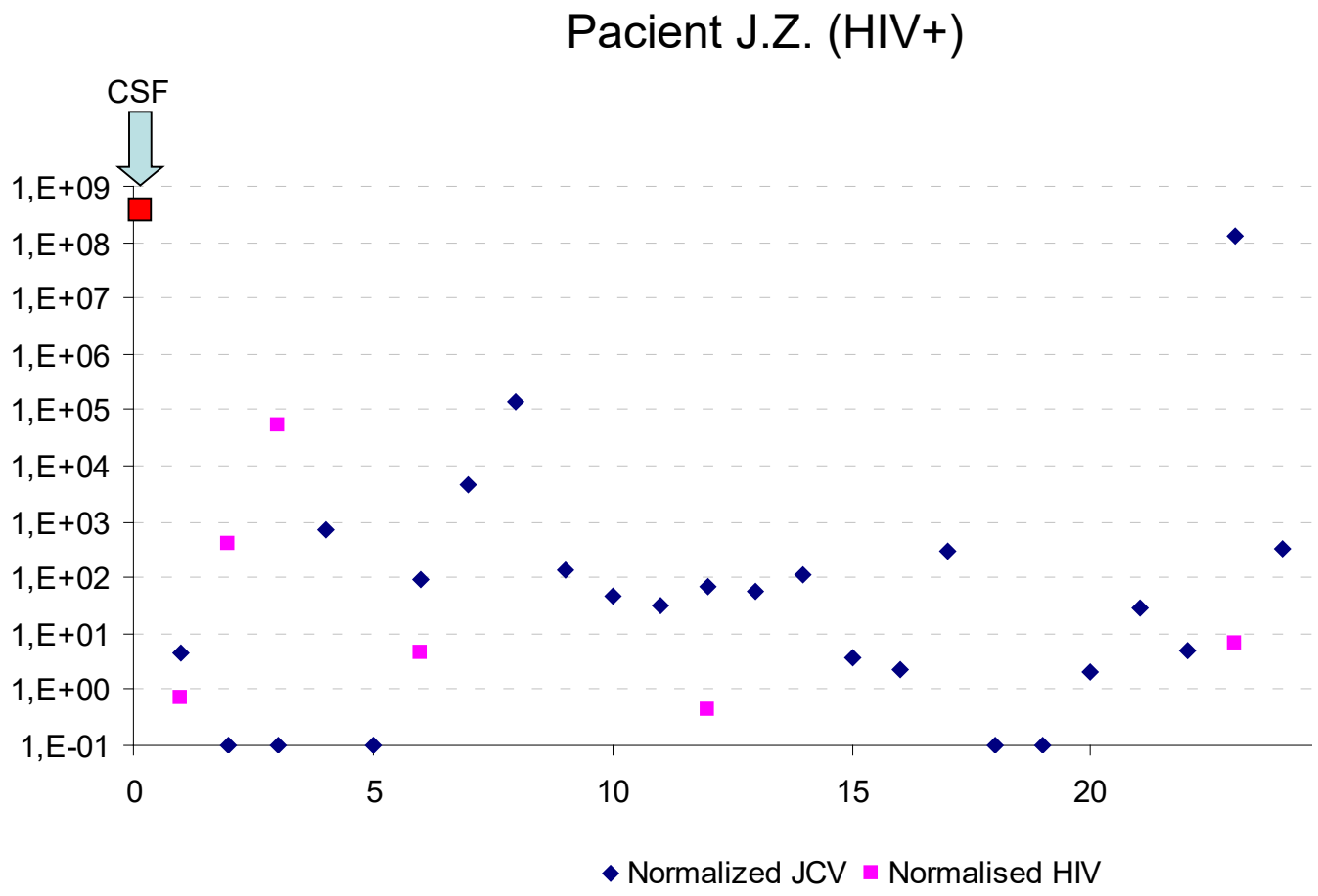
Age at HSCT.:18 let, Fanconi anemia, MUD 9/10, BM, aGvHD grade I.

Normalised viral load in 10,000 g.e. of the tissue

tissue specificity

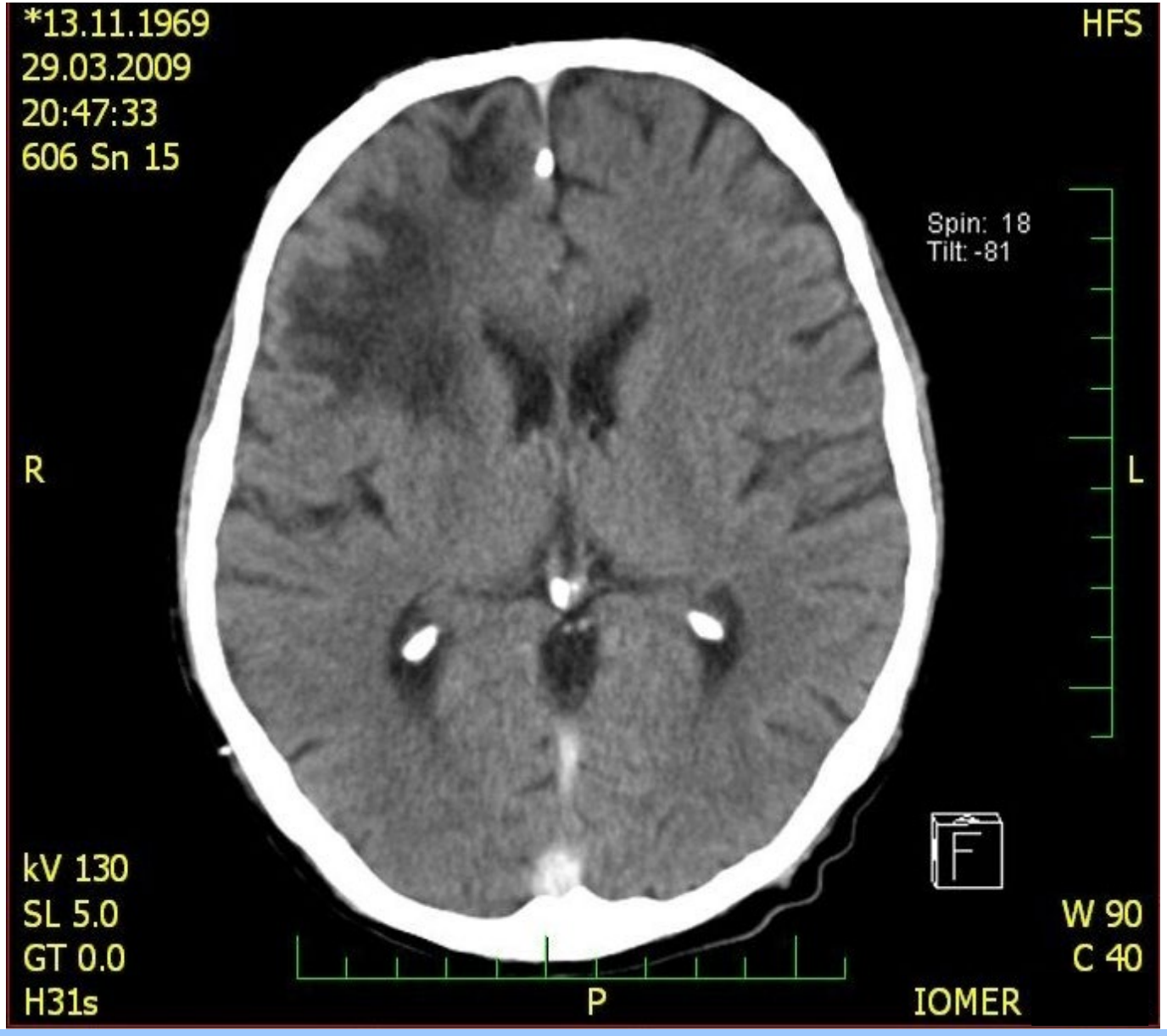


JCV



1	Colon
2	Lung Left Upper Lobe
3	Myocard
4	Testis
5	Kidney - Left Cortex
6	Kidney - Right Medulla
7	Stomach
8	Thyroid gland
9	Lung Left Lower Lobe
10	Lung Right Upper Lobe
11	Kidney - Right Cortex
12	Liver
13	Lung Right Middle Lobe
14	Duodenum
15	Jejunum
16	Spleen
17	Urinary Bladder
18	Ileum
19	Suprarenal gland - Right
20	Pancreas
21	Suprarenal gland - Left
22	Lung Right Lower Lobe
23	Brain
24	Kidney - Left Medulla

JCV



40 yrs. HIV+ patient deceased of PML.

Papilomavires

- ds DNA virus
- DNA lenght approx. 8 kb
- > 100 serotypes
- causing – warts
  - Condylomata accuminata
  - Epitelial carcinoma
    - cervix
    - larynx
    - penis …

- genital warts around 30 types
- most of the people gets infected in first 2-3 years of sexual activity (2/3 within 1st 3 months)

http://www.healthyeatingandyou.com/wp-content/uploads/2016/02/types-of-warts.jpg

Papilomaviruses

HPV-LR low risk

6, 11, 40, 42, 43, 44, 54, 61, 70, 72 a 82

(condylomata accuminata, ca.)

- 2-8 months after infection is neccesary for lesion development on 50% of infected women
- non-oncogenic
- devected ussually around 25 yrs.

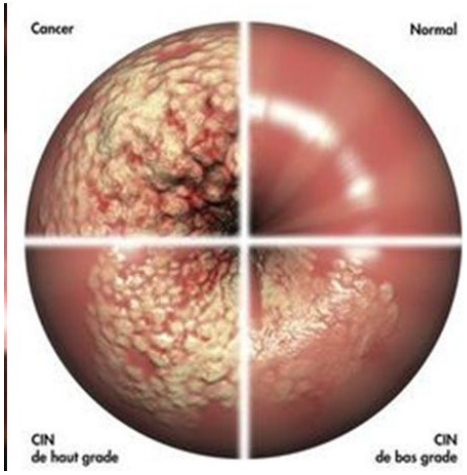
HPV-HR high risk

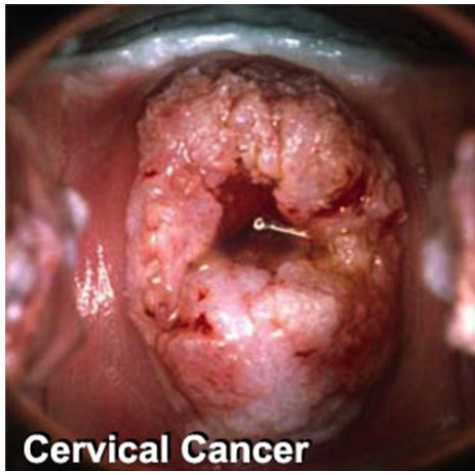
High risk: 16, 18, 31, 33, 35, 39, 45,51, 52, 56 a 86

- unifocal lesion (CIN 1–3 a ca.)
- transmission by sex. contact
- highly protective specific immunity
- in 35 years (CIN 3) and 45 yrs. (ca.)
- CIN 3 after 18M-5 yrs. after infection
- 80–90 % of women eliminate virus spontanuously within 8–16 months
- from 10–20 % of women with lasting infection :
  - 20 % develops CIN 3 within 5 yrs.
  - 5 % develops ca. until 15–20 yrs.

(in women with regular preventive testing only 1 % really develops ca.)

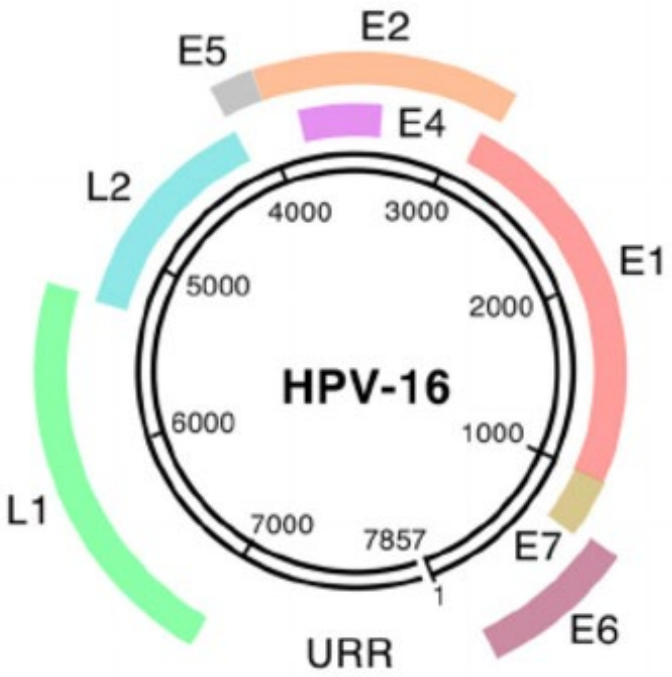
http://images.medicinenet.com/images/slideshow/stds-s1-photo-of-genital-warts.jpg





http://andryrasamindrakotroka.e-monsite.com/medias/album/papillomaviridae-7.jpg

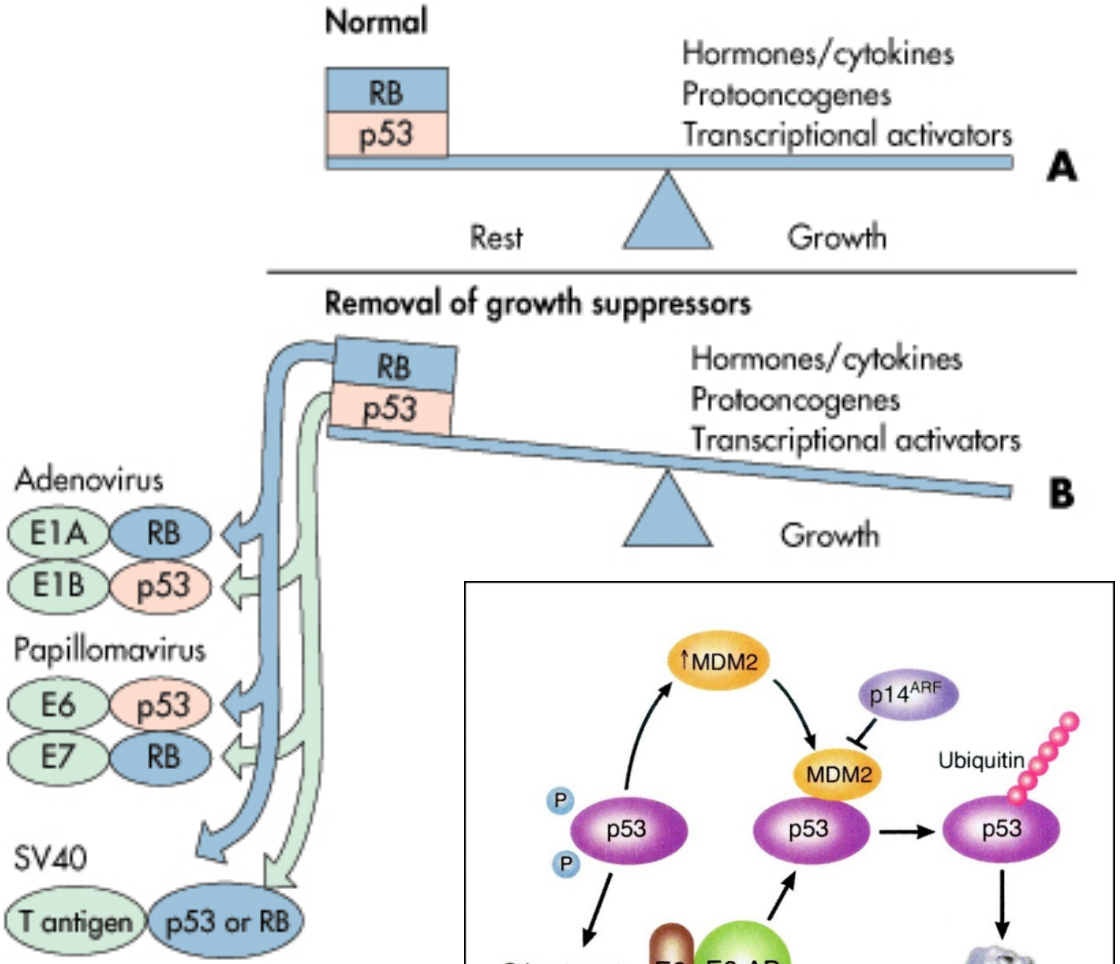
Papilomaviruses – genome

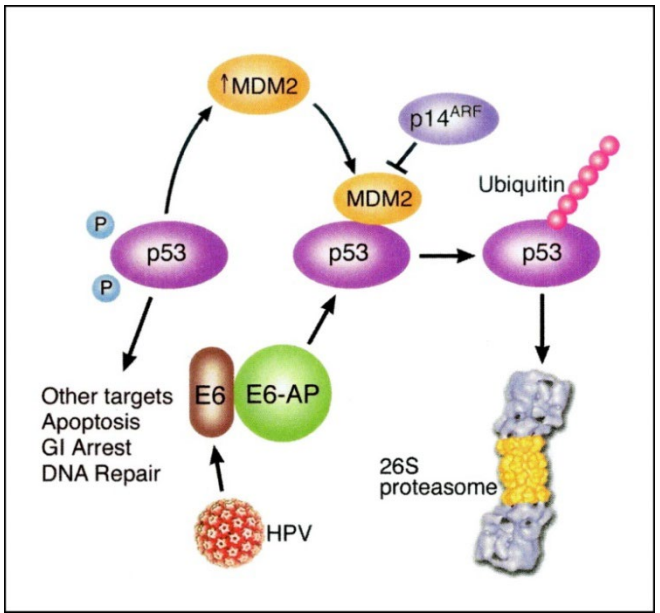


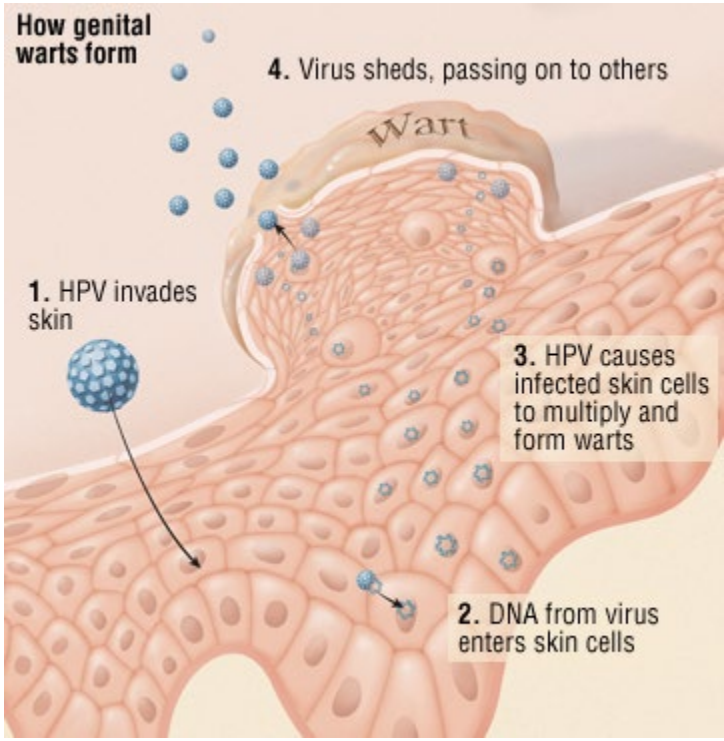
Gene	Function
L1	Major capsid protein.
L2	Minor capsid protein.
E1	Transcription factor, helicase activity. Mediates episomal DNA replication.
E2	Transcription factor. Regulates viral copy number.
E4	Facilitates virion release.
E5	Stimulates cell proliferation and prevents differentiation. Downregulates surface MHC class I expression.
E6	Deregulates cell cycle control through p53 inactivation/degradation. Induces malignant transformation together with E7.
E7	Keeps cells active in the cell cycle through Rb inactivation. Induces malignant transformation alone and together with E6.

https://www.researchgate.net/profile/Angelika_Riemer/publication/45113419/figure/fig1/AS:307360930254856@1450291964254/FIGURE-1-HPV-16-genome-and-transforming-activity-of-E6-and-E7-The-left-panel-shows-the.png

Papilomaviruses – oncogenic potential







Ubiquitinilation and proteolysis

HPV 16 a 18

Causes up to:

70% of cervical carcinoma
80% rectal ca.
60% ca. of vaginy
40% ca. of vulva
90% of genital warts

- HPV is most frequently transmitted STD in MSW adults
  (> 80% of american women got at least 1 HPV typ at the age of 50)
- 529,000 of new cervical ca. cases and 275,000 deaths/year
- **VACCINATION!!!**

HPV vaccines: Gardasil9 (9 types)
Gardasil (previously Silgard, 4 types)
Cervarix (2 types)

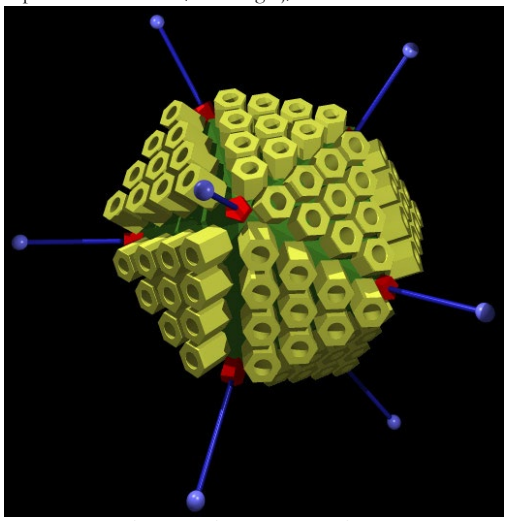
Adenoviruses

- non enveloped ds DNA viruses
  ikosahedral structure70-75 nm
  genome: 35 kbp
  according to similarity– 7 subgenes A-G
  accorging to antigenic specificity– more than 60 serotypes

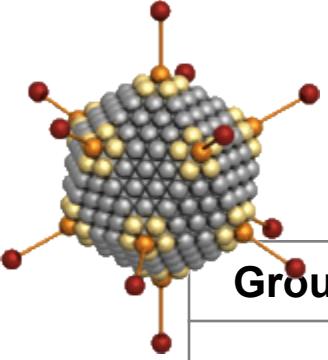
www-personal.umich.edu/ ~metzgerj/andeo.html

- **Acute faryngitis, Faryngoconjunctivitis, Acute respiratory tract infection, Pneumonia, Acute hemorrhagic cystitis, Keratokonjunktivitis, Pertussis-like sy., Hepatitis, Gastroenteritis, Meningoencefalitis, Myokarditis**

- Persistence in BMT, patients with immunodeficiencies or immunosupression – in colon, and urinary tract



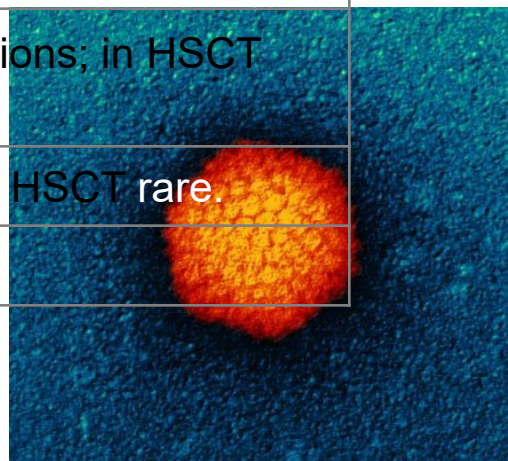
www.snof.org/maladies/imagesmaladie/ adenoviruslarg.jpg

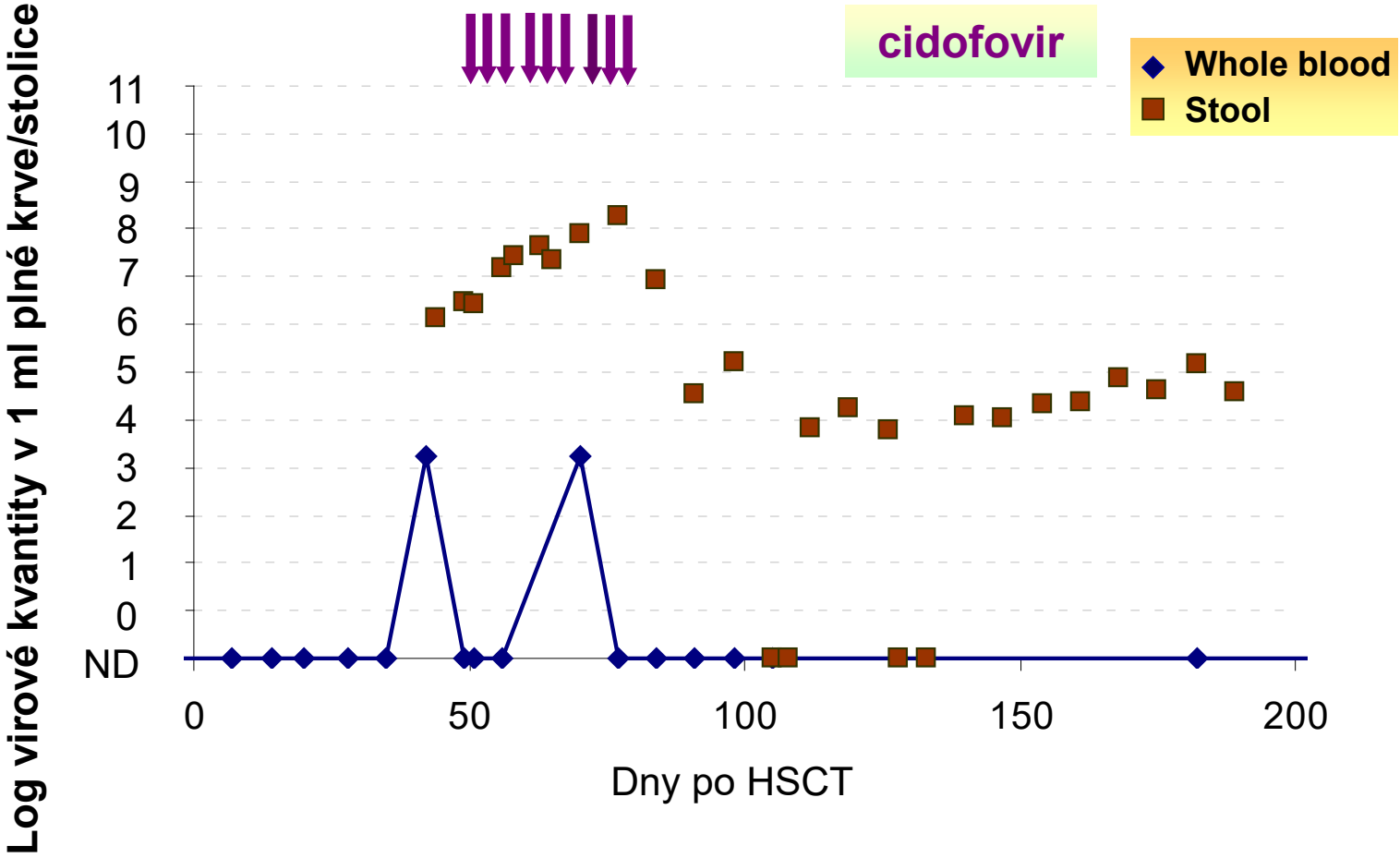


Serotypes

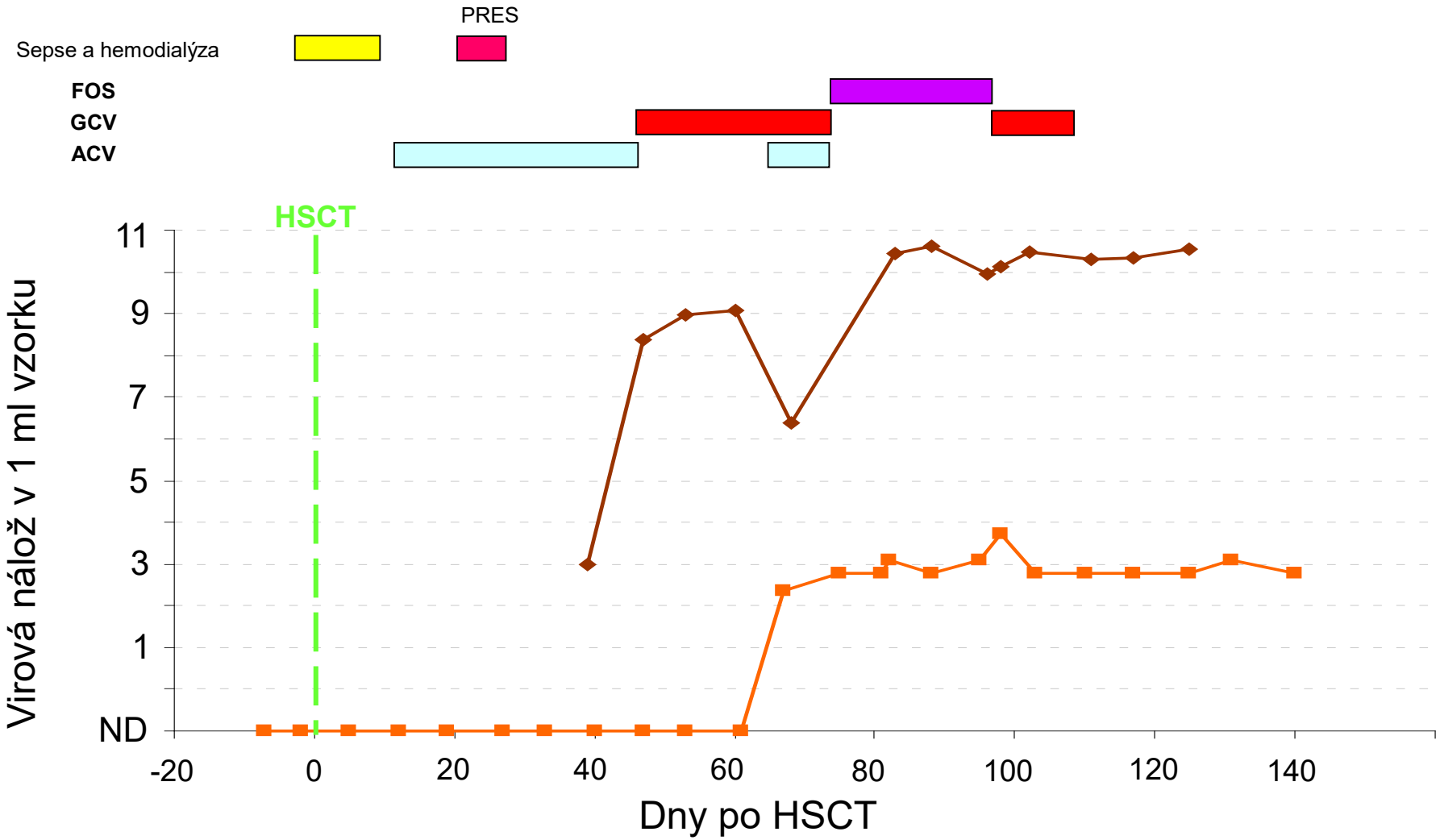
Group	Serotypes	Localisation of the infection
A	12, 18, **31**	Respiratory, urinary, GIT infections and CNS infections; in HSCT patients rare.
B	3, 7, 11, 14, 16, 21, 34, 35, 50	Respiratory, eye, urinary, GIT and CNS infections.
C	**1, 2, 5, 6**	**Respiratory, urinary and GIT infections – hepatitis too.**
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51	Eye, GIT and CNS infections; in HSCT patients rare.
E	4	Eye and respiratory tract infections; in HSCT patients rare.
F	41	GIT infections; in patients after HSCT rare.
G	52	GIT infections.

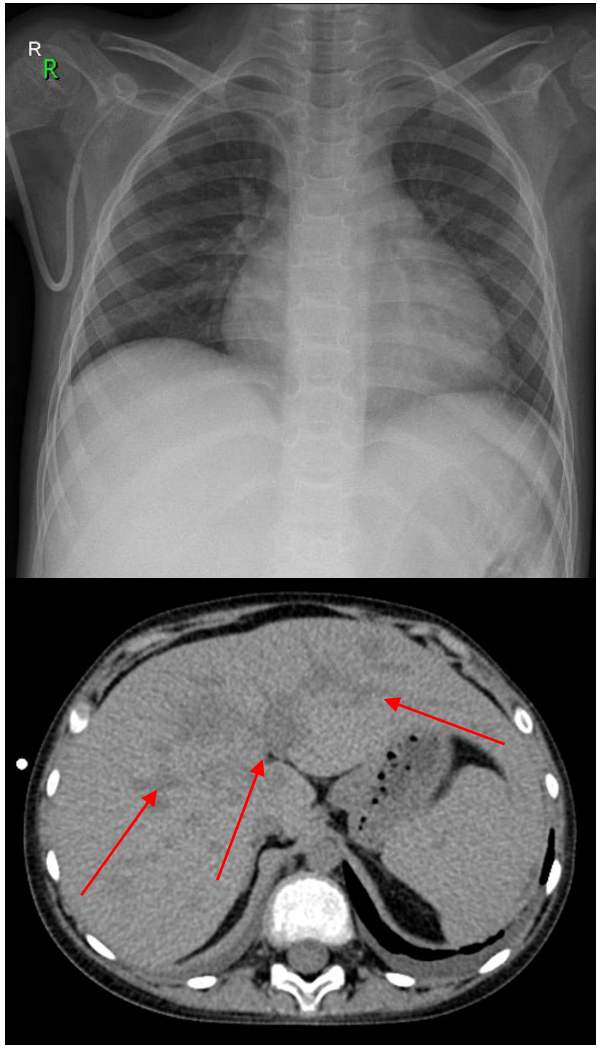
Rozdělení adenovirových infekcí do skupin (upraveno dle Fields Virology 5th edition, Kapitola 63).

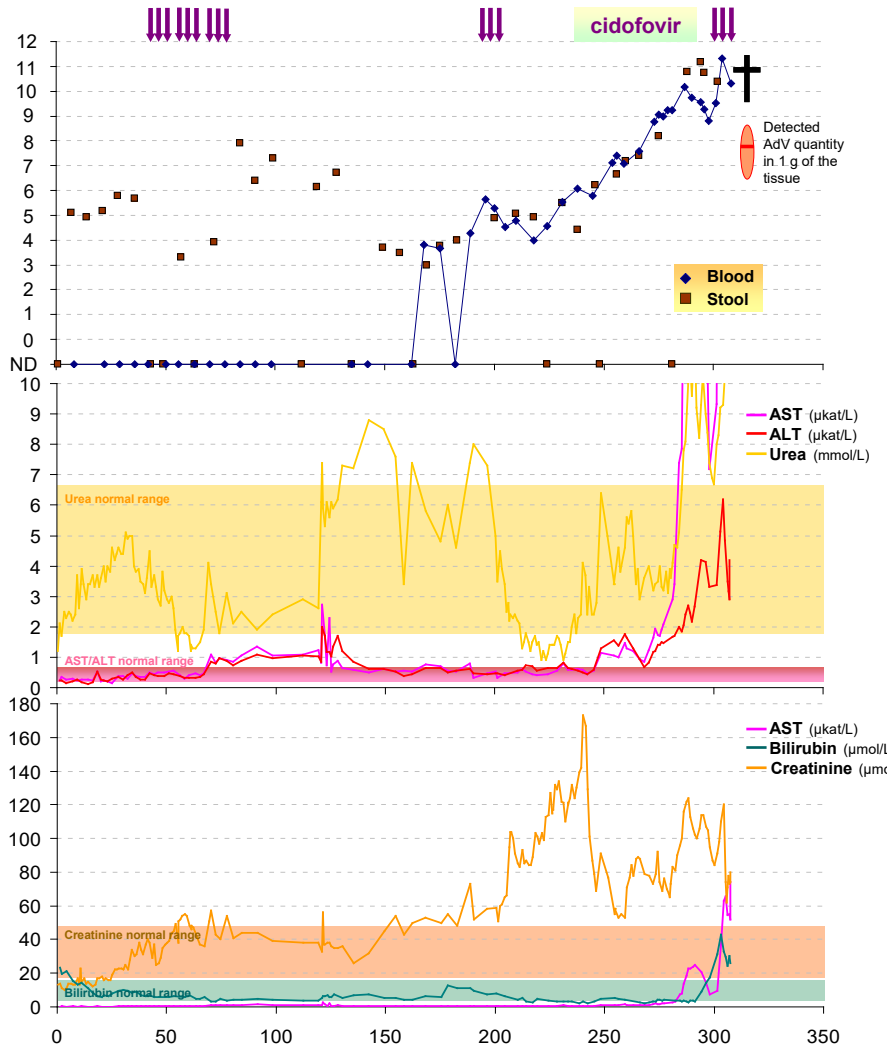


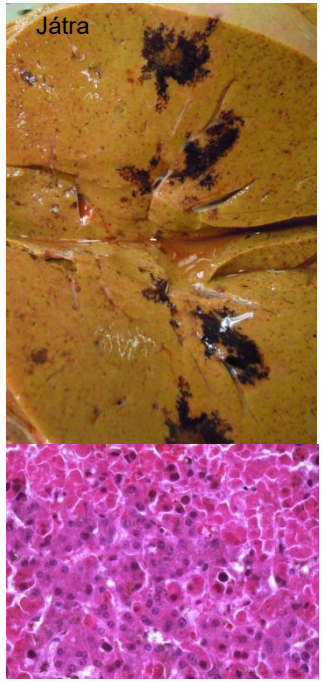


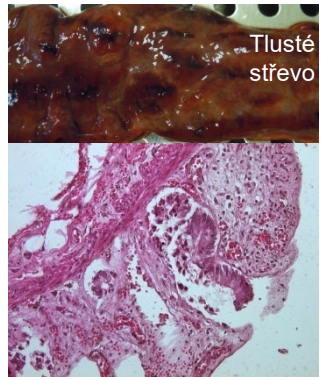
Dívka Věk při HSCT: 1 rok Dg.: ALL v CR2 Štěp: CB (5/6)
Příprava: busulfan, cyklofosfamid, melfalan a ATG Přihojení D+25.
GvHD grade II (GIT1, kůže 3) léčená kortikoidy.
Kompletní chiméra ode D+14.


PRES
Sepse a hemodialýza
FOS
GCV
ACV
HSCT
11
9
7
5
3
1
ND
Virová nálož v 1 ml vzorku
-20
0
20
40
60
80
100
120
140
Dny po HSCT


R
R


cidofovir
Log of viral load in 1 ml of whole blood / Stool
12
11
10
9
8
7
6
5
4
3
2
1
0
ND
Detected AdV quantity in 1 g of the tissue
Blood
Stool
Biochemical values during the time of infection and treatment
AST (μkat/L)
ALT (μkat/L)
Urea (mmol/L)
Urea normal range
AST/ALT normal range
AST (μkat/L)
Bilirubin (μmol/L)
Creatinine (μmol/L)
Creatinine normal range
Bilirubin normal range
0
50
100
150
200
250
300
350


Játra


Tlusté střevo

Possibilities of influence of viral infection

Immune system

(immunosupresive treatment, chemotherapy, …)

Patogens

Lymfocyte regulated – viruses , mykoses

1. Prevention – vaccination
2. Decrease of immunosupresive therapy
4. Improvement of lymfocyte function
6. Improvement/adding specific lymfocytes

3. Decrease of viral proliferation by virostatic therapy
5. Destruction of permissive cells

1. Prevention - Vaccination

TBE

Influenza

Rotaviruses

Human papilomaviruses

Hepatitis A

Travel and special vaccin

Lyssa

Yellow fever

…

2. Decrease of the immunosupression treatment intensity

Autoimmune disease

Transplant patients

Iatrogenic immunosupression

STEROIDS

> 2 mg/kg leads to lymfopenia

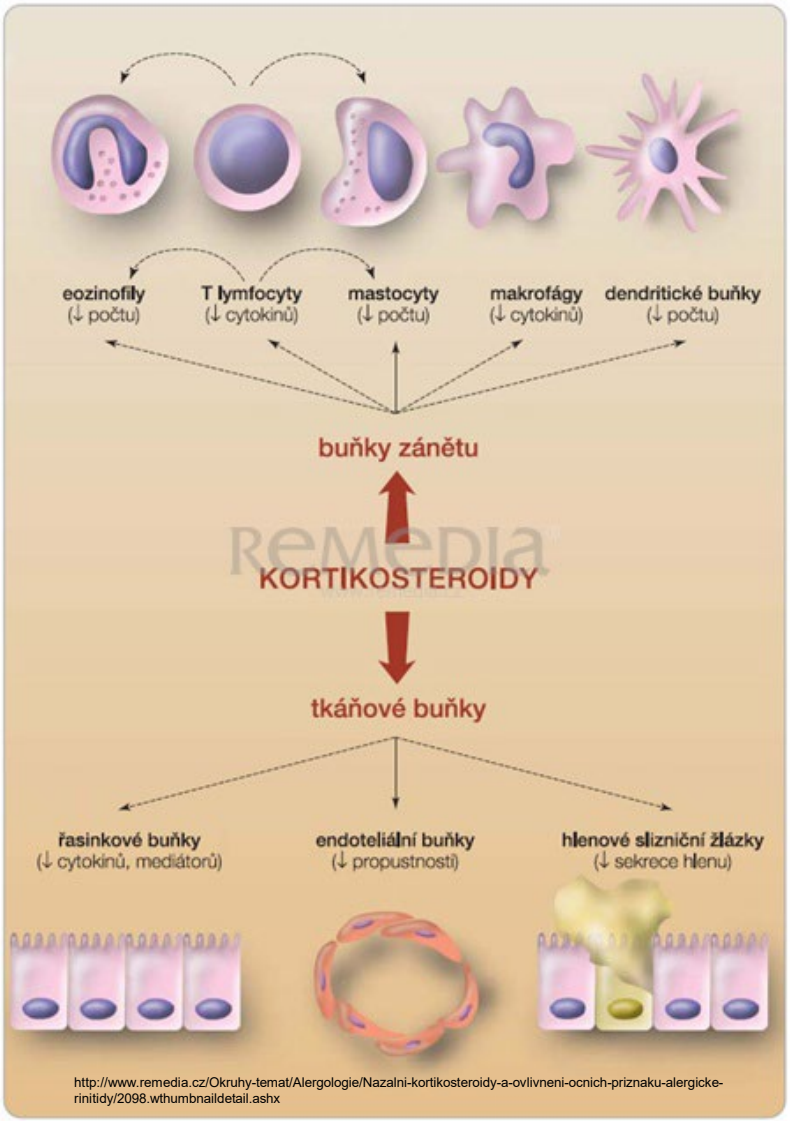
„BIOLOGIC TREATMENT “

infliximab (anti TNF-α)

basiliximab (anti CD25 – α řetězec IL-2R)

Campath (anti CD-52)

Antithymocytární globulin (ATG) …



http://www.remedia.cz/Okruhy-temat/Alergologie/Nazalni-kortikosteroidy-a-ovlivneni-ocnich-priznaku-alergicke-rinitidy/2098.wthumbnaildetail.ashx

3. Decrease of viral proliferation

Aiming proliferation improtant viral genes

DNA/RNA polymerase

herpesviruses, AdV

Reverse transkriptase

Protease

Neuraminidase

HCV, HBV, HIV

herpesviruses

Antibodies against permissive cells

anti CD20 - rituximab

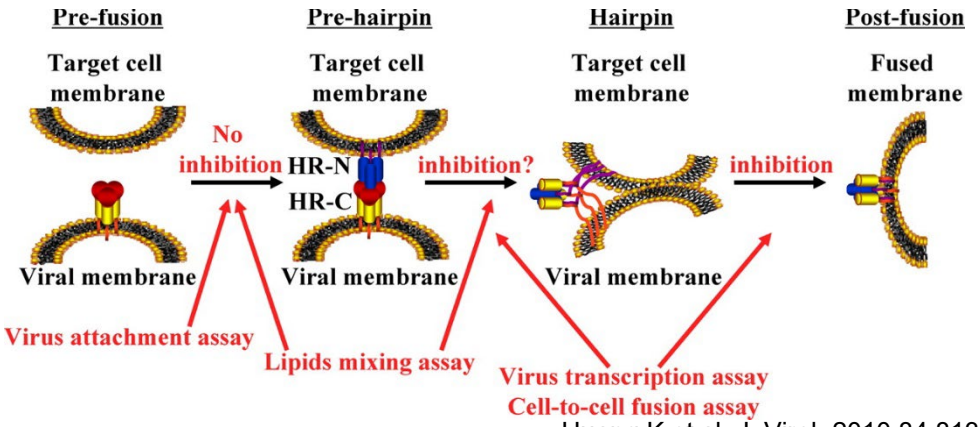
Neutralising antibodies

Profylactic prevention

motavizumab

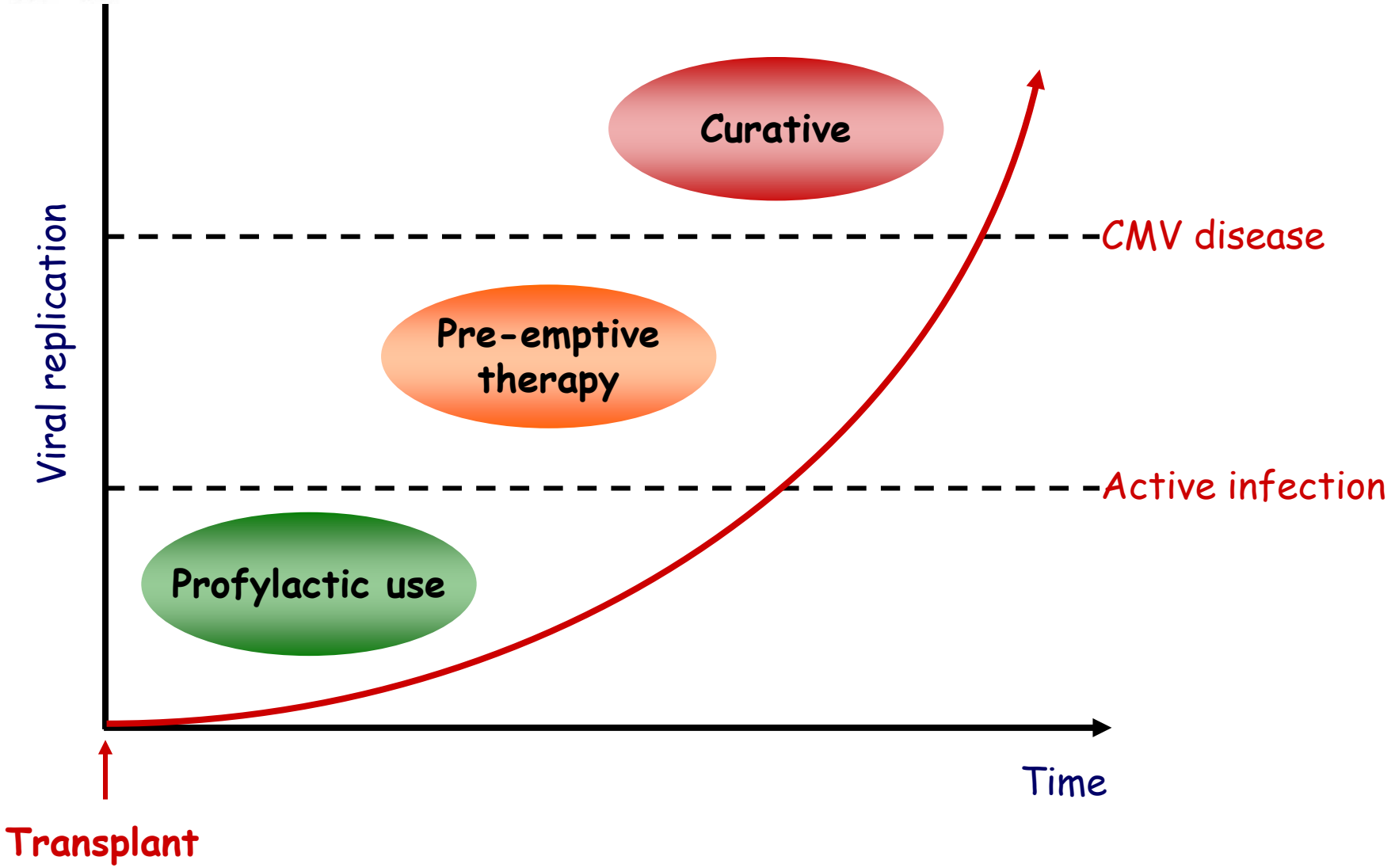
palivizumab (Synagis)

Humanisided neutralizating antibody against F- protein of RSV



Huang K et al. J. Virol. 2010;84:8132-8140

3. Decrease of viral proliferation

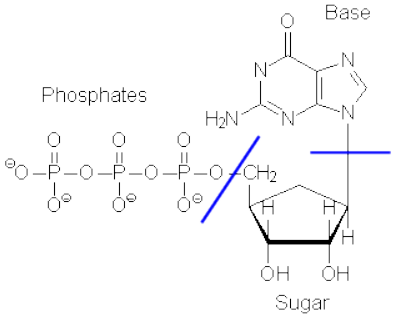


In HSCT recipient: pre-emptive and curative therapy

Virostatic drugs impact

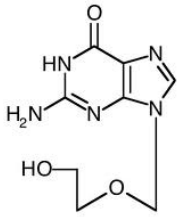
Virostatics

usually cellular nucleotides analogues blocking (more or less specifically) viral polymerase (acyklovir, ganciklovir, cidofovir…), or polymerase directly blocking drugs without similarity to nucleosides (e.g. foscarnet) or viral protein blocking drugs (neuraminidase inhibitors..)

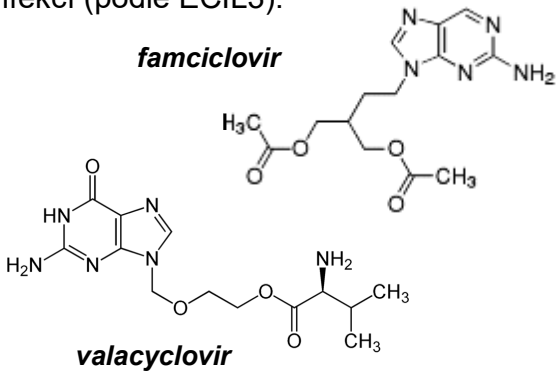


Guanidine trifosfát (GTP)

Nejčastěji používaná virostatika používaná při léčbě α-herpesvirových infekcí (podle ECIL3).



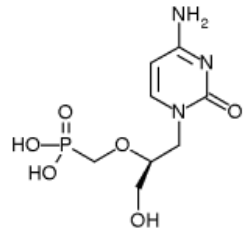
acyclovir



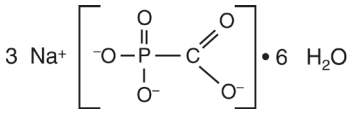
famciclovir

valacyclovir

Léky první volby.



cidofovir



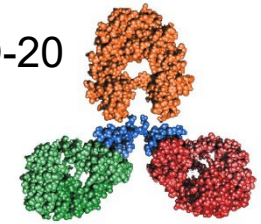
foscarnet

Léky používané při HSV rezistentní k léčbě.

Antibodies with virostatic effect

Neutralising antibodies against certain proteins important in pathogenesis of viral disease (F protein in RSV) or aimed against target cells (anti-CD20 in EBV).

Anti CD-20



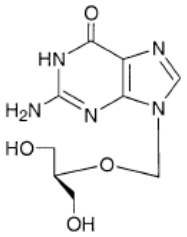
http://www.curaxys.com/images/img1B.jpg

3. Decrease of viral proliferation

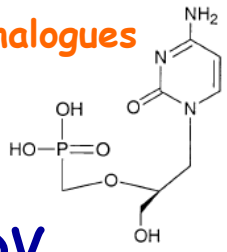
➔ **Inhibitors of CMV DNA polymerase UL54**

Nucleoside/nucleotide analogues

(V)GCV

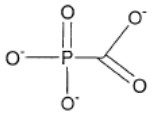


CDV



Pyrophosphate analogue

FOS



GCV → **UL97 Phosphotransferase** → **GCV-P** → **GCV-PP** → **GCV-PPP**

CDV → **CDV-P** → **CDV-PP**

FOS → **FOS**

Cellular kinases (GCV-PP, CDV-P)

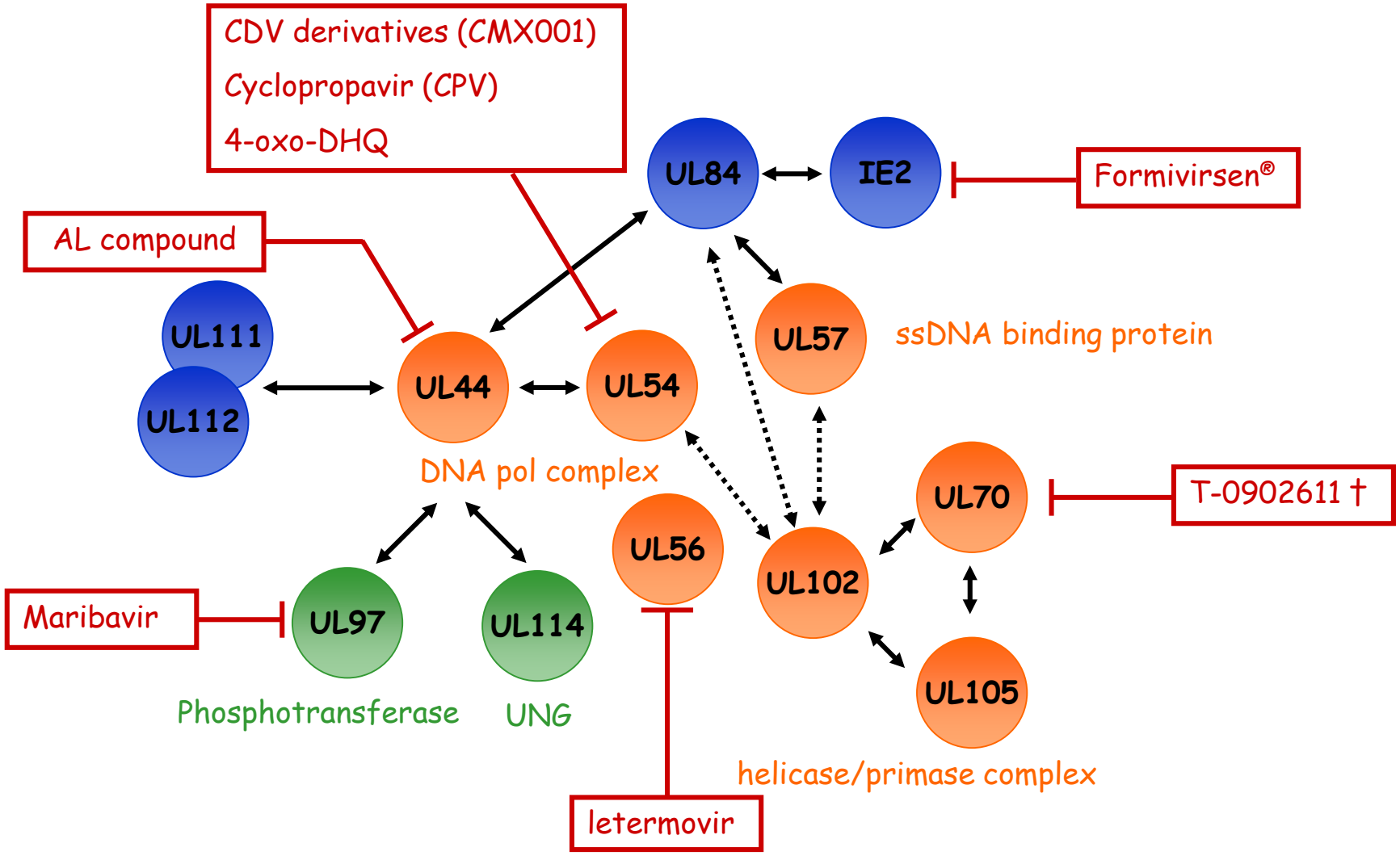
Competition with dNTP (GCV-PPP, CDV-PP, FOS)

UL54 DNA polymerase

Inhibition of viral DNA synthesis

Se svolením D. Boutolleau

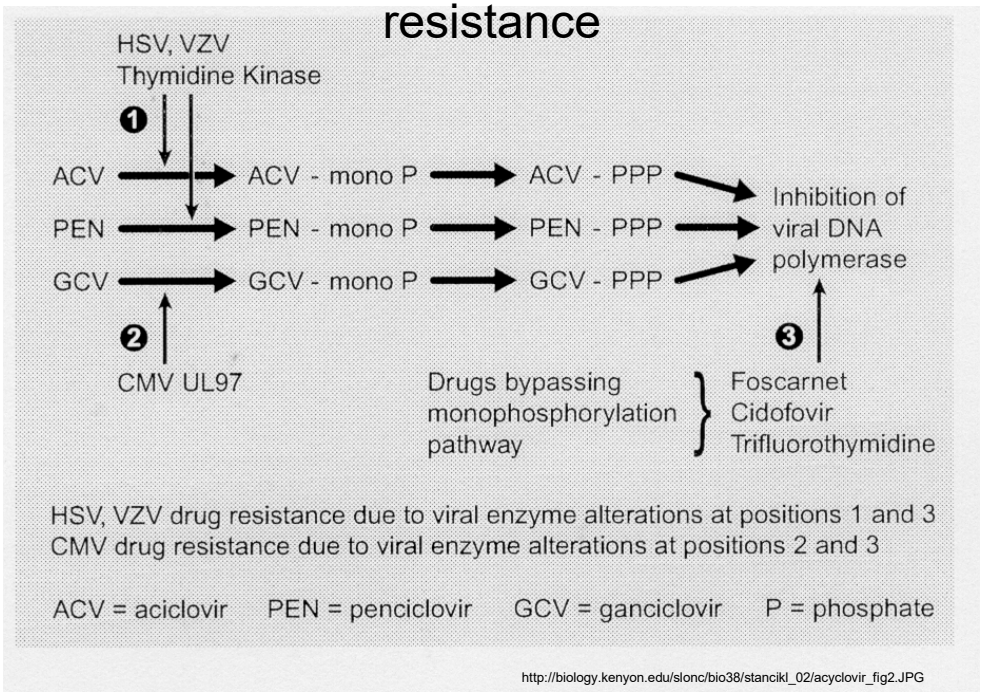
CMV replication complex



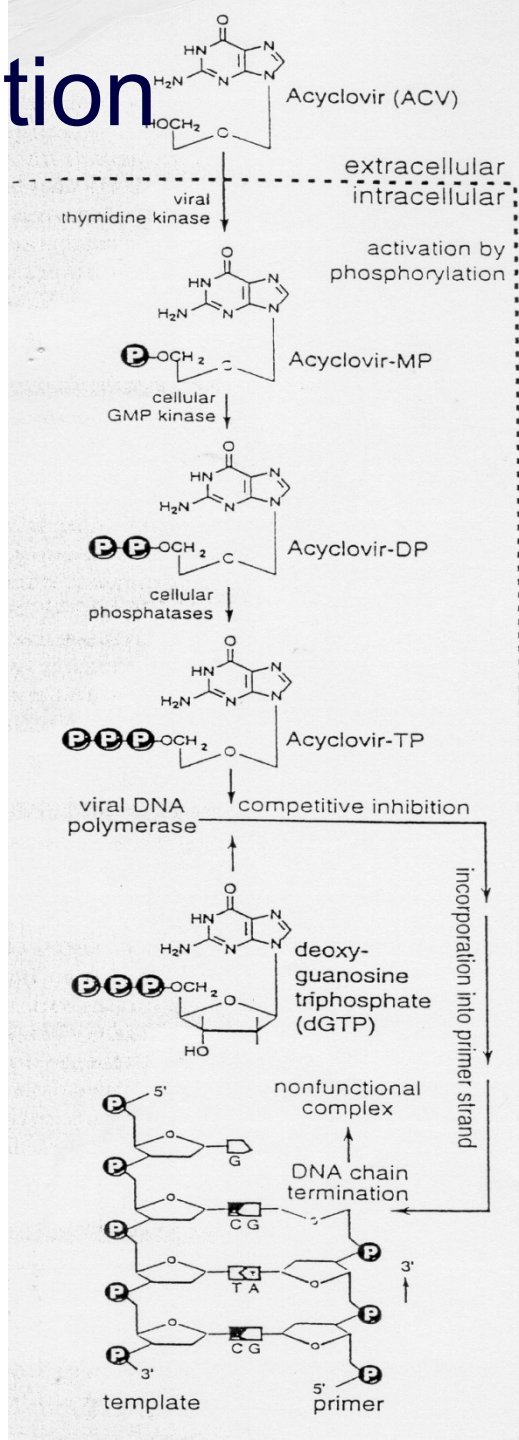
Se svolením D. Boutolleau

3. Decrease of viral proliferation

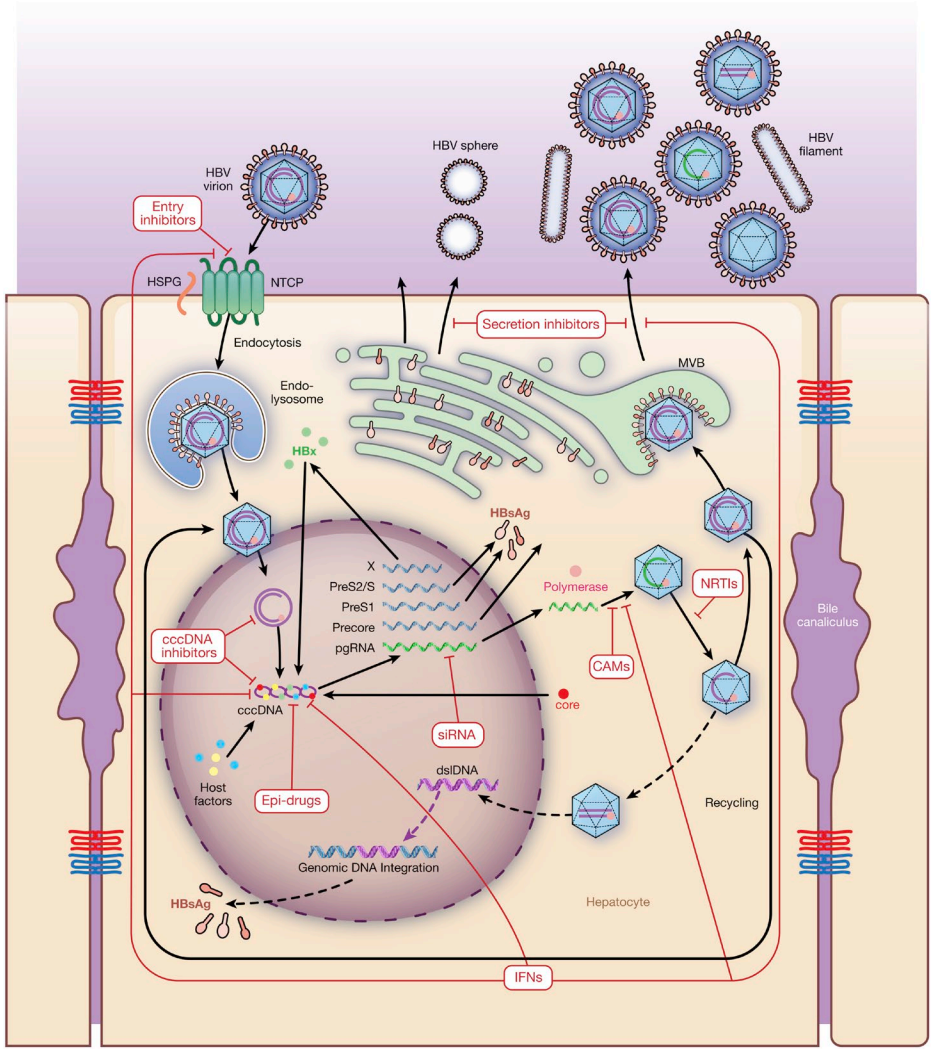
Inhibition of herpesviruses and the possible resistance



http://biology.kenyon.edu/slonc/bio38/stancikl_02/acyclovir_fig2.JPG

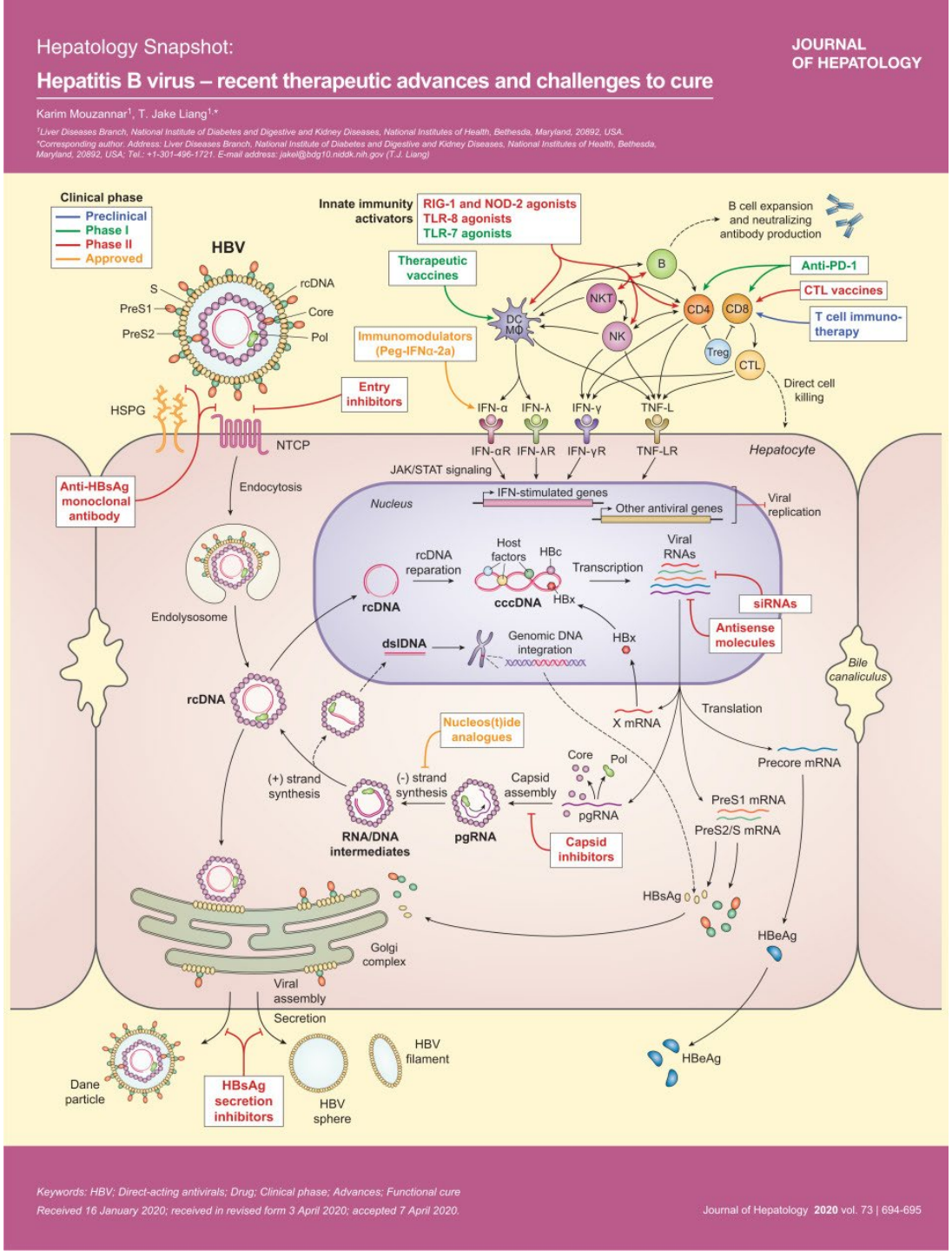


3. Direct acting antivirals (DAA) and future in HBV

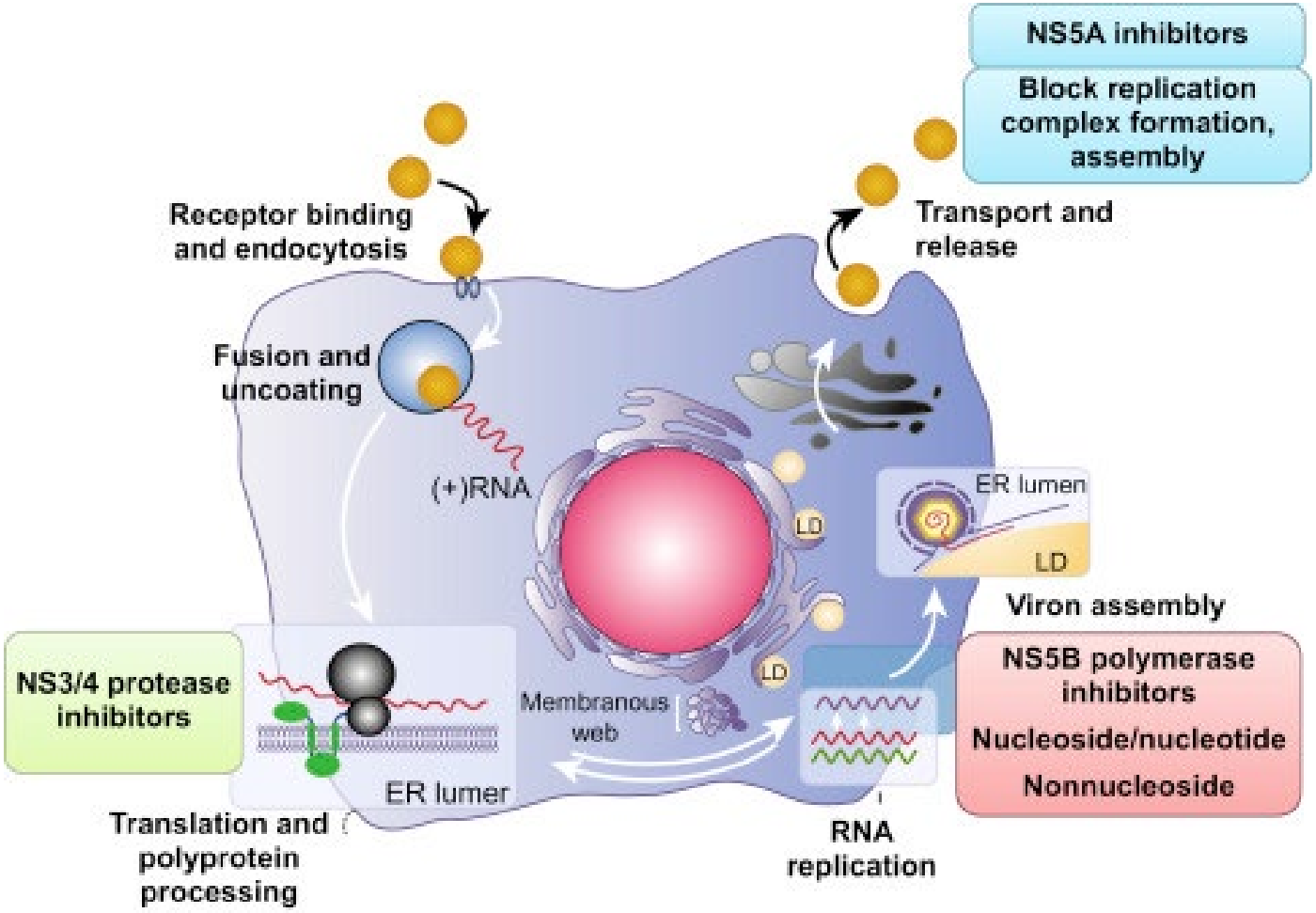


Gastroenterology 2019 156311-324DOI: (10.1053/j.gastro.2018.07.057)

3. Direct acting antivirals (DAA) and future in HBV



3. Direct acting antivirals (DAA) in HCV



https://encrypted-tbn0.gstatic.com/images?q=tbn:ANd9GcRM29bqBUDnkR6P_elbLbb60CcjXtdooocaKB_5doVmMtfuM4dcdi_jco2gRPVUzh-AYnQ&usqp=CAU

3. Direct acting antivirals (DAA) in HCV

PROTEASE Inhibitors (PI)
Block active site viral enzyme
-PREVIR

LOW BR
1st Gen.1st wave (GT1):
TELAPREVIR *
BOCEPREVIR *
1st Gen.2nd wave (all GTs except 3)
SIMEPREVIR *
ASUNAPREVIR
PARITAPREVIR/ritonavir *
VANIPREVIR (MK-7009)
DANOPREVIR

HIGHER BR
2nd Generation. Pangenotyping less effective for GT3:
MK-5172 (Grazoprevir-GRZ)
ACH-2684

NS5A Inhibitors (NS5AI)
Block Replication complex, particle assembly & release
-ASVIR

LOW BR
1st Gen. (GT1 and GT4. Other genotypes variable)
DACLATASVIR *
LEDIPASVIR *
OMBITASVIR (ABT-267) *

Slighlty **HIGHER** BR
2nd Gen. (Pangenotyping)
MK-8742 (Elbavir-EBR)
GS-5816
ACH3102
SAMATASVIR (IDX719)

ORAL IFN-free treatment (+/- Ribavirin)
HARVONI: LEDIPASVIR+SOFOSBUVIR

NS5B Nucleos(t)ide Inhibitors (Nucs or NI)
Block active site.
Inhibits RNA elongation
-BUVIR

HIGH BR
Pangenotyping (less SVR GT3)
SOFOSBUVIR *
MERACITABINE
ACH-3422
IDX21437
IDX21459

NS5B Non-Nucleos(t)ide Inhibitors (Non-Nucs or NNI)
Allosteric site.
Change tridimensional structure
-BUVIR

LOW BR
Narrow genotyping coverage
DASABUVIR (ABT-333) *
DELEOBUVIR
BMS-791325 (Beclabuvir)
PPI-383
GS-9669
TMC647055

ORAL IFN-free treatment (+/- Ribavirin)
VIEKIRAX: OMBITASVIR-PARITAPREVIR-ritonavir +
EXVIERA: DASABUVIR

BR= barrier to resistance. * Approved

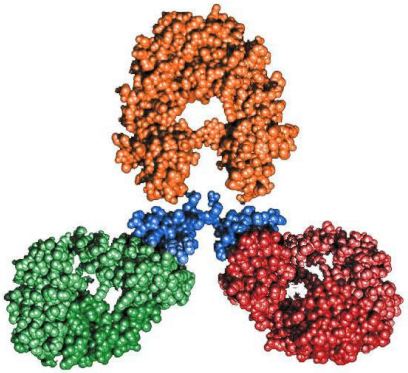
Resistance of Hepatitis C Virus to Inhibitors: Complexity and Clinical Implications. 2015 Viruses 7(11):5746-5766

3. Decrease of viral proliferation

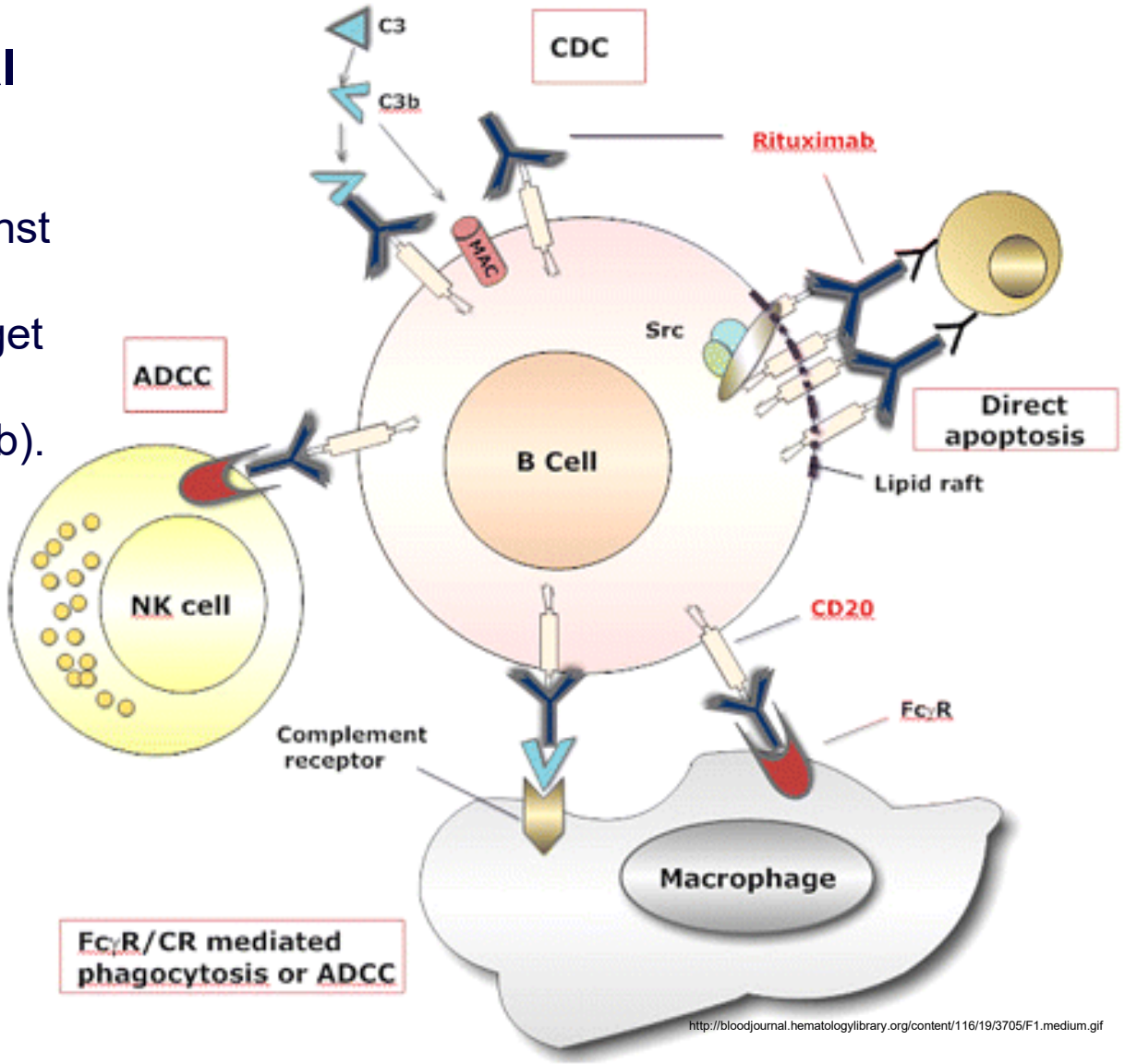
Antibodies

Antibodies with antiviral effect

Neutralising antibodies against proteins important in viral pathogenesis or against target cells of virus
(anti-CD20 u EBV - rituximab).



http://www.curaxys.com/images/img1B.jpg



http://bloodjournal.hematologylibrary.org/content/116/19/3705/F1.medium.gif

Dosing of most frequently used virostatic drugs

- **acyclovir** (HSV, VZV)
  - ***Prophylactical dosing*** – 500 mg/m^2/dose in infusion for 60 minut twice daily with maximum 750 mg/dose
  - ***Therapeutical dosing*** – for 7–10 days

    250 mg/m^2/dose in infusion for 60 minutes á 8 hours with maximum of 500 mg/dose (resp. 10-15 mg/kg/dose)
- **ganciclovir** (CMV, HHV-6, HHV-7)
  - ***Therapeutical dosing*** – at least 3 weeks

    2 weeks 5 mg/kg/dose in infusion for 60 min á 12 hours, 2 týdny; subsequently

    5 mg/kg/dose in infusion for 60 min/ day
- **foscarnet** (CMV, HHV-6, HHV-7, HSV, VZV)
  - ***Therapeutical dosing*** – for 3 weeks

    60 mg/kg/dose in infusion for 60 min (or i.v.) á 12 hours, 1- 2 weeks; subsequently 90 mg/kg/dose in infusion for 60 min (or i.v.) á 24 hours
- **cidofovir** (CMV, HHV-6, HHV-7, HSV, VZV, adenoviruses, BKV, …)
  - In case of CMV disease 5 mg/kg/dose in infusion (1/1 fysiological solution) 1x week
- **oseltamivir** (Influenza)
  - ***Prophylactical dosing*** - 30-60 mg in children younger 12 yrs. according to the weight (>15 kg - 30 mg, 15 to 23 kg - 45 mg, 23 to 40 kg – 60 mg), in patients older 13 yrs. and heavier 40 kg then 75 mg for at least 10 dní.
  - ***Therapeutical dosing*** – at least 10 days in children and adults; dvojnásobek prophylactic dosing – in adults 75 mg 2x day, in very severe cases 150 mg 2x day.

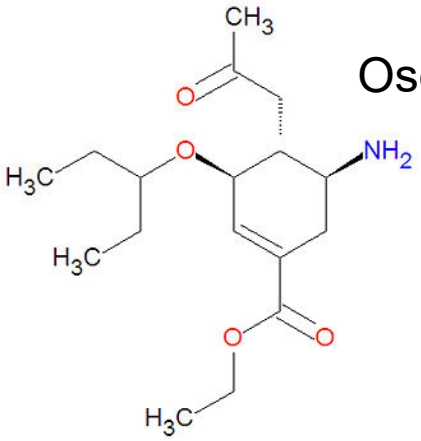
Adverse effects of the virostatic drugs

- **Acyclovir/valaciclovir**
  - ***AE usually reversible***, usually in patients with hepatopathy.
  - rarely haematopoietic and lymphatic system disorders (anaemia, leucopenia, thrombocytopenia), hepatitis, nefrotoxicity.
- **Ganciclovir/valganciclovir**
  - ***myelosupressive effects*** (neutropenia (25–40 %), thrombocytopenia (9-20 %
  - nauzea, vomiting and diarrhea, increase of the liver enzymes: confusion and seizures; renal insufficiency (rarely in patients after heart tx.); enormously rare exanthema or eosinophilia
- **Foscarnet**
  - ***Nephrotoxicity***- rarely acute renal failure (uremia and polyuria), potencially metabolic acidosis and diabetes insipidus
  - Increase of the liver enzymes, LDH, ALP and amylasis; often nauzea, vomitting nad diarrhea, rash (exanthema), tremor, muscle weakness and increase in body temperature, thrombocytopenia, hypokalemia, hypomagnezemia, hypo- or hyperfosfataemia, ***hypocalcemia*** (shortly after infusion or tonic-clonic seizures) – increased risk in CNS disorder or ciprofloxacine administration
  - Headache, tiredness, paresthesia, tremor, ataxia. Neuropathy, hypestazia, confusion, depression, psychosis, agressive reactions, psychosis, agressive reactions; changes in ECG, hyper- hypotension, rarely even chamber arythmias
  - Often Phlebitis (thrombophlebitis) in addministration of concentrated solutions (> 12 mg/ml) to peripheral vein.

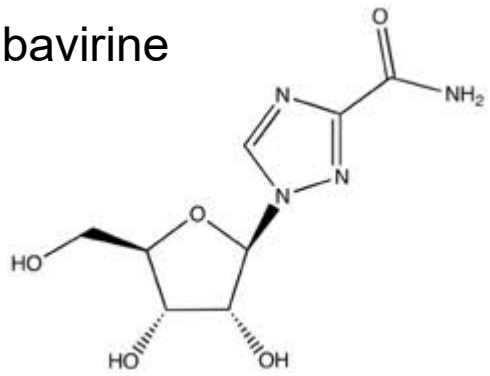
Adverse effects of the virostatic drugs

- **Cidofovir**
  - ***nephrotoxicity*** – proteinuria, creatinine increase; acute and even with delay; - good hydration, together with probenecid
  - potencially to ***chronic renal failure*** with dialysis
  - other more common neutropenia, headache, nauzea, vomitting, alopecia, rash, weakness and fever. Described also occular toxicity.
- **Oseltamivir**
  - most frequent AE are nausea, vomitting and belly pain
- **Ribavirine**
  - ***Haematopoietic disorders, depression, teratogenic effect (inhalation)*** from that reason there must not be exponed men or women about the conception. In case of higher cumulative dose risk of teratogenicity lasts for months; nausea, pain in belly….

Oseltamivir



Ribavirine



http://en.citizendium.org/images/thumb/6/68/Oseltamivir_structure.jpg/350px-Oseltamivir_structure.jpg

http://www.google.cz/imgres?q=ribavirine&hl=cs&biw=1010&bih=753&tbm=isch&tbnid=1XrThtPFBz_TBM:&imgrefurl=http://en.wikipedia.org/wiki/Ribavirin&docid=jA2kEFQ7b6AShM&imgurl=http://upload.wikimedia.org/wikipedia/commons/thumb/e/e8/Ribavirin2.png/300px-Ribavirin2.png&w=300&h=230&ei=E1rGT6yYKsmaOtPJmdAF&zoom=1&iact=hc&vpx=531&vpy=152&dur=635&hovh=184&hovw=240&tx=116&ty=116&sig=111538063890619018153&page=1&tbnh=129&tbnw=168&start=0&ndsp=22&ved=1t:429,r:3,s:0,i:74

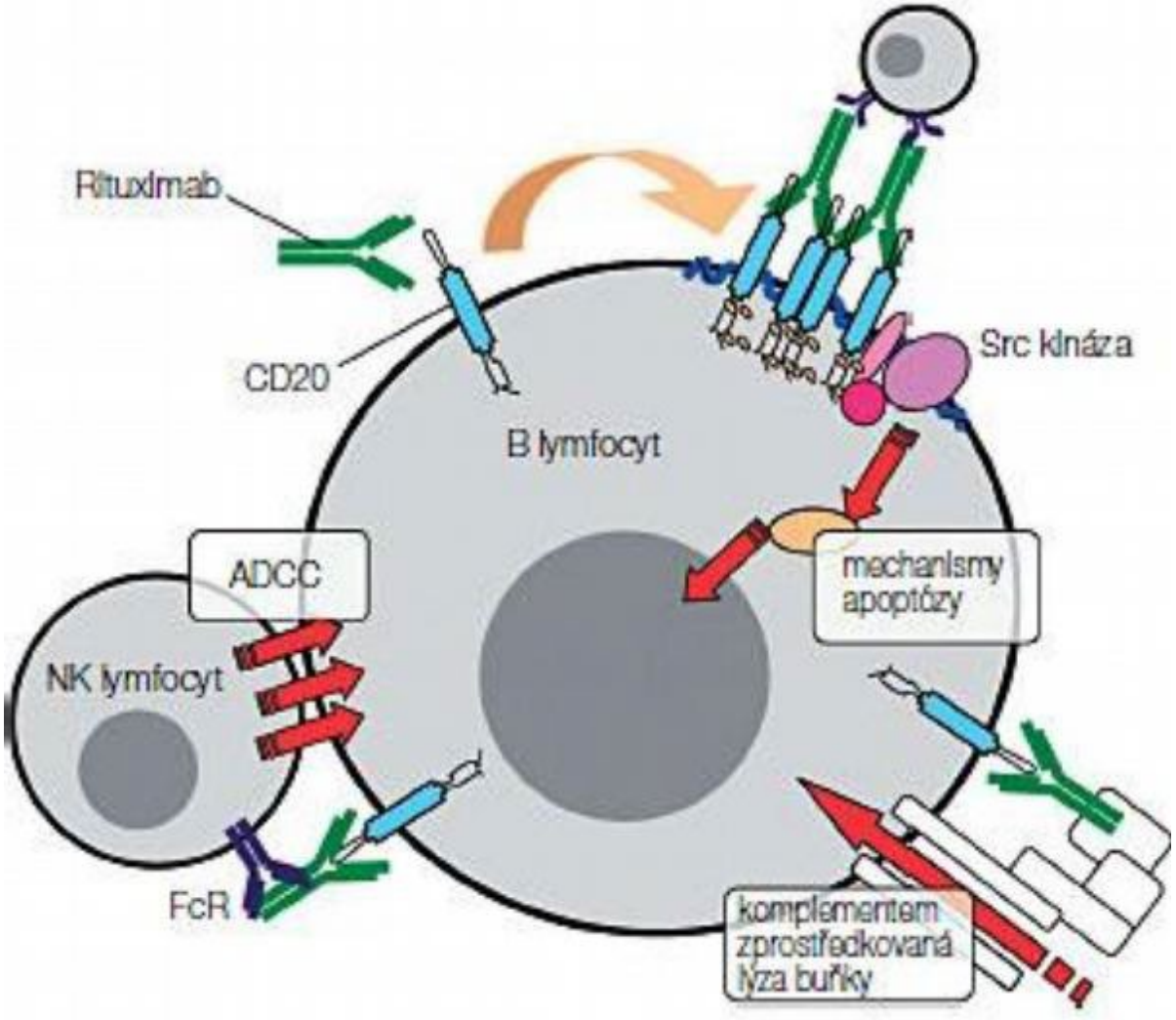
4. Improvement of the lymfocyte function

Antibodies

ADCC

– antibody dependent cellular cytotoxicity

Both „non-specific “ IVIG, and hyperimunne globulins (e.g. Cytotect®) might be used.



http://img.mf.cz/401/485/a.jpg

4. Improvement of the lymfocyte function

Interferon α

Používá se zejména při léčbě hepatitidy B.

Na trhu několik přípravků lišících se typem interferonu I – α2a

Např. (rekombinantní Roferon A), α2b (rekombinantní - INTRON A), případně pegylované interferony tj. s polyetylenglykolem (Pegasys^{TM}, PEG-INTRON)

Dávka: obvykle 2,5 - 5,0 milionů IU/m^2, resp. až 10 milionů IU/m^2 u dětí s.c., 3× týdně po dobu 4–6 měsíců.

Dávkování může být v případě nežádoucích účinků upraveno.

Není-li po 3–4 měsících léčby zlepšení, je třeba uvažovat o přerušení terapie.

Pacientům nad 18 let je v současnosti doporučen pegylovaný interferon- α2a v dávce 180 μg/týden v jedné dávce s.c.; délka léčby dle odpovědí na léčbu - při dobré odpovědi trvá 48 týdnů.

4. Improvement of the lymfocyte function

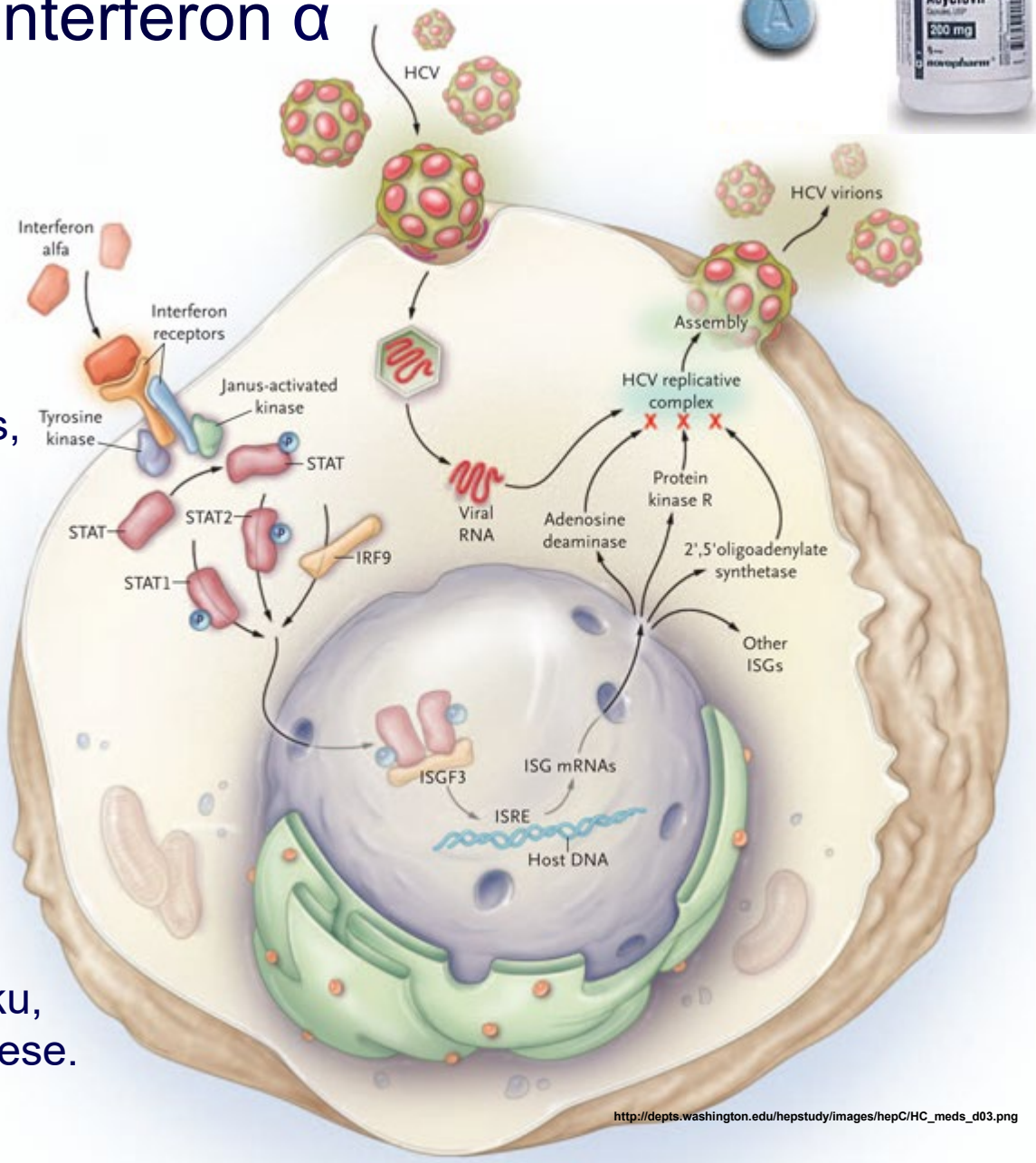
Interferon α

NÚ:

„flu-like“: únava, zimnice, bolest svalů nebo kloubů, bolest hlavy, pocení nebo horečka.

Vzácněji pneumonie a herpes, anémie, trombocytopenie, Leukopenie, autoimunitní stavy, sarkoidóza, poruchy štítné žlázy, zažívání, hypo- a hypertenze, proteinurie a poruchy renálních funkcí, glykémie a homeostázy.

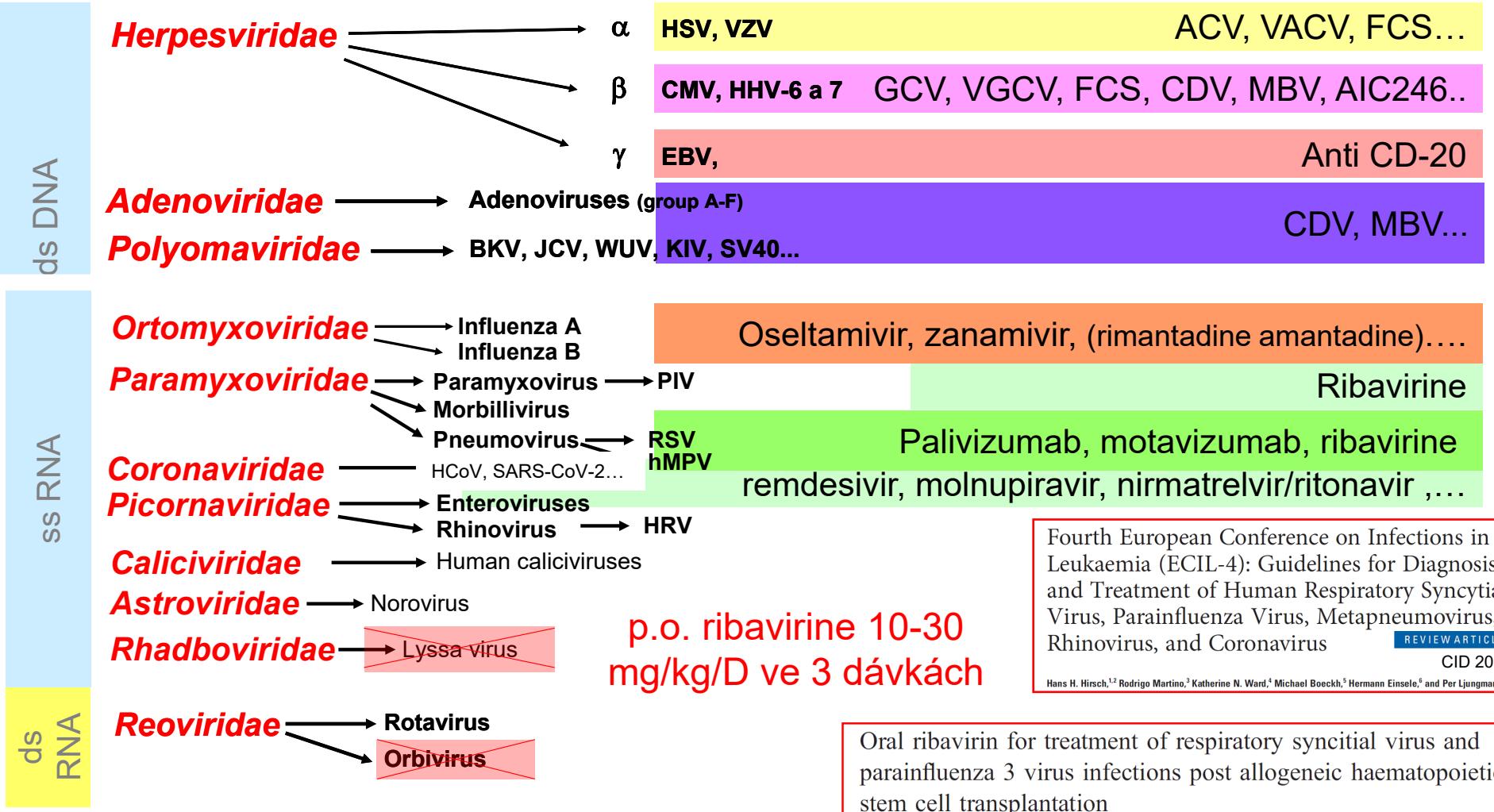
Případně účinky na CNS např. poruchy citlivosti, spánku, nervozita, stavy úzkosti, deprese.



http://depts.washington.edu/hepstudy/images/hepC/HC_meds_d03.png

Therapeutical possibilities of virostatics and specific antibodies

More or less specific for certain viral groups:



p.o. ribavirine 10-30 mg/kg/D ve 3 dávkách

Fourth European Conference on Infections in Leukaemia (ECIL-4): Guidelines for Diagnosis and Treatment of Human Respiratory Syncytial Virus, Parainfluenza Virus, Metapneumovirus, Rhinovirus, and Coronavirus

REVIEW ARTICLE

CID 2013

Hans H. Hirsch,[1,2] Rodrigo Martino,[3] Katherine N. Ward,[4] Michael Boeckh,[5] Hermann Einsele,[6] and Per Ljungman[7,8]

Oral ribavirin for treatment of respiratory syncitial virus and parainfluenza 3 virus infections post allogeneic haematopoietic stem cell transplantation

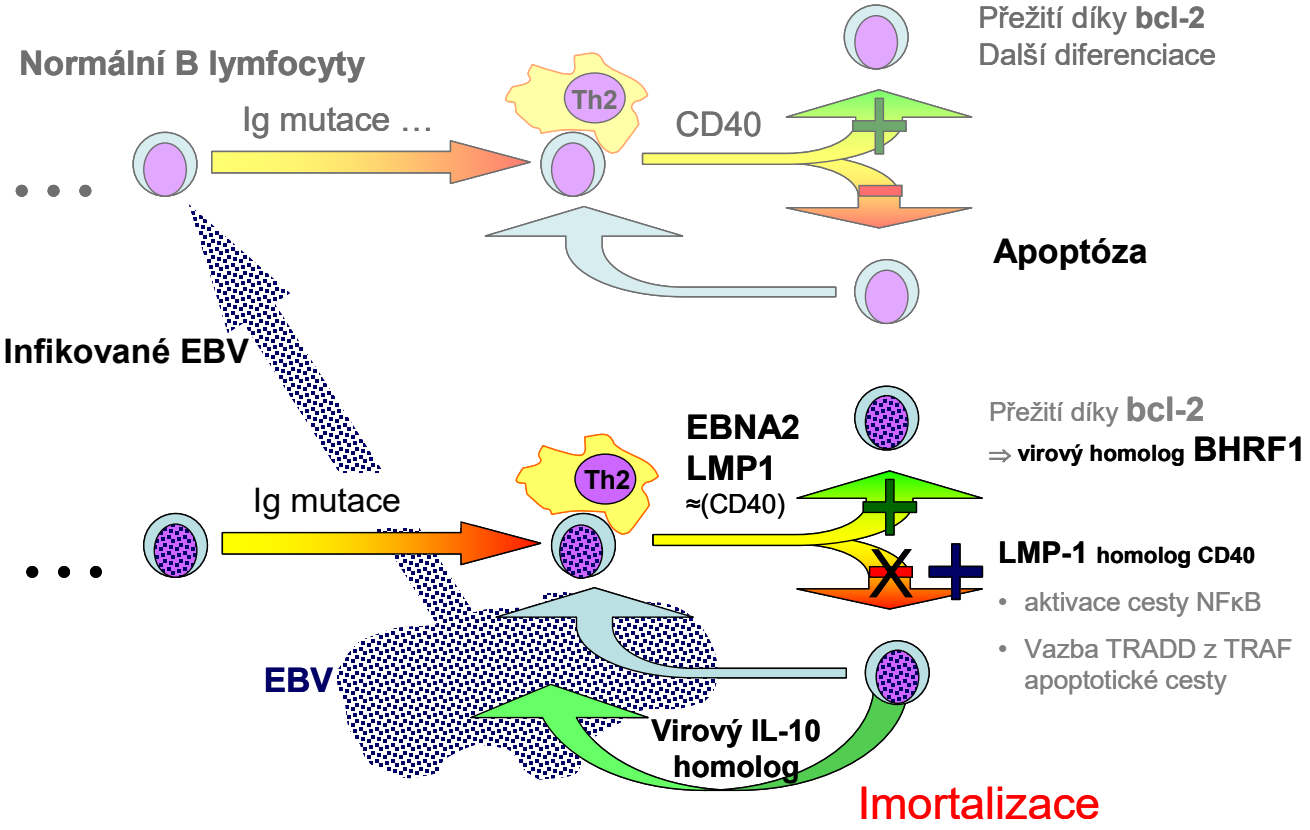
J Casey[1], K Morris[1], M Narayana[1], M Nakagaki[2] and GA Kennedy[1,3]

BMT 2011

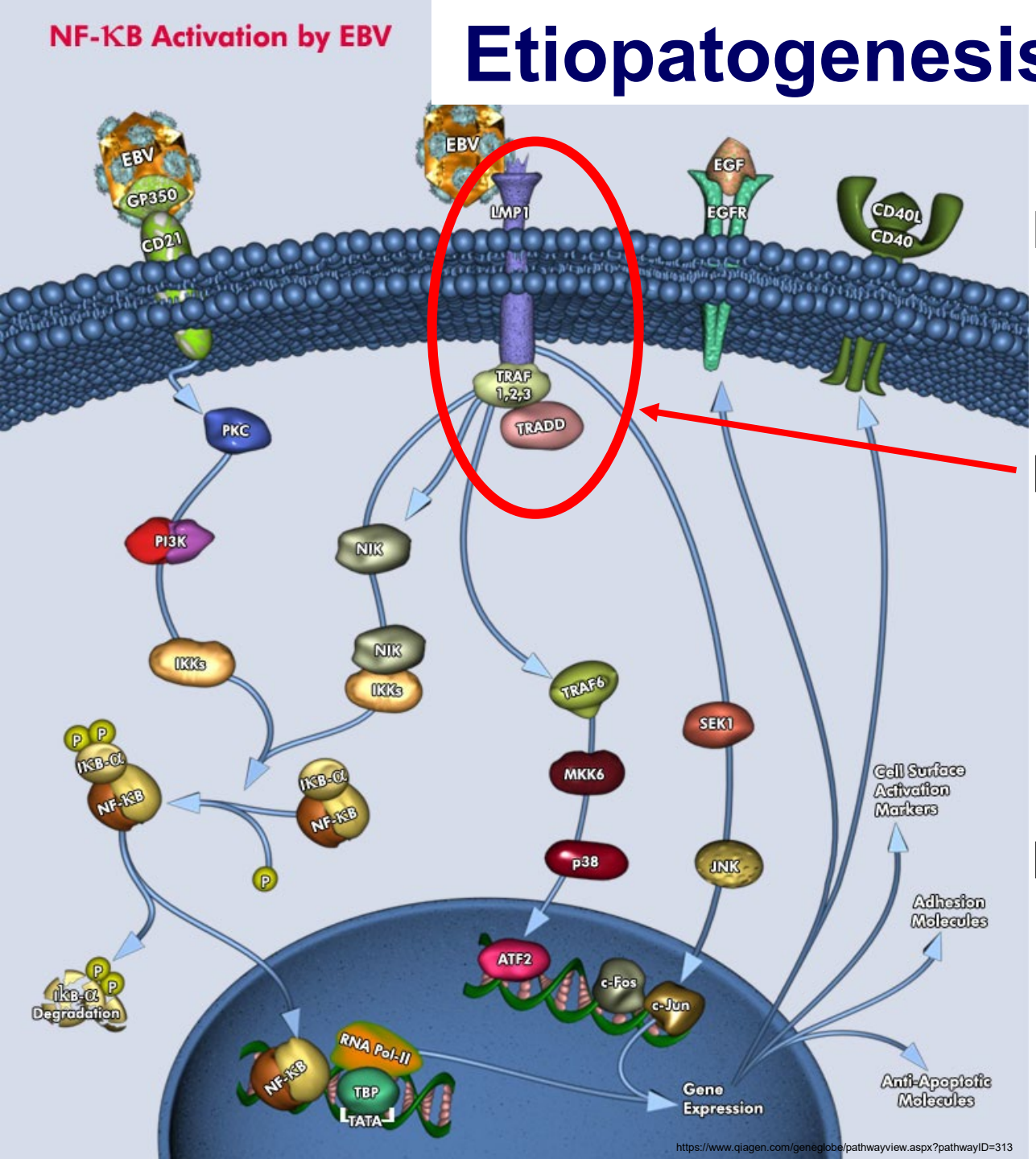
5. Destruction of permissive cells

Used in EBV associated malignant disease (HL, NHL), or post-transplant EBV-LPD.

Anti CD-20 – rituximab (MabThera)



Etiopatogenesis of EBV-LPD



EBER

– ↓kaspase 3, PARP

-↑ bcl-2 - ↓PKR fosf.

LMP1

– homologue CD40

- binds TRADD from TRAF apoptotic pathway

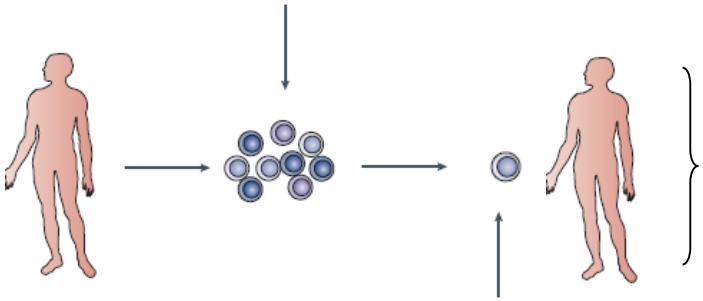
–NFkB activation, AP1, STAT1/3

BHRF1

– viral homologue bcl-2

6. Present possibilities of specific cell production and its limitations

Viral antigen stimulation

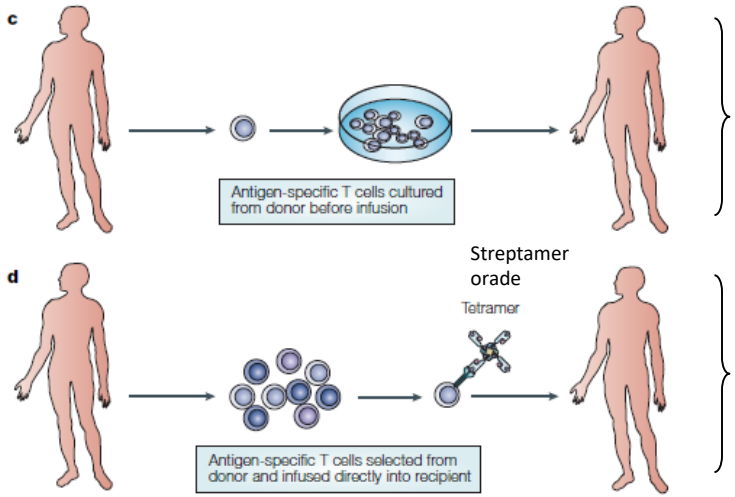


Specific IFNg producing T lymfocyte selection

Non-specific T cells, technically and financially difficult
ATMP (advanced therapeutic medicinal product)

Unrelated donors: preparation about 8-9 weeks from first demand to product

Feuchtinger et al BJH 2006
Feuchtinger et al Blood 2010



4-12 weeks of production,
ATMP (advanced therapeutic medicinal product)

So far - for prophylactic use only!

HLA-string, technically and financially difficult

Unrelated donors: preparation about 8-9 weeks from first demand to product

Literatura:

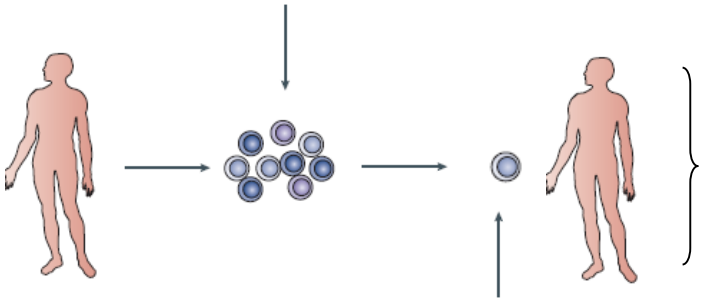
Leen et al Nature Medicine 2006
Leen et al Blood 2009

Cobbold et al J. Exp. Med. 2005
Schmitt et al Transfusion 2011

Moss P and Rickinson A Nature Reviews 2005 (5)

6. Present possibilities of specific cell production and its limitations

Viral antigen stimulation



Specific IFNg producing T lymfocyte selection

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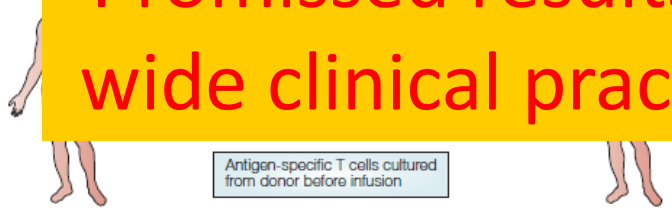
Feuchtinger et al Blood 2010

Promissed results, however so far not useful for wide clinical practice. Price approx. 8-14 000 Eur

c



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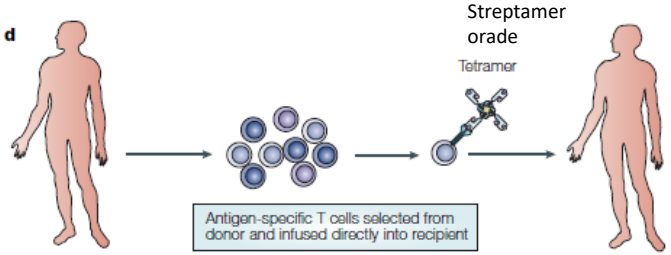
d



HLA-string, technically and financially difficult

Unrelated donors: preparation about 8-9 weeks from first demand to product

Cobbold et al J. Exp. Med. 2005
Schmitt et al Transfusion 2011

Moss P and Rickinson A Nature Reviews 2005 (5)

However – for success of the therapy is still crucial …

… reconstitution of immunity!

Thank you for you attention
Petr.Hubacek@Lfmotol.cuni.cz