DNA viruses

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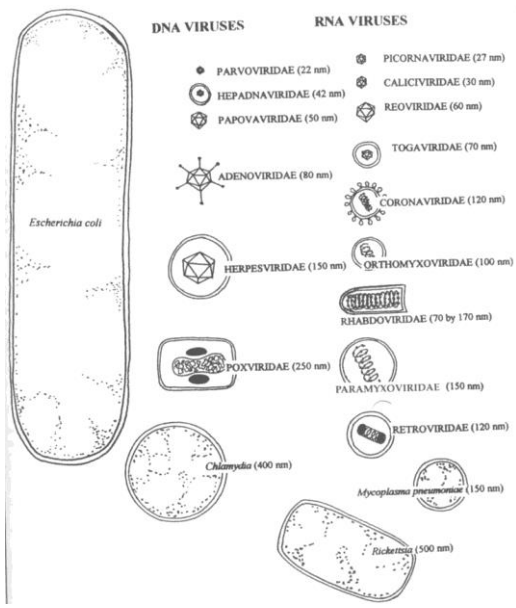
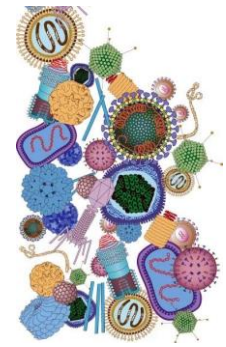
How they looks like?



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

- 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.

Balance in the (immunocompromised) patient



Immune system

(immunocompromised treatment, chemotherapy, …)

Pathogens

Lymfocyte regulated – viruses, mycoses

Methods of the viral detection

Detection methods in virology

- Microscopic — **Direct detection**
- Cultivation
- Detection of the antigen
- Detection of the nucleic acid

- Detection of the antibodies
- (Signs of disease)

Indirect detection

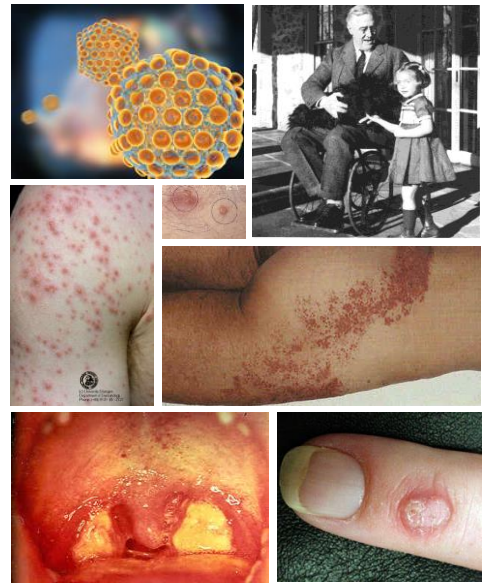
Methods of the viral detection - INDIRECT

Signs of the disease

Clinical signs of disease leading to suspition of viral infection (poliomyelitis) were described first 3 700 BC in Egypt.

Typical signs are e.g. in:

- varicella
- zoster
- fully deleloped IM
- papillomaviral infection (wart)
- also in HHV-8 and other viral infections

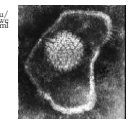


Why DNA viruses?

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

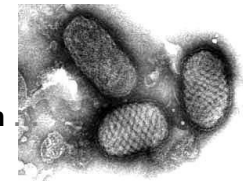
1. pneumocystis pneumonia
2. toxoplasma encefalitis
3. esofageal, tracheal, bronchial or lung candidiasis
4. **Chronic anal herpes simplex or herpetic bronchitis, pneumonia or esofagitis**
5. **CMV retinitis**
6. **generalized CMV infevtion (excluding liver and spleen)**
7. **progresive multifocal leukoencefalopatia**
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

DNA viruses



ds DNA

- ***Herpesviridae*** → α HSV, VZV; β CMV, HHV-6 a 7; γ EBV, HHV8
- ***Adenoviridae*** → Adenoviruses (group A-F)
- ***Polyomaviridae*** → BKV, JCV, WUV, KIV, SV40...
- ***Papillomaviridae*** → Papillomaviry
- ***Poxviridae*** → Variolla virus, vaccinia, molluscum contagiosum
- ***Hepadna*** → HBV

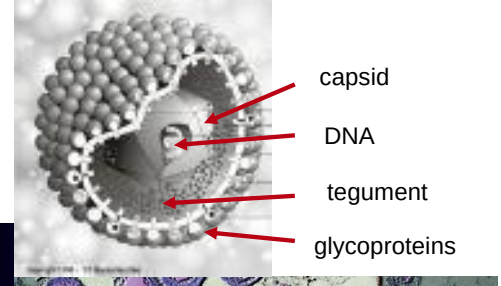


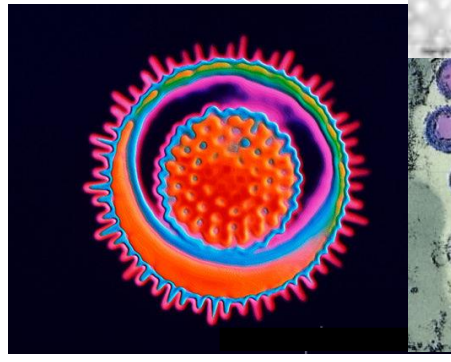
ss DNA

- ***Parvoviridae*** → Parvovirus B19, lidský bocavirus (HBoV)
- ***Anelloviridae*** → TTV, TTMV, TTMDV

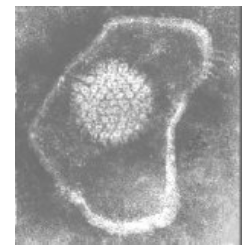
Herpesviruses

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

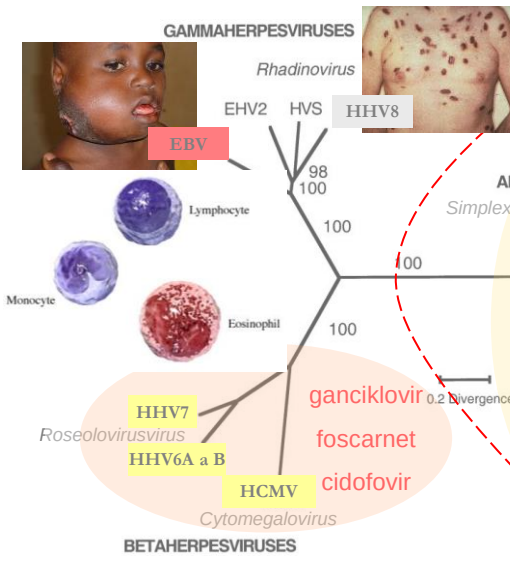


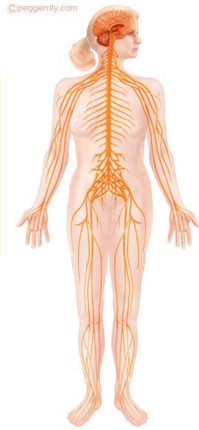


Taxonomy



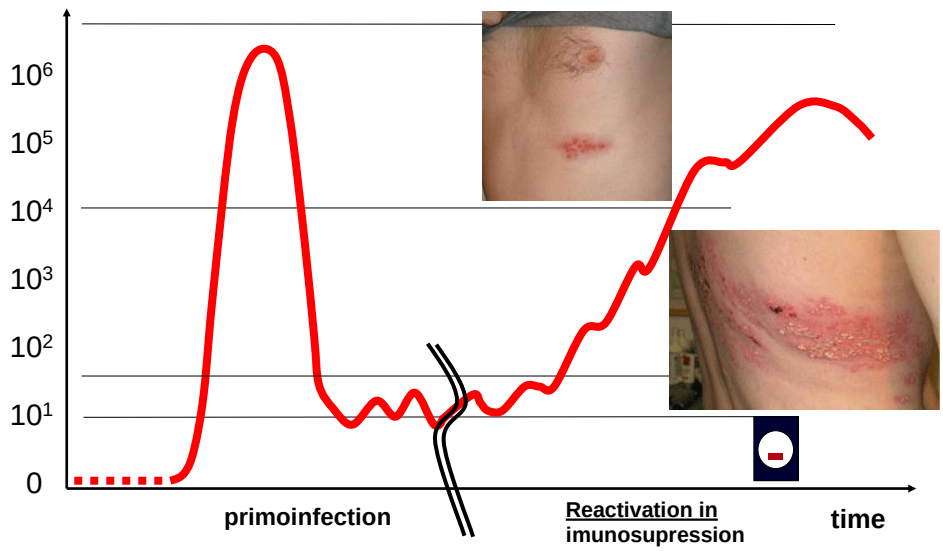
http://www.tulane.edu/~dmsander/garryfavweb.html

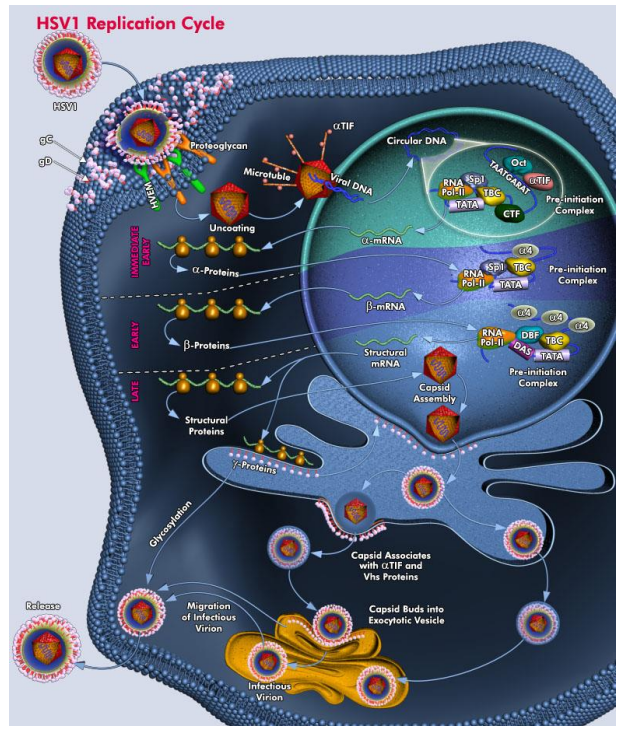


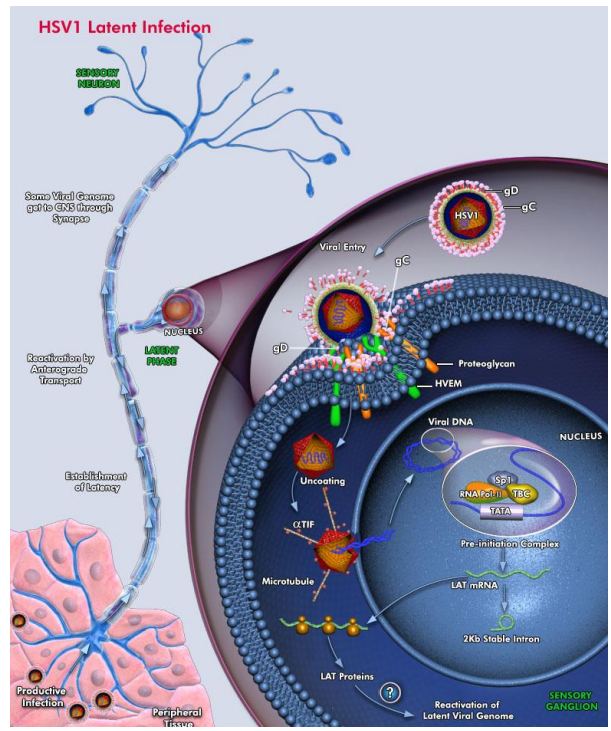


Latency with posibility of reactivation

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




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

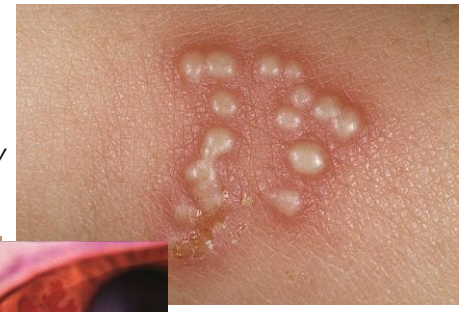

HSV1 Latent Infection
SENSORY NEURON
Some Viral Genome get to GNS through Synapse
NUCLEUS
LATENT PHASE
Reactivation by Anterograde Transport
Establishment of Latency
Productive Infection
Peripheral Tissue
HSV1
gD
gC
Viral Entry
Proteoglycan
HVEM
Viral DNA
NUCLEUS
Uncoating
Sp1
RNA Pol-II
TBC
TATA
Pre-initiation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

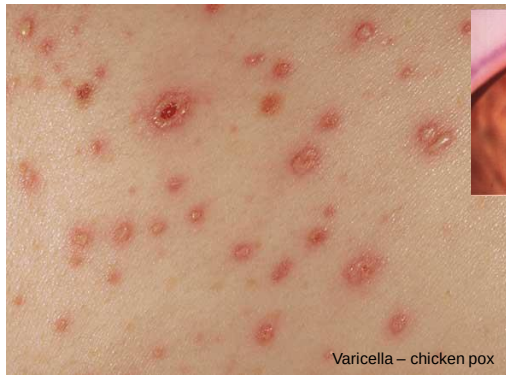
Pathological impact of HSV and VZV

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

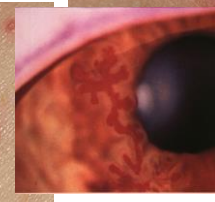
VZV – varicella, herpes zoster, encefalitis, pneumonie, hepatitis

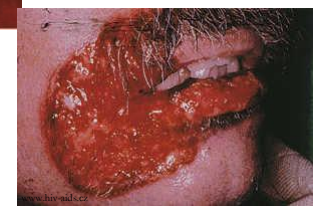
– *In allogeneic HSCT setting less frequently in case of acyclovir prophylaxis; reactiovation of HSV without ACV prophylaxis in 80% of patients*

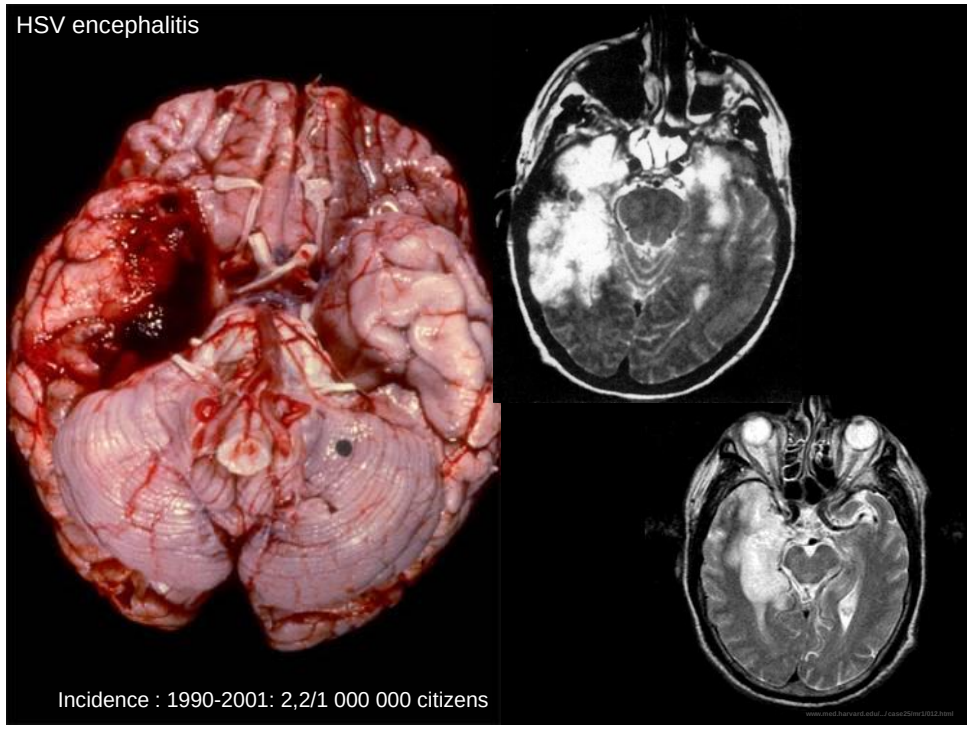




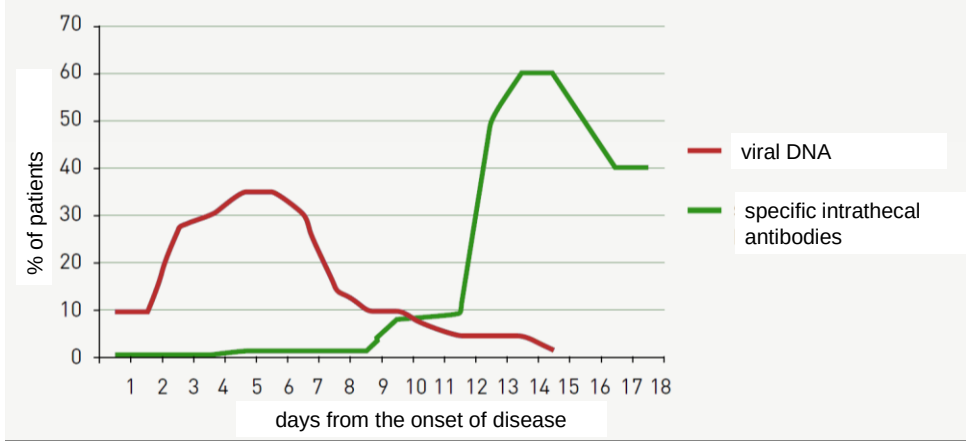
Varicella – chicken pox

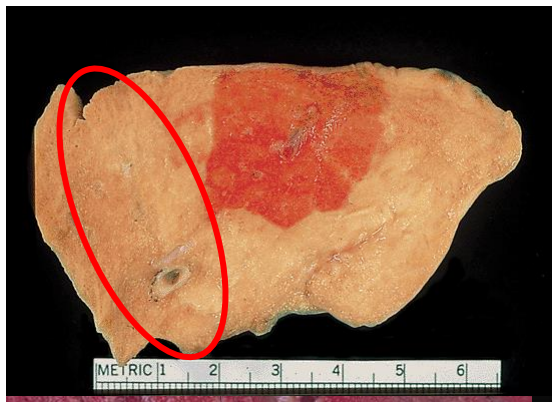


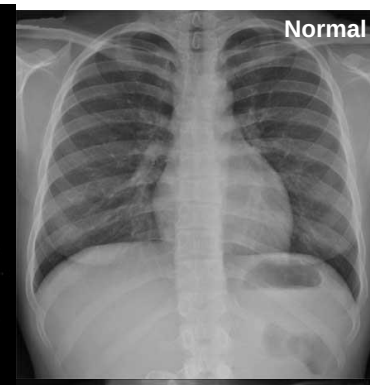


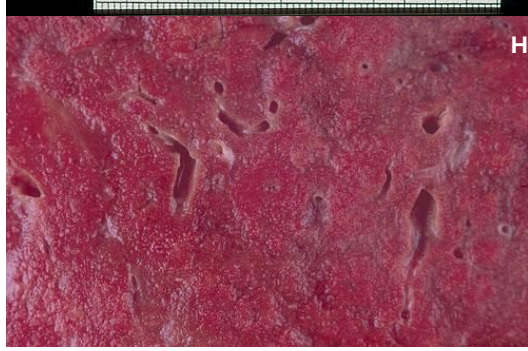


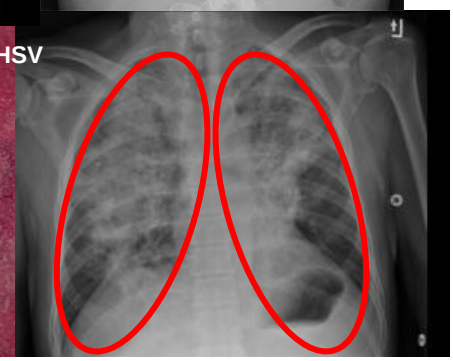
Antibody response to viral infection and detection of virus in CSF





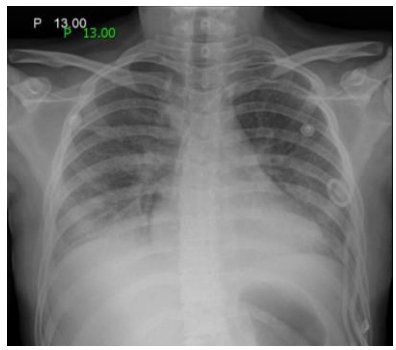




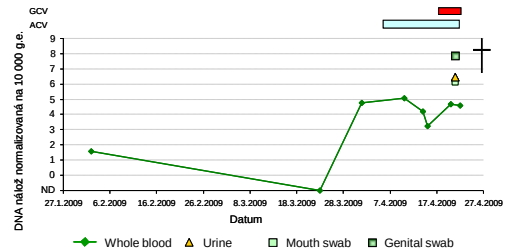


Different impact and destruction in different organs

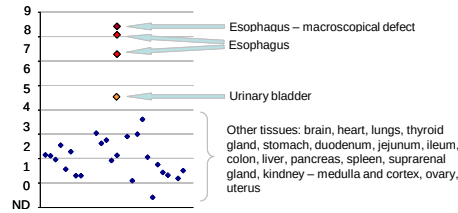
HSV (girl treated for ALL)



HSV pneumonia

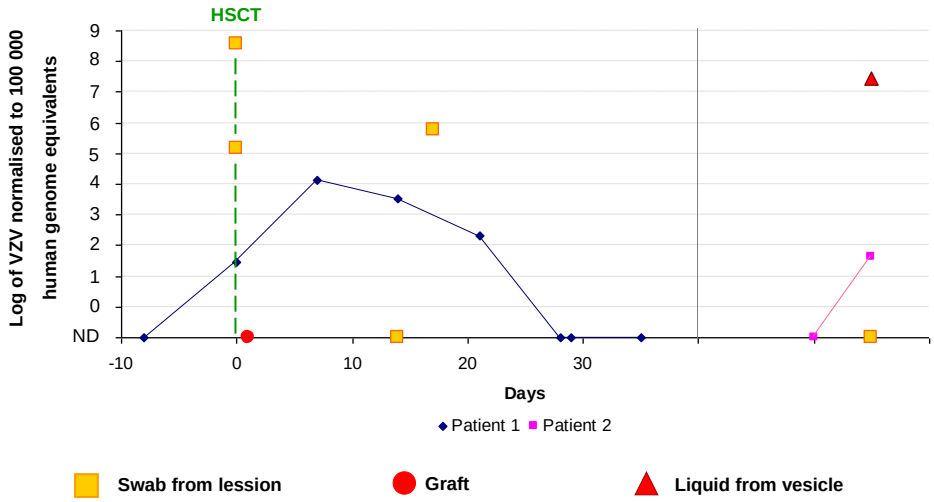


HSV quantity detected in the tissue at the authopsy

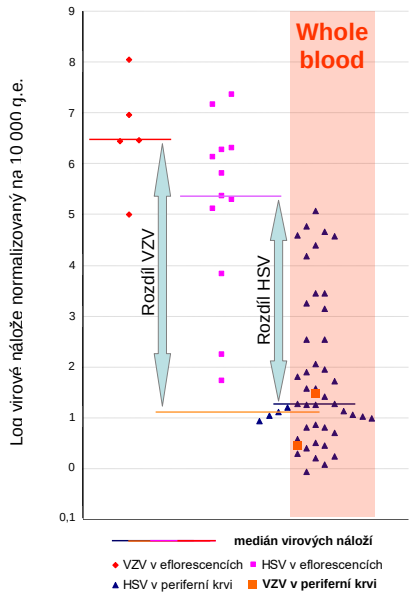


Difference in materials

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)**

Pathological impact of CMV

In immunocompetent

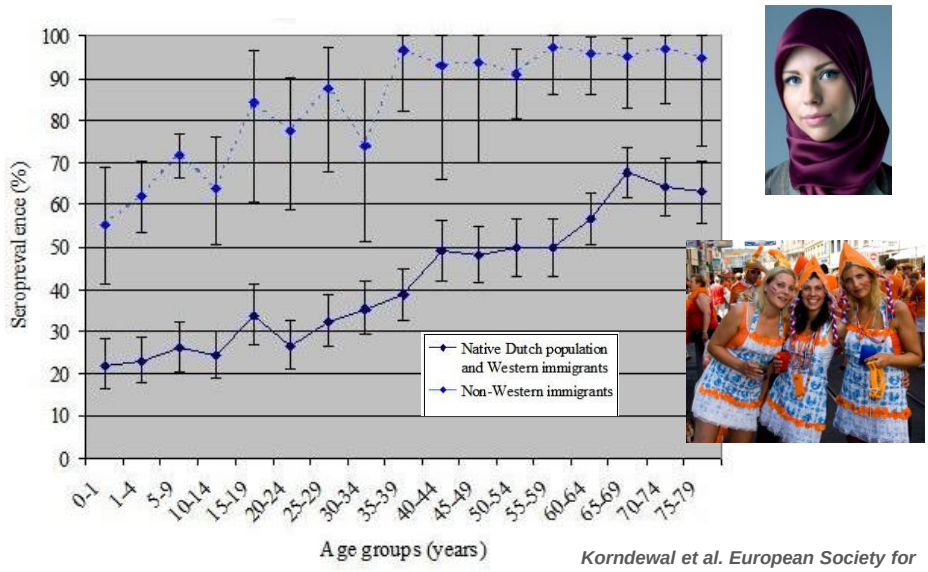
- Asymptomatic in 95% of children
- mononukleosis like sy.
- In pregnant woman teratogenic
- Associations with malignant glioma, ca. of breasts
- Possible association with Alzheimer‘s disease

In immunocompromised

mainly

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

CMV seroprevalence

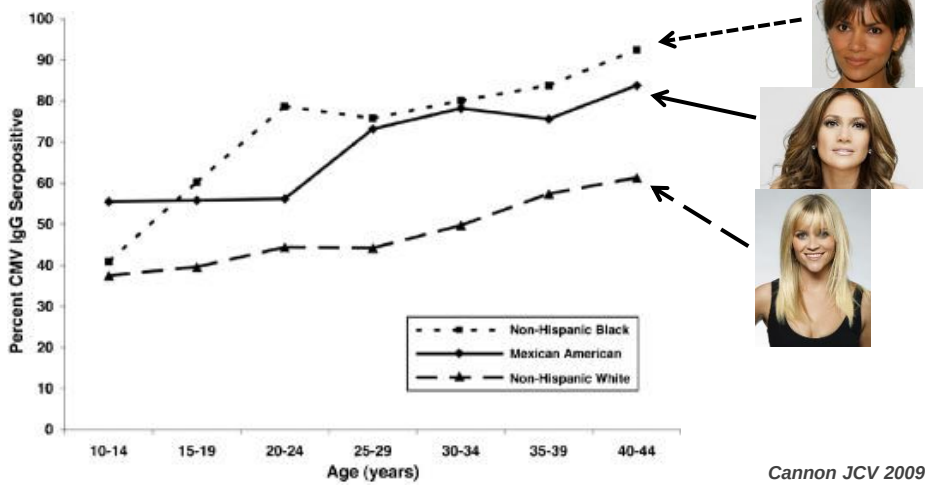


Korndewal et al. European Society for Paediatric Infectious Disease 2012

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

CMV seroprevalence

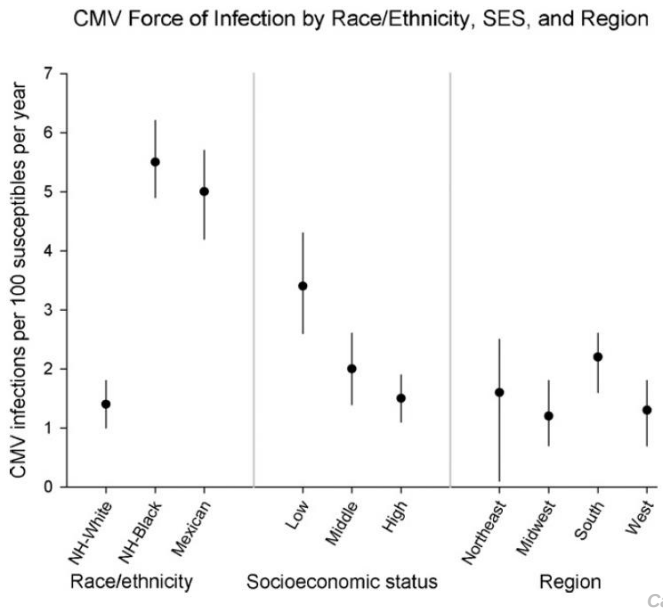
- **60-90% of healthy adult population** http://www.tulane.edu/~dmsander/garryfavweb.html
- **increases with age and decrease in developed countries**



Cannon JCV 2009

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

Incidence of CMV primoinfection

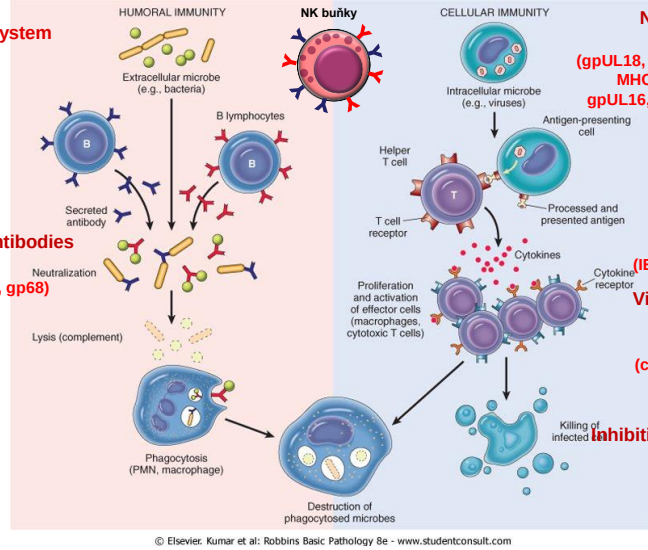


Cannon JCV 2009

How CMV manipulates with immunity?

Complement system inhibition (?)

Inhibition of antibodies by FcR homolog (gp34, gp68)



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

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

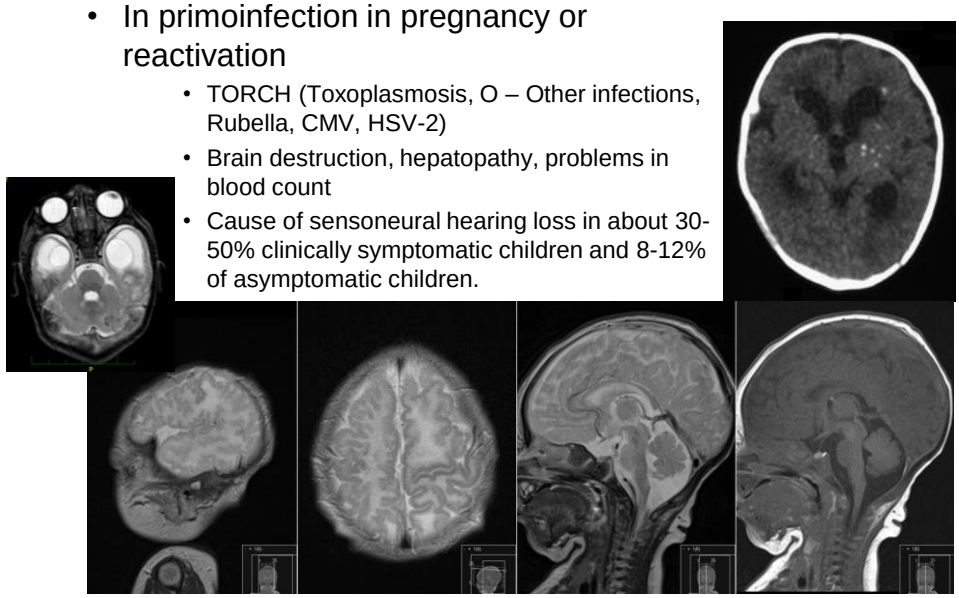
Viral homologs of cytokines and chemokines (cmvIL-10, vCXCL-1, pUL147, pUL128-131)

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

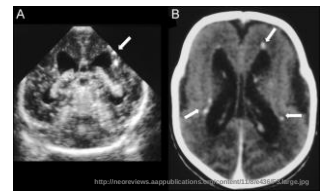
- bone marrow supression **petechia, „blueberry muffin baby“**
- CMV end-organ infection
- vasculitis – especially eyes and a CNS

Neurologic problems/seisures

Brain calcification/ cavity

CMV excretion to urine

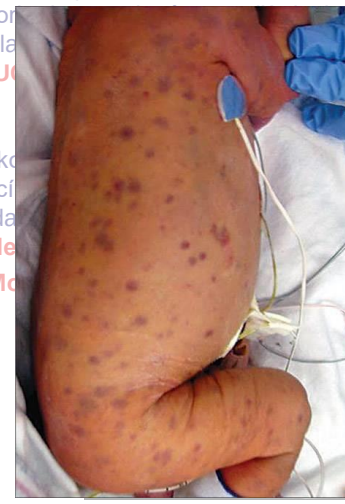
Premature delivery



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)

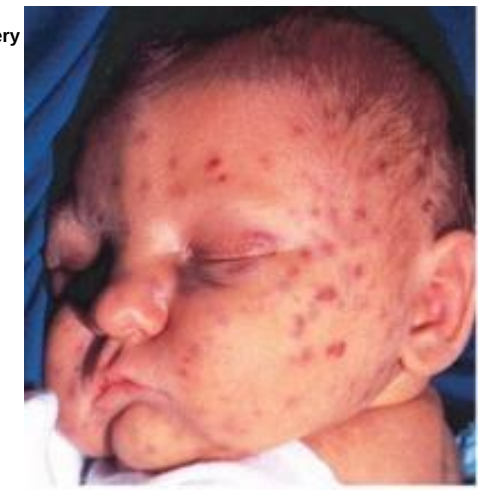
According to CDC

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

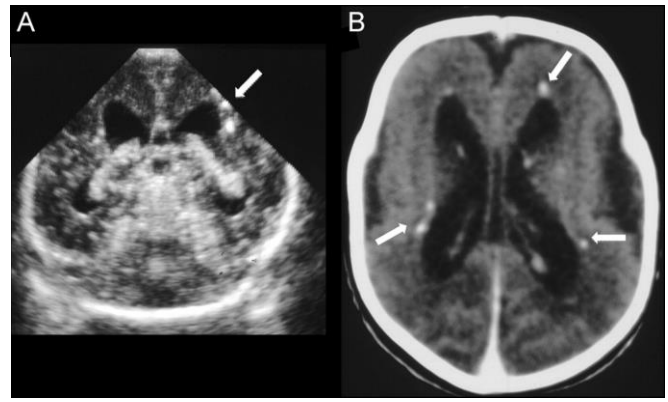
Transient Outcomes

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

Permanent Outcomes

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

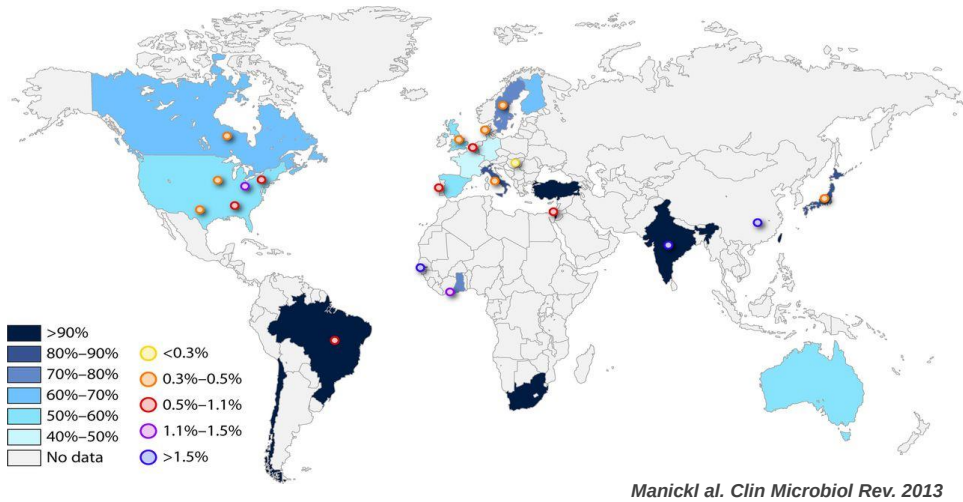
Brain calcification



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

What is the frequency of cCMV?

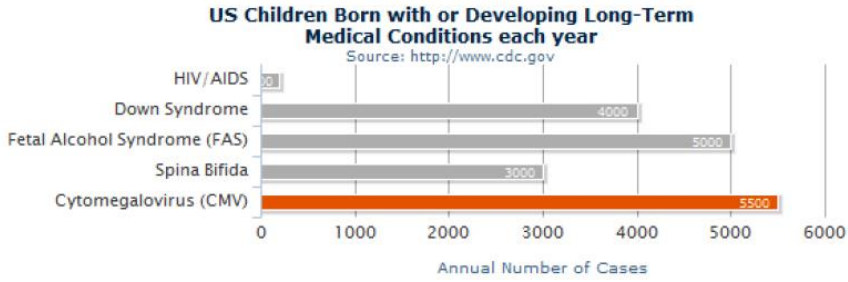
There is 370,000 children born every day in the world, representing 134 millions/year.
Average frequency approx. 1,5% of living birth – 2.01 millions of children with cCMV/year.
In Europe and Czech Republic is estimated frequence 0.5-1% cCMV of living neoborns.

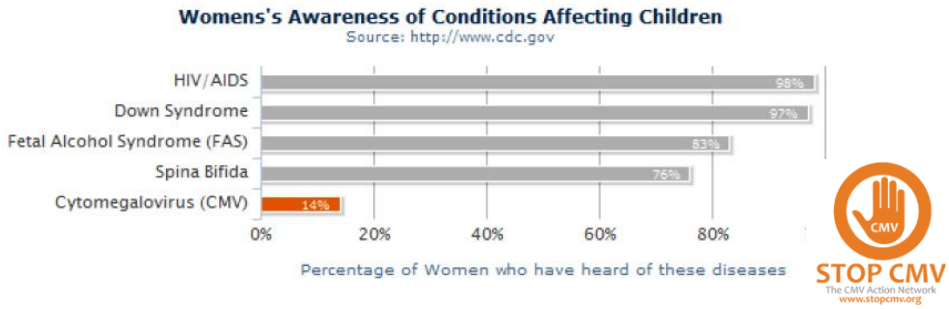


Manickl al. Clin Microbiol Rev. 2013

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

What is a knowlegde about cCMV and its impacts?





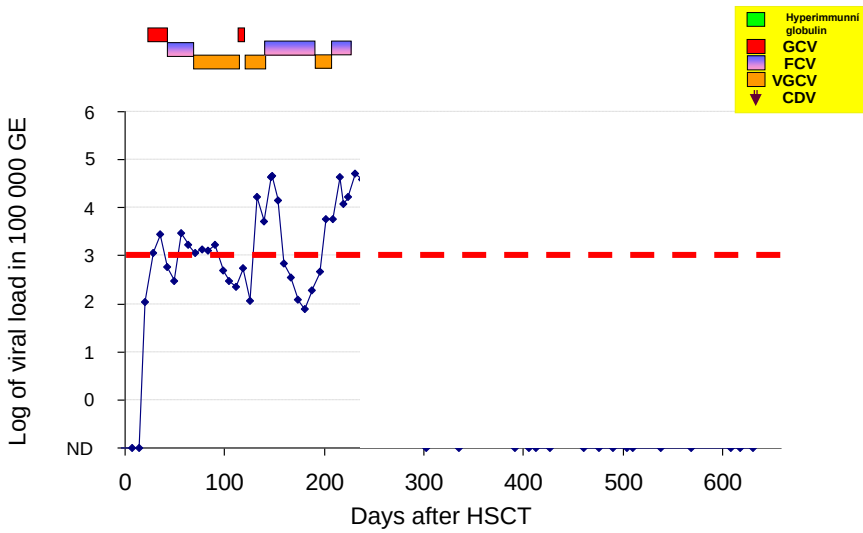
Patient 1

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

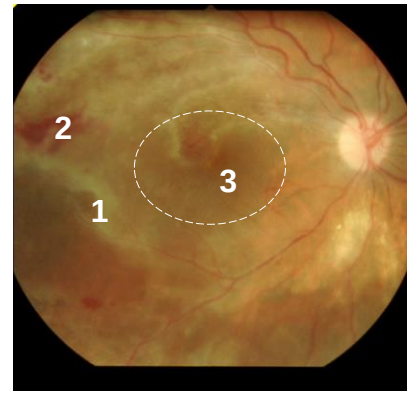


Patient 1

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

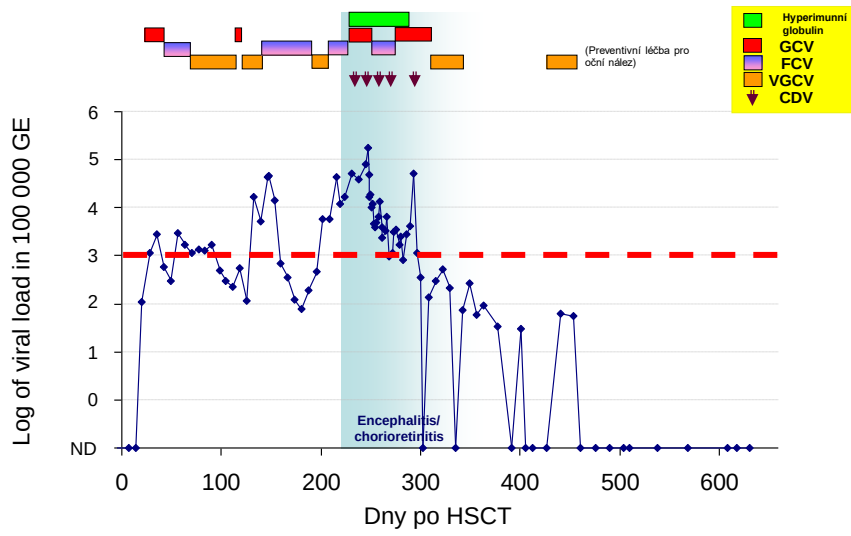
CMV detected in CSF (approx. 2 600 000 copies / ml) and increase of viral load in peripheral blood.

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



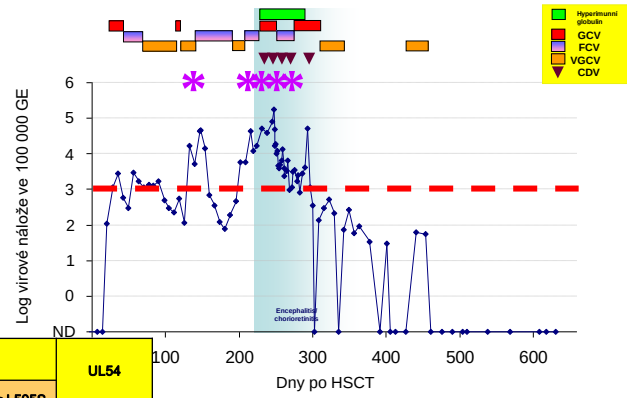
1 – retinal fibrotisation
2 – intraretinal bleeding
3- epiretinal pseudomembrane

Patient 1



Patient 1

Ganciclovir resistance

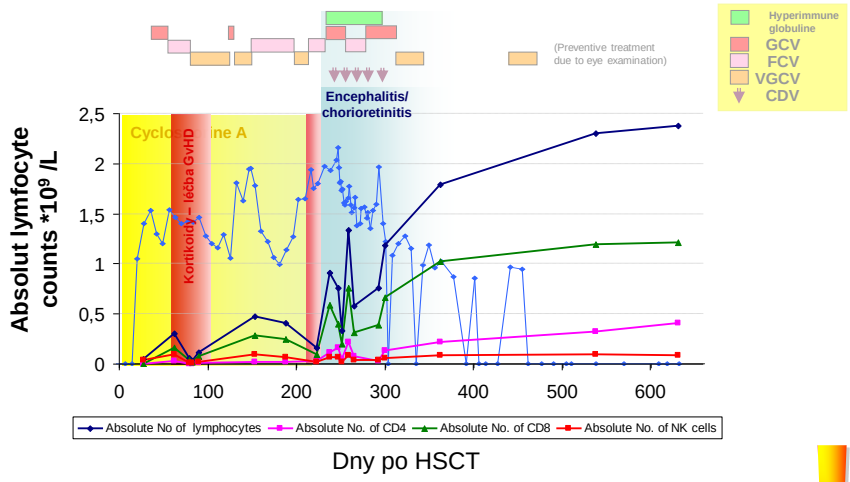


Den	Materiál	Gen UL97		UL54
		Mutace A594V	Mutace L595S	
146	*Krev*	WT+mutation	WT+mutation	ND
209	*Krev*	WT	Mutatiom	ND
231	*Krev*	WT	Mutatiom	ND
234	*CSF*	WT+mutation	Mutatiom	ND
238	*Krev*	ND	ND	Mutatiom
247	*Krev*	ND	ND	Mutatiom
248	*Krev*	WT+mutation	Mutatiom	ND
276	*Plazma*	WT	Mutatiom	ND

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

Patient 1

Lymfocyte counts

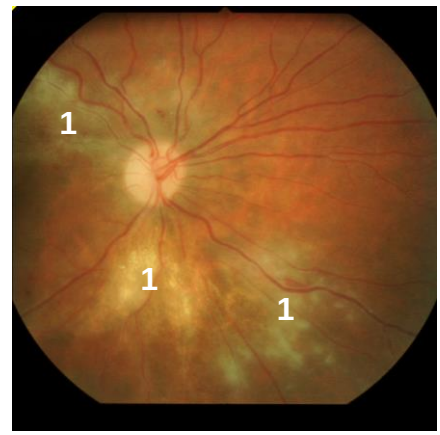


Even a short term steroid treatment leads to decrease of the lymfocyte count neccessary for infection control

Patient 1

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

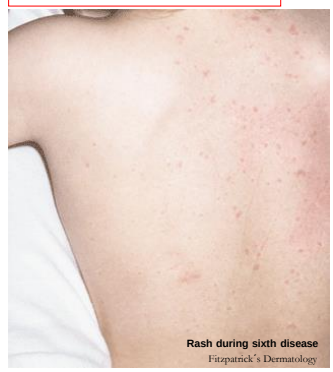
HHV-6

Recently 2 distinct viral species

HHV-6 A

Unknown

„Orphan virus“



HHV-6 B

Immunocompetent host

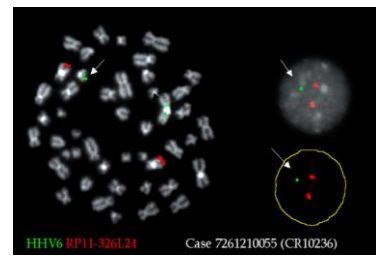
- **Sixth disease**
- **Febrile seizures**
- **Encephalitis**

Immunocompromised host

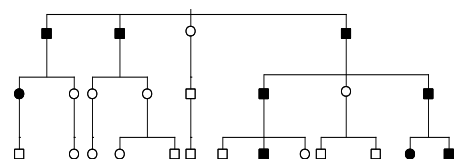
- **Encephalitis**
- Hepatitis
- Pneumonitis
- Pericarditis
- **Delayed engraftment after HSCT**

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**

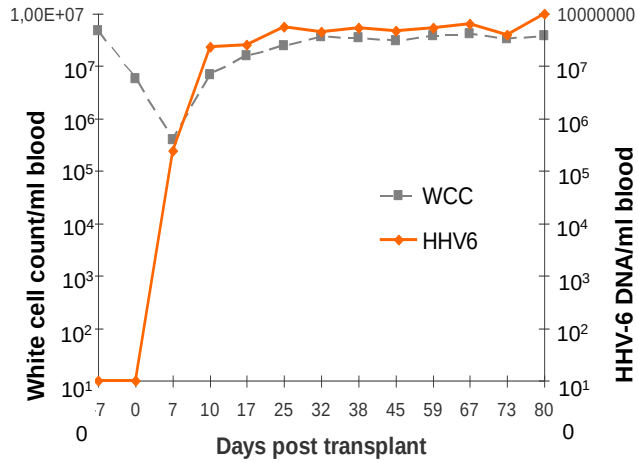


HHV-6 integration at 22q13.3 control probe on 9q34.4



HHV6 DNA in blood after HSCT

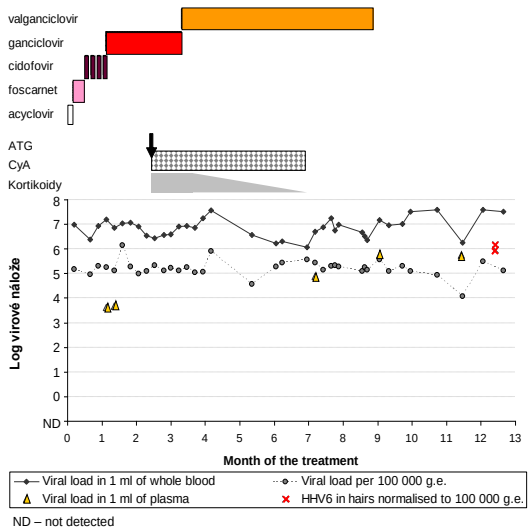
donor with Ci-HHV-6



Clark et al., JID 2006

Patient 2

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



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.

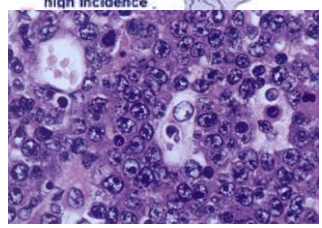
EBV discovery

surgeon

1958
„A sarcoma involving the jaws of African children.“British Journal of Surgery

1st described human oncogenic virus

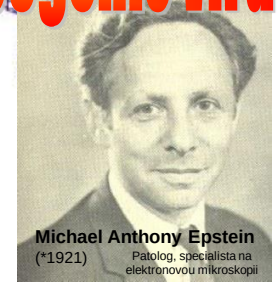
Burkitts lymphoma high incidence



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

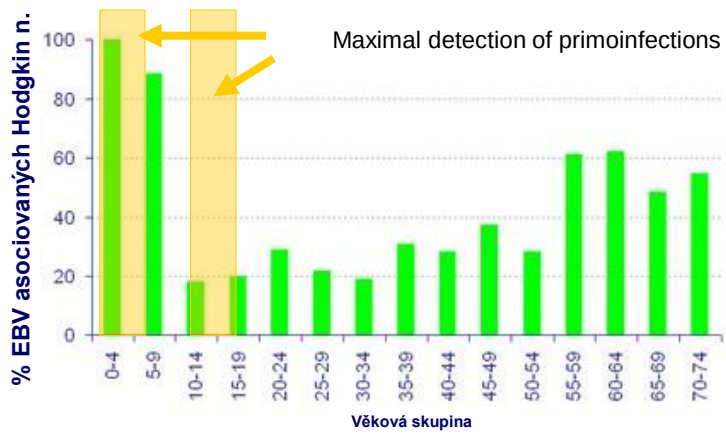
Yvonne M. Barr (*1932)

Bert Geoffrey Achong (1928-1996)

Transmission and epidemiology

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

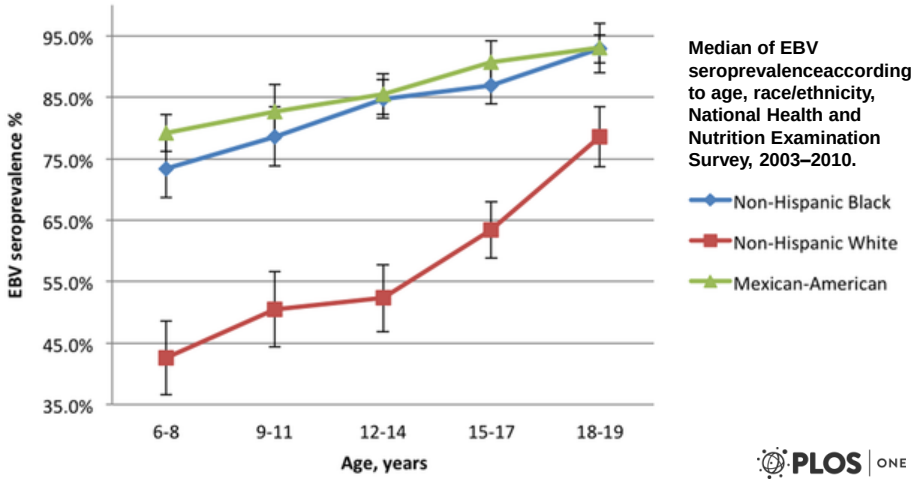
(in developing countries, it is 90% of children older 2 yrs)



Maximal detection of primoinfections

Transmission and epidemiology

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



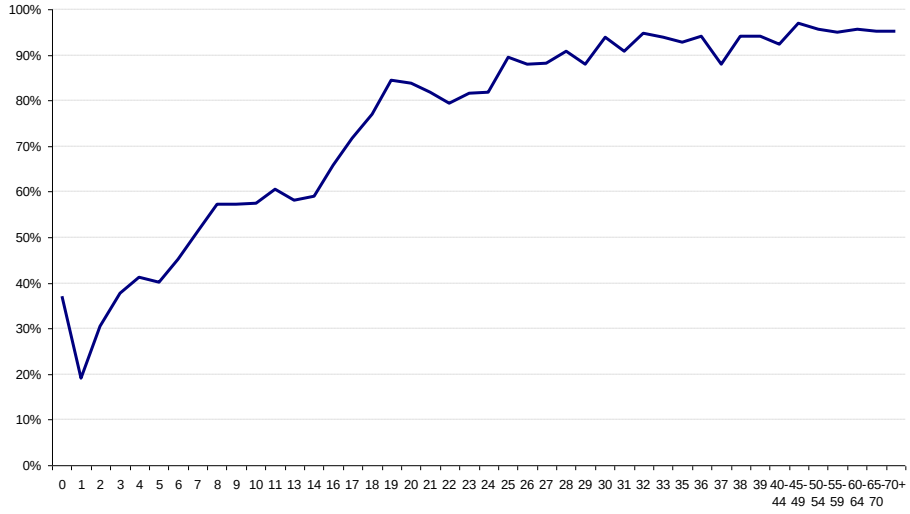
Median of EBV seroprevalenceaccording to age, race/ethnicity, National Health and Nutrition Examination Survey, 2003–2010.

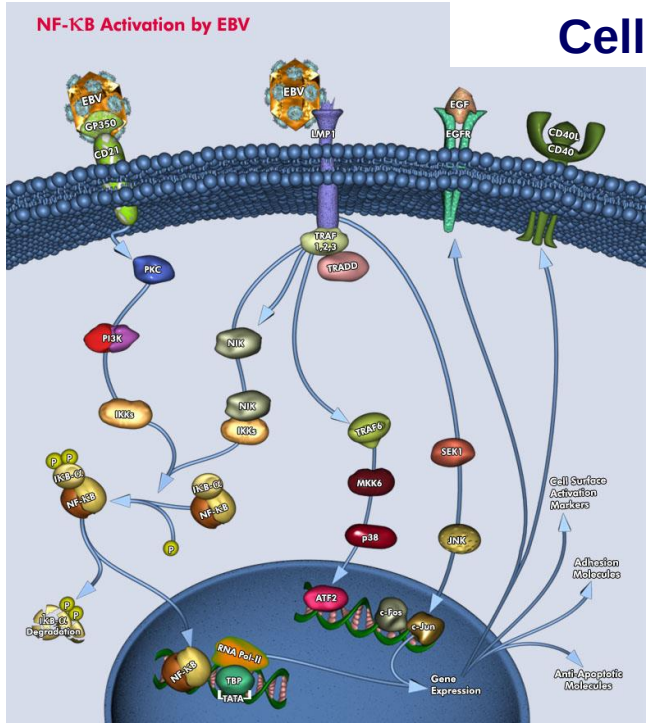
PLOS ONE

*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.0064921*http://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

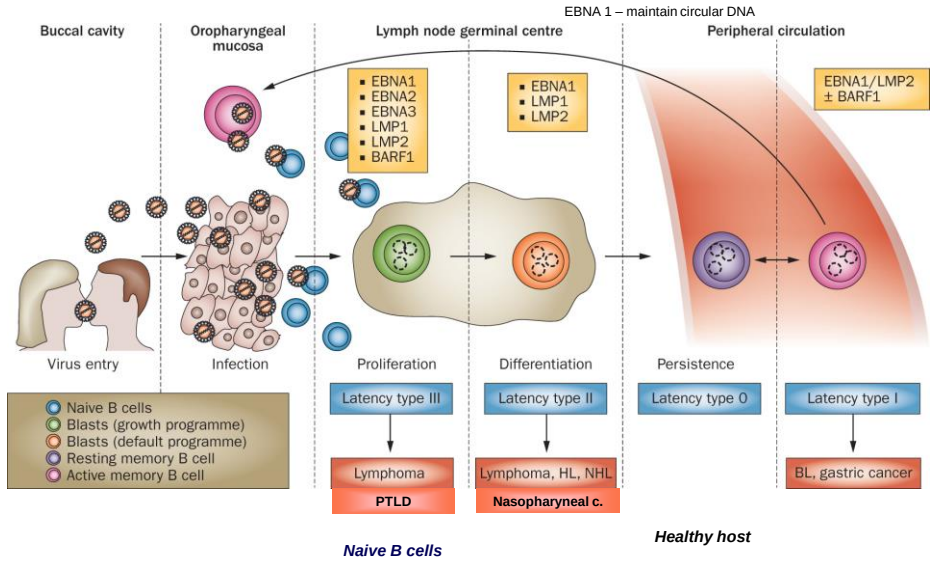
How EBV manipulates the immunity /prolipheration?

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

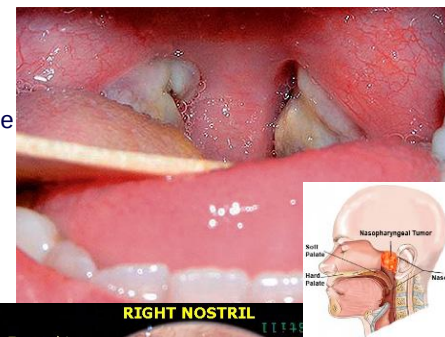
(antigens and latency)

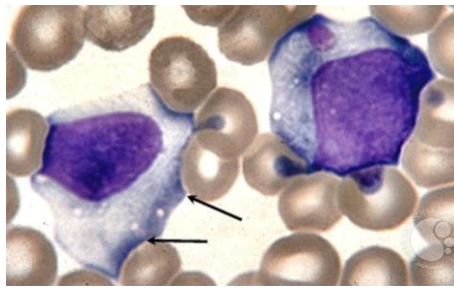


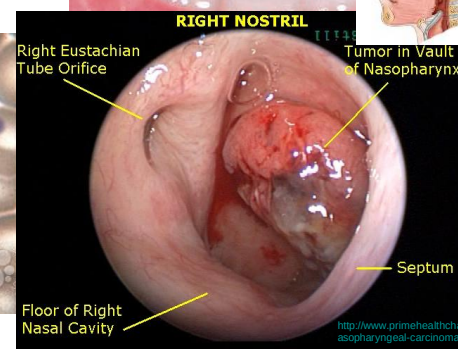
Bollard, C. M. et al. (2012) T-cell therapy in the treatment of post-transplant lymphoproliferative disease
Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2012.111

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

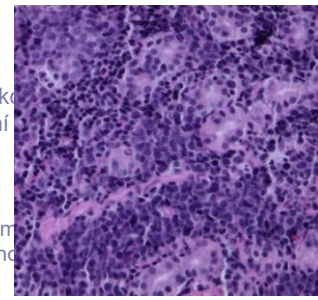


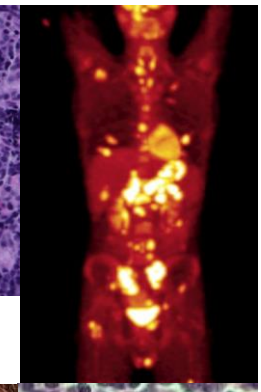




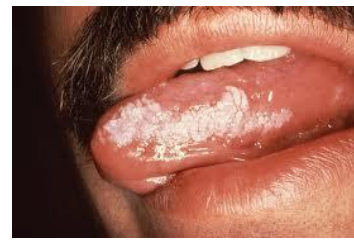
Patological activities of EBV

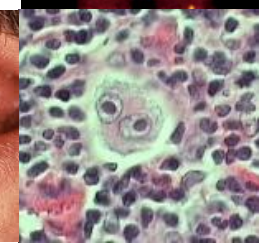
- **Imunokompetentní hostitel**
  - Infekční mononukleóza
  - Chronická aktivní EBV infek
  - X-vázaná lymfoproliferativní
  - Maligní onemocnění
    - Hodgkinova nemoc
    - Burkittův lymfom
    - non-Hodgkinský T/NK lym
    - Nasopharyngeální karcino
    - Karcinom žaludku
    - Angioblastický T lymfom





- **Immunocompromised host**
  - Hairy leukoplaky
  - Above listed malignant diseases
  - Post-transplant lymfoproliferative disease (EBV-LPD)
  - Encefalitis/myelitis
  - Pneumonie
  - Hepatopathy/hepatitis





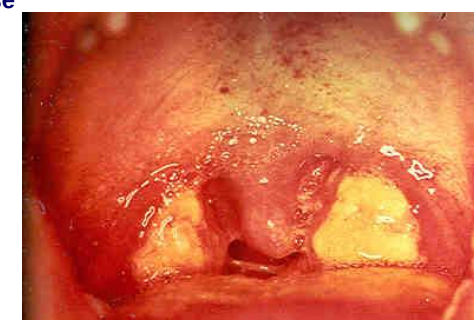
http://www.kcom.edu/faculty/chamberlain/Website/lectures/lecture/aids.htm www.med-ed.virginia.edu/courses/path/innes/wed/hodgkin

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



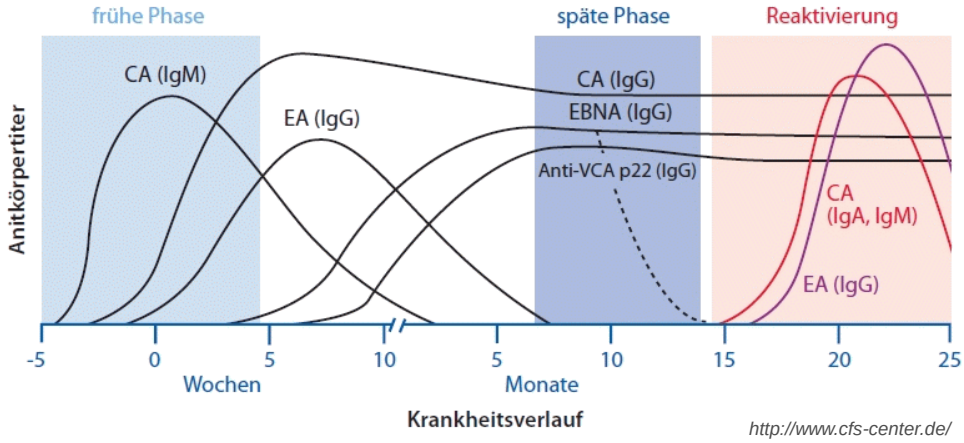
http://home.teleport.com/~bobh/InfectiousMononucleosis.htm

Diagnostics

Basic diagnosis of EBV is indirect – serological.

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

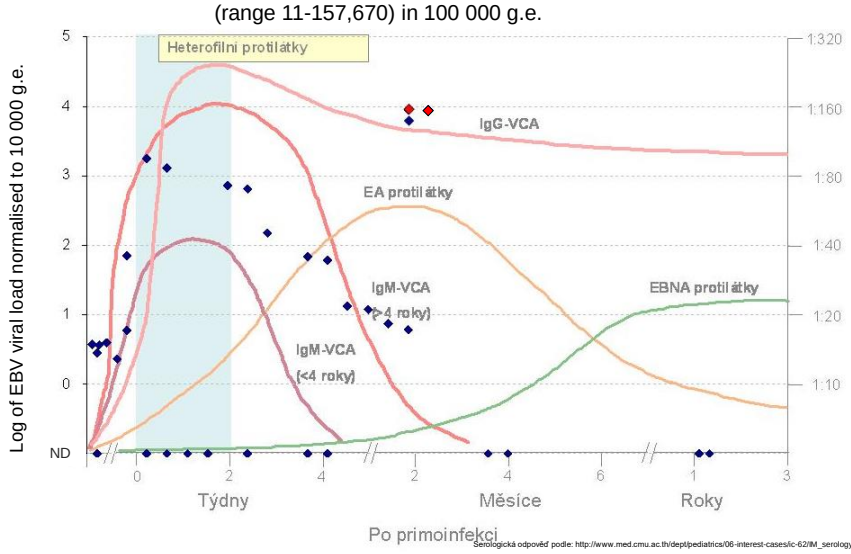
Heterofile antibodies Paul-Bunnelova reaction



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

Viral load in patients with dg. B27 - IM

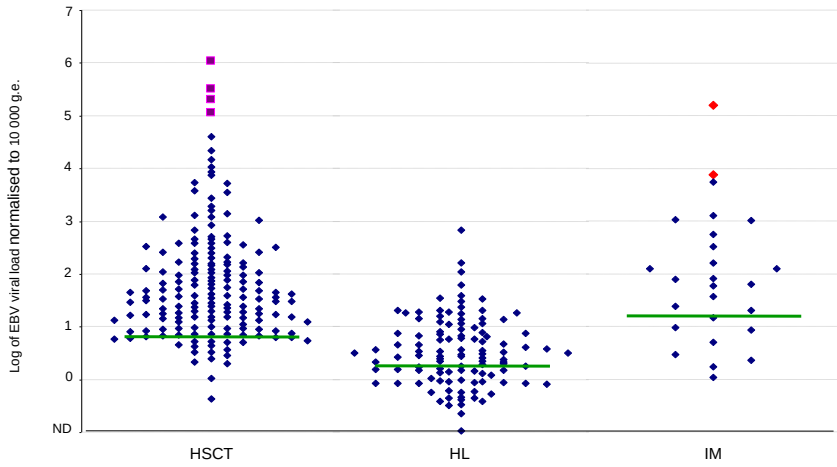
- Positive – 26 patients (62%)
  - 50 samples positive (65%); median of positivity 110 (range 11-157,670) in 100 000 g.e.



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, ≥5120; VCA IgA, positive; EA [D] IgG, ≥640; EA [D] IgA, positive; and EA [D] and EA [R] IgG, ≥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)	$10^{4.1 \pm 0.5}$	$10^{4.4 \pm 0.4}$	.09
Plasma (copies/mL, mean ± SD)	$10^{2.9 \pm 1.1}$	$10^{2.4 \pm 2.1}$	.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.

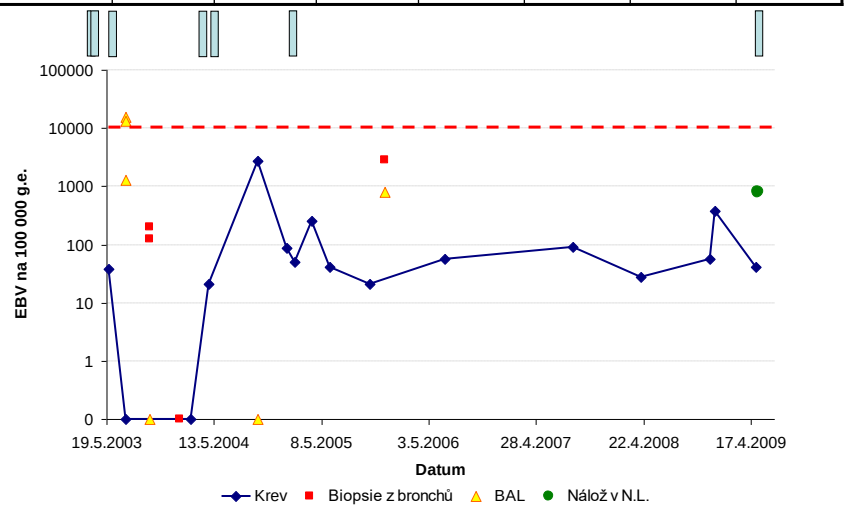
Table 2. Clinical features of 30 patients with chronic active Epstein-Barr virus infection

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		

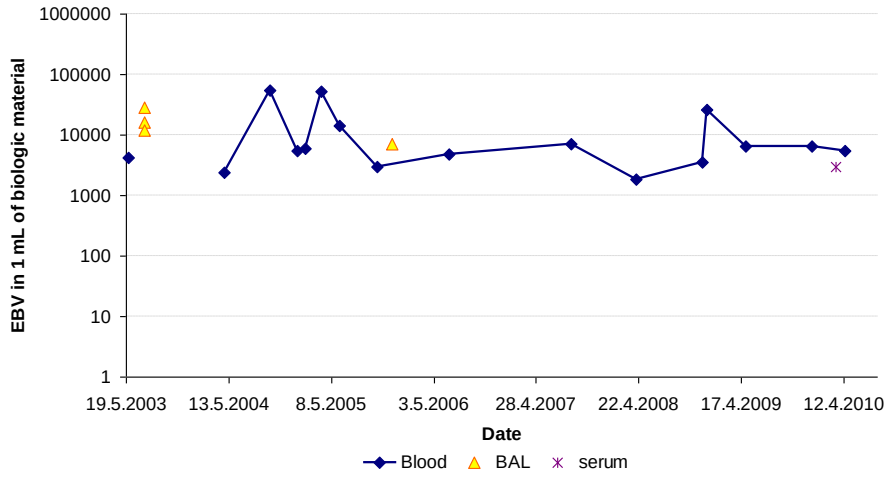
HMB indicates hypersensitivity to mosquito bites.

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

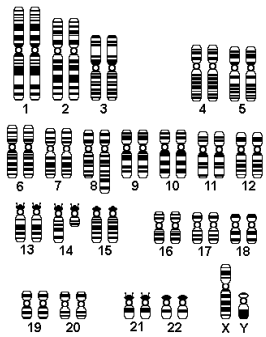
Very agressive

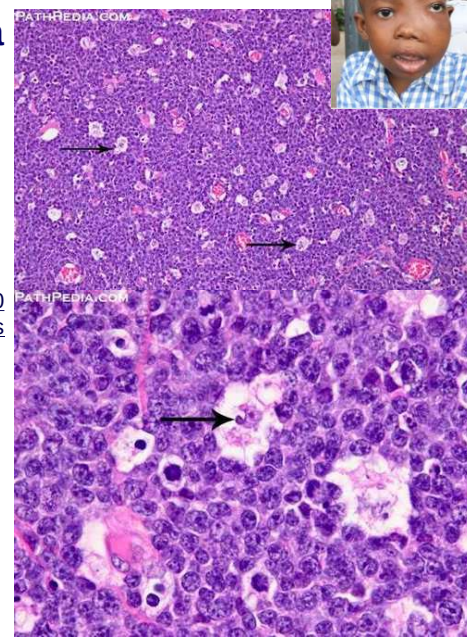
Picture of the **„Sky of stars “** – „stars“ are apoptotic tumor cells which are fagocyte by macrophages; „sky“ – represent tumor lymfocytes

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

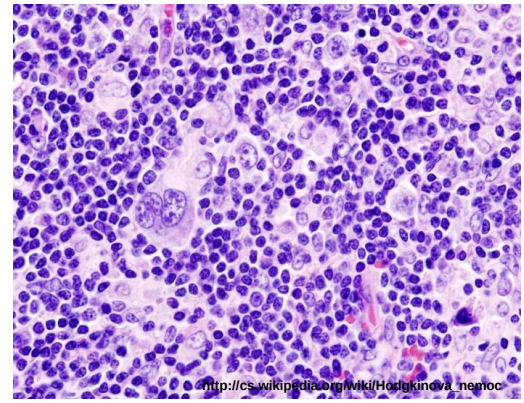
Malignant impact of EBV

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 ♂.

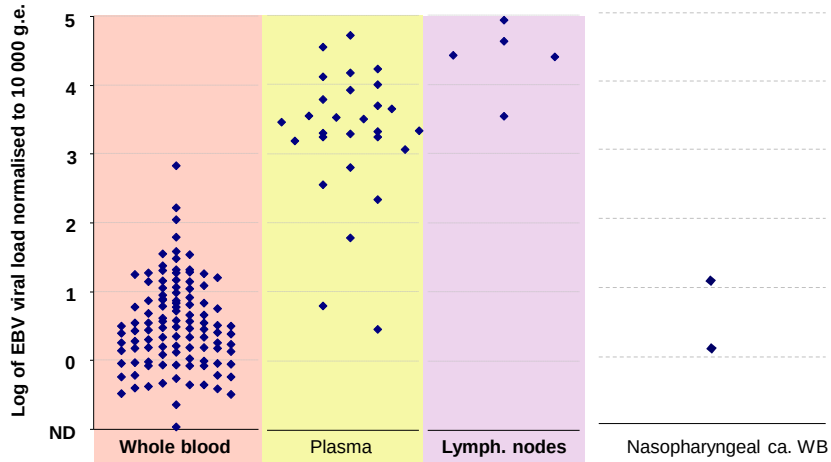


Histologically divided according to no. of **Reed-Sternberg‘s cells** (cells developed by mutation from B-cells) and according to the cellular frections:

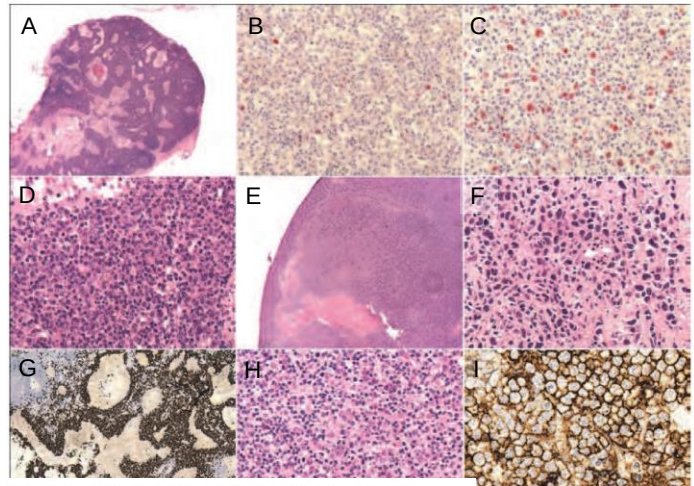
typ I with dominance of lymfocytes (few R-S cells, dominance of lymfocytes; best prognosis) (5 %);
typ II nodular-sclerotic (nodular centres, cells (reticular, lymphocytes, histiocytes) in colagen fibres) (70 %);
typ III mixed (20–25 %);
typ IV classical, few lymphocytes (No. of Sternberg‘s cells increased;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

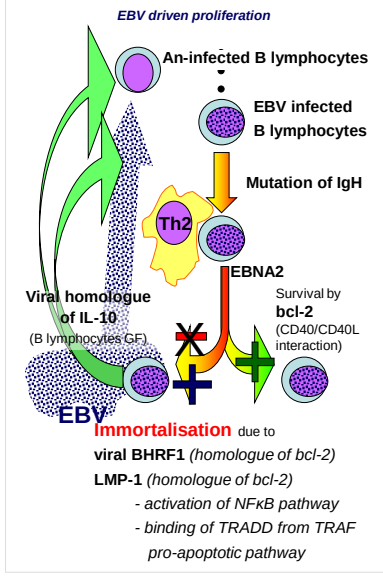


EBV associated posttransplant proliferative disease (EBV-LPD)



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:

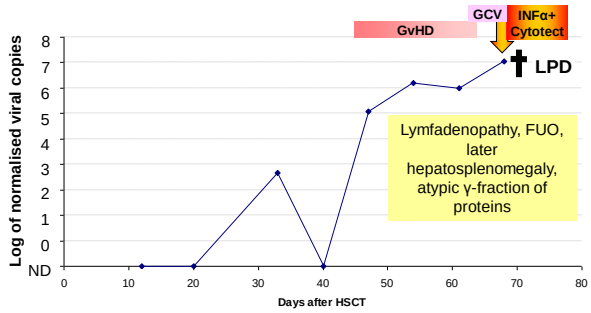
- **Change and/or destroy of the cell tissue culture by lymphoproliferative process**
- **Presence of monoclonal, or oligoclonal proliferation proven with cell and/or viral markers**
- **Evidence of EBV infection in many cells (e.g.. DNA, RNA, protein…**

EBV DNA detection in whole blood is not enough.

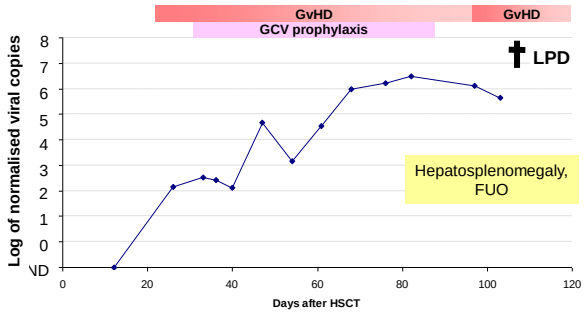
Dle definice EBMT IDWP, 2007

- 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

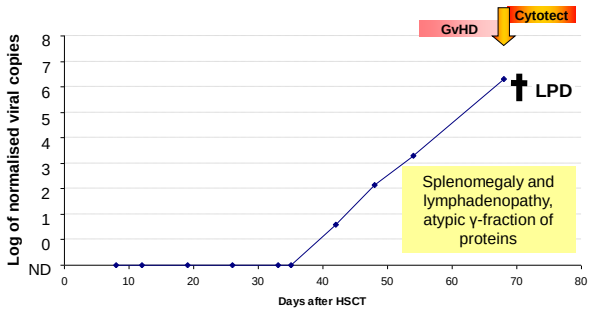


Girl, 5.5 yrs., HSCT for ALL CR2.
Donor: MMUD (8/10)
Graft: BM
Conditioning: TBI (12 Gy)
cyclophosphamide (120 mg/kg)
ATG (16 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade III (D+ 49; GIT)
Deceased on D+74 due to MOF.

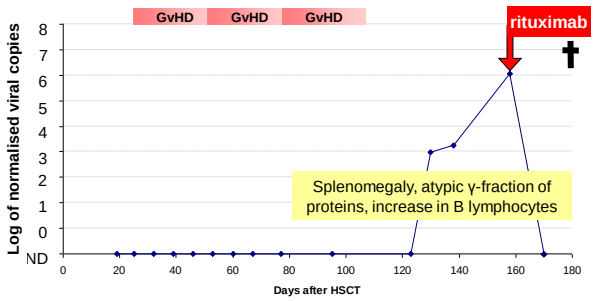


Girl, 4 yrs., HSCT for AML PR2
Donor: MMUD (9/10)
Graft: BM
Conditioning:busulphan(16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD: grade III (D+ 25; GIT)
Deceased on D+117 due to bleeding and respiratory failure.

Retrospectively tested patients

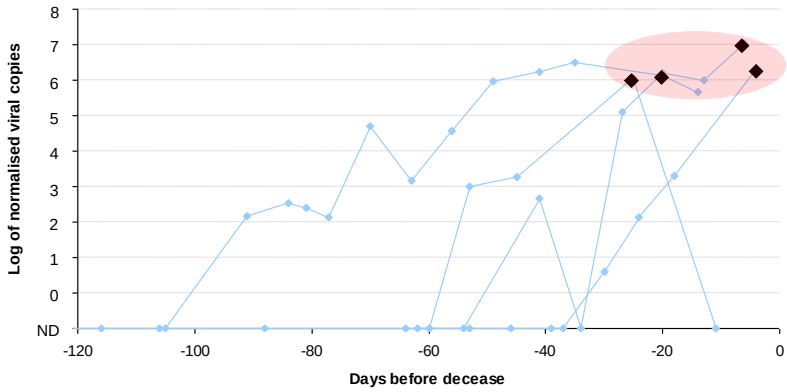


Boy, 5 yrs., HSCT for JMML
Donor: MUD
Graft: BM
Conditioning:busulphan(18 mg/kg)
cyclophosphamide (120 mg/kg)
ATG (16 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+ 56; GIT)
Deceased on D+72 due to MOF.



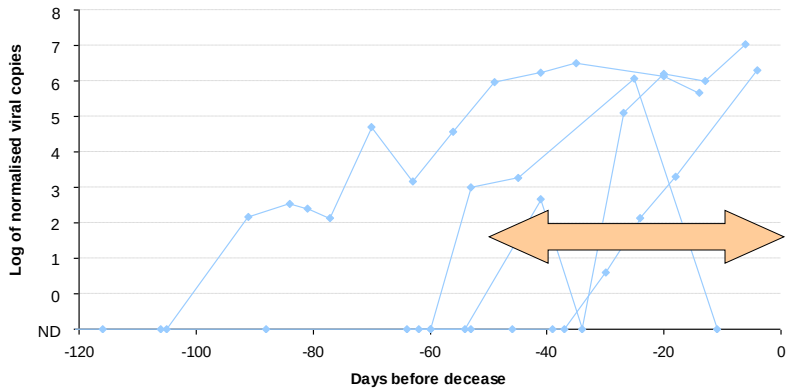
Boy, 11 yrs., HSCT for AML CR2.
Donor: MUD
Graft: BM
Conditioning: busulphan(16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD: grade III-IV (D+ 23; GIT)
Deceased on D+182 due to MOF.

Retrospectively tested patients



Maximum detected quantity was between
$1.16x10^6$ and **$1.17x10^7$ NVCs**

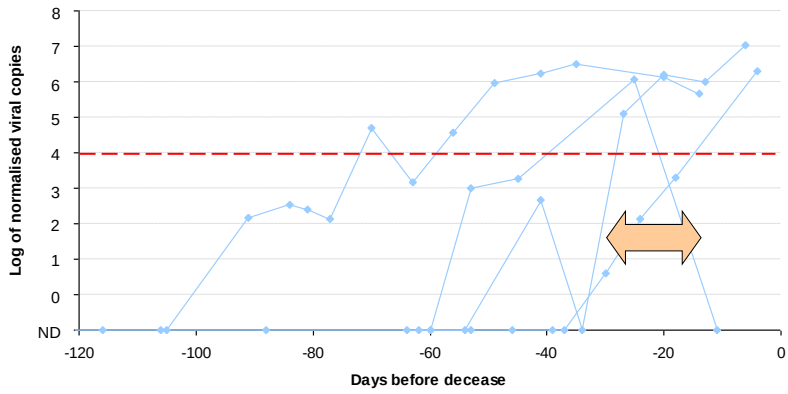
Retrospectively tested patients



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

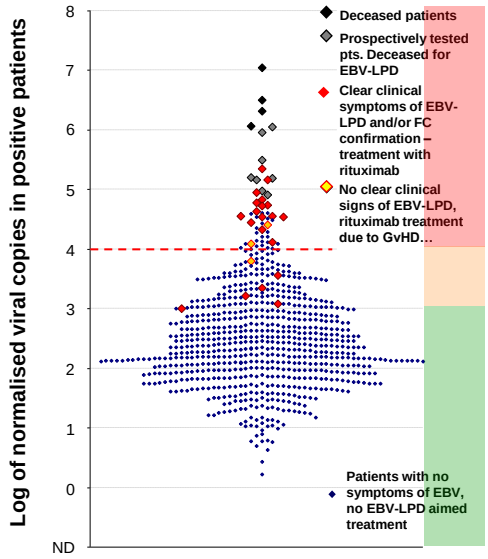
Detection preceded clinical signs of EBV-LPD with median of 35 days (-77 to -24)

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

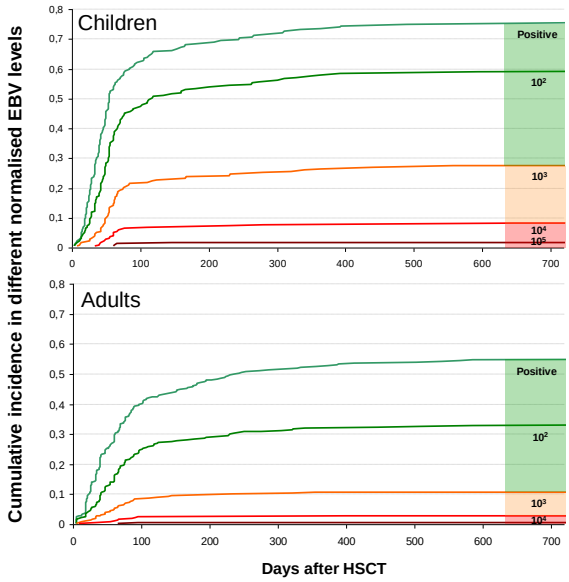


- **Very high risk level reactivation**
  > 10^4 NVCs – ≈ 50 IU/ng
  suspected and/or correlated with signs of EBV-LPD - 102 samples from 26 children (7.1%) and 22 adults (3.2%)
  EBV-LPD was confirmed in 22 (45%)

- **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.

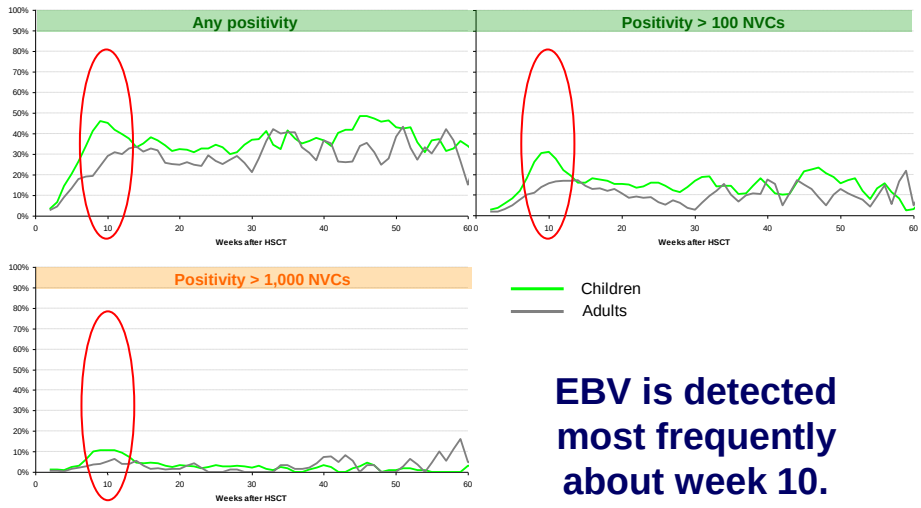
Prospective testing – incidence in time



Higher incidence in paediatric patients at every level

p<0.007

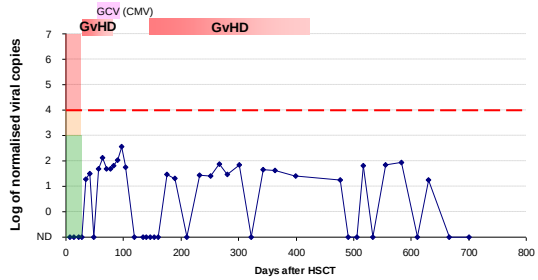
Proportion of positive patients by week and level



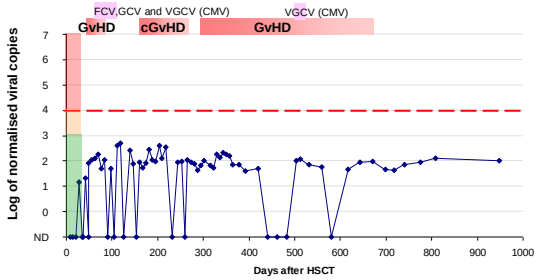
EBV is detected most frequently about week 10.

Benign level reactivation

< 10^3 NVCs ≈ 5 < IU/ng



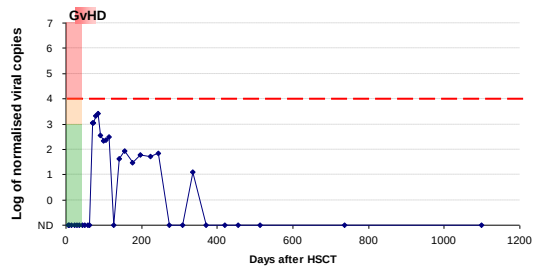
Boy, 13 yrs., HSCT for MDS-RAEB
Donor: MMUD (8/10)
Graft: PBSC
Conditioning: busulphan (16 mg/kg)
cyclophosphamide (120 mg/kg)
melphalan (140 mg/m²)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+28;GIT)
Other: BKV-HC(D+40), CMV(D+55)
Outcome: alive, no clin. problems



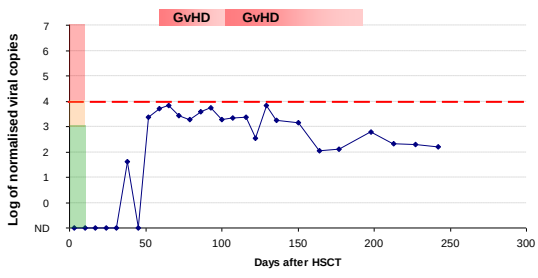
Girl, 13 yrs., HSCT for SAA
Donor: MMUD (9/10)
Graft: BM
Conditioning: TBI (5,4 Gy)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA+MTX
GvHD: grade II (D+40;GIT)
Other: CMV(D+46), lung affection of unknown ethiology, steroid DM
Outcome: alive, no clin. problems

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

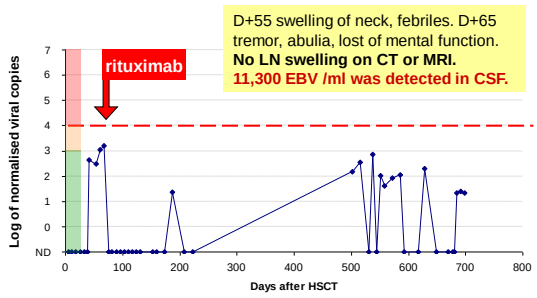
- **Detected: 28 patiens (2.65% of tx, 3.3% of EBV positive)**
  15 adults (1.98%) and 13 children (3.5%)
- **Mononucleosis like syndrom:** 1 adult
  peak at 54 days after HSCT (1,198 NVCs)
- **Localised :** 12 patients (9 adults, 3 children)
  median peak level at 68 days after HSCT
  median peak level 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.

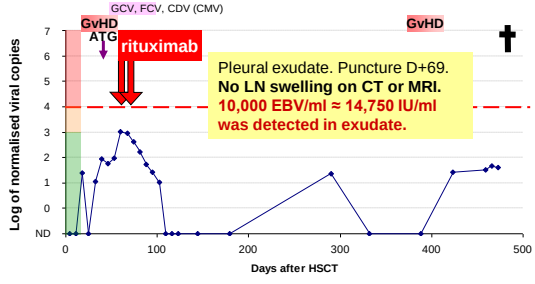
- **EBV 1 was detected in all but one patient with EBV 2.**

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)

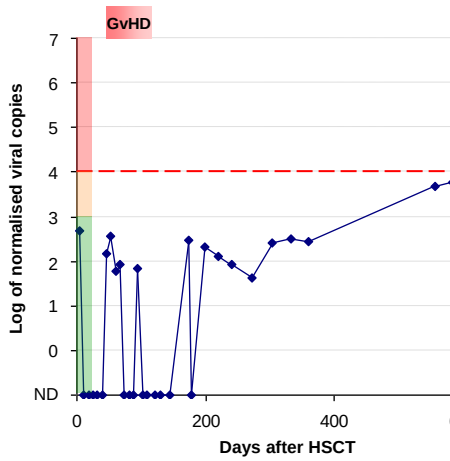
Outcome: rapid improvement,alive



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

Patient 3

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

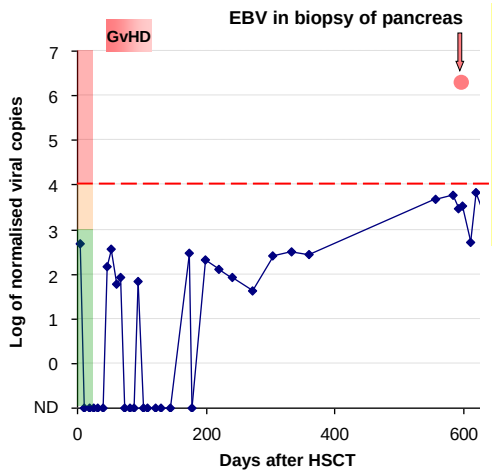
Engraftment: D+14
Chimaerism: CC D+28
D+95 – 20% autologous (negative MRD)
D+130 – 1% autologous (negative MRD)

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

D+280 – Herpes zoster (ACV)

7-12

Localised EBV-LPD (NHL)



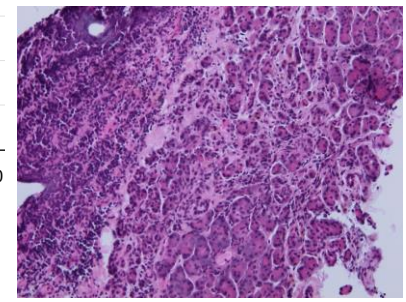
Pancreatopathy of unknown origin, confirmed by CT and US.
MRD negative.

↑CRP, ↑ IgG, no autoantibodies.

Laparotomy.

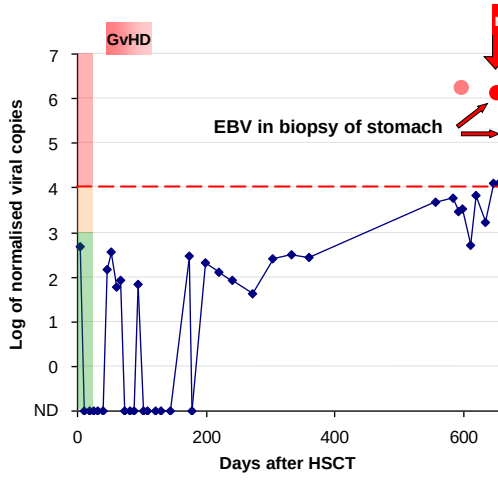
All types of lymphocytes detected in the biopsy. No malignant cells.

DR+ lymphocytes detected



Patient 3

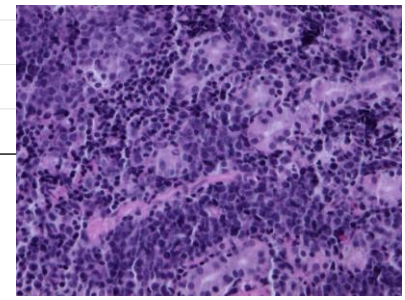
Localised EBV-LPD (NHL)



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

Gastric biopsy proves EBV+ Non Hodgkin Lymphoma (DLBCL)

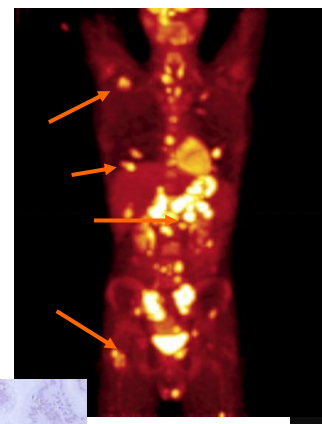
Malignant cells of donor origin.

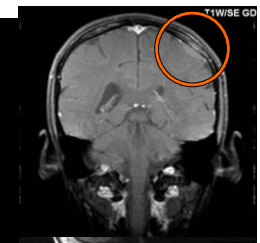


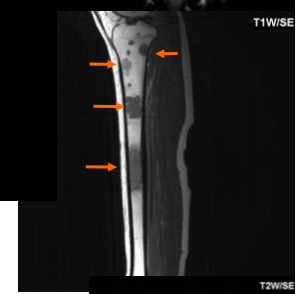
Treatment according to Protocol **BFM NHL 2004**

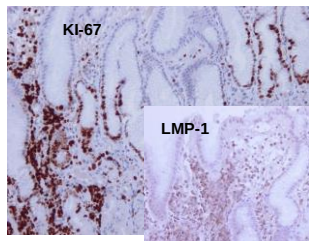
During last chemotherapy sepsis cased by Pseudomonas aeruginosa.

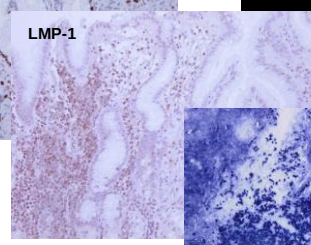
Last PET CT confirmed Remission of NHL.

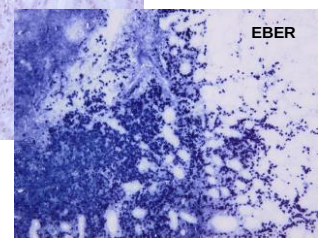


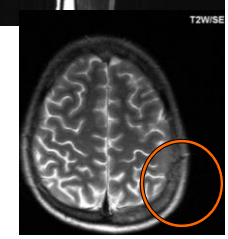






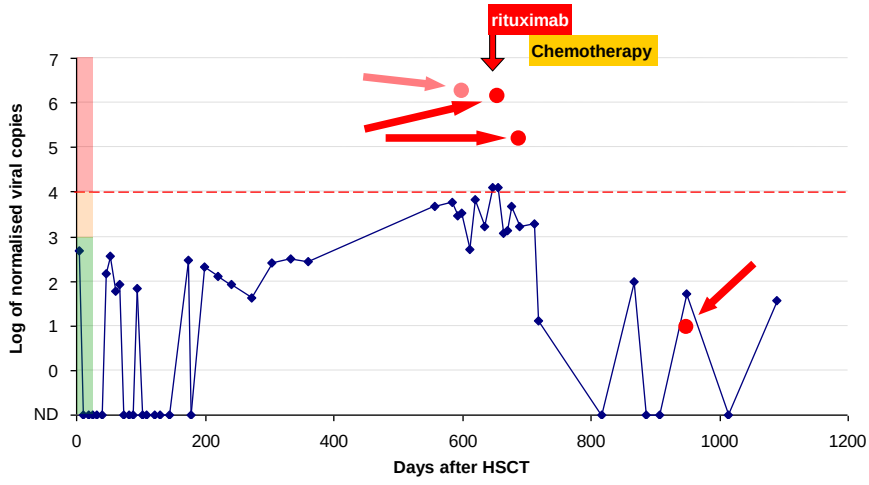






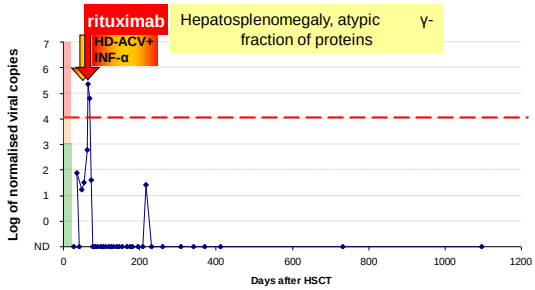
Patient 3

Localised EBV-LPD (NHL)

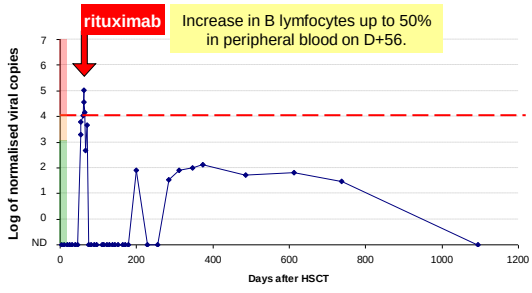


The patient remains in the remission of ALL and NHL.

Generalized EBV-LPD

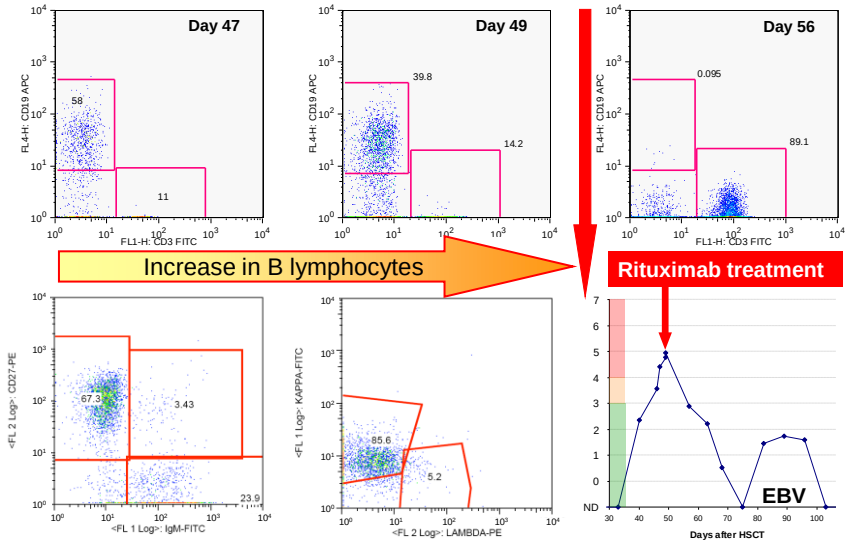


Boy, 3.3 yrs., HSCT for MPS type I
Donor: MMUD (5/6)
Graft: CB
Conditioning: busulphan (24.5 mg/kg)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA, MP
GvHD: grade II (D+49; GIT)
rituximab on D+69
Outcome: alive, no clin. problems



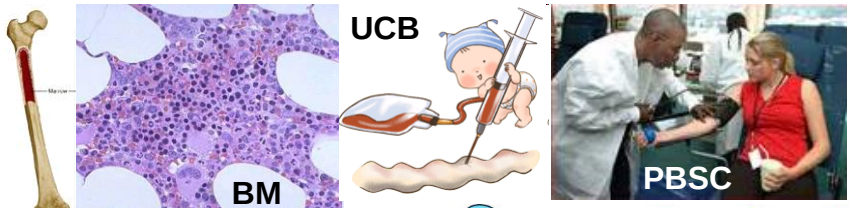
Boy, 4.4 yrs., HSCT for WAS
Donor: MUD
Graft: BM
Conditioning: busulfan (15.3 mg/kg)
cyclophosphamide (200 mg/kg)
ATG (40 mg/kg)
GvHD prophylaxis: CyA, MTX
GvHD: grade II (D+54; GIT)
rituximab on D+56.
Outcome: alive, no clin. problems

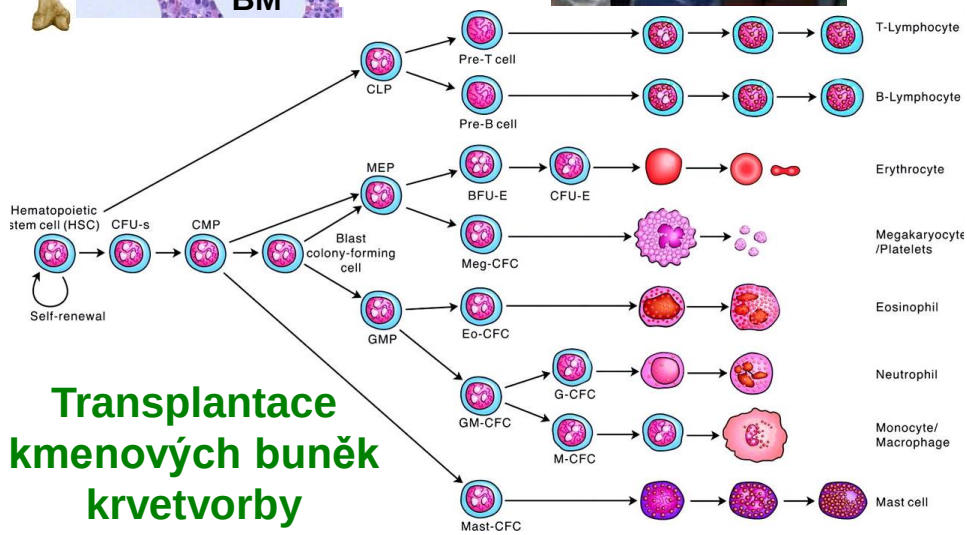
Flow cytometry EBV-LPD confirmation



Proliferation of memory B cells

KAPPA IgL+ cells





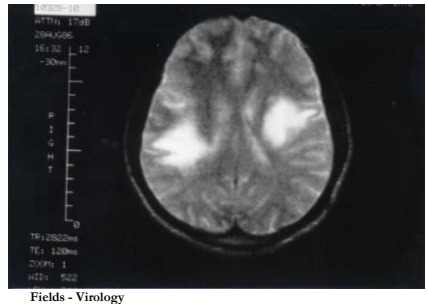
Transplantace kmenových buněk krvetvorby

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**

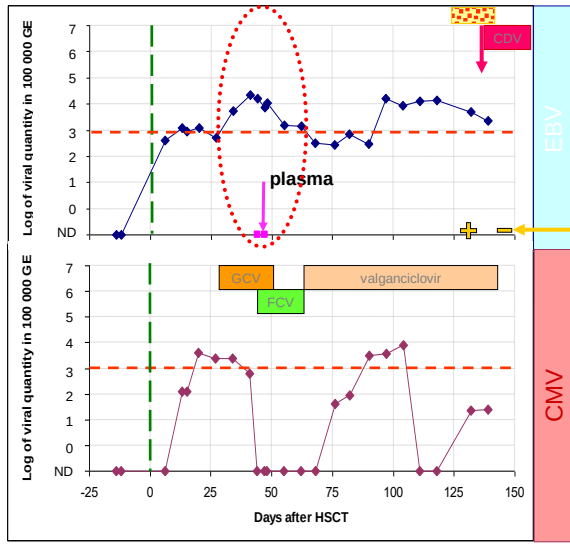


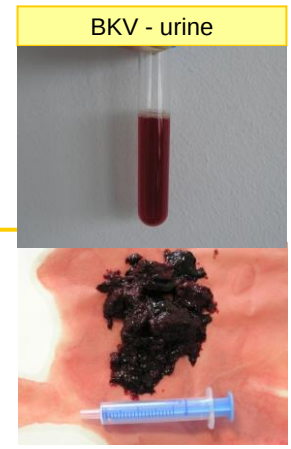
Fields - Virology

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

Patient 4

BKV – haemorrhagic cystitis

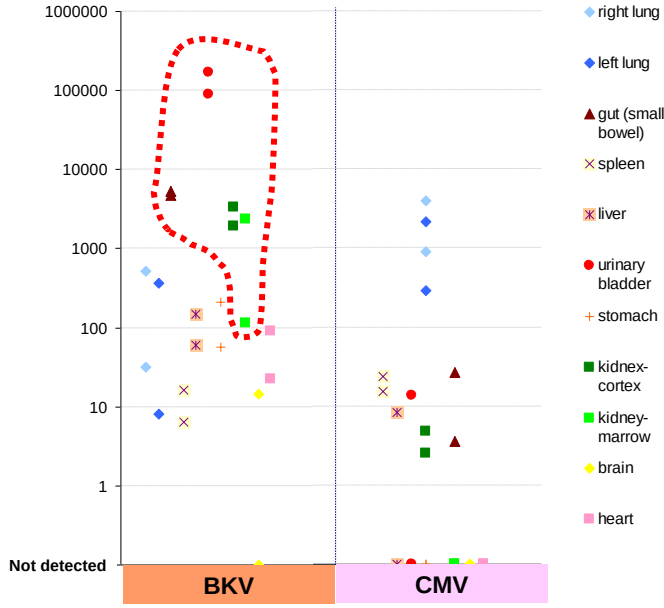




Age at HSCT.:18 let, Fanconi anemia, MUD 9/10, BM, aGvHD grade I.

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

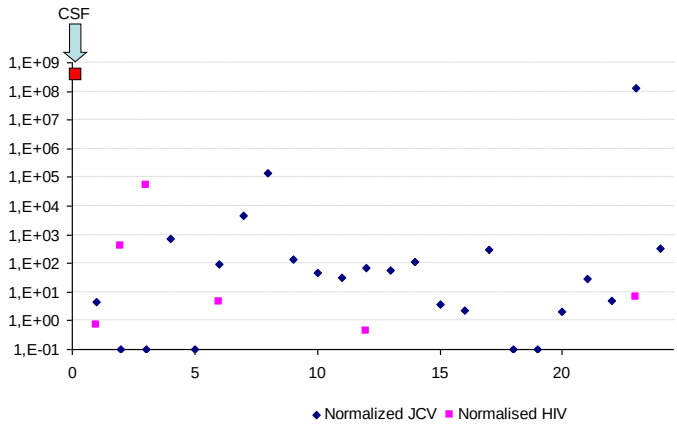
tissue specificity



Patient 5

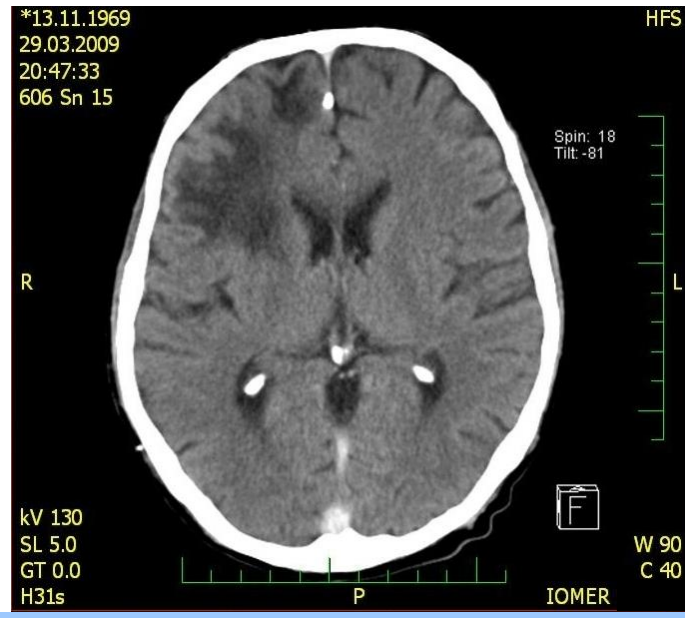
JCV

Pacient J.Z. (HIV+)



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

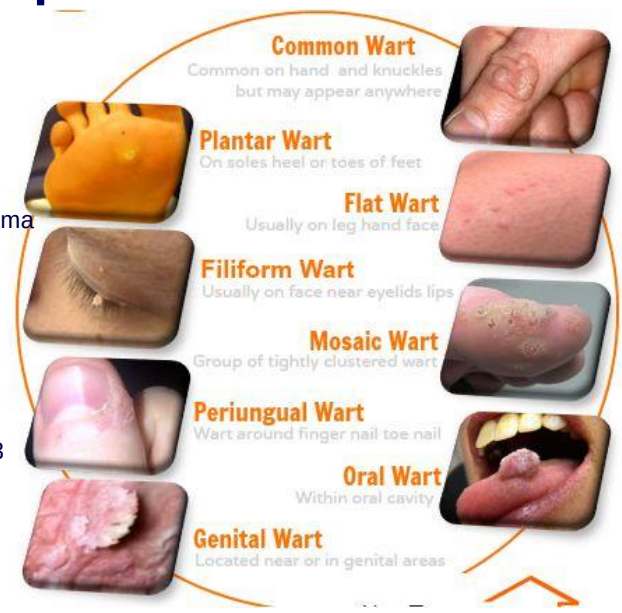


40 yrs. HIV+ patient deceased of PML.

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)

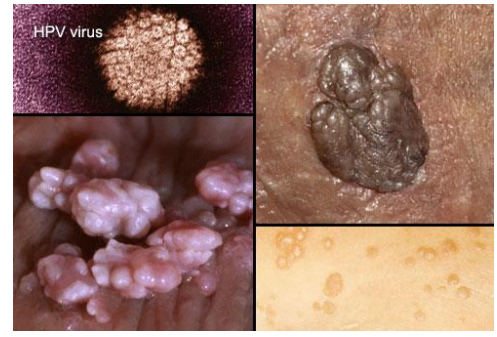


http://www.healthyeatingandyou.com/wp-content/uploads/2016/02/types-of-warts.jpg

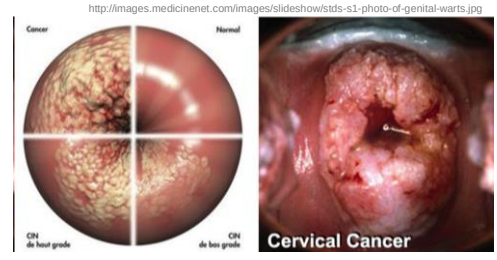
Papilomaviruses

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.)

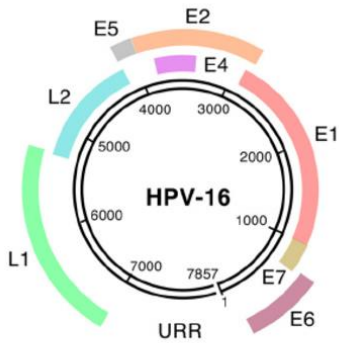


http://images.medicinenet.com/images/slideshow/stds-s1-photo-of-genital-warts.jpg



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

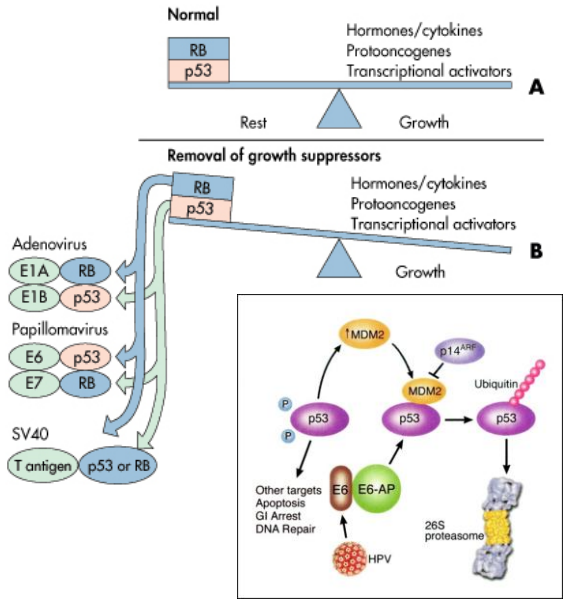
Papilomaviruses – genome

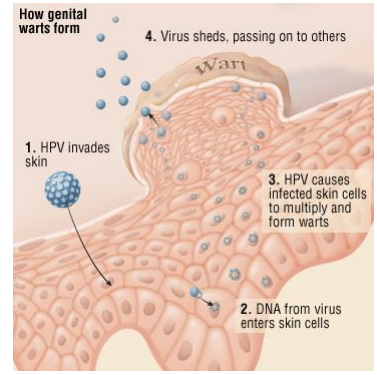


Gene	Function
L1	Major capsid protein.
L2	Minor capsid protein.
E1	Transcription factor, helicase activity. Mediates episomal DNA replication.
E2	Transcription factor. Regulates viral copy number.
E4	Facilitates virion release.
E5	Stimulates cell proliferation and prevents differentiation. Downregulates 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

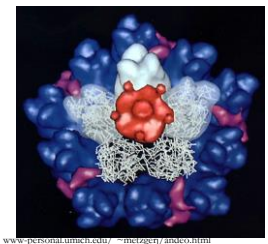
- HPV is most frequently transmitted STD in MSW adults

  (> 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: Gardasil(Silgard)
  Cervarix

Adenoviruses

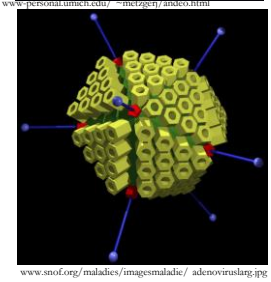
- non enveloped ds DNA viruses
  ikosahedral structure70-75 nm
  genome: 35 kbp
  according to similarity– 7 subgenes A-G
  accorging to antigenic specificity– more than 60 serotypes



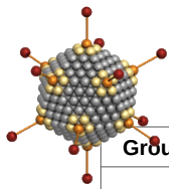
www-personal.umich.edu/ ~metzgerj/adeco.html

- **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



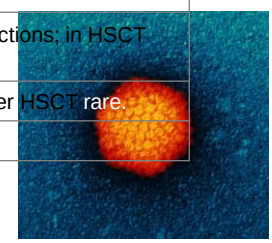
www.snof.org/maladies/imagesmaladie/ adenoviruslarg.jpg



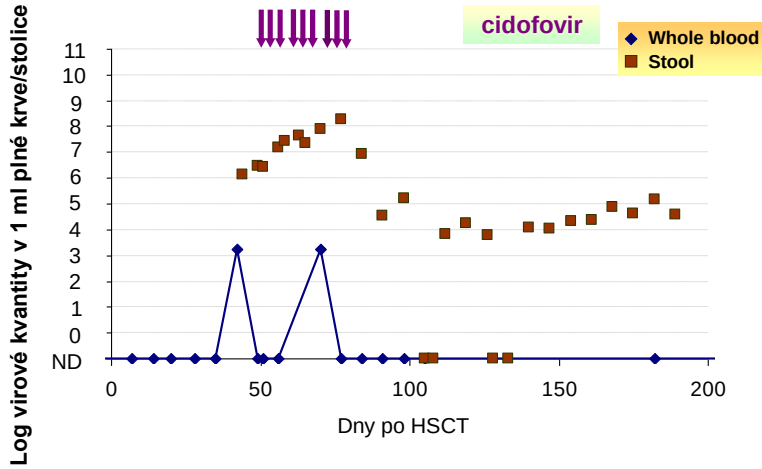
Serotypes

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).

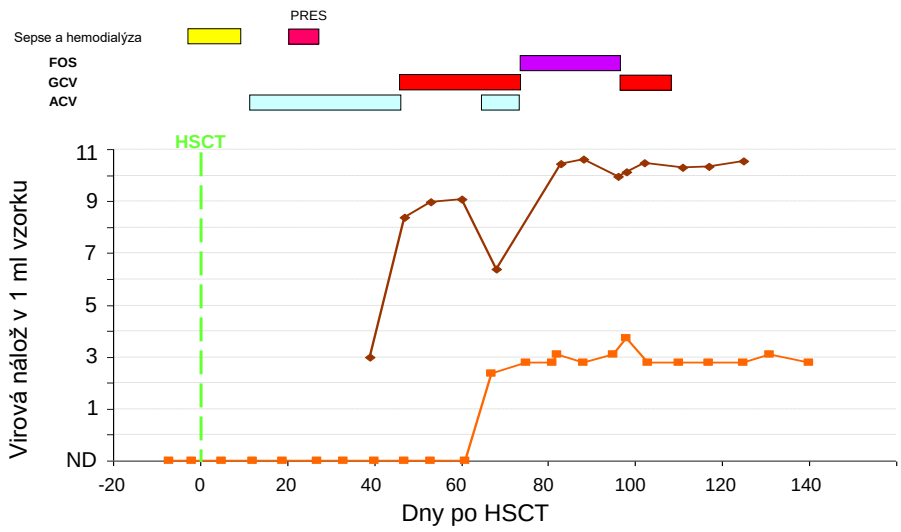


Patient 6

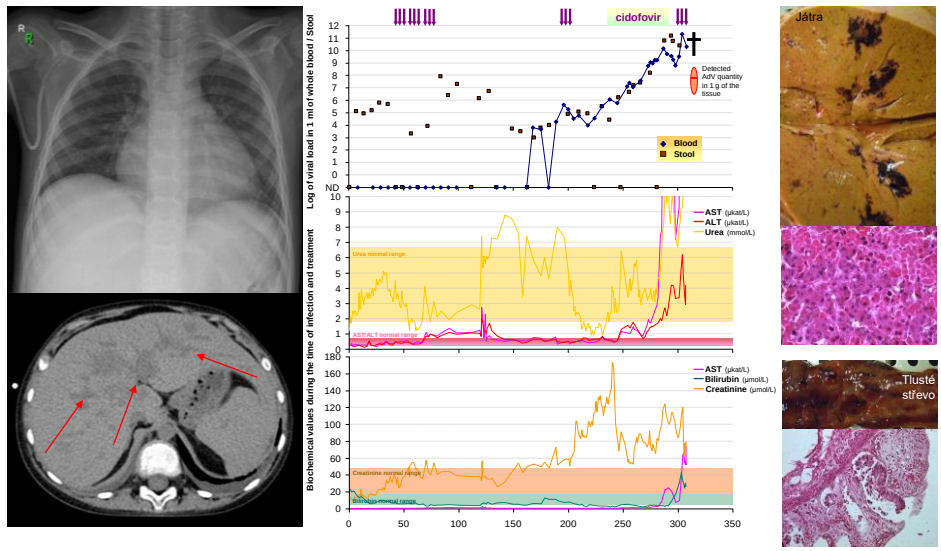


Dívka Věk při HSCT: 1 rok Dg.: ALL v CR2 Štěp: CB (5/6)
Příprava: busulfan, cyklofosfamid, melfalan a ATG Přihojení D+25.
GvHD grade II (GIT1, kůže 3) léčená kortikoidy.
Kompletní chiméra ode D+14.

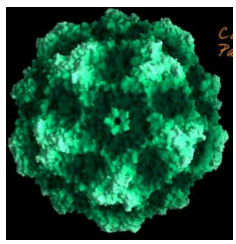
Patient 7



Patient 8

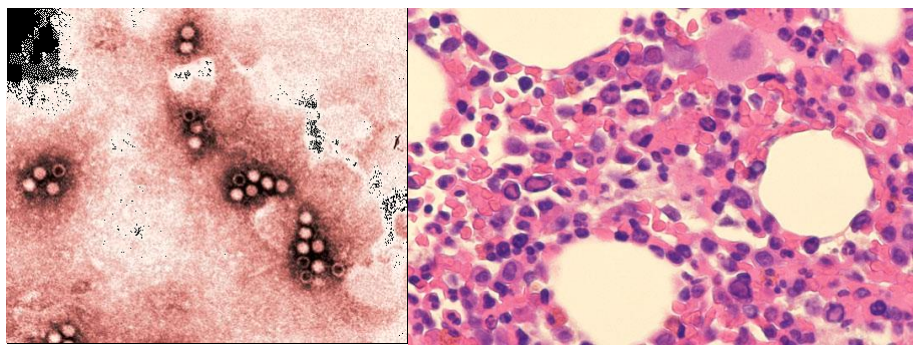


Parvovirus B19



http://fai.unne.edu.ar/biologia/virologia/images/virolo6.jpg

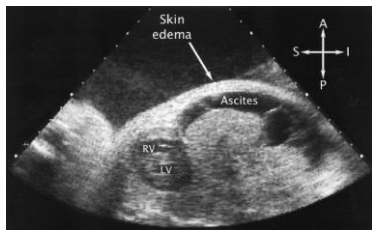
- small non-enveloped ss DNA +/-
- capsida in diameter 20-26 nm, genome: 5 kbp
- prolipheration in erythroid progenitors – transient stop of erythrocyte production and so it leads in certain clinical situations (e.g. Hereditary erythropoiesis disorders) to anaemia.
-
- E.g. aplastic crises, Bone marrow aplasia, teratogenicity-hydrops foetalis…
- ***Fiths exanthematic disease*** (see lecture)



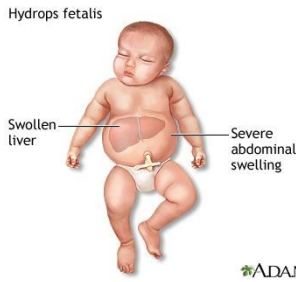
http://www.wadsworth.org/databank/hirez/gradyp2.gif

http://www.yamagiku.co.jp/pathology/image/210/1.jpg

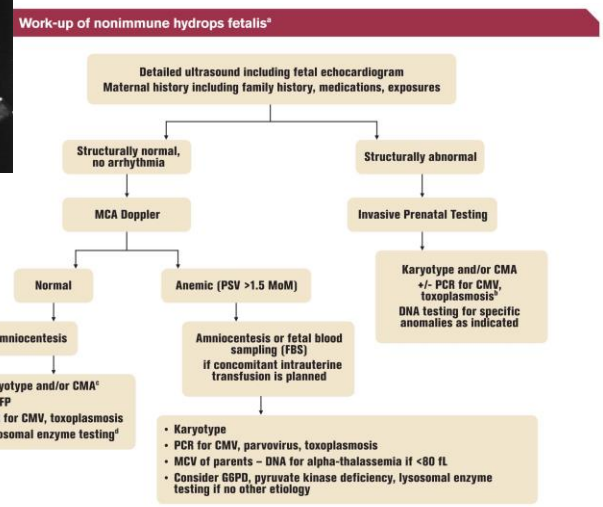
Parvovirus B19



http://www.parasfetalmedicine.com/wp-content/uploads/2014/01/nonimmune-hydrops-fetalis.jpg



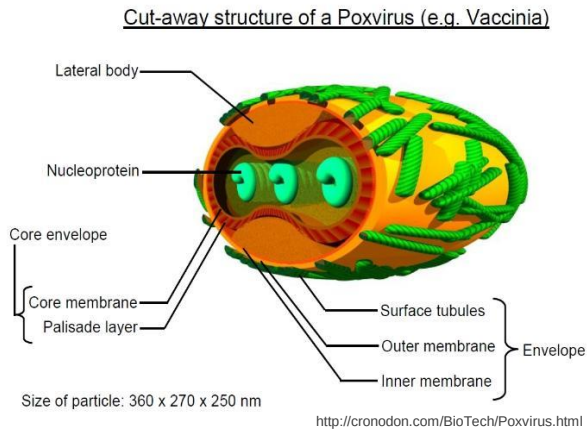
http://www.tibbiyardim.com/wp-content/uploads/non-immune-hydrops-fetalis.jpg



Source: SMFM. Nonimmune hydrops fetalis. *Am J Obstet Gynecol.* 2015.
[a]Assuming negative antibody screen and normal indirect Coombs to rule out alloimmunizaton; [b]CMV/toxo testing if fetal anomalies suggestive of infection; [c]Either amniocentesis or FBS; [d]Available in some laboratories.
Abbreviations: CMA, chromosomal microarray; CMV, cytomegalovirus; G6PD, glucose-6-phosphate dehydrogenase deficiency; PCR, polymerase chain reaction; PSV, peak systolic velocity; MCV, mean corpuscular volume.
http://images.alfresco.advanstar.com/alfresco_images/HealthCare/2015/02/10/d21bdf24-fc56-4a92-903f-f2a7ca4c1188/OBGYN0215_026_1.jpg

Poxviry

- Complex structure (symetria)
- Enveloped but resistant to inactivation
- linear ds DNA
- Genome 130–375 kb coding approx. 250 genes (>100 polypeptides-often immunogenic)
- Replication in cytoplasma
- Highly species specific
- Used for genome vector constructions
- Human pathology is associated with 4 genera:
  - Orthopoxvirus
  - Parapoxvirus
  - Yatapoxvirus
  - Molluscipoxvirus

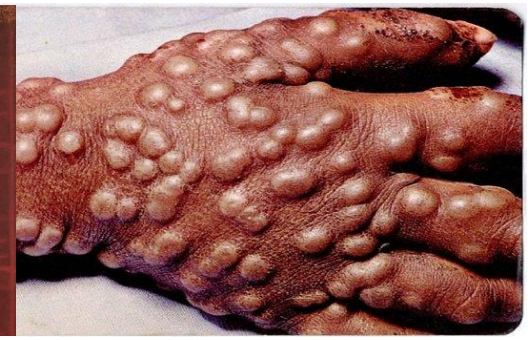


http://cronodon.com/BioTech/Poxvirus.html

Orthopoxvirus

- Variola virus
  - Variola major (mortality 20%), variola minor (mortality 1-2%)
  - Eradicated (last diagnosed in 1977)
  - All eruptions in same status of development
  - Primary replication in air-ways
- Vaccinia virus (used for vaccination and eradication of variola)
- Cow pox virus
  (first vaccination against variola – Edward Jenner – 1796)

http://www.smithsonianmag.com/ist/?next=/smart-news/queen-elizabeth-1-loved-live-action-role-playing-9151091/



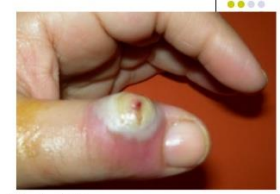
http://www.wikiihealth.com/wp-content/uploads/2014/07/rsz_smallpox.jpg

Parapoxvirus

- Zoonosis
- Human infections causes
  - Bovine papular stomatitis virus
  - Orf virus
  - Pseudocowpox virus
- Aftous eruptions on mucous and/or skin
  Clinically called
  -“farmyard pox“

Orf (Ecthyma contagiosum)

- **C/P:**
  - Typically presents as a papule/nodule on the dorsal index finger.
  - ***Progression through several stages:***
    - maculopapular
    - targetoid
    - weeping nodule
    - regenerative dry stage with black dots
    - papillomatosis
    - regression with a dry crust
  - ***Other Findings;*** Ascending *lymphangitis*, *lymphadenopathy*, *malaise*, and *fever* may occur.
  - Bacterial superinfection may occur.
  - Erythema multiforme occasionally occurs 10 to 14 ds. later



http://www.slideshare.net/HimaFarag/viral-diseases-of-the-skin-other

Yatapoxvirus

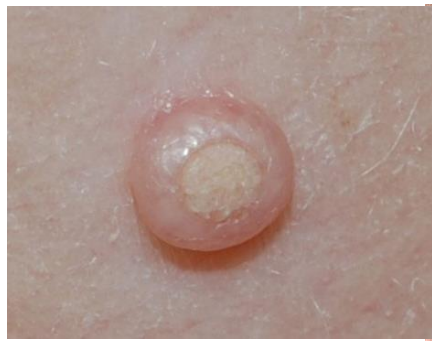
- Yaba monkey pox virus
  - Oncogenic virus – histiocytomas (tumour from macrophages) in humans and monkeys (e.g. *Macaca fascicularis*)
  - Presence by the river Niger

https://upload.wikimedia.org/wikipedia/commons/9/9f/Macaca_fascicularis.jpg

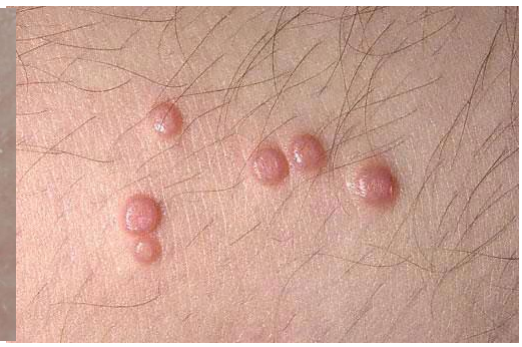
https://en.wikipedia.org/wiki/Monkeypox_virus#/media/File:Monkeypox.gif

Molluscipoxvirus

- Molluscum contagiosum
  - Viral infection of skin, rarely mucous membranes
  - Charakteristic skin lessions
  - Infection of human, primate and kangaroos
- 4 types
- Often STD (MCV 1,2)
- Incubation period – up to months

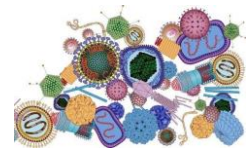


http://www.dermapics.com/molluscum%20contagiosum.html



http://www.molluscumrx.com/molluscum-contagiosum-pictures/

Possibilities of influence of viral infection



Immune system

(immunosupresive treatment, chemotherapy, …)

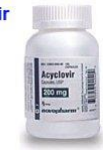
Patogens

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

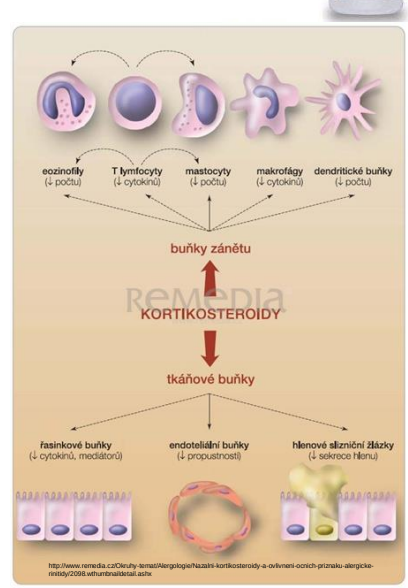
„BIOLOGIC TREATMENT “

infliximab (anti TNF-α)

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

Campath (anti CD-52)

Antithymocytární globulin (ATG)



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

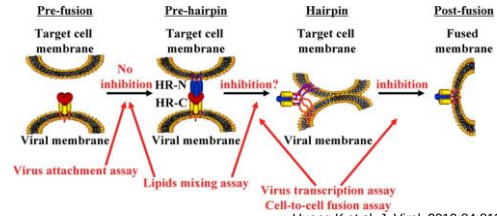
Neutralising antibodies

Profylactic prevention

motavizumab

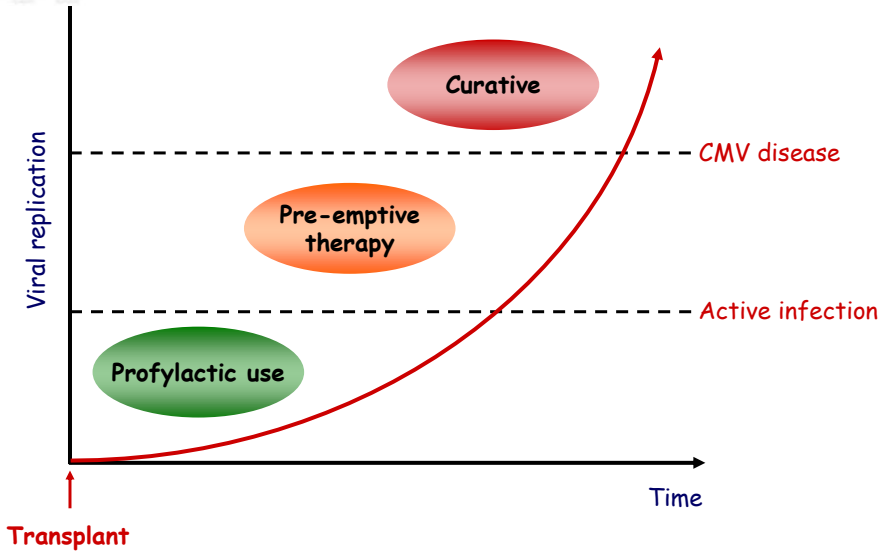
palivizumab (Synagis)

Humanisided neutralizating antibody against F- protein of RSV



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

3. Decrease of viral proliferation

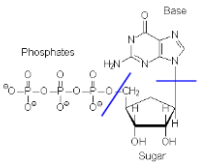


In HSCT recipient: pre-emptive and curative therapy

Virostatic drugs impact

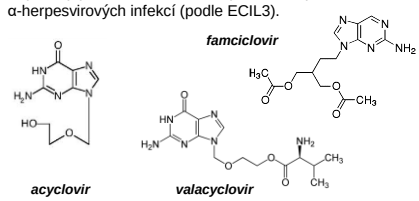
Virostatics

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..)



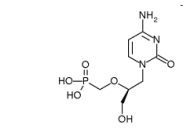
Guanidine trifosfát (GTP)

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

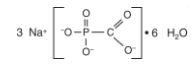


acyclovir ***valacyclovir*** ***famciclovir***

Léky první volby.



cidofovir



foscarnet

Léky používané při HSV rezistentní k léčbě.

Antibodies with virostatic effect

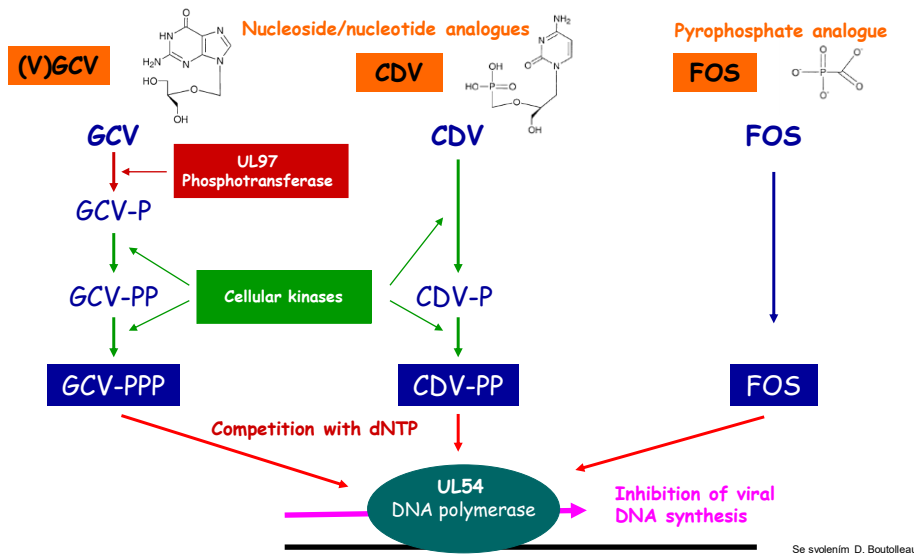
Neutralising antibodies against certain proteins important in pathogenesis of viral disease (F protein in RSV) or aimed against target cells (anti-CD20 in EBV).

Anti CD-20

http://www.curaxys.com/images/img18.jpg

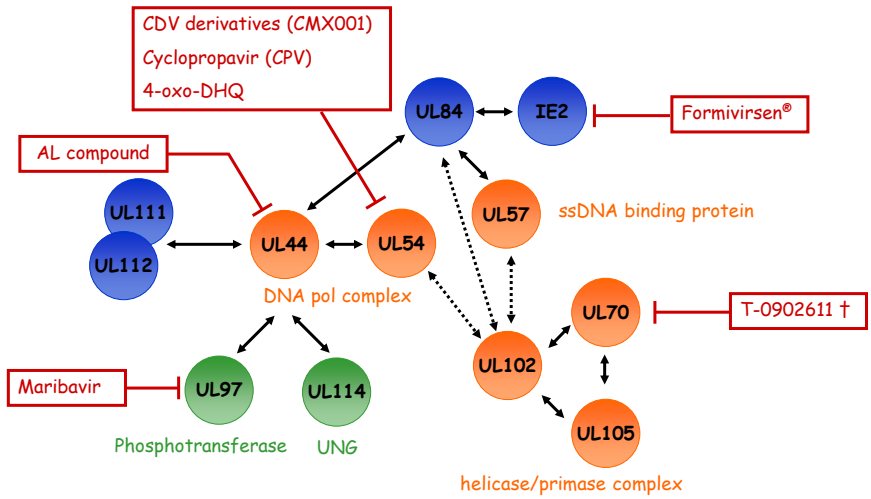
3. Decrease of viral proliferation

➔ **Inhibitors of CMV DNA polymerase UL54**



Se svolením D. Boutolleau

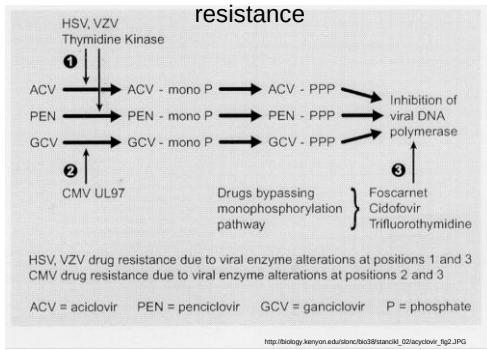
CMV replication complex

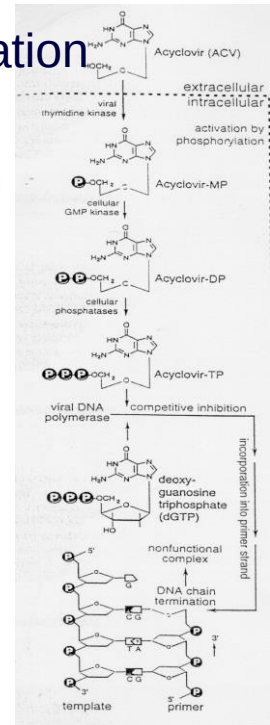


Se svolením D. Boutolleau

3. Decrease of viral proliferation

Inhibition of herpesviruses and the possible resistance



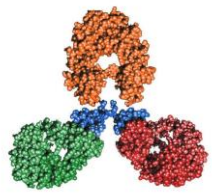


3. Decrease of viral proliferation

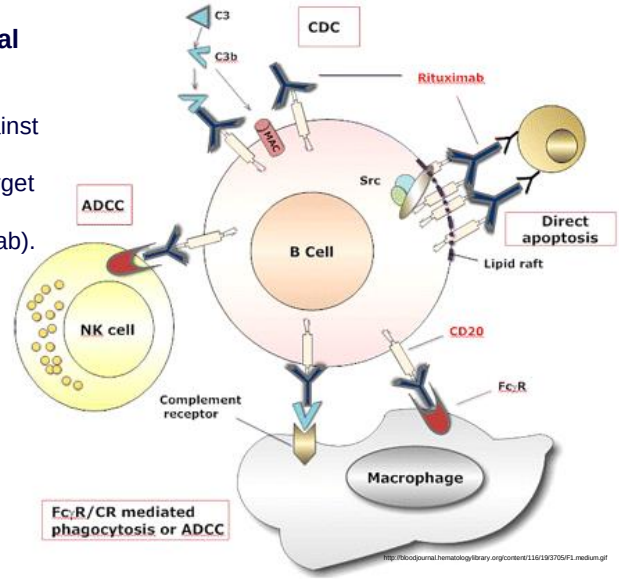
Antibodies

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.

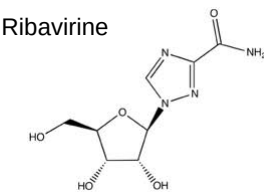
Adverse effects of the virostatic drugs

- **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

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

Ribavirine



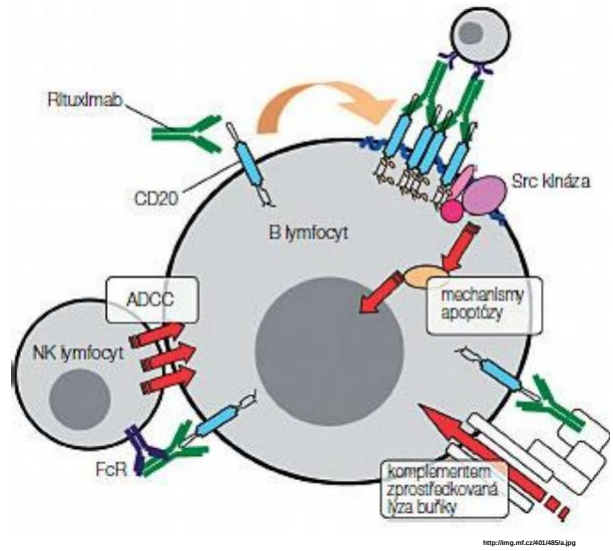
4. Improvement of the lymfocyte function

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™, 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.

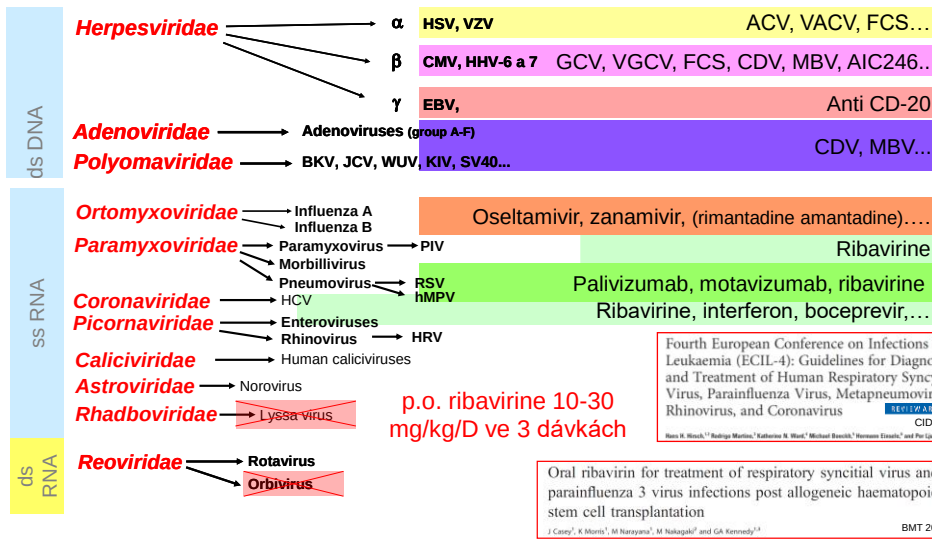
4. Improvement of the lymfocyte function

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.

Therapeutical possibilities of virostatics and specific antibodies

More or less specific for certain viral groups:

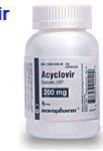


p.o. ribavirine 10-30 mg/kg/D ve 3 dávkách

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

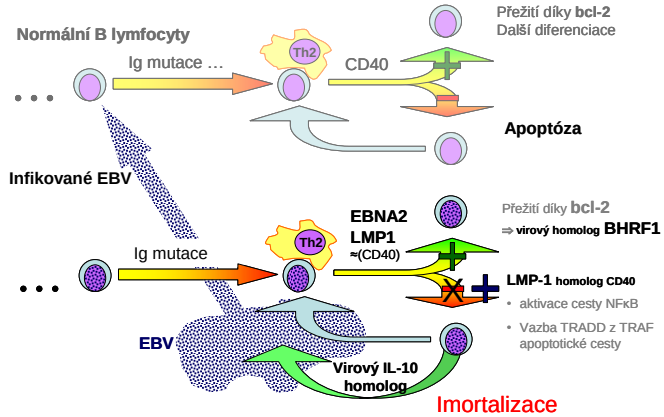
Oral ribavirin for treatment of respiratory syncitial virus and parainfluenza 3 virus infections post allogeneic haematopoietic stem cell transplantation
BMT 2011

5. Destruction of permissive cells

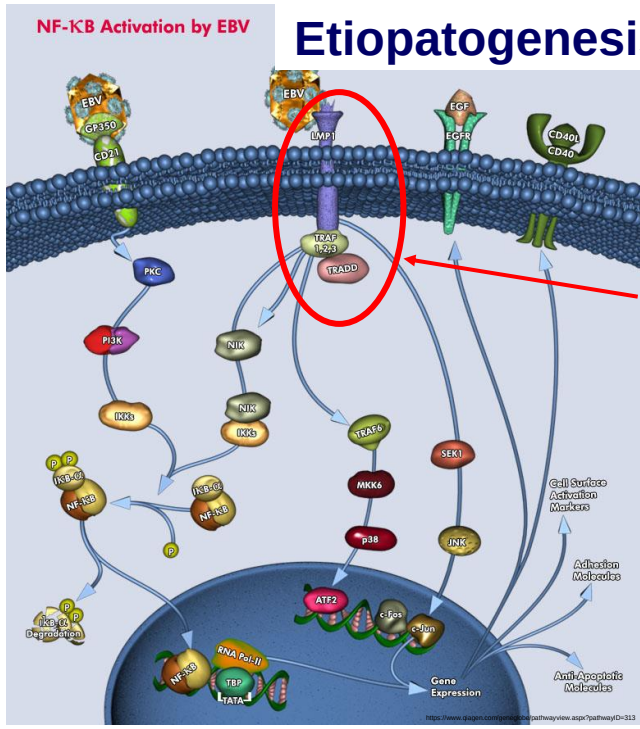


Used in EBV associated malignant disease (HL, NHL), or post-transplant EBV-LPD.

Anti CD-20 – rituximab (MabThera)



Etiopatogenesis of EBV-LPD



EBER

– ↓kaspase 3, PARP

-↑ bcl-2 - ↓PKR fosf.

LMP1

– homologue CD40

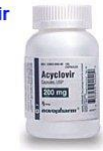
- binds TRADD from TRAF apoptotic pathway

–NFkB activation, AP1, STAT1/3

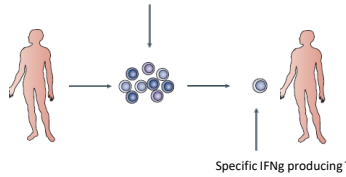
BHRF1

– viral homologue bcl-2

Acyclovir



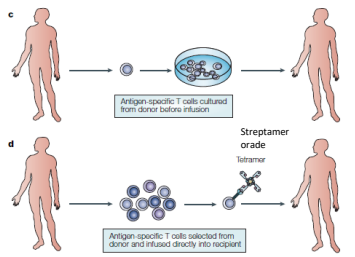
6. Present possibilities of specific cell production and its limitations



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

Feuchtinger et al BJH 2006
Feuchtinger et al Blood 2010

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



Literatura:

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

Leen et al Nature Medicine 2006
Leen et al Blood 2009

So far - for prophylactic use only!

HLA-string, technically and financially difficult

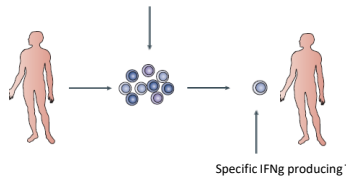
Unrelated donors: preparation about 8-9 weeks from first demand to product

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

Moss P and Rickinson A Nature Reviews 2005 (5)

Acyclovir

6. Present possibilities of specific cell production and its limitations

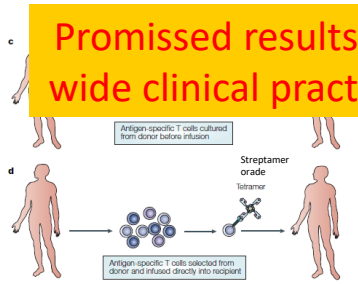


Non-specific T cells, technically and financially difficult
ATMP (advanced therapeutic medicinal product)

Feuchtinger et al BJH 2006
Feuchtinger et al Blood 2010

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

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



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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!