

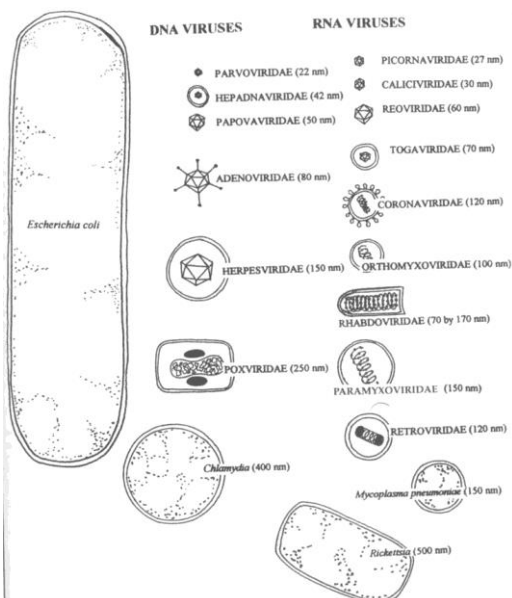
## DNA viruses



Department of Medical Microbiology and Paediatric Haematology and Oncology,  
2<sup>nd</sup> Medical Faculty of Charles University and Motol University Hospital



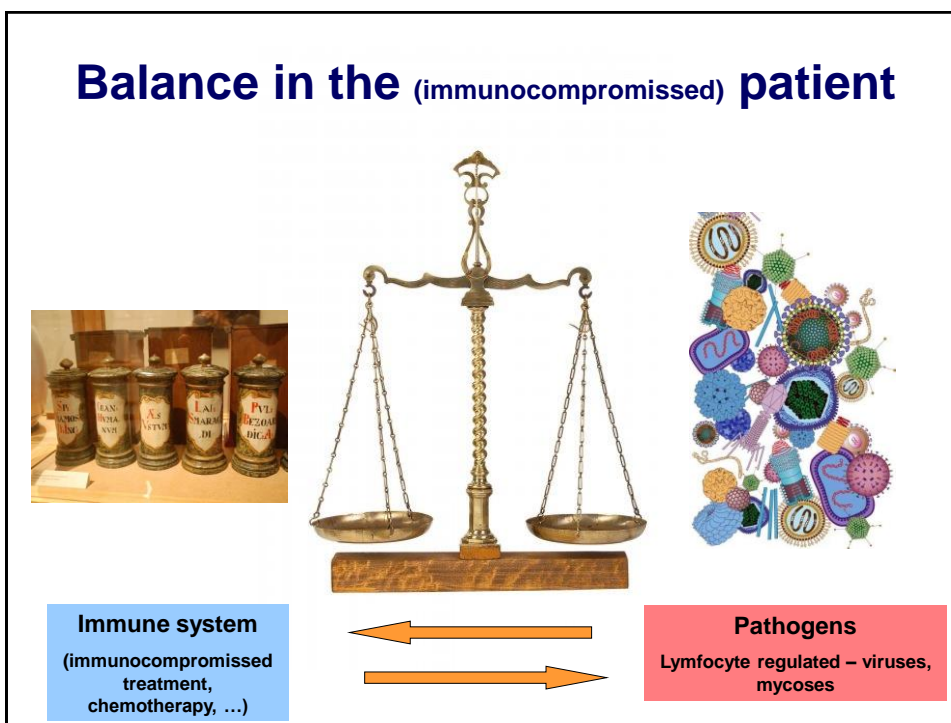
## How they look like?



<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



### 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 suspicion of viral infection (poliomyelitis) were described first 3 700 BC in Egypt.

Typical signs are e.g. in:

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

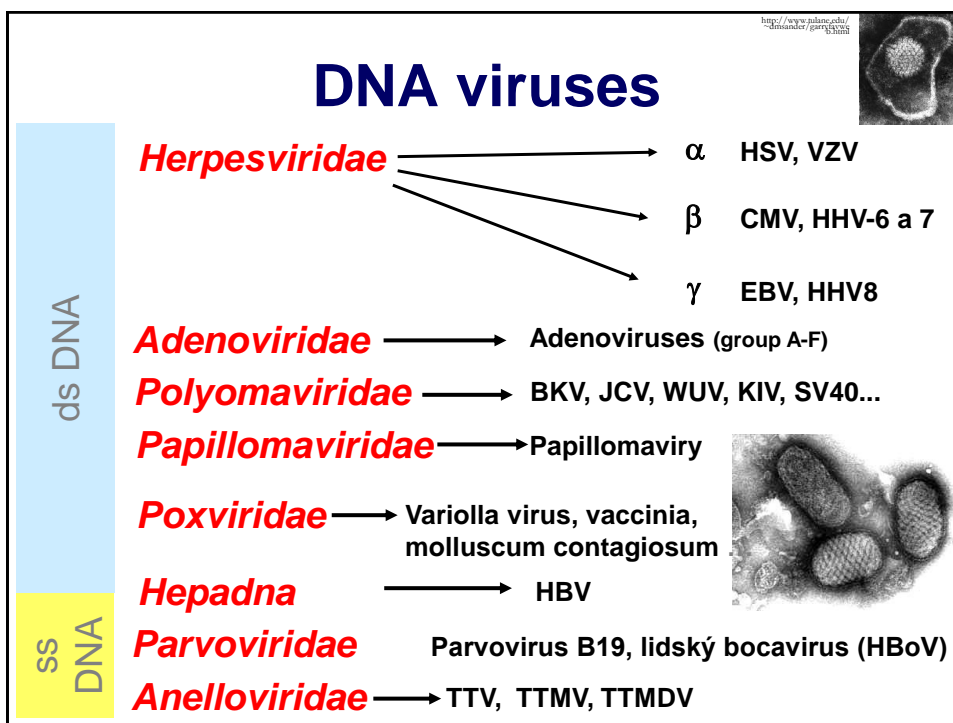


## Why DNA viruses?

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

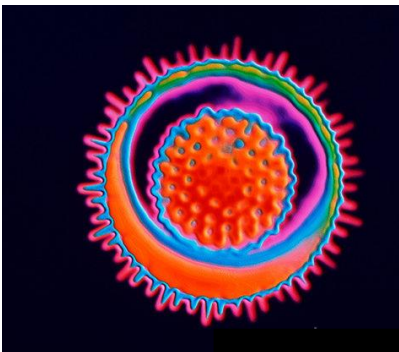
1. pneumocystis pneumonia
2. toxoplasma encefalitis
3. esophageal, tracheal, bronchial or lung candidiasis
4. **Chronic anal herpes simplex or herpetic bronchitis, pneumonia or esofagitis**
5. **CMV retinitis**
6. **generalized CMV infevtion (excluding liver and spleen)**
7. **progressive multifocal 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. **Kaposi sarkoma**
18. **malignant lymphoma (Burkitt's lymphoma, imunoblastic and primary cerebelar lymphoma)**
19. Invasi carcinoma of cervix
20. HIV encefalopatia
21. wasting syndrom

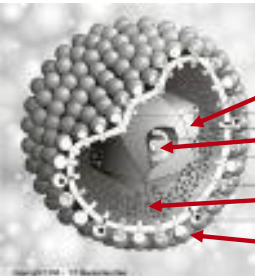




## Herpesviruses

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



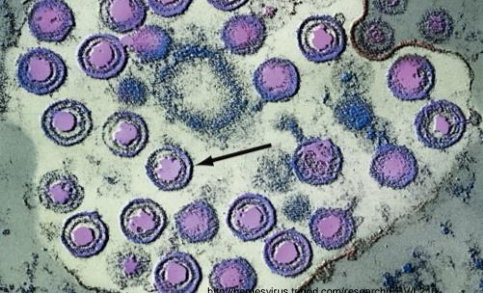


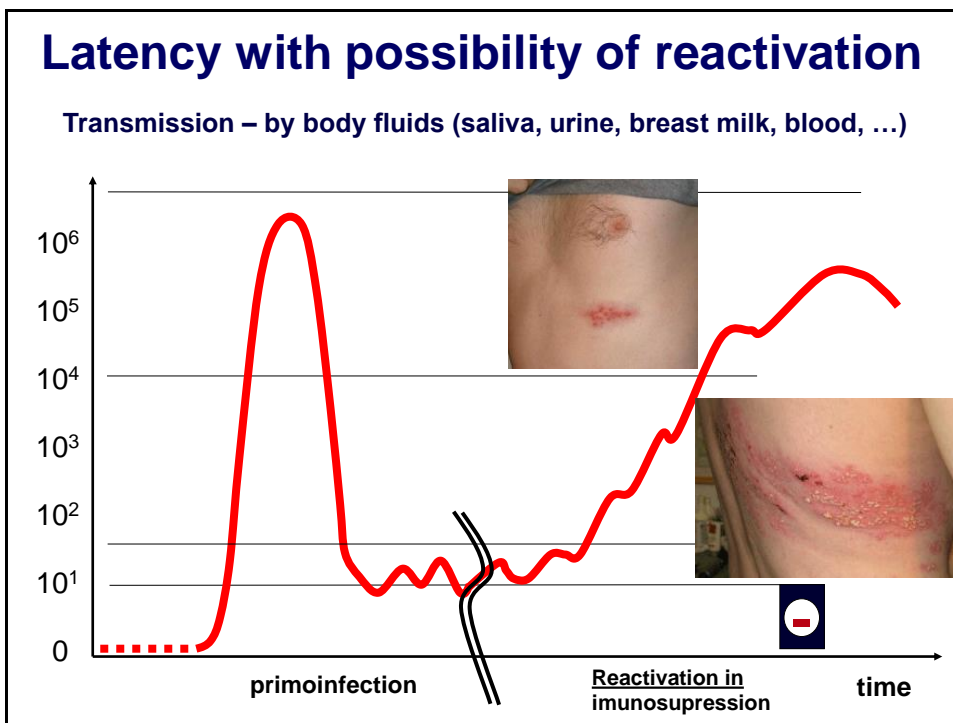
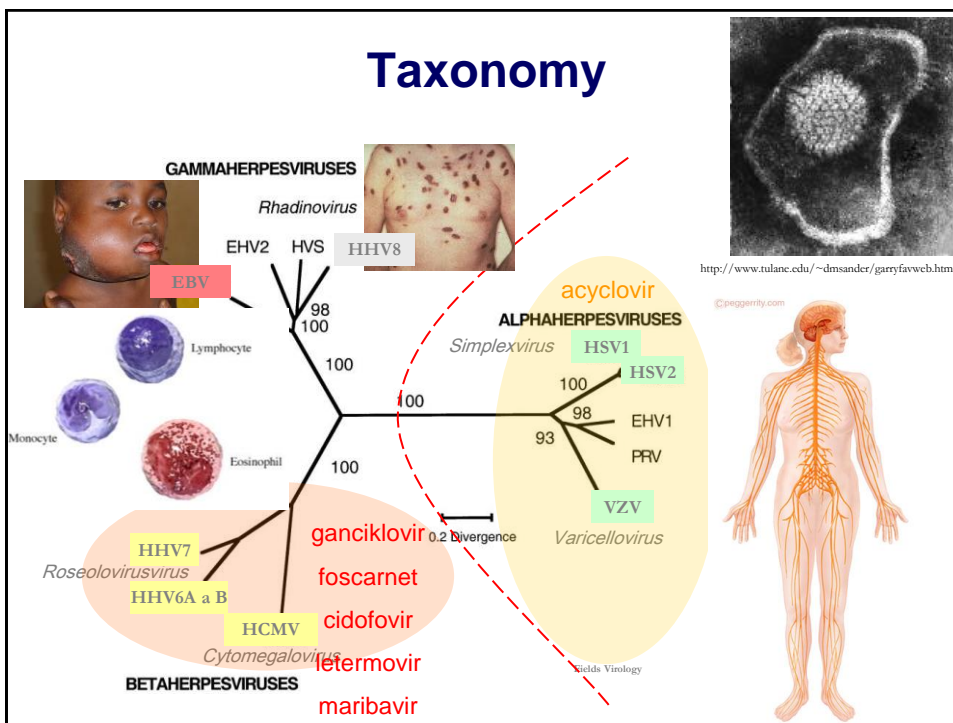
capsid

DNA

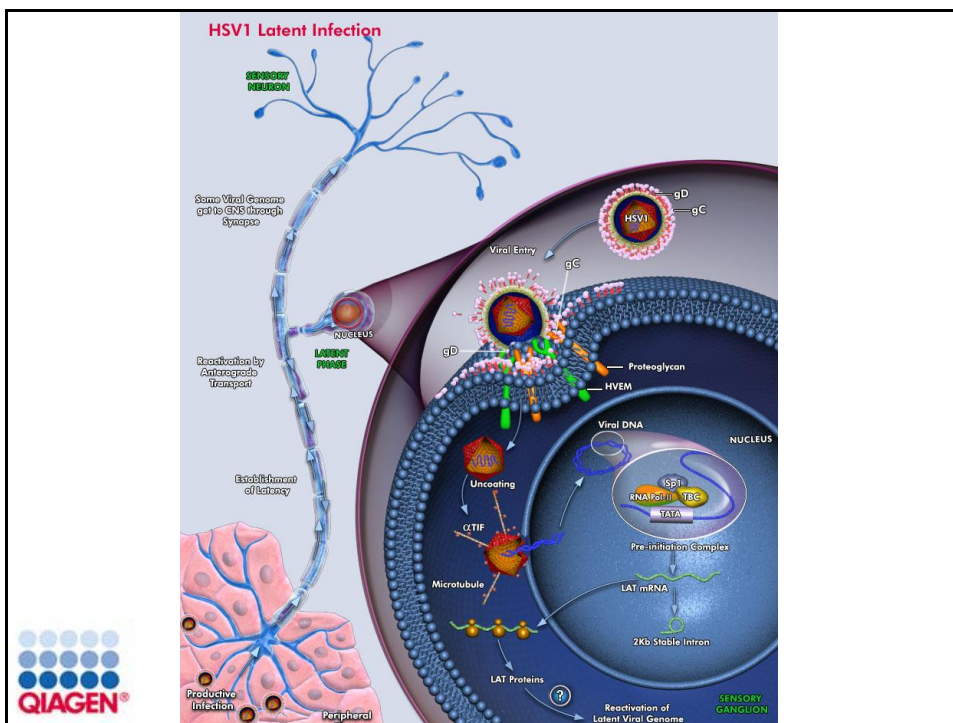
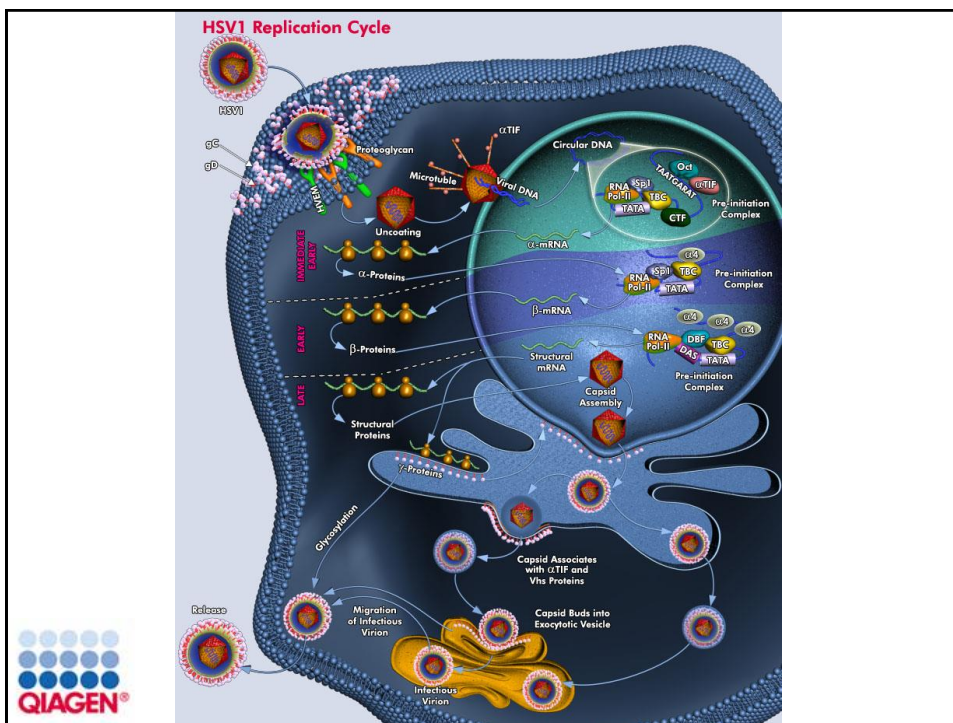
tegument

glycoproteins







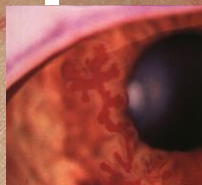


## Pathological impact of HSV and VZV

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

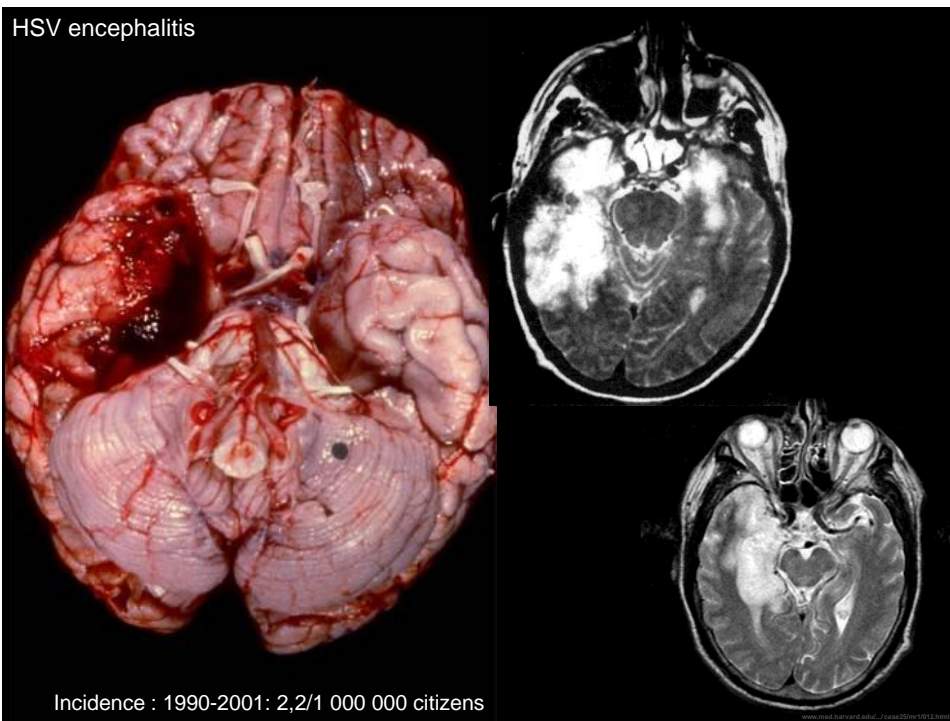
VZV – varicella, herpes zoster, encefalitis, pneumonie, hepatitis

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



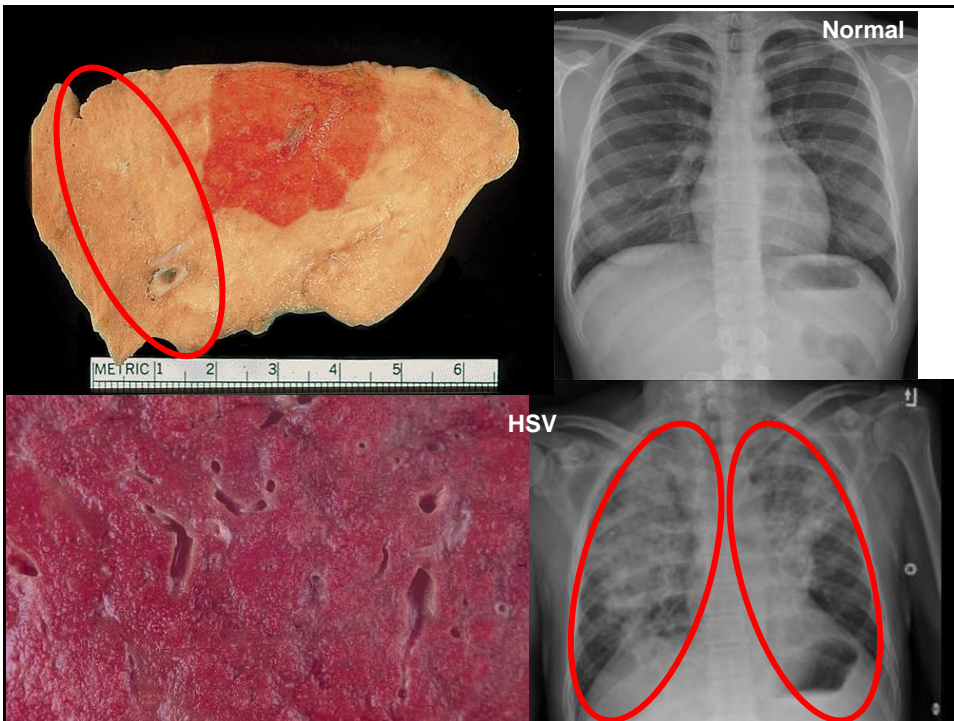
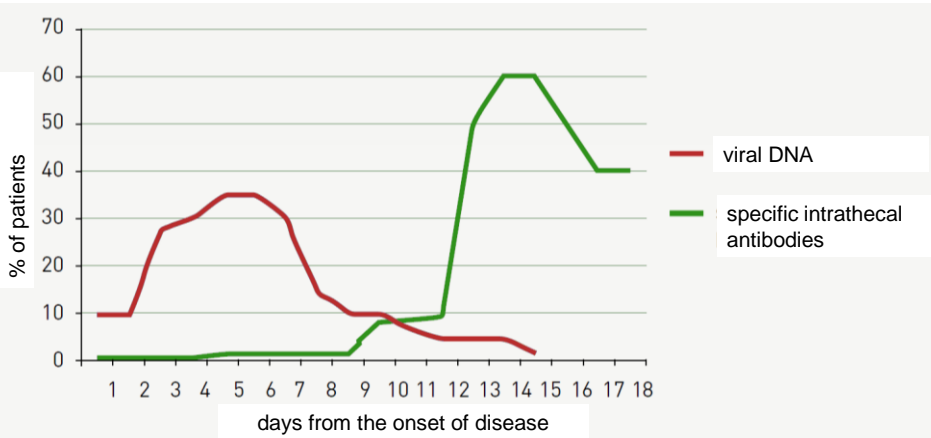
Varicella – chicken pox

HSV encephalitis



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

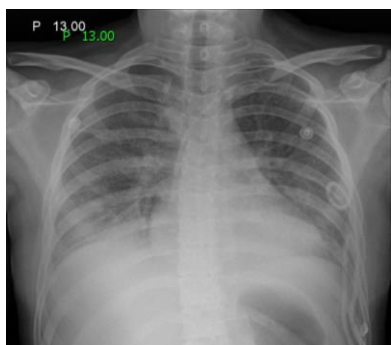
## Antibody response to viral infection and detection of virus in CSF



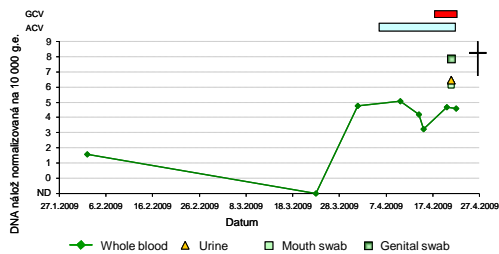


## Different impact and destruction in different organs

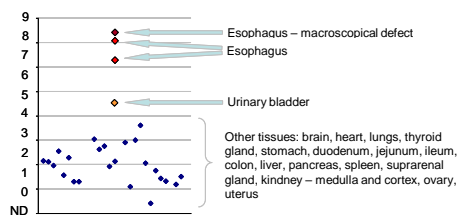
**HSV** (girl treated for ALL)



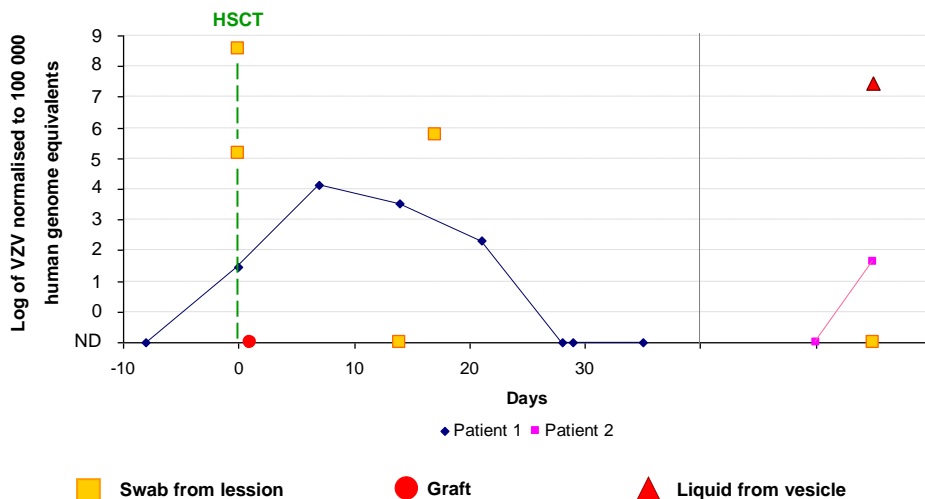
HSV pneumonia



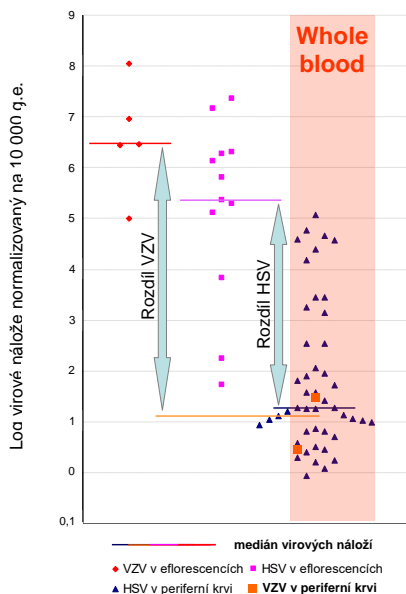
### HSV quantity detected in the tissue at the autopsy



## 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 efluorescence from **9** pts; median 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 mononucleosis like sy.

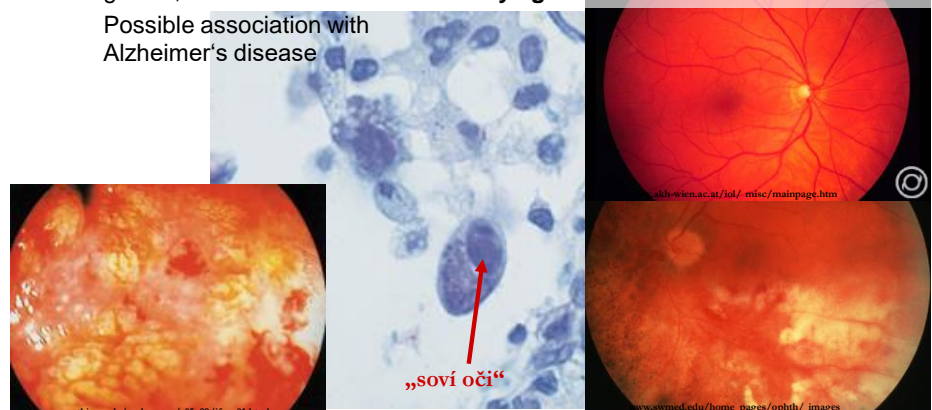
In pregnant woman teratogenic

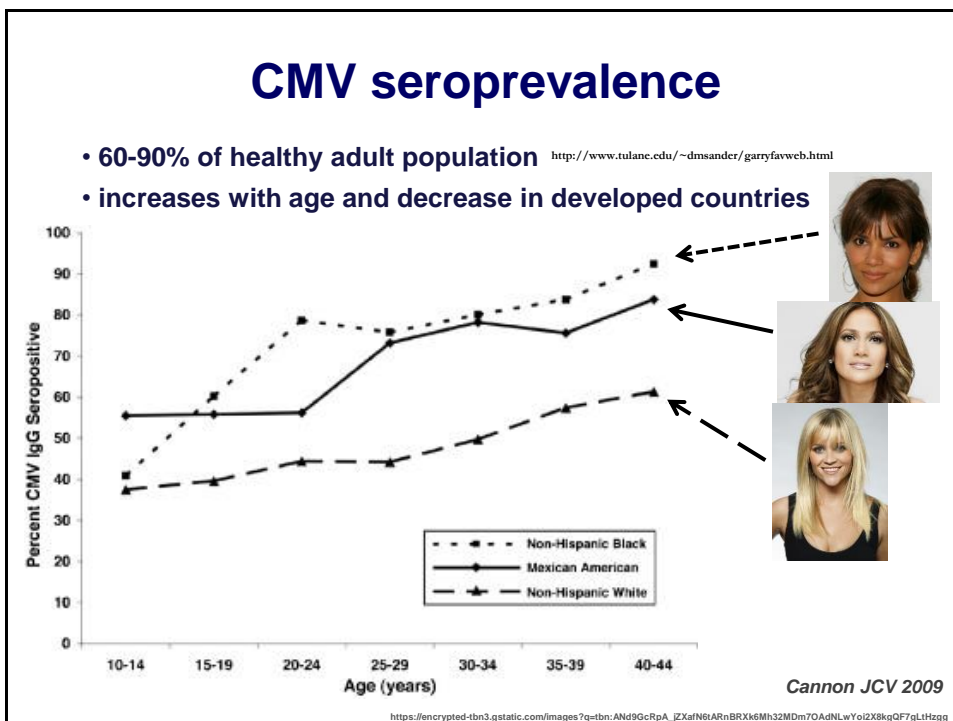
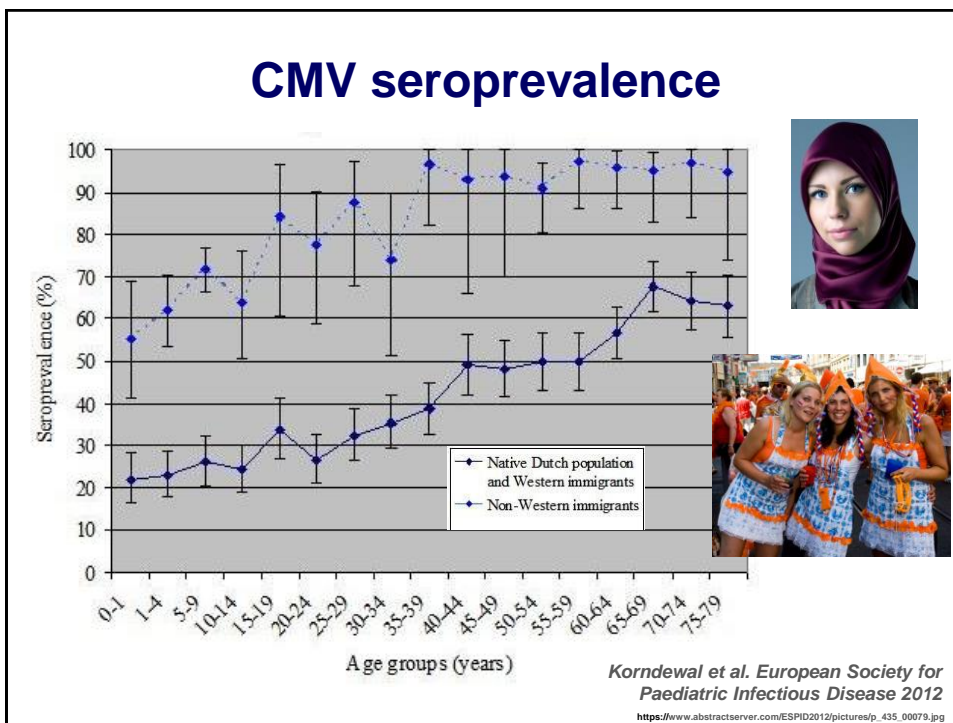
Associations with malignant glioma, ca. of breasts

Possible association with Alzheimer's disease

In immunocompromised mainly

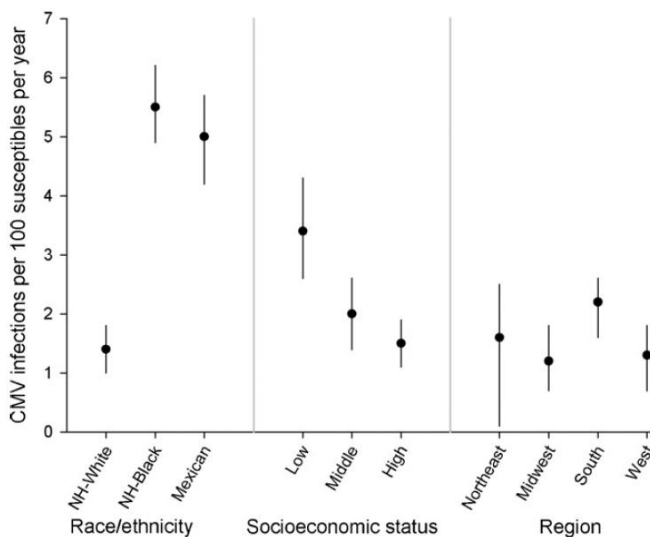
**trombocytopenia, pneumonitis, hepatitis, encefalitis, retinitis, colitis, esofagitis, pankreatitis, vasculitis, malaise, vomiting, artralgia, myalgia**





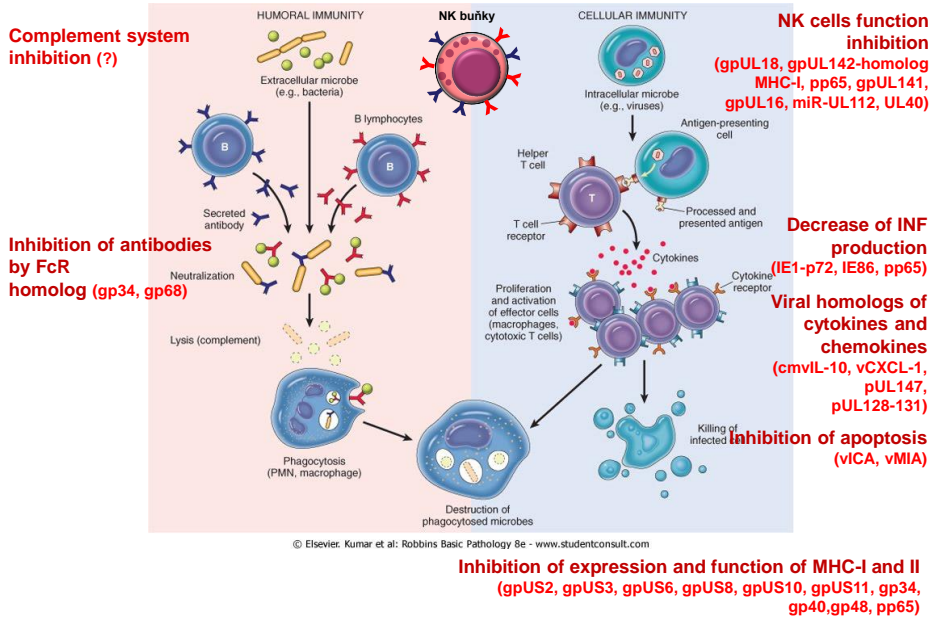
# Incidence of CMV primoinfection

CMV Force of Infection by Race/Ethnicity, SES, and Region



Cannon JCV 2009

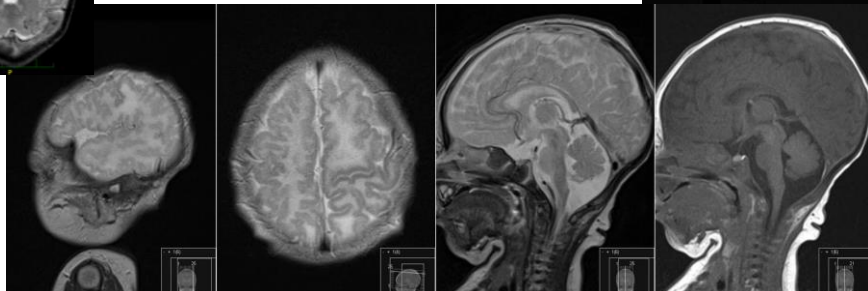
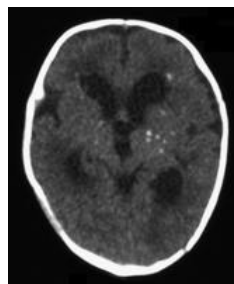
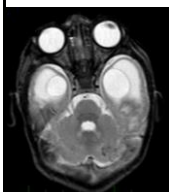
# How CMV manipulates with immunity?





## 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 sensorineural hearing loss in about 30-50% clinically symptomatic children and 8-12% of asymptomatic children.



## Symptoms and impact of cCMV



### Placental infection

- swelling of the placenta – worse diffusion characteristics
- smaller cotyledon development – smaller placental surface

**IUGR**

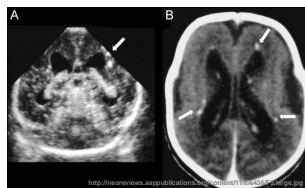
### Fetal infection

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

**Neurologic problems/seizures**  
**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 hematopoiesis.

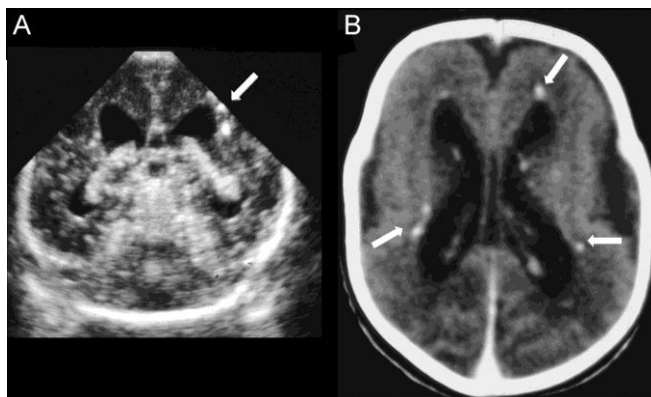
## Symptoms and impact of cCMV

### Transient Outcomes

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

### Permanent Outcomes

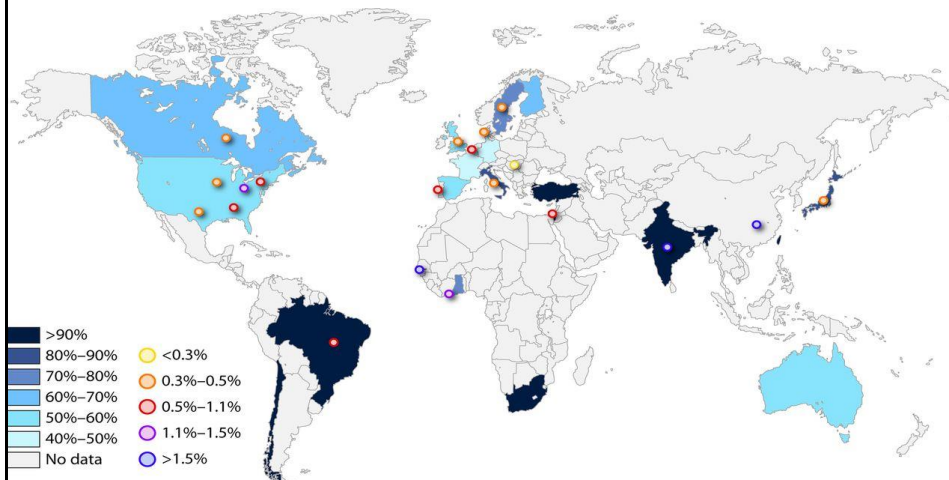
- Microcephaly
- Vision loss
- Hearing loss
- Mental retardation
- Motor disabilities
- Seizures
- Death



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

## What is the frequency of cCMV?

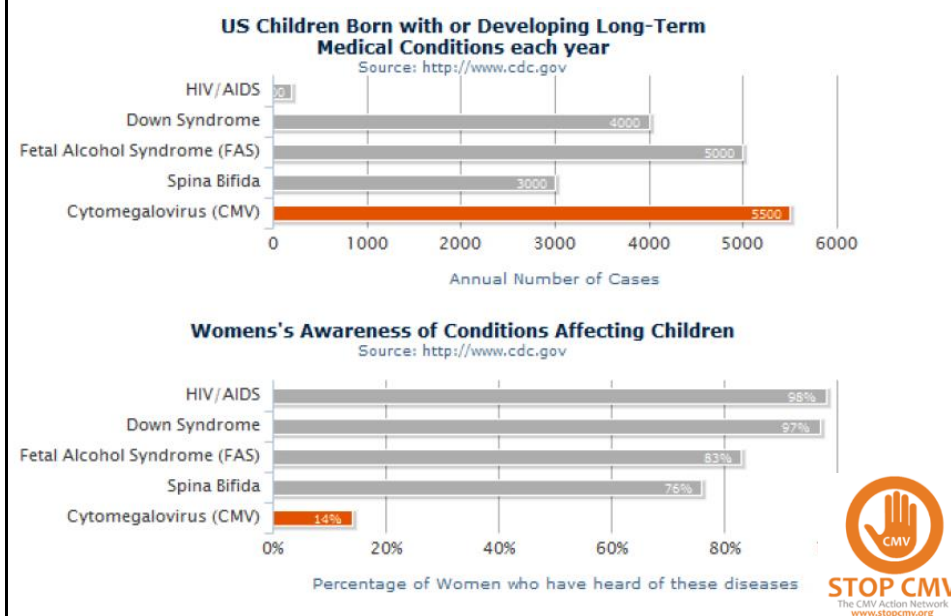
There is 370,000 children born every day in the world, representing 134 millions/year.  
Average frequency approx. 1,5% of living birth – 2.01 millions of children with cCMV/year.  
In Europe and Czech Republic is estimated frequency 0.5-1% cCMV of living newborns.



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?



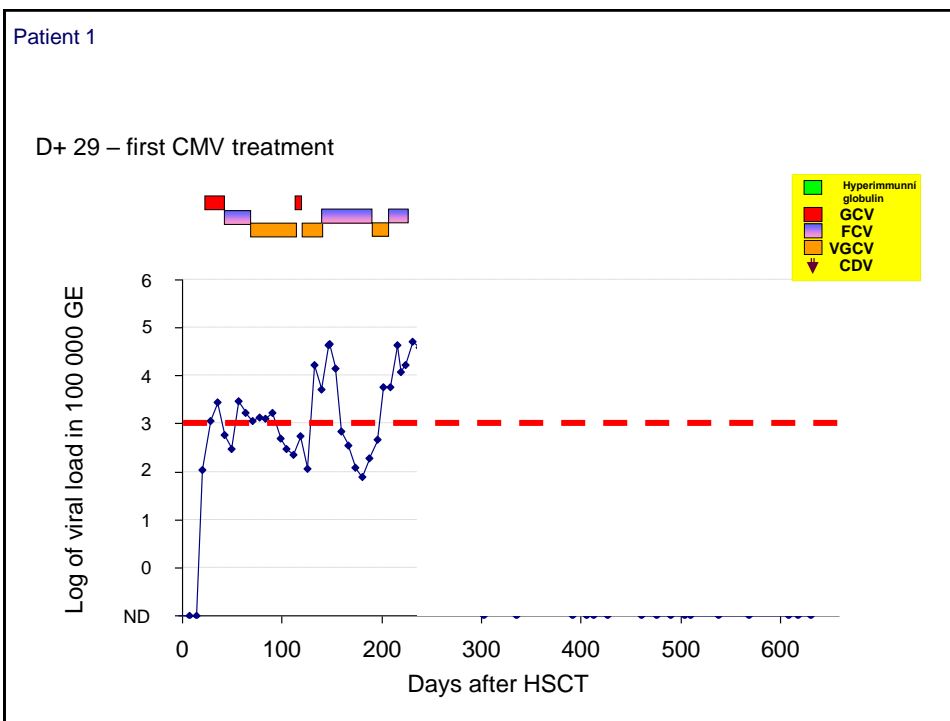
### Patient 1

Girl, 16.5 yrs of age at HSCT  
 Allogeneic HSCT for AML M2 (AML1/ETO+) in 2<sup>nd</sup> CR  
 MMUD – 7/10  
 Conditioning: Busulphan, Cyclofosamid, Melphalan, ATG  
 Graft: Peripheral stem cells  
 CD34+: 11,12 x 10<sup>6</sup> /kg; CD3+: 302,1 x 10<sup>6</sup> /kg; NC: 12,09x10<sup>8</sup> /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

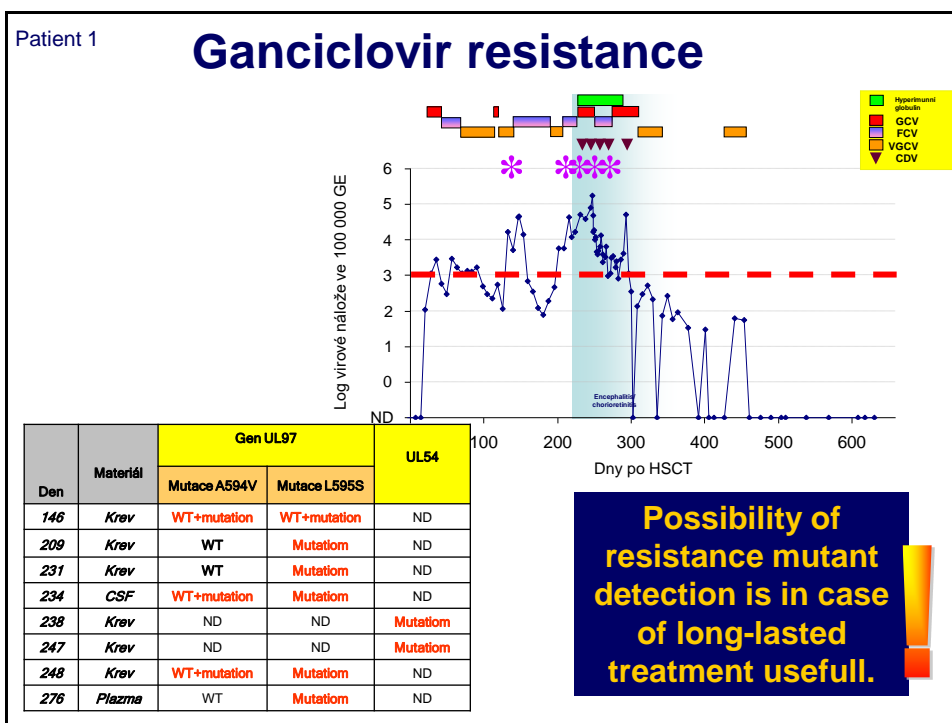
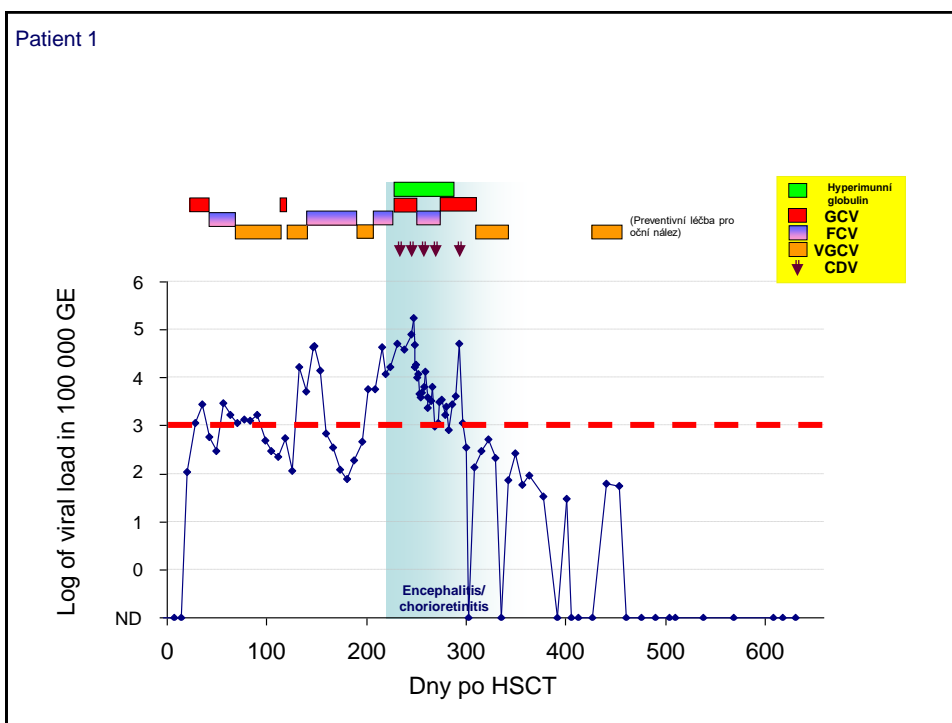
D+230 – during foscarnet treatment patient presented diplopy, headache, vomiting and sleepness.

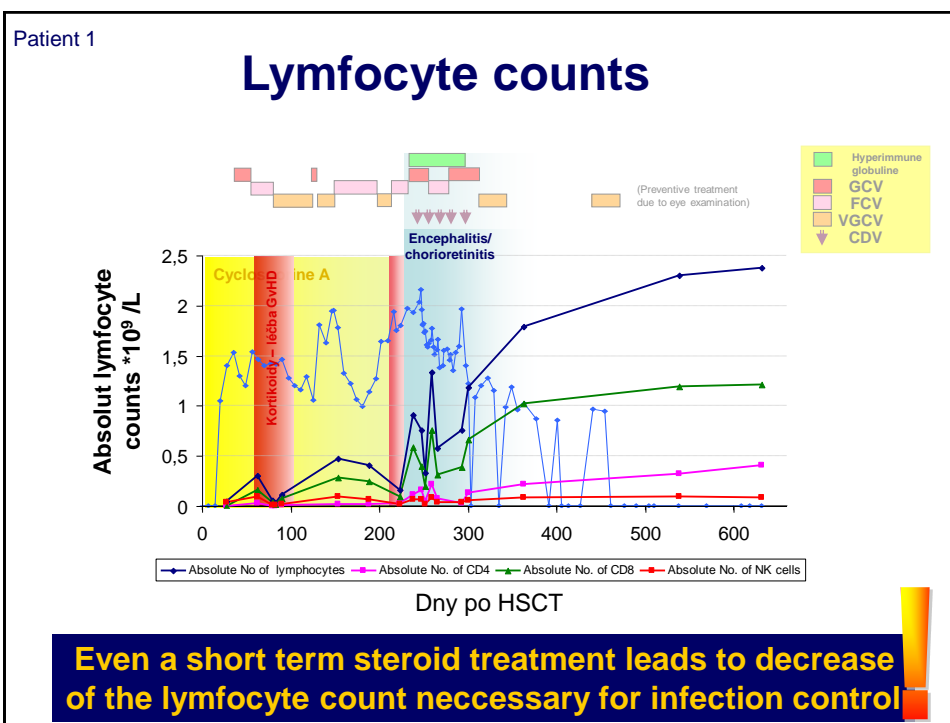
**CMV detected in CSF** (approx. 2 600 000 copies / ml) and increase of viral load in peripheral blood.

↓

Results confirmed **encephalitis and bilateral chorioretinitis.**

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





Patient 1

## Outcome

- Recently the patient is regularly controlled by ophthalmologists. Visus in one eye is very limited, however the second eye is healthy. In both 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

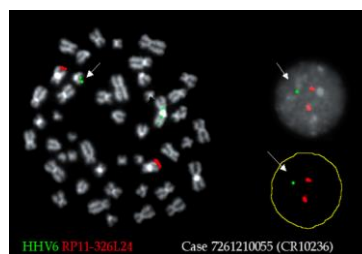
- Sixth disease
- Febrile seizures
- Encephalitis

##### Immunocompromised host

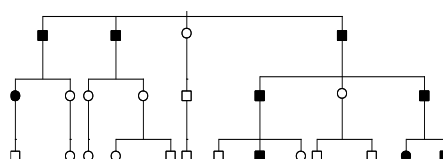
- Encephalitis
- Hepatitis
- Pneumonitis
- Pericarditis
- Delayed engraftment after HSCT

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

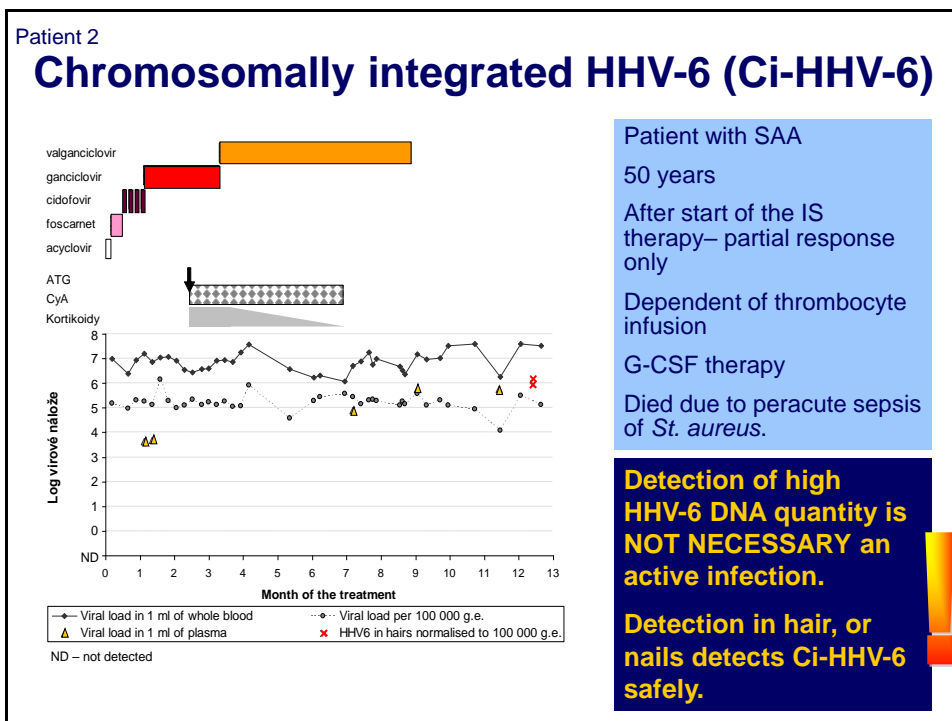
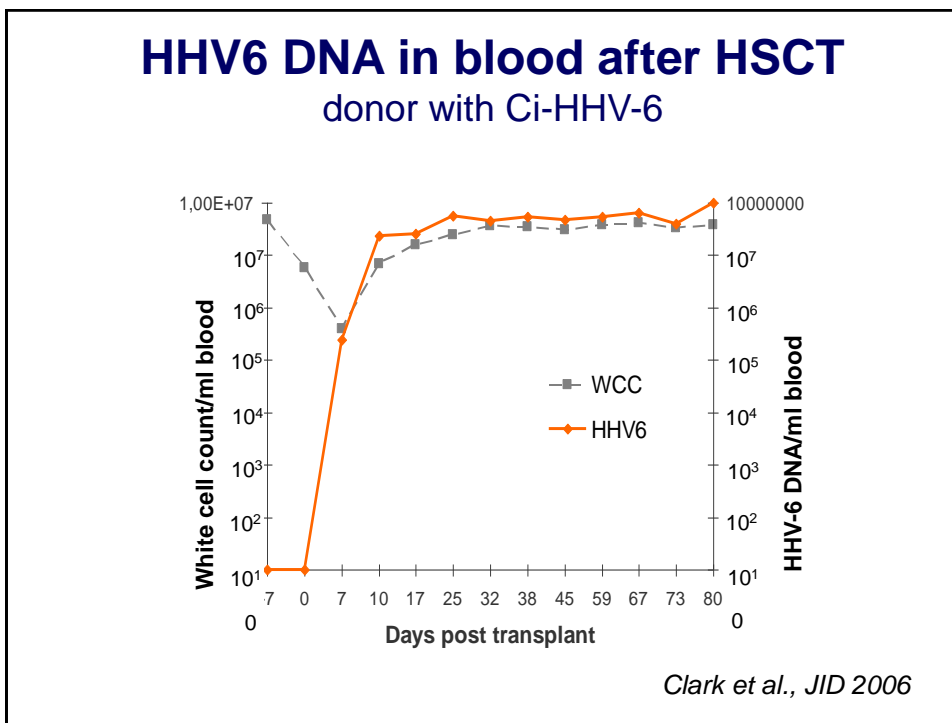
- Viral DNA integrated into human chromosomes
  - Inherited from parents to child
  - Viral DNA is present in every body cell (e.g. hair roots, nails)
  - Ratio of viral DNA : human DNA = 1:1
- Described frequency in population between 0.2-2.9% (Tanaka-Taya 2004, Ward 2007)
- Both variants (A or B) integrates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



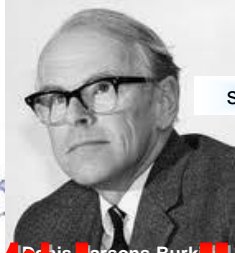
HHV-6 integration at 22q13.3 control probe on 9q34.4







## EBV discovery




**surgeon**

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



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“



Burkitt's lymphoma high incidence

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

1st described human oncogenic virus

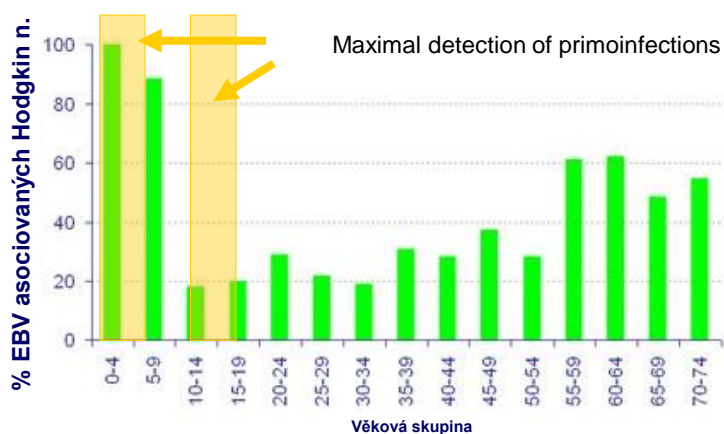
**Yvonne M. Barr**  
(\*1932)

**Bert Geoffrey Achong**  
(1928-1996)

## Transmission and epidemiology

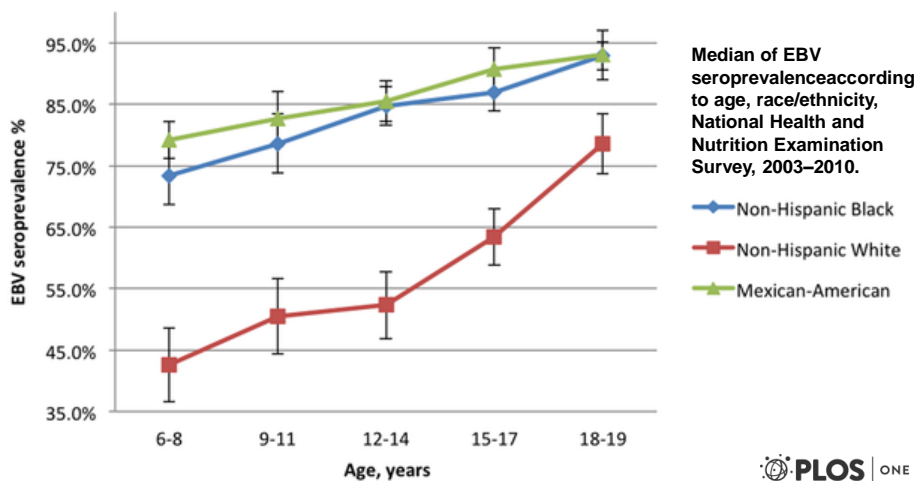
- **Transmission through saliva by oral route**
- **80 - 90% adult population is seropositive**

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



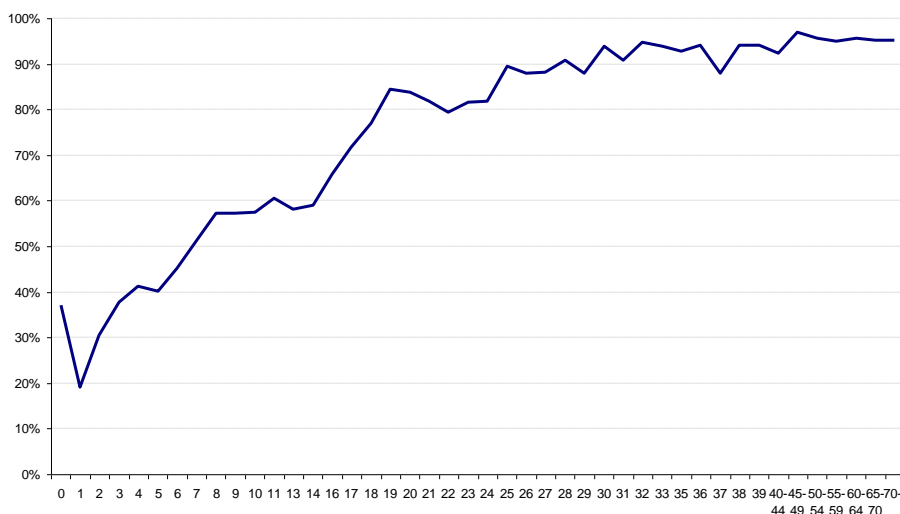
## Transmission and epidemiology

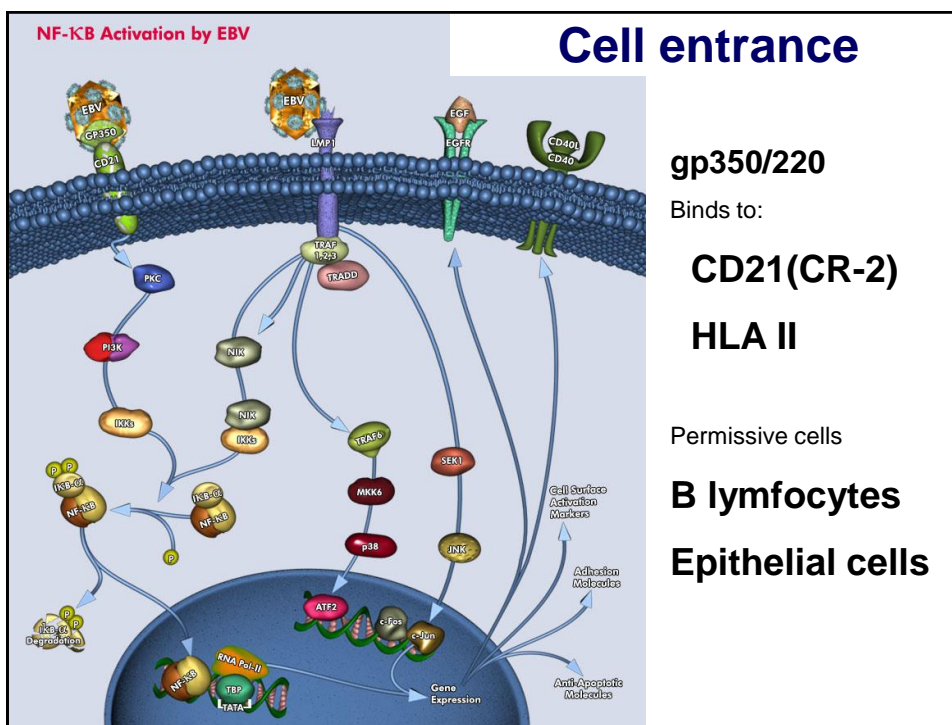
- Transmission through saliva and oral route
- (permissive cells: B lymphocytes and epithelial cells)
- 80 - 90% of adults population is seropositive



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

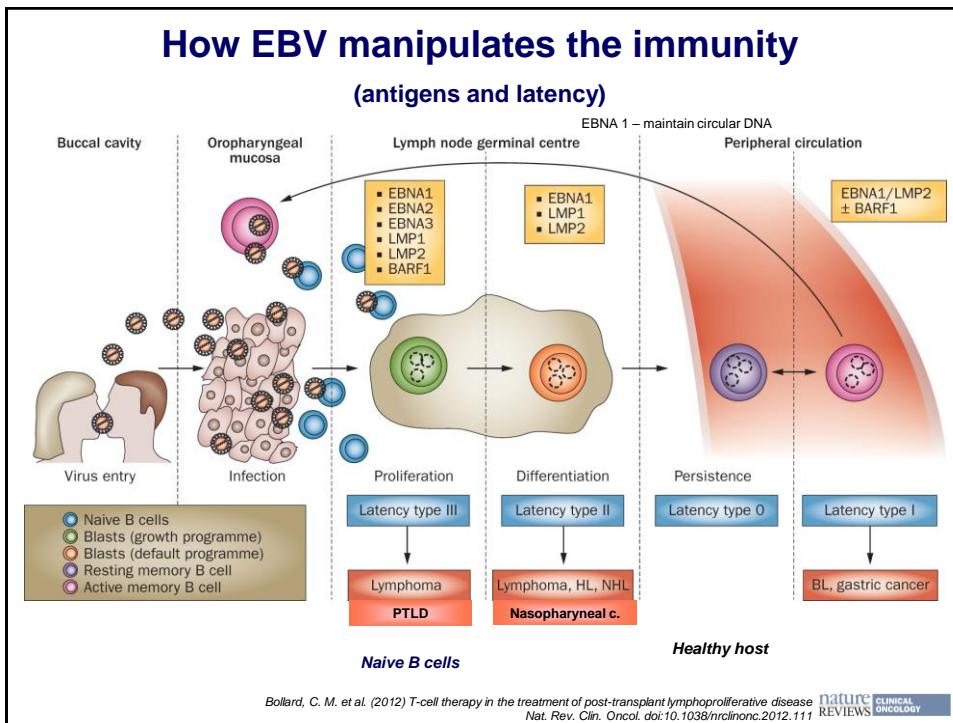




## How EBV manipulates the immunity /proliferation?

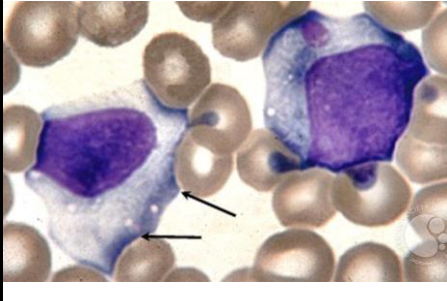
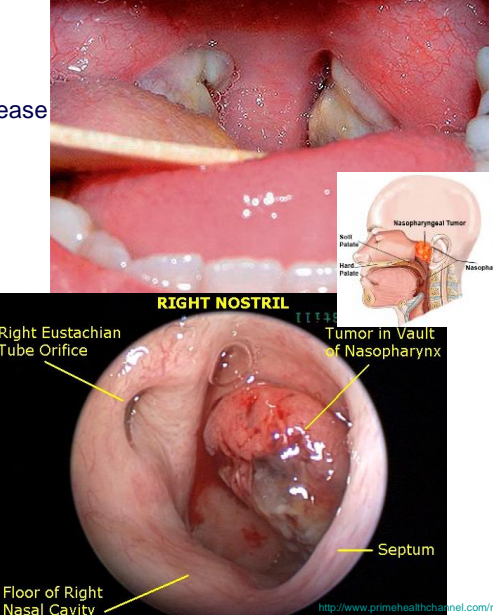
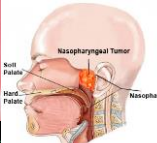
<b>EBNA-1</b>	Sequence-specific DNA-binding protein to EBV element; sequence-nonspecific chromosome association protein; transactivator of viral latent genes and host genes; responsible for episome replication, segregation and persistence of viral genome; <u>involved in p53 degradation and oncogenesis</u>
<b>EBNA-LP</b>	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; <u>dismisses repressor complex from promoter or enhancer sites; is essential for EBV-mediated B-cell transformation</u>
<b>EBNA-2</b>	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; <u>is critical for EBV-mediated B-cell transformation</u>
<b>EBNA-3A</b>	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 <u>induce CDKN2 and chemokines. Induces G1 arrests, which is essential for EBV-mediated B-cell transformation</u>
<b>EBNA-3B</b>	A coactivator of EBNA-2; dispensable for B-cell transformation; <u>viral tumor suppressor; and upregulates CXCL10. EBNA-3B-knockout induces DLBCL-like tumors</u>
<b>EBNA-3C</b>	Coactivates with EBNA-2 host <u>CXCR4 and CXCL12</u> genes; induces CDKN2, chemokines and aurora kinase B; mediates <u>RB degradation</u> ; attenuates H2AX expression and overcomes EBV-infection-mediated DNA damage response; promotes cell proliferation; <u>induces G1 arrests; essential for EBV-mediated B-cell transformation</u>
<b>LMP-1</b>	Mimics the <u>constitutively active form of CD40, a major EBV-encoded oncogene</u> ; 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
<b>LMP-2A</b>	Mimics <u>constitutively active, antigen-independent BCR signaling through constitutive activation of the ERK/MAPK pathway</u> ; blocks antigen-dependent BCR signaling; induces B-cell lymphoma in transgenic condition; is important but not essential for <i>in vitro</i> primary B-lymphocyte growth transformation; rescues the LMP-1-generated impairment in germinal center in the response to antigen in animals; confers <u>resting B cells sensitive to NF-κB inhibition and apoptosis</u> ; suppresses differentiation and promotes epithelial cell spreading and motility in epithelial cells; and <u>enriches cancer stem cell-like population</u>
<b>EBER</b>	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
<b>miRNAs</b>	Transcribed from BART and BHRF1; validated targets include Bim, BRUCE, CXCL11, DICER1, PUMA; has a role in sustaining latently infected cells. <u>BHRF1 miRNA and BART miRNAs interfere with apoptosis. The miR-BART15-3p promoted apoptosis</u> 331

Experimental & Molecular Medicine (2015) 47,



## Patological activities of EBV

- Immunocompetent host**
  - Infectious mononucleosis
  - Chronic active EBV infection
  - X-binded lymphoproliferative disease
  - Malignant diseases
    - Hodgkin disease
    - Burkitt's lymphoma
    - non-Hodgkin T/NK lymphoma
    - Nasopharyngeal carcinoma
    - Gastric carcinoma
    - Angioblastic T lymphoma

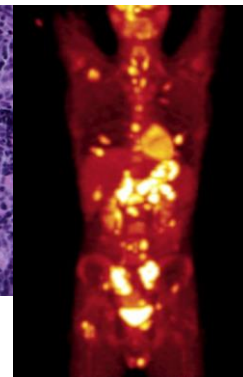
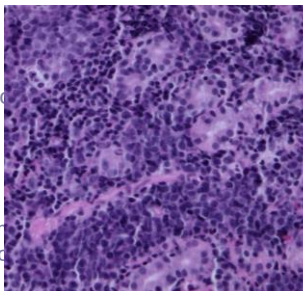
<http://www.primahealthjournal.com/nasopharyngeal-carcinoma.html>



## Patological activities of EBV

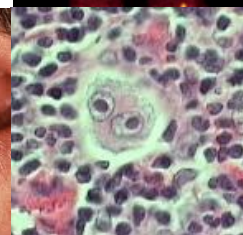
- **Imunokompetentní hostitel**

- Infekční mononukleóza
- Chronická aktivní EBV infekce
- X-vázaná lymfoproliferativní onemocnění
- Maligní onemocnění
  - Hodgkinova nemoc
  - Burkittův lymfom
  - non-Hodgkinský T/NK lymfom
  - Nasopharyngeální karcinom
  - Karcinom žaludku
  - Angioblastický T lymfom



- **Immunocompromised host**

- Hairy leukoplakia
- Above listed malignant diseases
- Post-transplant lymphoproliferative disease (EBV-LPD)
- Encefalitis/myelitis
- Pneumonie
- Hepatopathy/hepatitis



<http://www.keom.edu/faculty/chamberlain/Website/lectures/lecture/aid.htm>

[www.med-ed.virginia.edu/courses/path/imes/wcd/hodgkin](http://www.med-ed.virginia.edu/courses/path/imes/wcd/hodgkin)

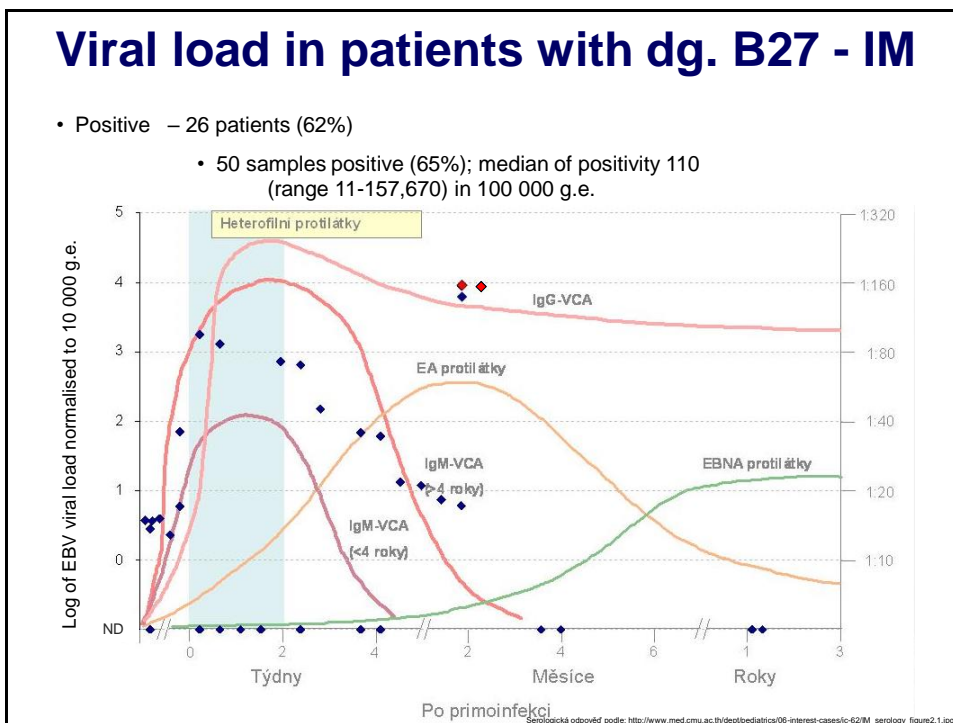
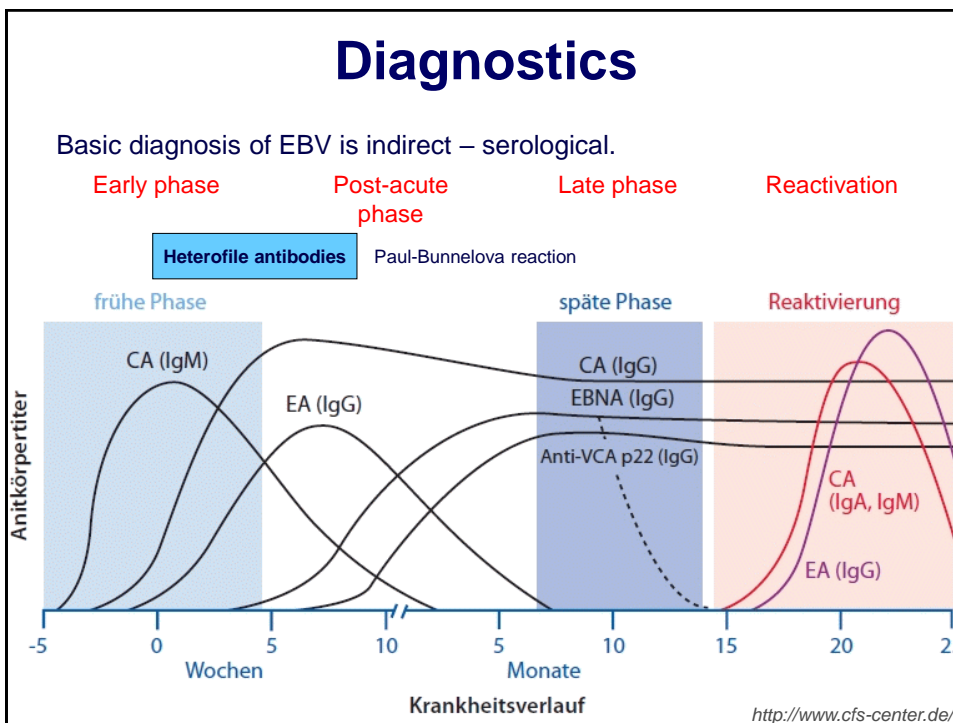
## Infectious mononucleosis

- „Kissing disease“
- Proliferation affects spleen, liver and lymph nodes
- Tiredness lasting for weeks, increased temperature and fevers (often approx. 39 °C), pharyngitis and swelling of the lymph nodes (submandibular and cervical); hepatosplenomegaly, hepatopathy, swelling of the eye lashes and face, malaise
- Inkubation period 4-6 weeks
- At the beginning seems like „tonsillitis“
- Transmission by saliva
- Treatment approx. 6 months  
Relax and diet (2-3 months);  
Subsequently it is necessary to have some relax in physical activity



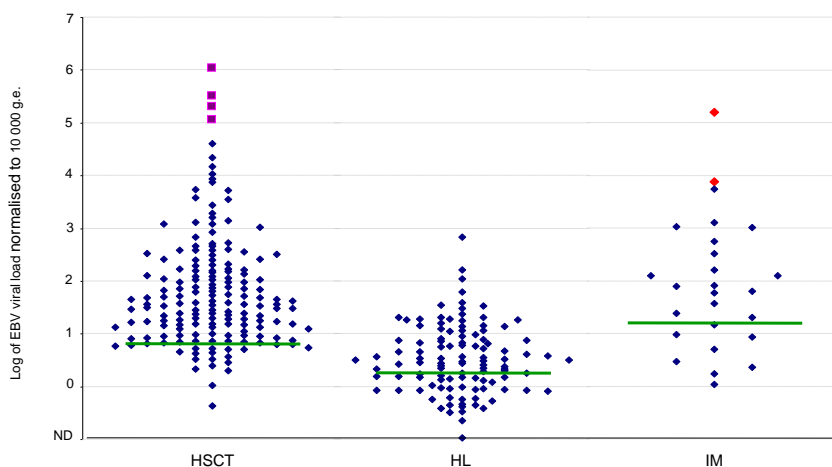
<http://home.teleport.com/~bobh/InfectiousMononucleosis.htm>





## 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 lymphocytes and NK cells

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

Diagnostic criteria of a case definition for SCAEBV [15]

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

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

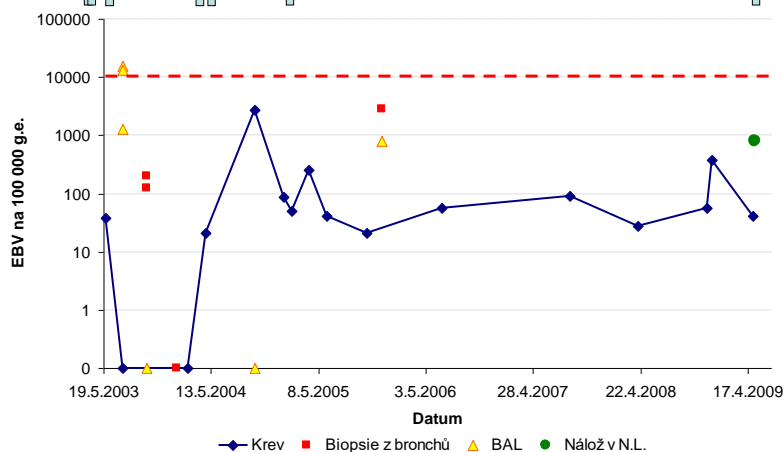
## Chronic active EBV infection

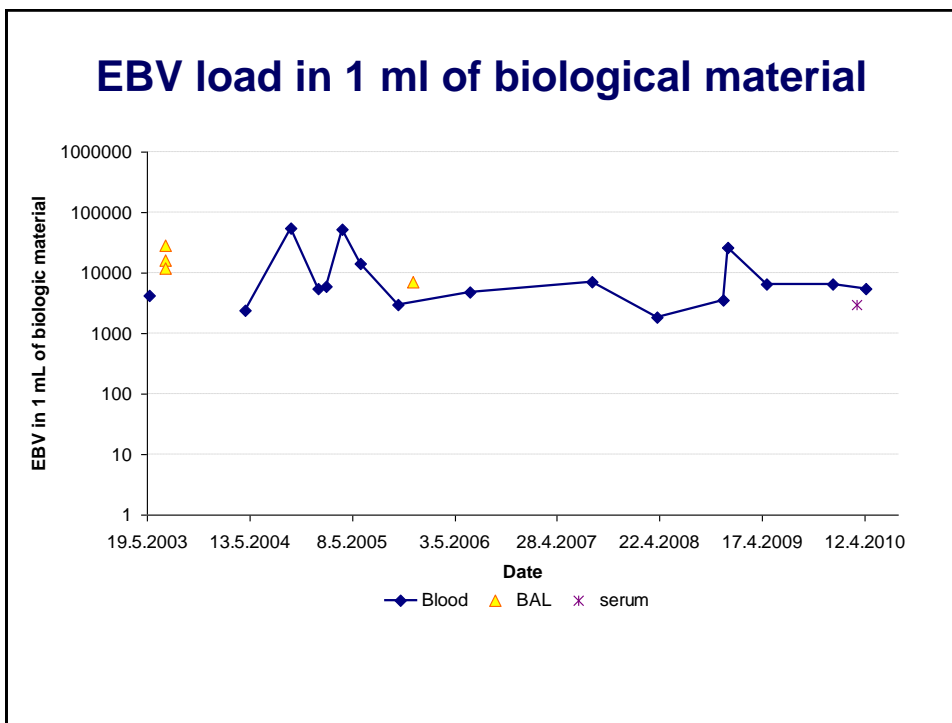
	T-cell type (n = 16)	NK-cell type (n = 12)	P	Table 2. Clinical features of 30 patients with chronic active Epstein-Barr virus infection			
Symptoms				Symptoms and signs	(%)	Life-threatening complications	(%)
Fever, > 1 d/wk (%)	67	25	.04	Fever	100	Hemophagocytic syndrome	21
HMB (%)	13	<b>75</b>	.002	Liver dysfunction	90	Coronary artery aneurysm	21
Splenomegaly (%)	73	100	.08	Splenomegaly	90	Hepatic failure	18
Large granular lymphocytosis (%)	13	83	.0004	Lymphadenopathy	50	Malignant lymphoma	16
Calcification in basal ganglia (%)	7	33	.10	Thrombocytopenia	50	Interstitial pneumonia	12
Laboratory data				Anemia	48	Central nervous system involvement	7
IgG (mg/dL, mean ± SD)	2213 ± 1104	1682 ± 464	.11	HMB	43	Sepsis	7
IgE (IU/mL, mean ± SD)	282 ± 298	<b>2774 ± 3774</b>	.04	Skin rash	28	Pulmonary hypertension	4
VCA IgG (geometric mean titer)	2405	446	.01	Calcification in basal ganglia	18	Intestinal perforation	4
EA IgG (geometric mean titer)	831	119	.02	Oral ulcer	18	Myocarditis	4
EBNA (geometric mean titer)	30	45	.24	Hydroa vacciniforme	14		
Viral load				HMB indicates hypersensitivity to mosquito bites.			
PBMC (copies/μg DNA, mean ± SD)	10 <sup>4.1±0.5</sup>	10 <sup>4.4±0.4</sup>	.09				
Plasma (copies/mL, mean ± SD)	10 <sup>2.9±1.1</sup>	10 <sup>2.4±2.1</sup>	.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.

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





## Malignant impact of EBV

### NHL - Burkitt lymphoma

**Very aggressive**

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

**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://myphotos4usmile.tumblr.com/post/53262736354/burkitts-lymphoma#.VPgrFSx5vU4>

## Malignant impact of EBV

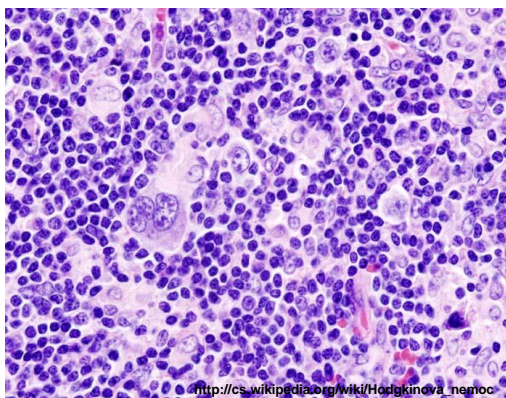
### Hodgkin lymphoma

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 ♂.

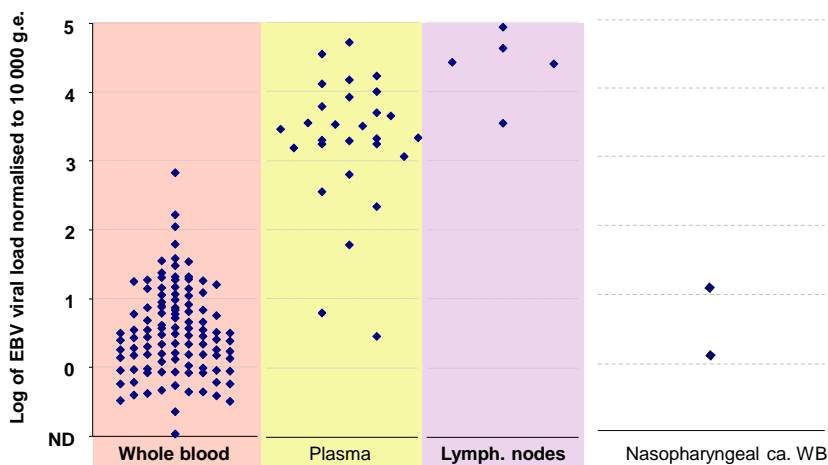


Histologically divided according to no. of **Reed-Sternberg's cells** (cells developed by mutation from B-cells) and according to the cellular freccions:

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

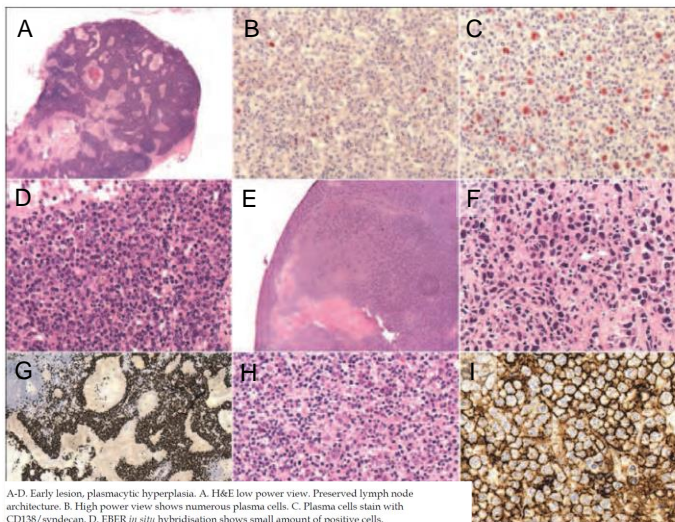
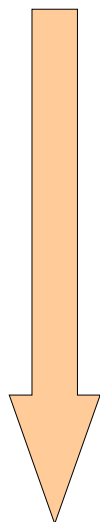
## Patients with Hodgkin L. and NF ca.

- 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)



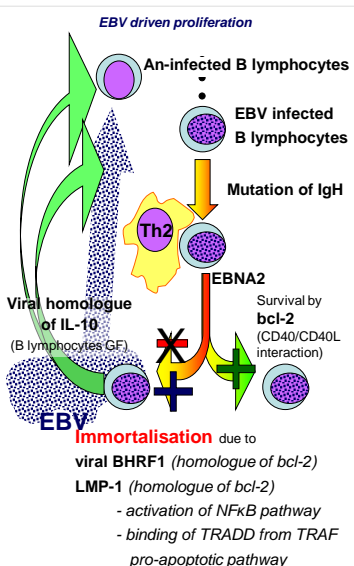


# EBV associated posttransplant proliferative disease (EBV-LPD)



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 Lousserat)

## Etiopathogenesis and classification EBV-LPD



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

Category	Subtype
Early lesions	Plasmacytic hyperplasia Infectious mononucleosis-like lesion
Polymorphic PTLD	B-cell neoplasms
Monomorphic PTLD (classify according to lymphoma they resemble)	- Diffuse large B-cell lymphoma - Burkitt lymphoma - Plasma cell myeloma - Plasmacytoma-like lesion - Other <sup>a</sup>
	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
<b>Type of immune suppression</b>		
– Tacrolimus	2–5 × risk with cyclosporine	1,16,17
– OKT3 and/or ATG	3–4 × risk without these drugs	1
<b>Type of organ transplant</b>		
Kidney	1%–3% of all transplant patients	9
Liver	1%–3% of all transplant patients	
Heart	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
<b>De novo CMV infection:</b>		
CMV-positive recipient of a CMV-positive organ	4–6 × risk of CMV-negative recipient	21

**In allogeneic HSCT incidence 2-25%.**

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

- Cumulative intensity of immunosuppressive treatment
- Use of anti-T lymphocytic 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 neoplastic 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...

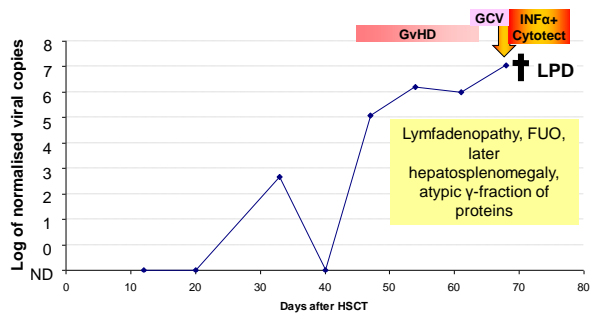
**EBV DNA detection in whole blood is not enough.**

*De definice EBMT IDWP, 2007*

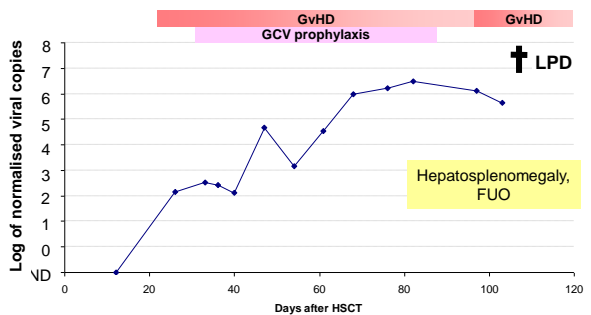
- Clinical symptoms
- Imagine methods
- Immunology (Flow cytometry, Ig levels, clonality)
- Histology N.L. (detecting the presence of EBV)
- Direct detection of virus
  - EBV load (based mainly on NA detection)
    - Sample type: plasma, whole blood, MNC
    - Different methods of PCR – most frequently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



## Retrospectively tested patients

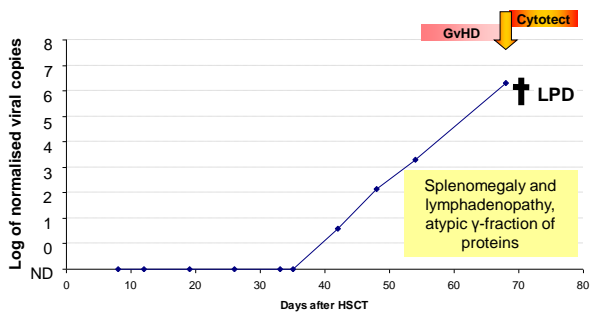


**Girl, 5.5 yrs., HSCT for ALL CR2.**  
**Donor:** MMUD (8/10)  
**Graft:** BM  
**Conditioning:** TBI (12 Gy)  
 cyclophosphamide (120 mg/kg)  
 ATG (16 mg/kg)  
**GvHD prophylaxis:** CyA+MTX  
**GvHD:** grade III (D+ 49; GIT)  
**Deceased on D+74 due to MOF.**

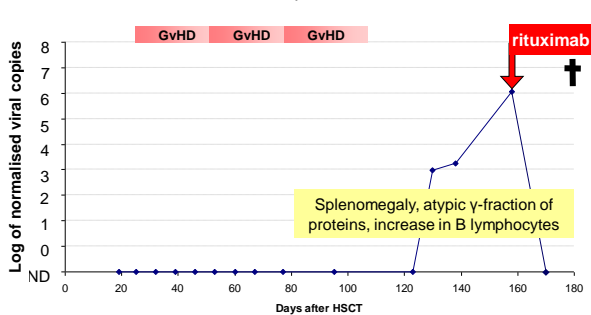


**Girl, 4 yrs., HSCT for AML PR2**  
**Donor:** MMUD (9/10)  
**Graft:** BM  
**Conditioning:** busulphan (16 mg/kg)  
 cyclophosphamide (120 mg/kg)  
 melphalan (140 mg/m<sup>2</sup>)  
 ATG (40 mg/kg)  
**GvHD:** grade III (D+ 25; GIT)  
**Deceased on D+117 due to bleeding and respiratory failure.**

## Retrospectively tested patients

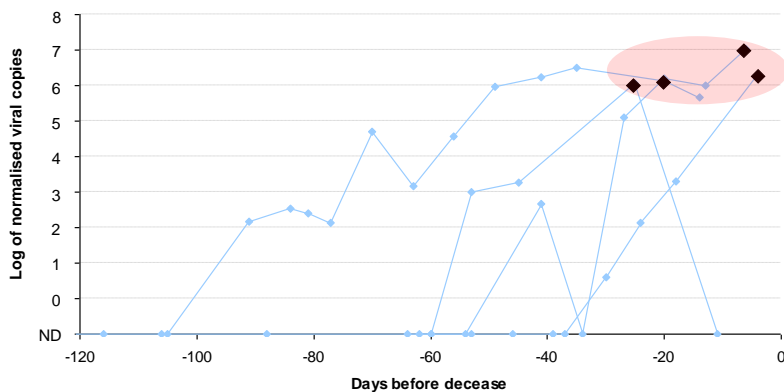


**Boy, 5 yrs., HSCT for JMML**  
**Donor:** MUD  
**Graft:** BM  
**Conditioning:** busulphan (18 mg/kg)  
 cyclophosphamide (120 mg/kg)  
 ATG (16 mg/kg)  
**GvHD prophylaxis:** CyA+MTX  
**GvHD:** grade II (D+ 56; GIT)  
**Deceased on D+72 due to MOF.**



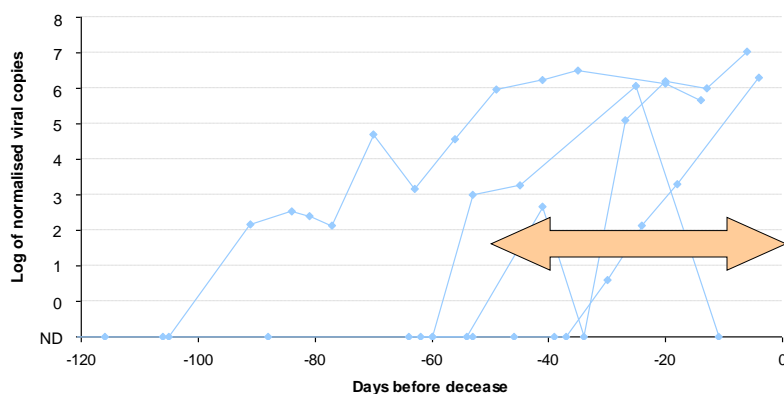
**Boy, 11 yrs., HSCT for AML CR2.**  
**Donor:** MUD  
**Graft:** BM  
**Conditioning:** busulphan (16 mg/kg)  
 cyclophosphamide (120 mg/kg)  
 melphalan (140 mg/m<sup>2</sup>)  
 ATG (40 mg/kg)  
**GvHD:** grade III-IV (D+ 23; GIT)  
**Deceased on D+182 due to MOF.**

## Retrospectively tested patients



Maximum detected quantity was between  
 $1.16 \times 10^6$  and  $1.17 \times 10^7$  NVCs

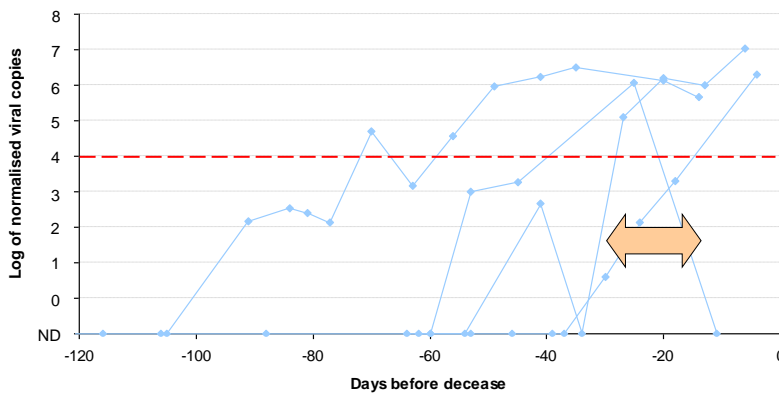
## Retrospectively tested patients



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

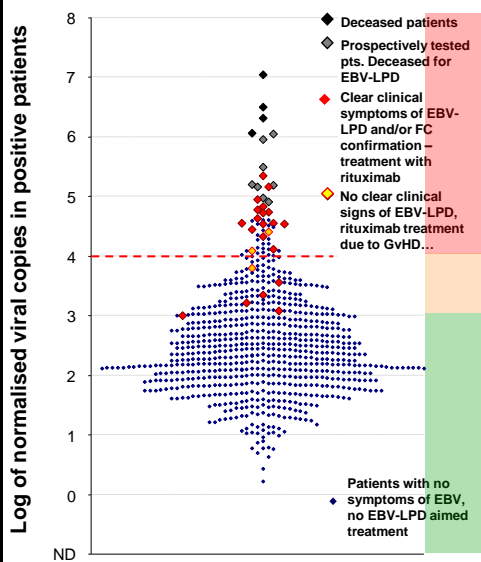
Detection preceded clinical signs of EBV-LPD with median of 35 days (-77 to -24)

## Retrospectively tested patients

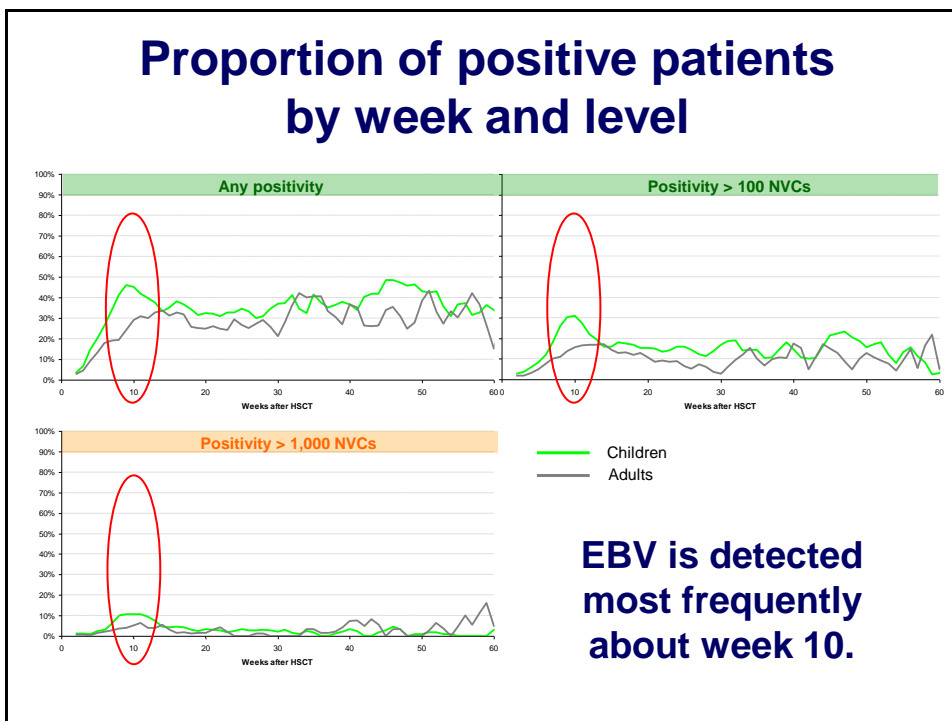
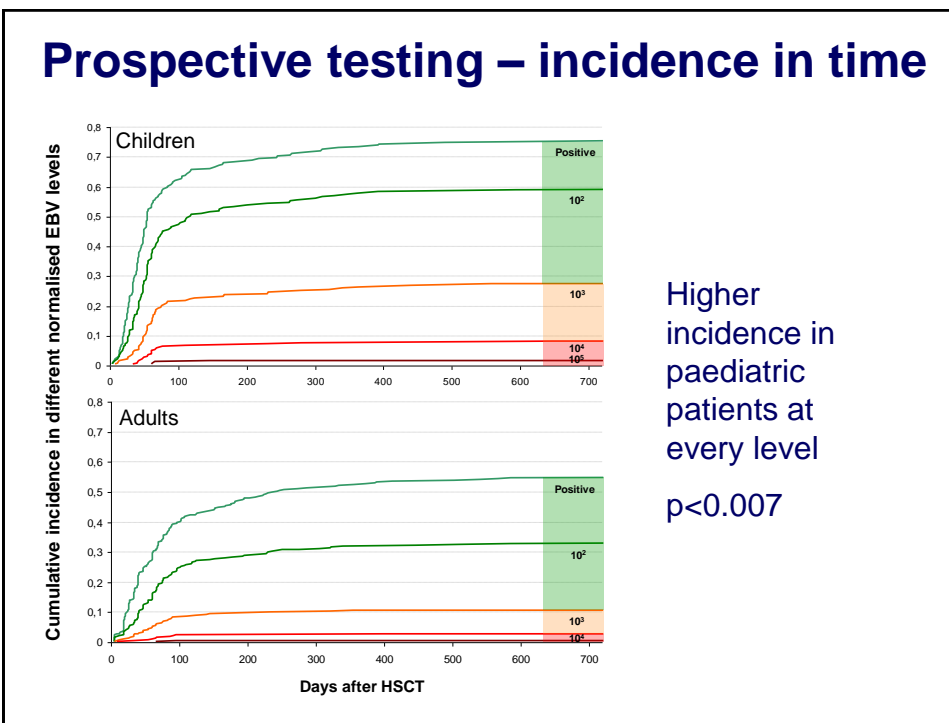


Quantity > 10<sup>4</sup> NVCs preceded clinical signs of EBV-LPD with median of 14 days (-56 to 2)

## Prospective testing – maximal quantity

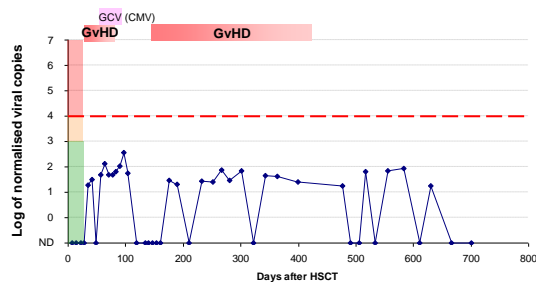


- **Very high risk level reactivation**  
 > 10<sup>4</sup> NVCs – ≈ 50 IU/ng  
 suspected and/or correlated with signs of EBV-LPD - 102 samples from 26 children (7.1%) and 22 adults (3.2%)  
**EBV-LPD was confirmed in 22 (45%)**
- **Risk level reactivation**  
 > 10<sup>3</sup> and < 10<sup>4</sup> NVCs – ≈ 5-50 IU/ng  
 687 samples from 95 (26%) children and 104 adults (15%)
- **Benign level reactivation**  
 < 10<sup>3</sup> NVCs ≈ 5 < IU/ng  
 low quantities with no clinical signs of EBV were detected in majority of samples from 275 children and 481 adults.



## Benign level reactivation

$< 10^3$  NVCs  $\approx 5 < \text{IU/ng}$



**Boy, 13 yrs., HSCT for MDS-RAEB**

**Donor:** MMUD (8/10)

**Graft:** PBSC

**Conditioning:** busulphan (16 mg/kg)

cyclophosphamide (120 mg/kg)

melphalan (140 mg/m<sup>2</sup>)

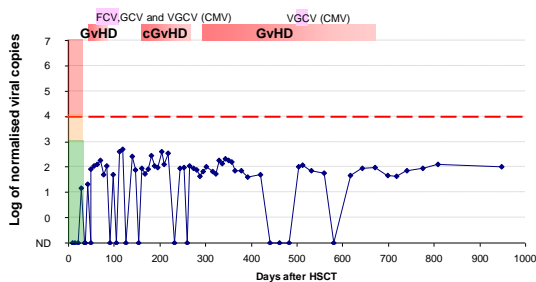
ATG (40 mg/kg)

**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

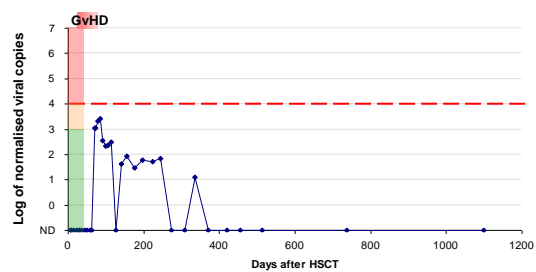
**GvHD:** grade II (D+40;GIT)

**Other:** CMV(D+46), lung affection of unknown etiology, steroid DM

**Outcome:** alive, no clin. problems

## Risk level reactivation

$> 10^3 < 10^4$  NVCs  $\approx 5 - 50 \text{ IU/ng}$



**Boy, 6.5 yrs., HSCT for ALL**

**Donor:** RD (10/10)

**Graft:** BM

**Conditioning:** TBI (12 Gy)

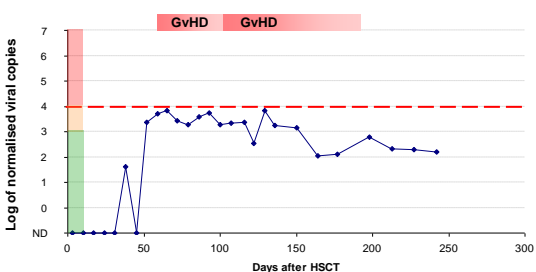
etoposide (60 mg/kg)

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

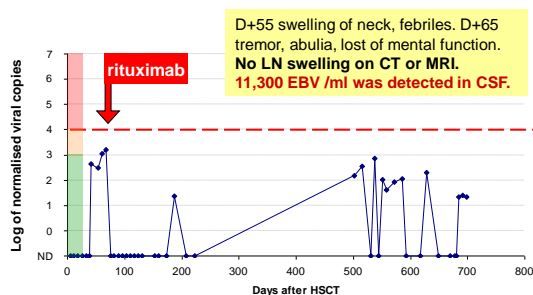
- **Detected: 28 patients** (2.65% of tx, 3.3% of EBV positive)  
15 adults (1.98%) and 13 children (3.5%)
- **Mononucleosis like syndrome:** 1 adult  
peak at 54 days after HSCT (1,198 NVCs)
- **Localised :** 12 patients (9 adults, 3 children)  
median peak level at 68 days after HSCT  
median peak 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/m<sup>2</sup>)

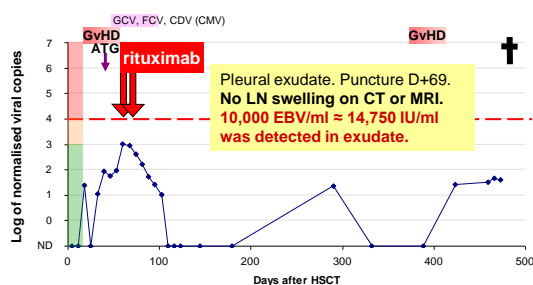
fludarabine (25 mg/m<sup>2</sup>)

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/m<sup>2</sup>)

fludarabine (25 mg/m<sup>2</sup>)

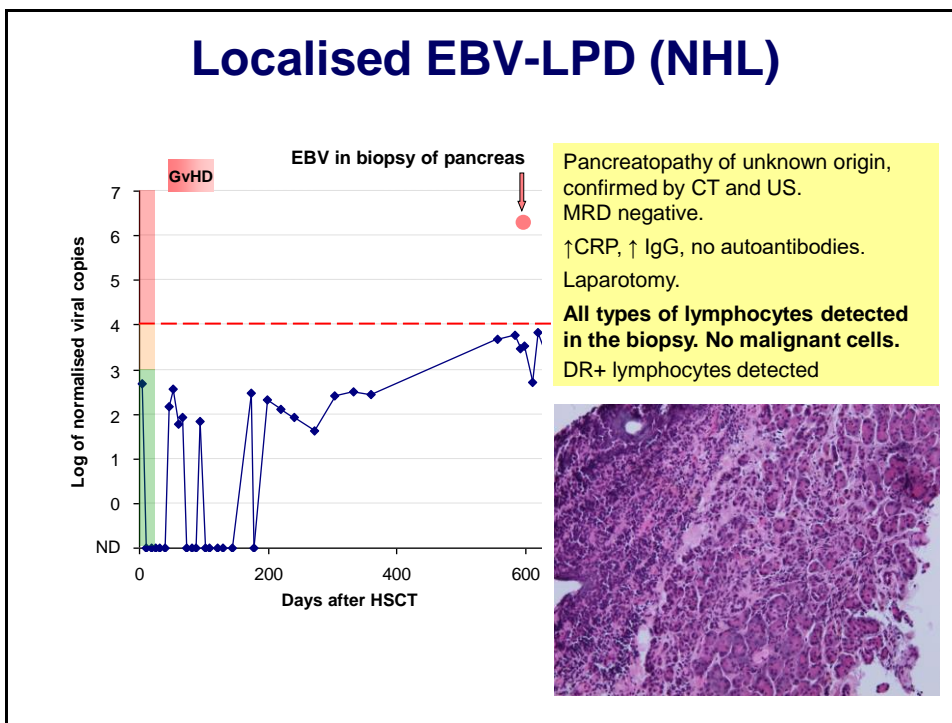
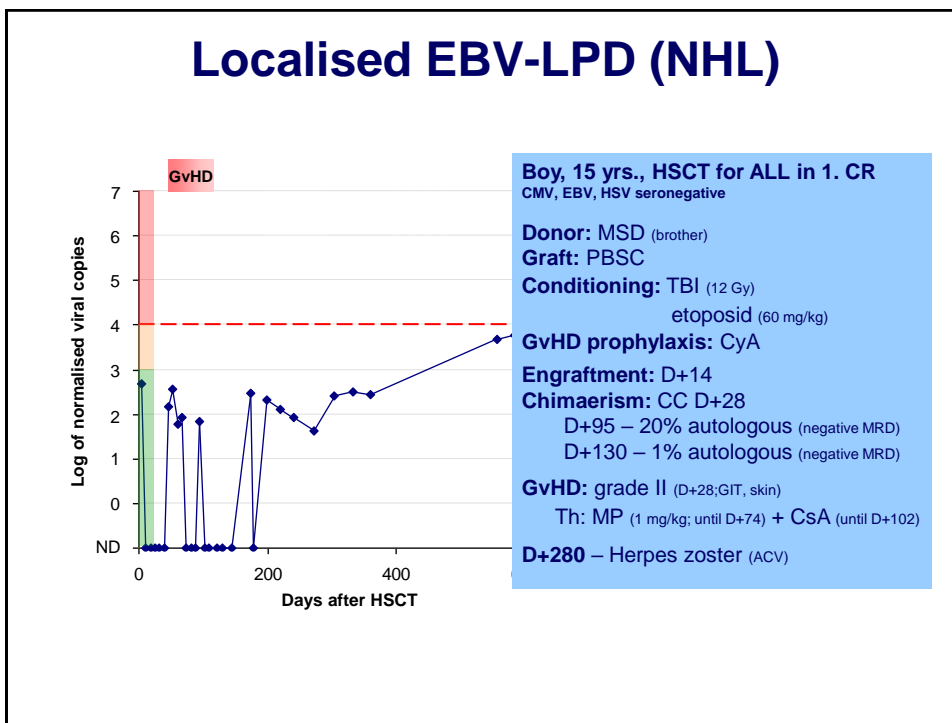
TBI (12 Gy) ATG (40 mg/kg)

GvHD prophylaxis: CyA+MMF

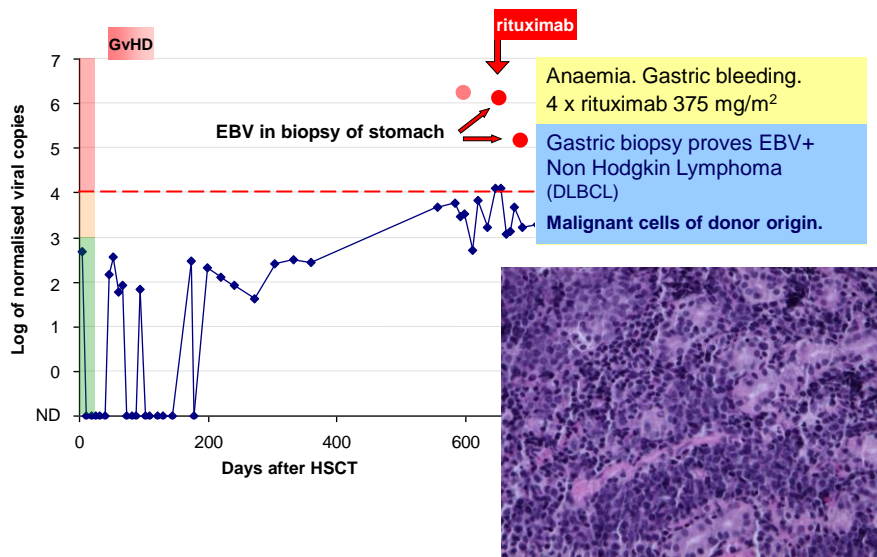
GvHD: grade III-IV (D+15)

Other: pulmonary proces of unknown origin

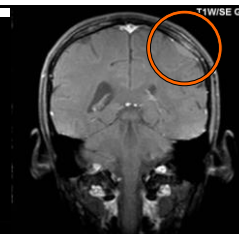
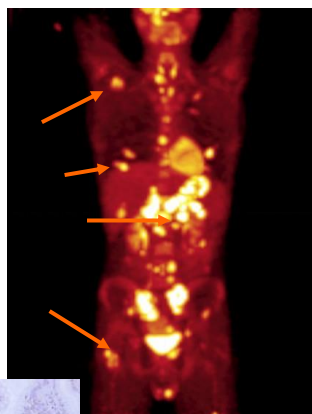
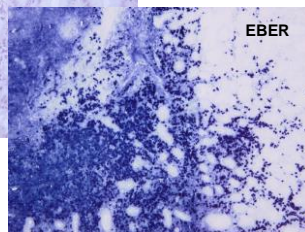
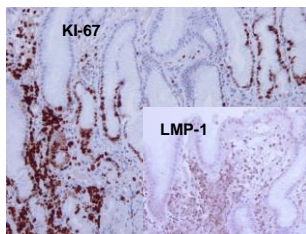
**Outcome: deceased on D+478 due to MOF**

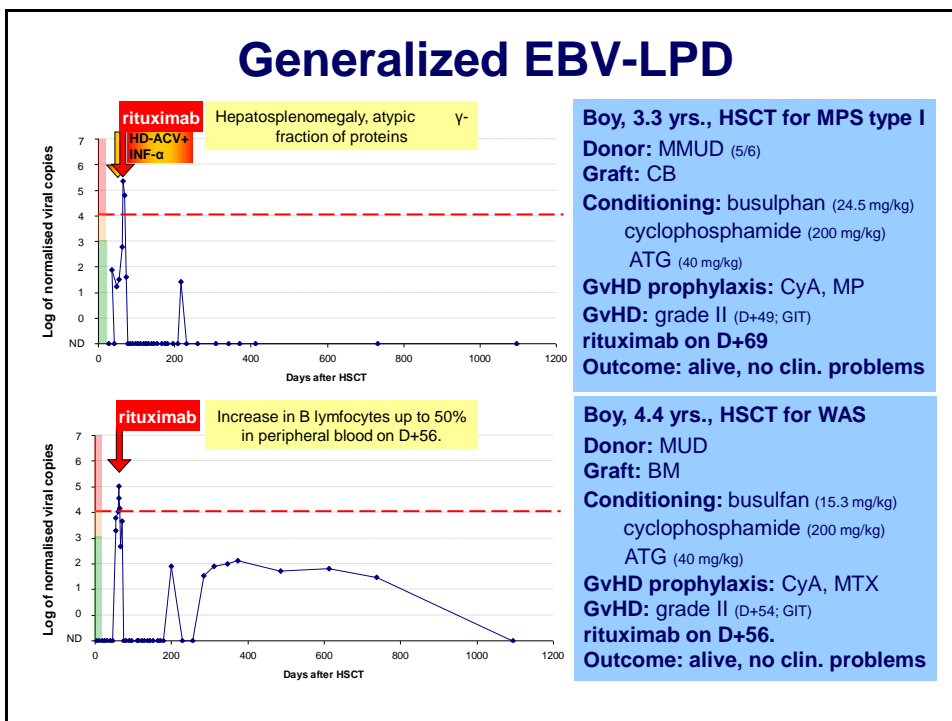
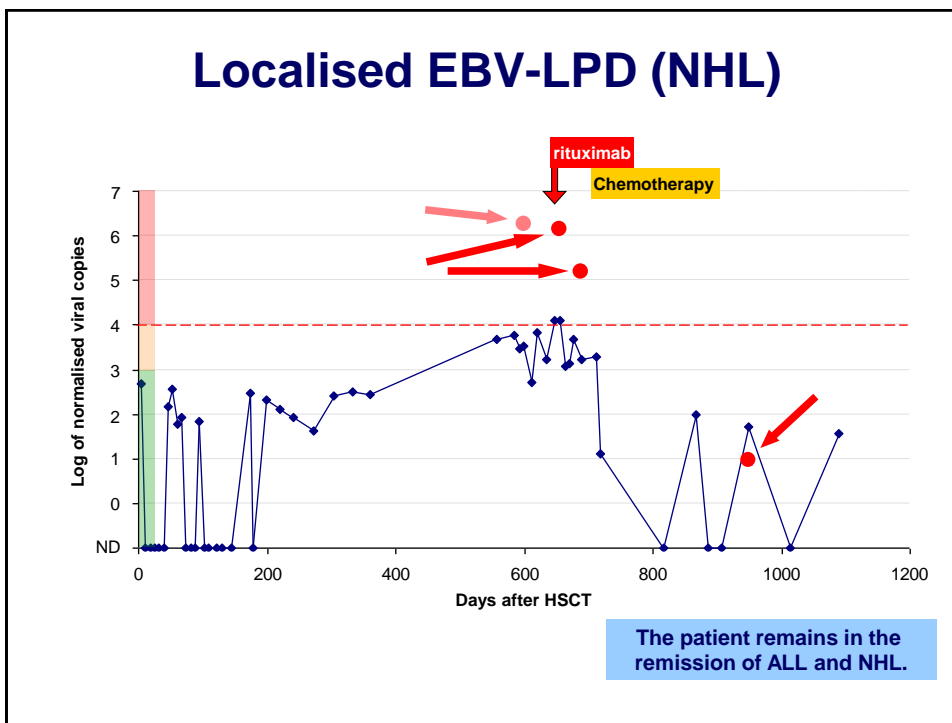


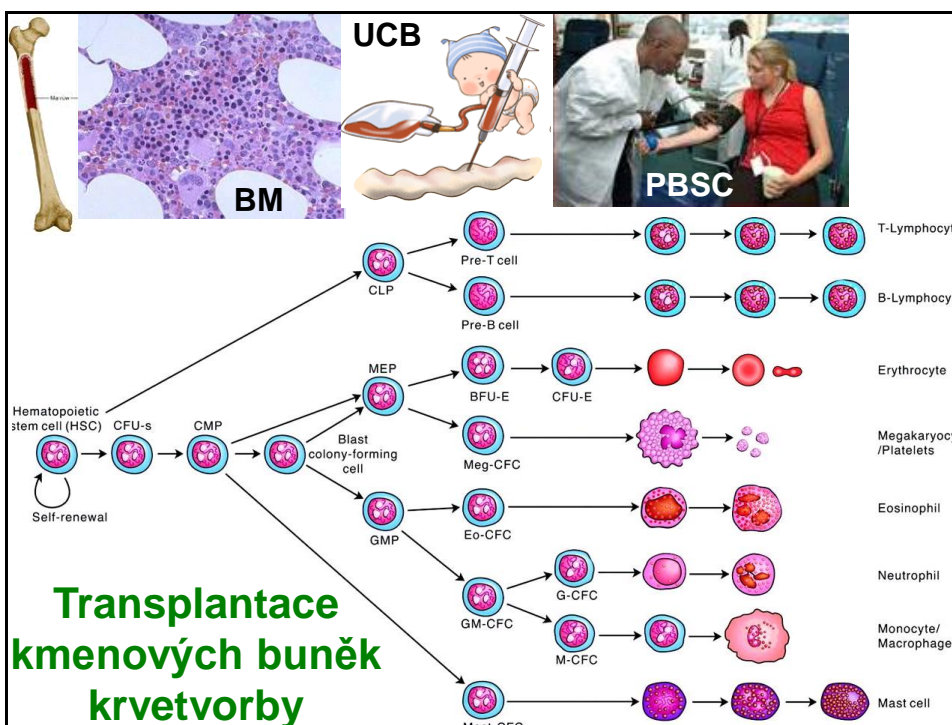
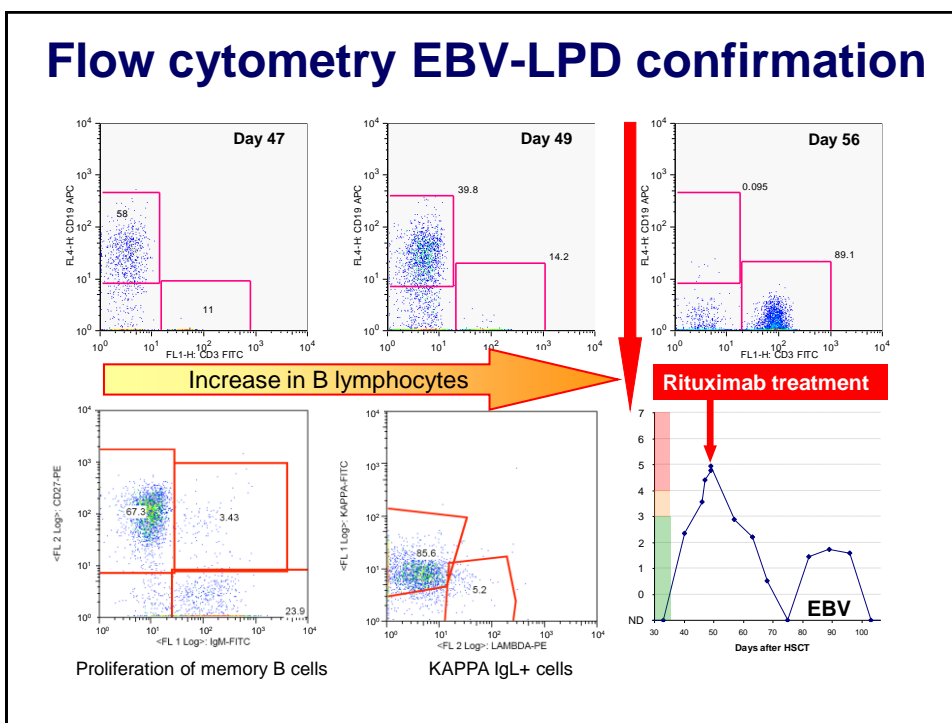
## Localised EBV-LPD (NHL)



Treatment according to Protocol **BFM NHL 2004**  
 During last chemotherapy sepsis caused by *Pseudomonas aeruginosa*.  
 Last PET CT confirmed Remission of NHL.

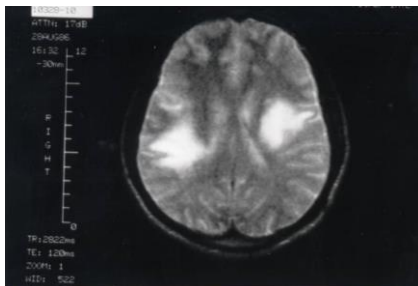




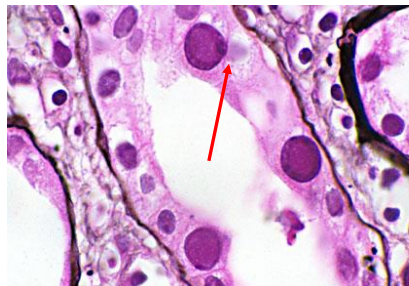


## Polyomaviruses

- small ds DNA viruses with circular NA
- Capsid diameter 42-45 nm, genome: 5 kbp
- Transmission by fecal-oral route
- **JC virus** – progressive multifocal leukoencephalopathy 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
- Potentially treatable with **cidofovir**



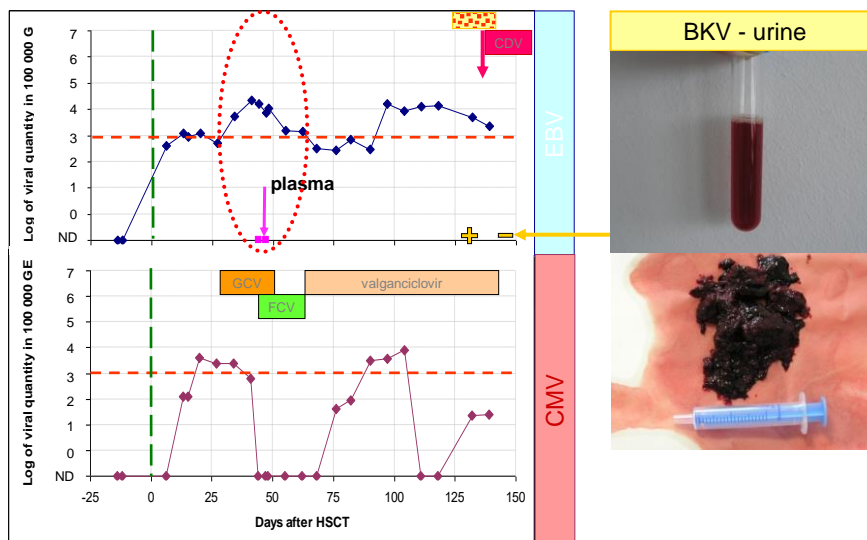
Fields - Virology



Copyright © 2001 by the National Kidney Foundation

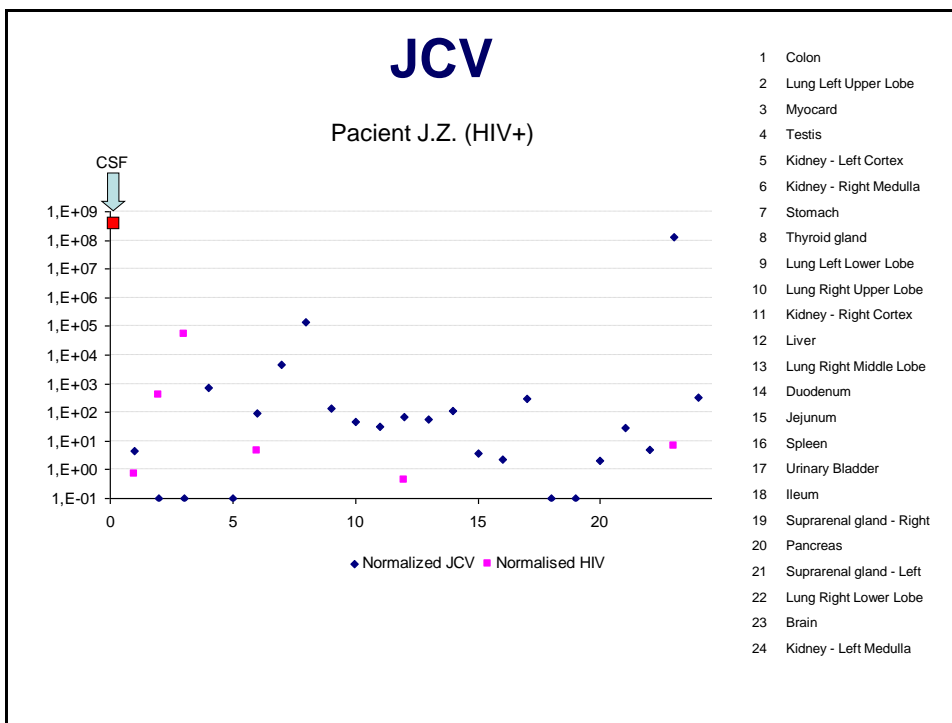
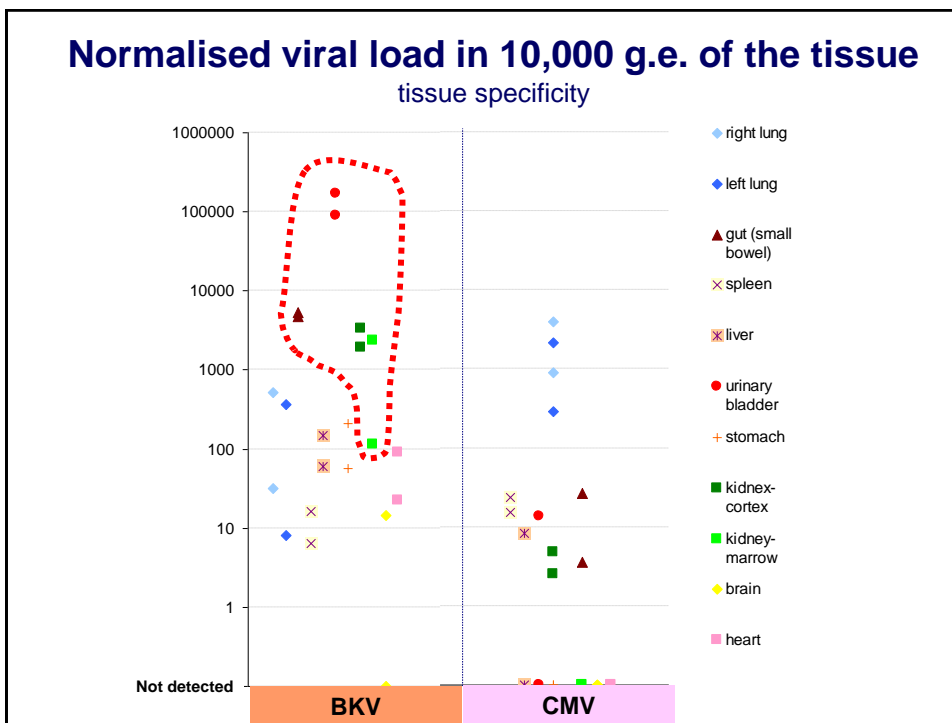
[www3.us.elsevierhealth.com/aiak4/atlas/37/1/atlas02.htm](http://www3.us.elsevierhealth.com/aiak4/atlas/37/1/atlas02.htm)

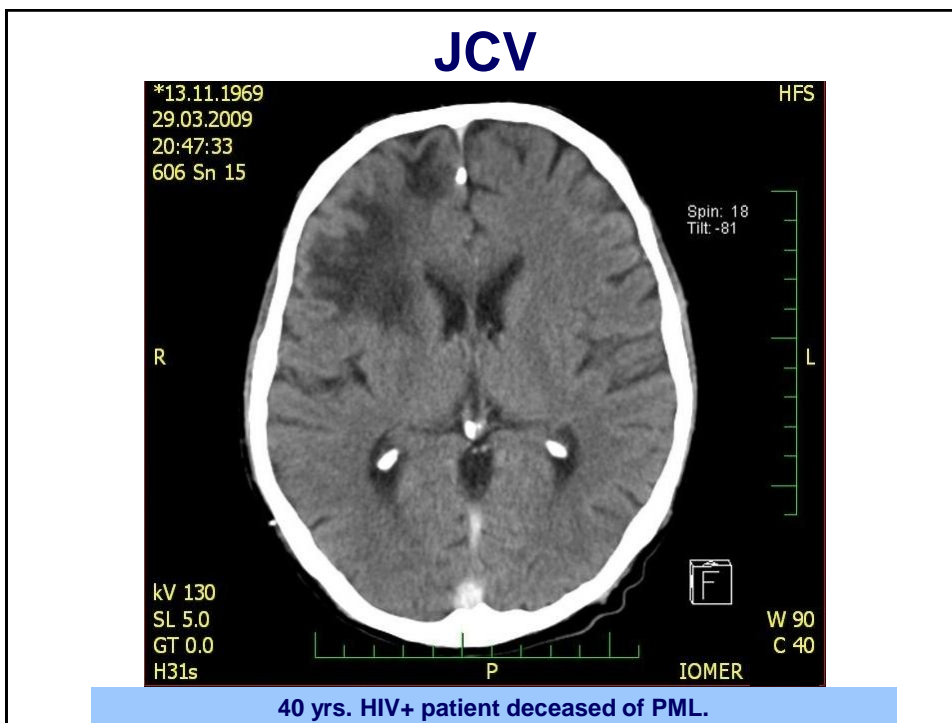
## BKV – haemorrhagic cystitis



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









## Papilomavires


- ds DNA virus
- DNA lenght approx. 8 kb
- > 100 serotypes
- causing – warts
  - Condylomata acuminata
  - 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 1<sup>st</sup> 3 months)




**Common Wart**  
Common on hand and knuckles but may appear anywhere




**Plantar Wart**  
On soles heel or toes of feet




**Flat Wart**  
Usually on leg hand face




**Filiform Wart**  
Usually on face near eyelids lips




**Mosaic Wart**  
Group of tightly clustered wart



**Periungual Wart**  
Wart around finger nail toe nail



**Oral Wart**  
Within oral cavity



**Genital Wart**  
Located near or in genital areas

<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.)

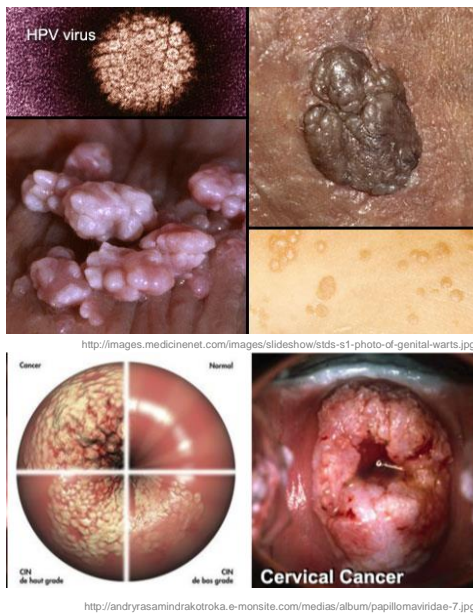
• 2-8 months after infection is necessary for lesion development on 50% of infected women

- non-oncogenic
- delected usually around 25 yrs.

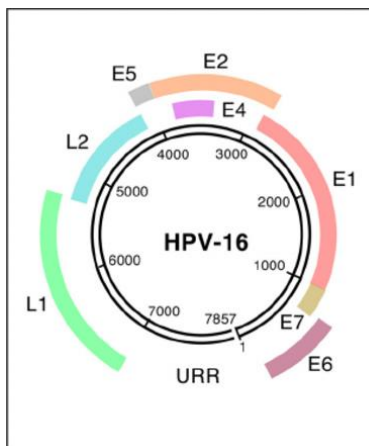
## HPV-HR high risk

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 spontaneously 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.)



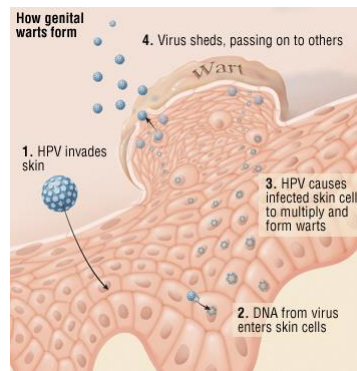
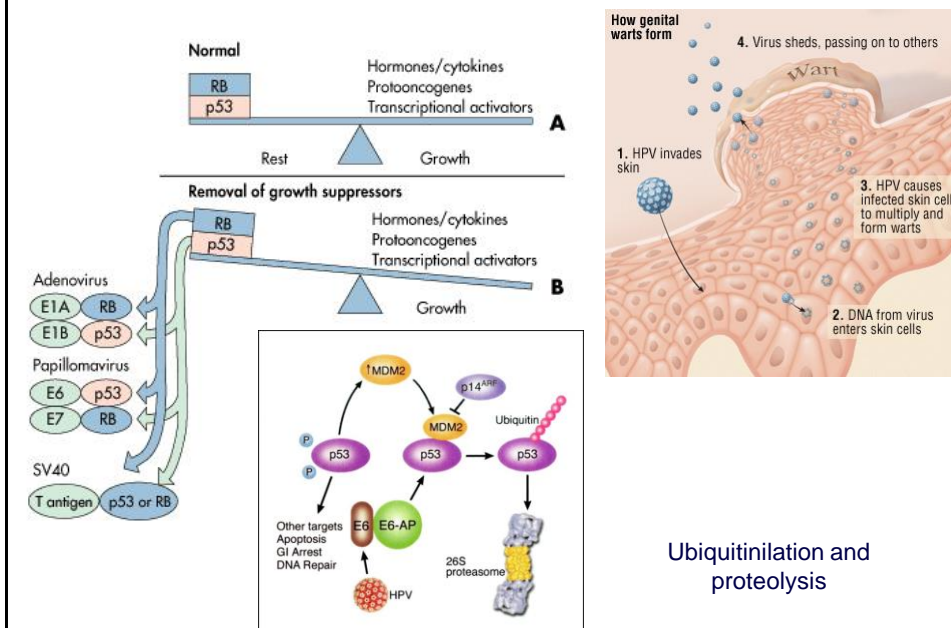
# Papilomaviruses – genome



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/fig/1/AS:30736030254856@1450291964254/FIGURE-1-HPV-16-genome-and-transforming-activity-of-E6-and-E7-The-left-panel-shows-the.png](https://www.researchgate.net/profile/Angelika_Riemer/publication/45113419/figure/fig/1/AS:30736030254856@1450291964254/FIGURE-1-HPV-16-genome-and-transforming-activity-of-E6-and-E7-The-left-panel-shows-the.png)

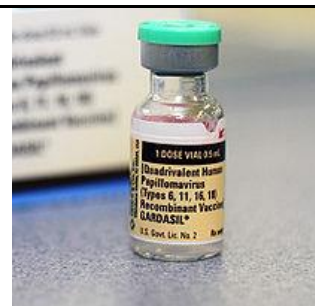
## Papilomaviruses – oncogenic potential



## HPV 16 a 18

Causes up to:

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



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

## • VACCINATION!!!

HPV vaccines: Gardasil9 (9 types)

Gardasil (previously

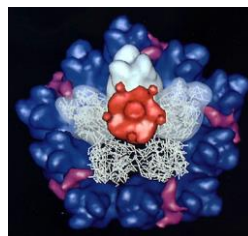
Silgard, 4 types)

Cervarix (2 types)

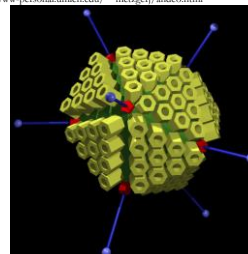


## Adenoviruses

- non enveloped ds DNA viruses  
 ikosahedral structure 70-75 nm  
 genome: 35 kbp  
 according to similarity– 7 subgenes A-G  
 according to antigenic specificity– more than 60 serotypes
- **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 immunosuppression – in colon, and urinary tract

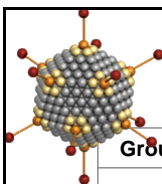


[www.fic.nih.gov/understanding\\_the\\_image/adenovirus.html](http://www.fic.nih.gov/understanding_the_image/adenovirus.html)



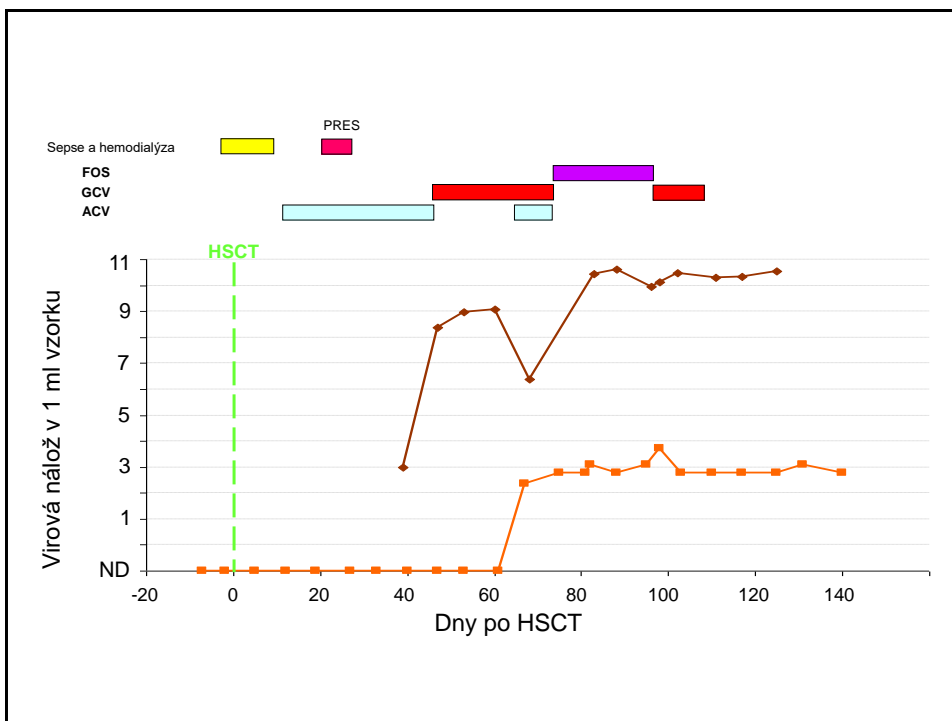
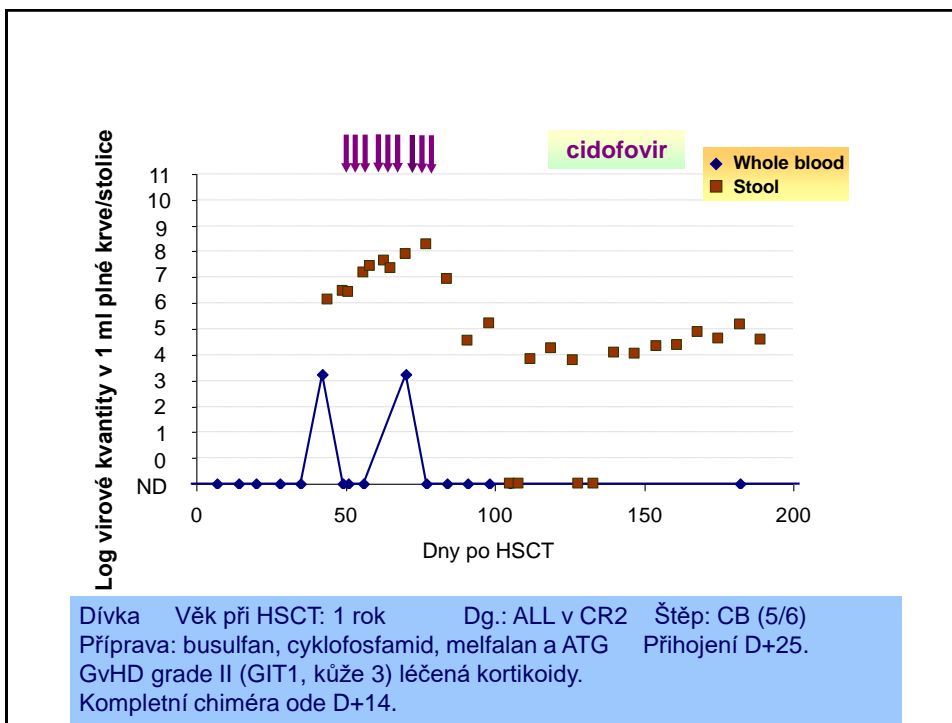
[www.stof.org/maladies/images/maladie/adenoviruslang.jpg](http://www.stof.org/maladies/images/maladie/adenoviruslang.jpg)

## Serotypes

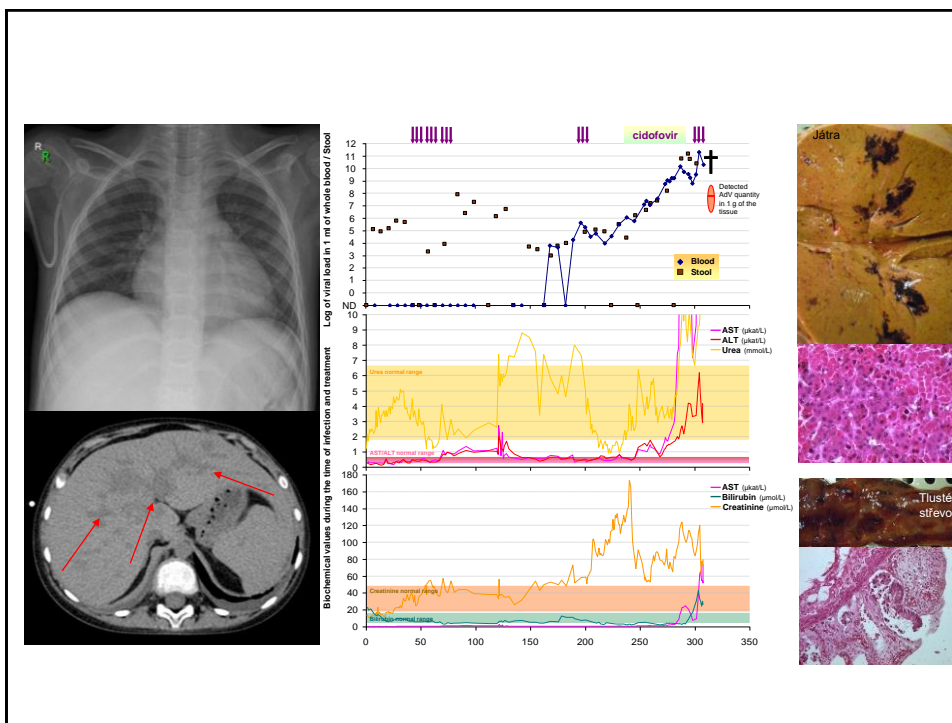


Group	Serotypes	Localisation of the infection
A	12, 18, 31	Respiratory, urinary, GIT infections and CNS infections; <u>in HSCT patients rare.</u>
B	3, 7, 11, 14, 16, 21, 34, 35, 50	Respiratory, eye, urinary, GIT and CNS infections.
C	1, 2, 5, 6	<b>Respiratory, urinary and GIT infections – hepatitis too.</b>
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; <u>in HSCT patients rare.</u>
F	41	GIT infections; in patients after <u>HSCT rare.</u>
G	52	GIT infections.

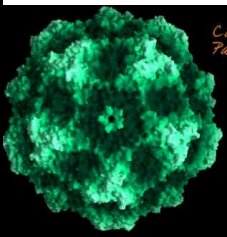
Rozdělení adenovirových infekcí do skupin (upraveno dle Fields Virology 5th edition, Kapitola 63).





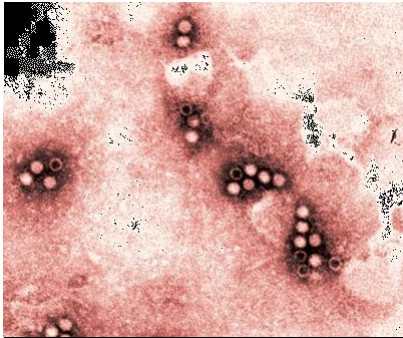


## Parvovirus B19

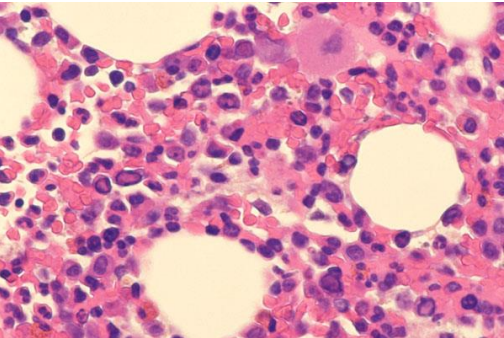


<http://fa.ammc.edu.ar/biologia/virologia/images/virolo6.jpg>

- small non-enveloped ss DNA +/-
- capsida in diameter 20-26 nm, genome: 5 kbp
- proliferation 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...
- **Fifth exanthematic disease** (see lecture)

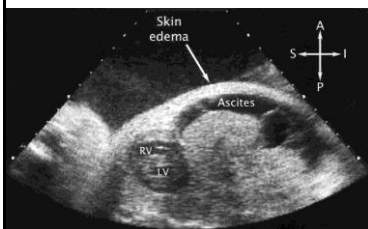


<http://www.wadsworth.org/databank/hircz/grady2.gif>



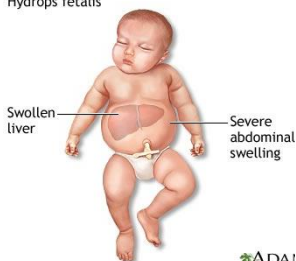
<http://www.yamagiku.co.jp/pathology/image/210/1.jpg>

# Parvovirus B19



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

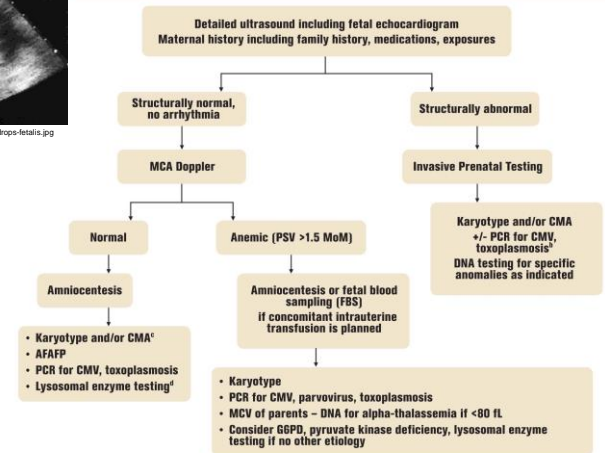
Hydrops fetalis



ADAJ

<http://www.abbeyardim.com/wp-content/uploads/non-immune-hydrops-fetalis.jpg>

## Work-up of nonimmune hydrops fetalis\*

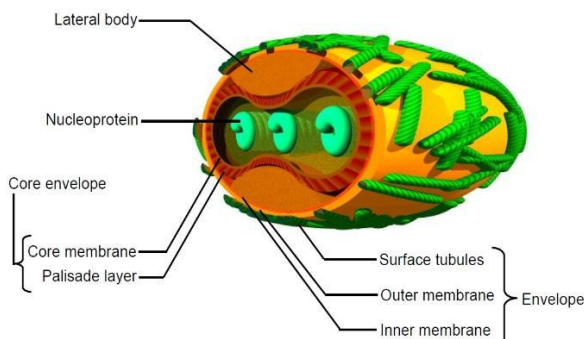


Source: SMFM. Nonimmune hydrops fetalis. *Am J Obstet Gynecol*. 2015.  
 \*Assuming negative antibody screen and normal indirect Coombs to rule out alloimmunization; \*CMV/toxo testing if fetal anomalies suggestive of infection;  
 †Either amniocentesis or FBS; ‡Available in some laboratories.  
 Abbreviations: 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/d21bd24-656-4a92-903f-f2a7c4c1188/OBGYN0215\\_026\\_1.jpg](http://images.alfresco.advanstar.com/alfresco_images/HealthCare/2015/02/10/d21bd24-656-4a92-903f-f2a7c4c1188/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

Cut-away structure of a Poxvirus (e.g. Vaccinia)



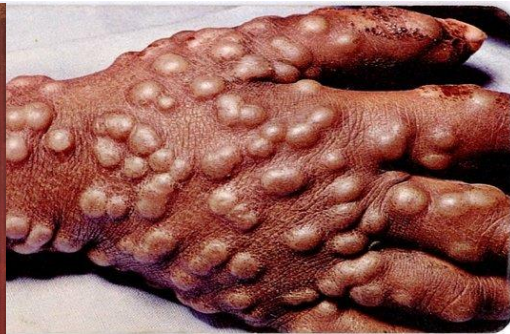
<http://cronodon.com/BioTech/Poxvirus.html>

## 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->



[http://www.wikihealth.com/wp-content/uploads/2014/07/rsz\\_smallpox.jpg](http://www.wikihealth.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://upload.wikimedia.org/wikipedia/commons/9/9f/Macaca_fascicularis.jpg)

[https://en.wikipedia.org/wiki/Monkeypox\\_virus#/media/File:Monkeypox.gif](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

(immunosuppressive treatment, chemotherapy, ...)



### Patogens

Lymfocyte regulated – viruses , mykoses



1. Prevention – vaccination
2. Decrease of immunosuppressive therapy
4. Improvement of lymphocyte function
6. Improvement/adding specific lymphocytes

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


## 1. Prevention - Vaccination

TBE  
Influenza  
Rotaviruses  
Human papillomaviruses  
Hepatitis A

Travel and special vaccin  
Lyssa  
Yellow fever  
...


**CHŘÍPKOVÉ VAKCÍNY**  
Dnes jediná vysoce účinná prevence chřípky

celovirionové vakcíny




obsahují kompletní viry

vakcíny typu „split“



obsahují virové částice ve vysoce purifikované formě

subjednotkové vakcíny



obsahují pouze purifikované HA a NA antigeny

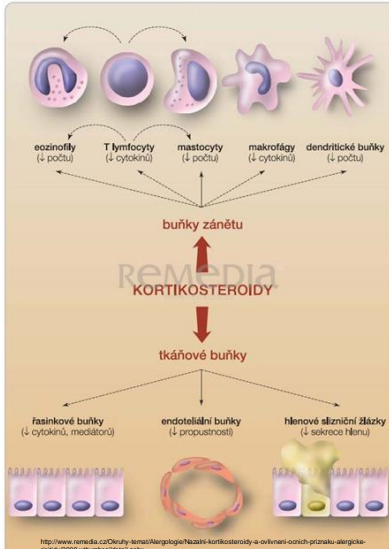
Vakcíny sezónní i pandemické s adjuvantním prostředkem nebo bez něj, injekční do svalu či kůže nebo ve spreji na sliznici nosní

## 2. Decrease of the immunosuppression treatment intensity

Autoimmune disease  
Transplant patients  
iatrogenic immunosuppression

**STEROIDS**  
> 2 mg/kg leads to lymphopenia

„BIOLOGIC TREATMENT“  
infiximab (anti TNF- $\alpha$ )  
basiliximab (anti CD25 –  $\alpha$  řetězec IL-2R)  
Campath (anti CD-52)  
Antithymocytární globulin (ATG) ...



**KORTIKOSTEROIDY**



buňky zánětu  
tkáňové buňky

eozinofily (+ pociť)    T lymfocyty (+ cytokiny)    mastocyty (+ pociť)    makrofágy (+ cytokiny)    dendritické buňky (+ pociť)

plazmatické buňky (+ cytokiny, mediátor)    endoteliální buňky (+ propustnost)    hlenové slizniční žlázy (+ sekrece hlenů)



### 3. Decrease of viral proliferation

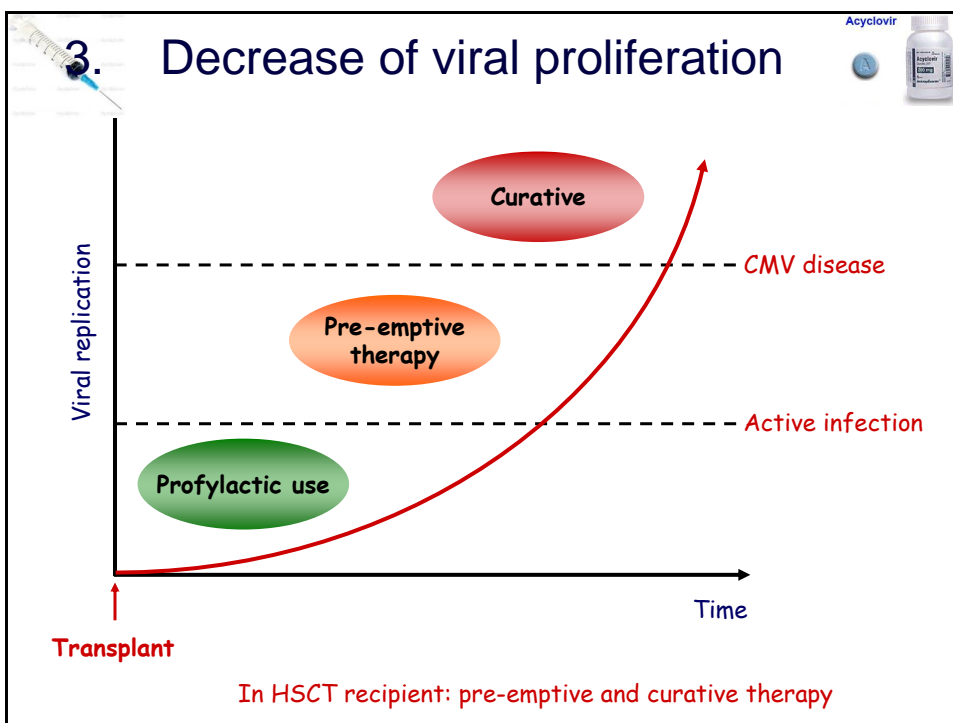



<p><b>Aiming proliferation important viral genes</b></p> <p>DNA/RNA polymerase herpesviruses, AdV</p> <p>Reverse transkriptase Protease</p> <p>Neuraminidase</p>	<p><b>Antibodies against permissive cells</b></p> <p>anti CD20 - rituximab</p>	<p><b>Neutralising antibodies</b></p> <p><b>Profylactic prevention</b></p> <p>motavizumab</p> <p>palivizumab (Synagis)</p> <p>Humanised neutralizing antibody against F- protein of RSV</p>
--	--	---

**HCV, HBV, HIV**

**herpesviruses**

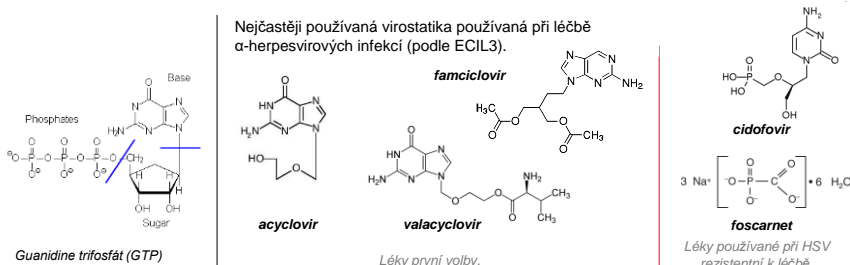
Huang K et al. J. Virol. 2010;84:8132-8140



# 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..)



### 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).

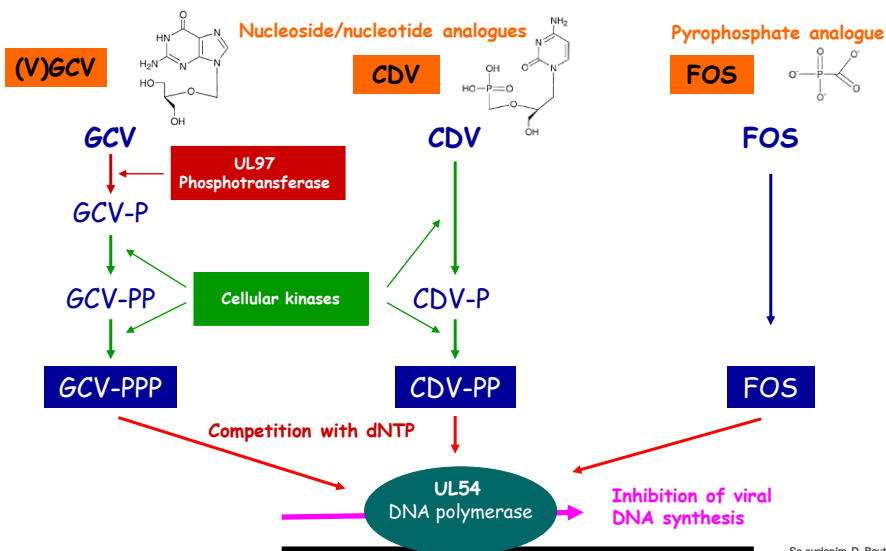
### Anti CD-20

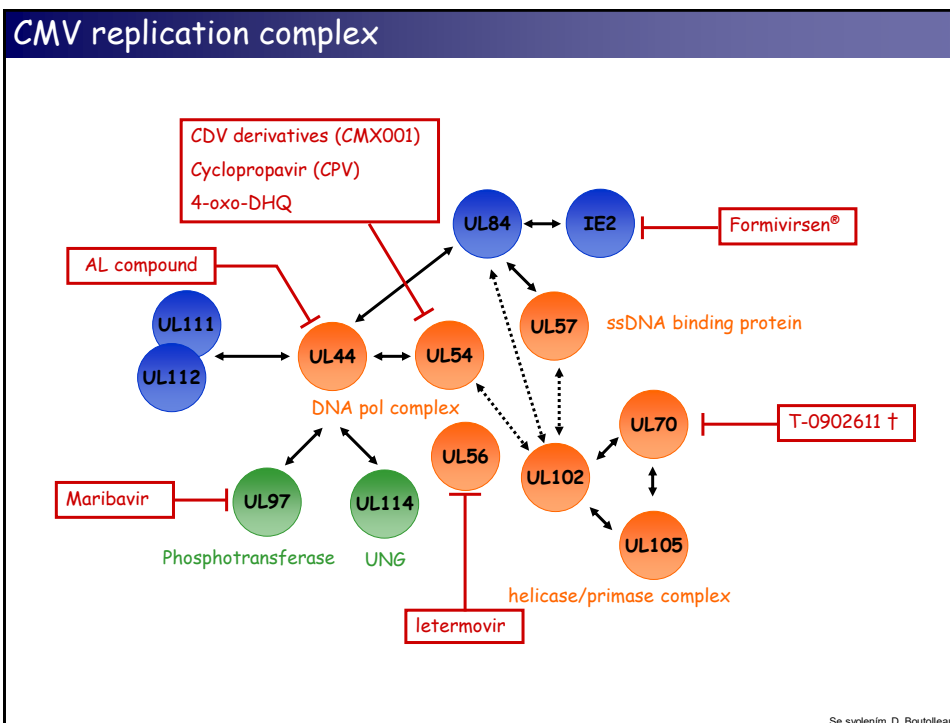


<http://www.curavys.com/images/mgr/B.jpg>

## 3. Decrease of viral proliferation

### → Inhibitors of CMV DNA polymerase UL54





### 3. Decrease of viral proliferation

#### Inhibition of herpesviruses and the possible resistance

HSV, VZV Thymidine Kinase

ACV → ACV - mono P → ACV - PPP → Inhibition of viral DNA polymerase

PEN → PEN - mono P → PEN - PPP → Inhibition of viral DNA polymerase

GCV → GCV - mono P → GCV - PPP → Inhibition of viral DNA polymerase

CMV UL97

Drugs bypassing monophosphorylation pathway: Foscarnet, Cidofovir, Trifluorothymidine

HSV, VZV drug resistance due to viral enzyme alterations at positions 1 and 3  
CMV drug resistance due to viral enzyme alterations at positions 2 and 3

ACV = aciclovir PEN = penciclovir GCV = ganciclovir P = phosphate

[http://biology.kerryon.edu/stenc/bc33stanckl\\_02acyclovir\\_fig2.JPG](http://biology.kerryon.edu/stenc/bc33stanckl_02acyclovir_fig2.JPG)

Acyclovir (ACV)

extracellular intracellular

activation by phosphorylation

thymidine kinase

Acyclovir-MP

GMP kinase

Acyclovir-DP

cellular phosphatases

Acyclovir-TP

viral DNA polymerase

competitive inhibition

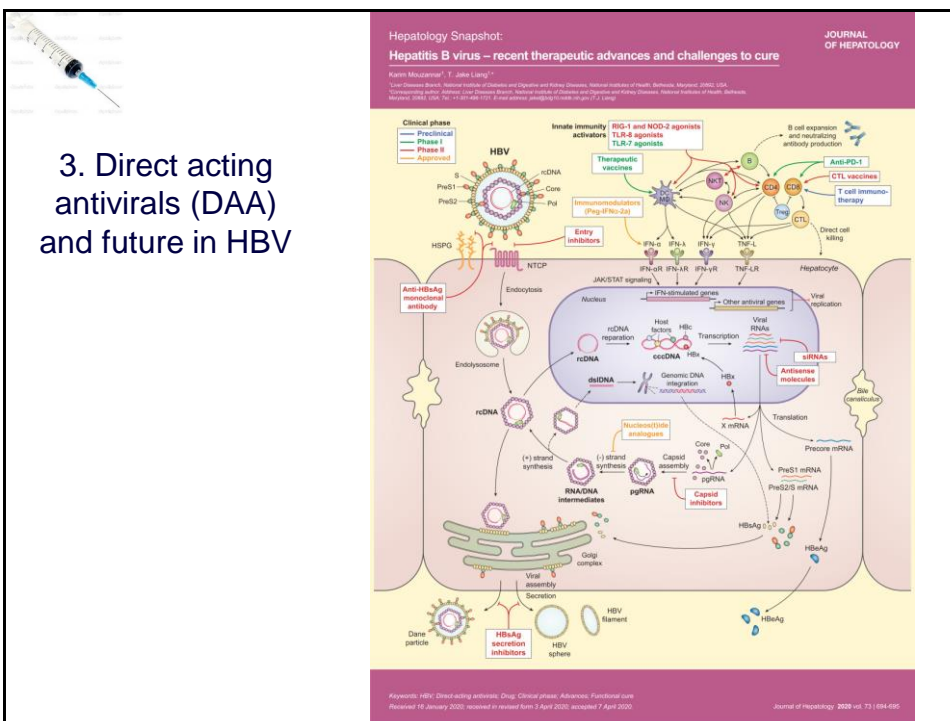
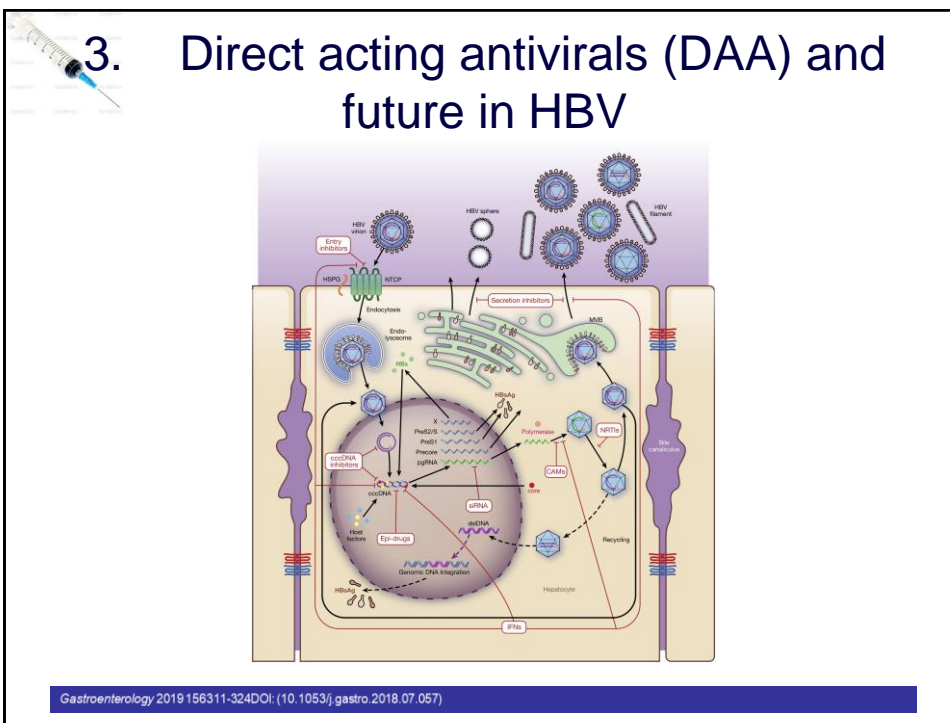
deoxy-guanosine triphosphate (dGTP)

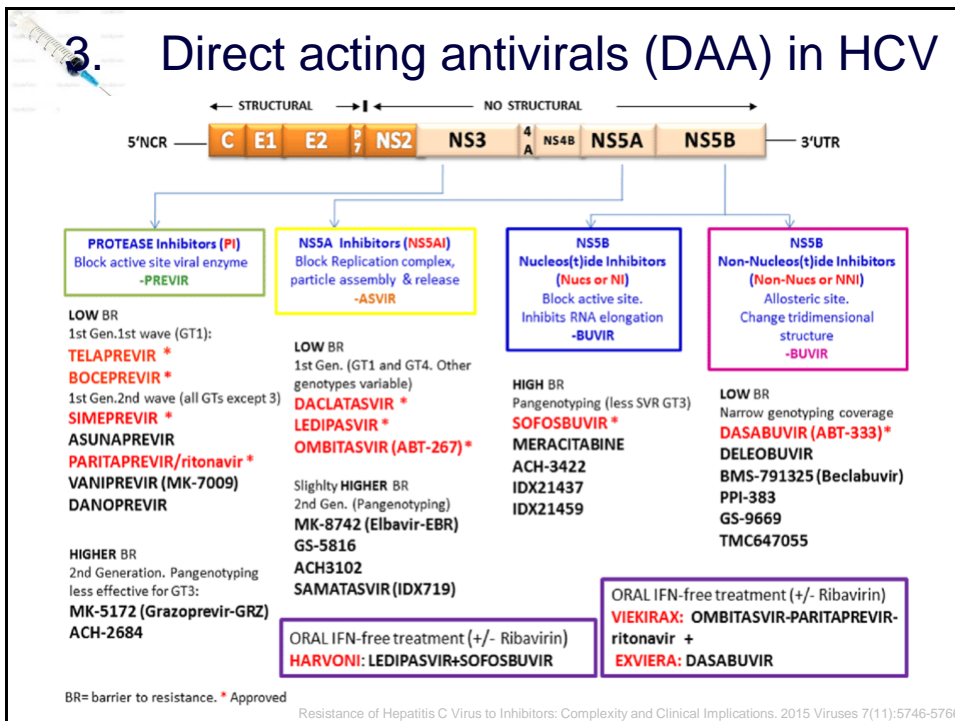
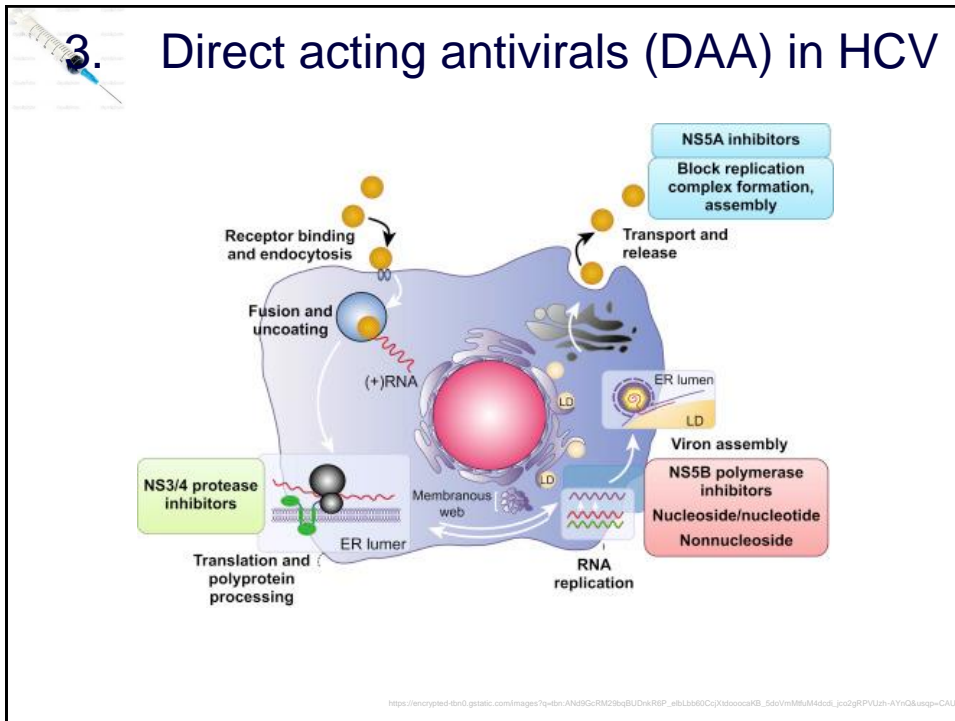
nonfunctional complex

DNA chain termination

template primer

phosphate strand ends (nucleotides)





**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).

**ADCC**

**Direct apoptosis**

**Fc<sub>γ</sub>R/CR mediated phagocytosis or ADCC**

**CDC**

**Rituximab**

**CD20**

**Fc<sub>γ</sub>R**

**Macrophage**

**B Cell**

**NK cell**

**Complement receptor**

**Src**

**Lipid raft**

**C3**

**C3b**

**Fc<sub>γ</sub>C**

<http://www.courtesy.com/images/img18.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<sup>2</sup>/dose in infusion for 60 minut twice daily with maximum 750 mg/dose
  - **Therapeutical dosing** – for 7–10 days  
250 mg/m<sup>2</sup>/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 physiological 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 dni.
  - **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.

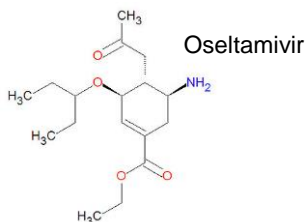


## Adverse effects of the virostatic drugs

- **Acyclovir/valaciclovir**
  - **AE usually reversible**, usually in patients with hepatopathy.
  - rarely haematopoietic and lymphatic system disorders (anaemia, leucopenia, thrombocytopenia), hepatitis, nephrotoxicity.
- **Ganciclovir/valganciclovir**
  - **myelosuppressive 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), potentially metabolic acidosis and diabetes insipidus
  - Increase of the liver enzymes, LDH, ALP and amylase; often nauzea, vomiting 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 ciprofloxacin administration
  - Headache, tiredness, paresthesia, tremor, ataxia. Neuropathy, hypestazia, confusion, depression, psychosis, agresive reactions, psychosis, agresive reactions; changes in ECG, hyper- hypotension, rarely even chamber arhythmias
  - Often Phlebitis (thrombophlebitis) in administration 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
  - potentially to **chronic renal failure** with dialysis
  - other more common neutropenia, headache, nauzea, vomiting, alopecia, rash, weakness and fever. Described also ocular toxicity.
- **Oseltamivir**
  - most frequent AE are nausea, vomiting 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....



[http://en.ck12.com/organic/chem/1188/Oseltamivir\\_structure.jpg?500x-Oseltamivir\\_structure.jpg](http://en.ck12.com/organic/chem/1188/Oseltamivir_structure.jpg?500x-Oseltamivir_structure.jpg)

[http://www.sigmaaldrich.com/catalog/images/structures/1188/Ribavirine\\_structure.jpg?500x-Ribavirine\\_structure.jpg](http://www.sigmaaldrich.com/catalog/images/structures/1188/Ribavirine_structure.jpg?500x-Ribavirine_structure.jpg)

## 4. Improvement of the lymphocyte function

### Antibodies

**ADCC**  
– antibody dependent cellular cytotoxicity

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

<http://img.mf.cz/014656a.jpg>

## 4. Improvement of the lymphocyte function

### Interferon $\alpha$

Používá se zejména při léčbě hepatitidy B.

Na trhu několik přípravků lišících se typem interferonu I –  $\alpha 2a$

Např. (rekombinantní Roferon A),  $\alpha 2b$  (rekombinantní - INTRON A), případně pegylované interferony tj. s polyetylglykolem (Pegasys™, PEG-INTRON)

Dávka: obvykle 2,5 - 5,0 milionů IU/m<sup>2</sup>, resp. až 10 milionů IU/m<sup>2</sup> u dětí s.c., 3x 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-  $\alpha 2a$  v dávce 180  $\mu\text{g}$ /týden v jedné dávce s.c.; délka léčby dle odpovědi na léčbu - při dobré odpovědi trvá 48 týdnů.

## 4. Improvement of the lymphocyte function

**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.

**Interferon  $\alpha$**

<http://dx.doi.org/10.1002/hep.21904>

## Therapeutical possibilities of virostatics and specific antibodies

More or less specific for certain viral groups:


	Virus Family	Virus	Antiviral	Antibody
ds DNA	<b>Herpesviridae</b>	$\alpha$ HSV, VZV	ACV, VACV, FCS...	
		$\beta$ CMV, HHV-6 a 7	GCV, VGCV, FCS, CDV, MBV, AIC246..	
		$\gamma$ EBV,	Anti CD-20	
	<b>Adenoviridae</b>	Adenoviruses (group A-F)	CDV, MBV...	
	<b>Polyomaviridae</b>	BKV, JCV, WUV, KIV, SV40...		
ss RNA	<b>Orthomyxoviridae</b>	Influenza A, Influenza B	Osetamivir, zanamivir, (rimantadine amantadine)....	
	<b>Paramyxoviridae</b>	Paramyxovirus	PIV	Ribavirine
		Morbillivirus		
	<b>Coronaviridae</b>	Pneumovirus	RSV, hMPV	Palivizumab, motavizumab, ribavirine
	<b>Picornaviridae</b>	Enteroviruses		remdesivir, molnupiravir, nirmatrelvir/ritonavir, ...
		Rhinovirus	HRV	
	<b>Caliciviridae</b>	Human caliciviruses		
ds RNA	<b>Astroviridae</b>	Norovirus		
	<b>Rhabdoviridae</b>	Lyssa-virus		
	<b>Reoviridae</b>	Rotavirus, Orbivirus		

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  
ALEXIA ABILETTI  
CID 2013

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

J. Cases<sup>1</sup>, K. Morris<sup>1</sup>, M. Narayani<sup>1</sup>, M. Nakagaki<sup>2</sup> and GA Kennedy<sup>3\*</sup> BMT 2011



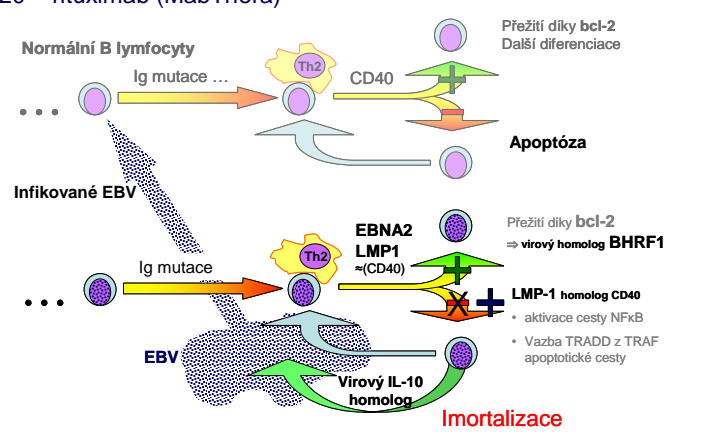
## 5. Destruction of permissive cells



Acyclovir

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

Anti CD-20 – rituximab (MabThera)



**Normální B lymfocyty**

Ig mutace ... → Th2 → CD40 → Přežití díky bcl-2  
Další diferenciace

Apoptóza

**Infikované EBV**

Ig mutace → Th2 → EBNA2, LMP1 ≈(CD40) → Přežití díky bcl-2  
⇒ virový homolog BHRF1

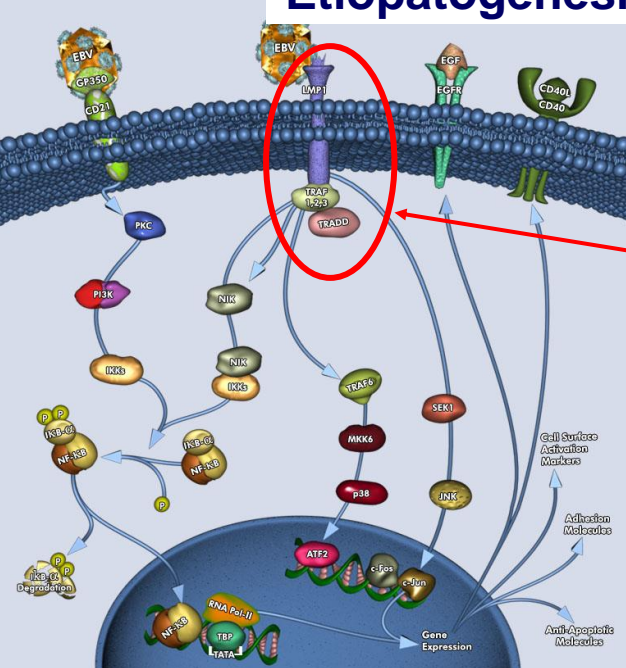
EBV → Virový IL-10 homolog → Imortalizace

LMP-1 homolog CD40

- aktivace cesty NFkB
- Vazba TRADD z TRAF apoptotické cesty

### NF-κB Activation by EBV

## 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



Cell Surface Activation Markers

Adhesion Molecules

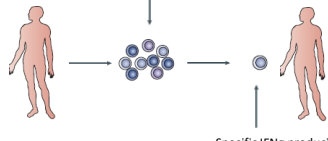
Anti-Apoptotic Molecules

Gene Expression

## 6. Present possibilities of specific cell production and its limitations

Viral antigen stimulation



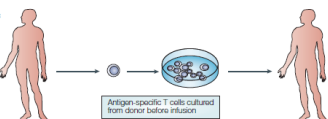
Non-specific T cells, technically and financially difficult  
ATMP (advanced therapeutic medicinal product)

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

Specific IFN $\gamma$  producing T lymphocyte selection

Feuchtinger et al BJH 2006  
Feuchtinger et al Blood 2010

**c**



Antigen-specific T cells cultured from donor before infusion

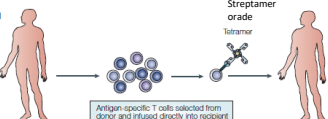
4-12 weeks of production, ATMP (advanced therapeutic medicinal product)

So far - for prophylactic use only!

Literatura:

Leen et al Nature Medicine 2006  
Leen et al Blood 2009

**d**



Antigen-specific T cells selected from donor and infused directly into recipient

Streptamer orade Tetramer



HLA-string, technically and financially difficult

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

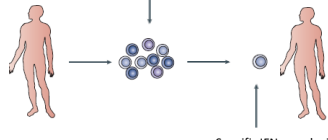
Cobbold et al J. Exp. Med. 2005  
Schmitt et al Transfusion 2011

Moss P and Rickinson A Nature Reviews 2005 (5)

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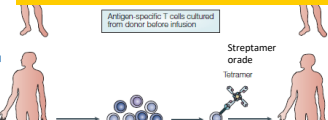
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Feuchtinger et al Blood 2010

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

**c**



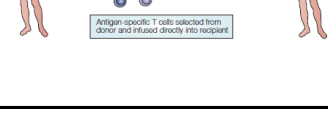
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Unrelated donors: preparation about 8-9 weeks from first demand to product

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Schmitt et al Transfusion 2011

Moss P and Rickinson A Nature Reviews 2005 (5)

**However – for success of the therapy is still crucial ...**



**... reconstitution of immunity!**

**Thank you for you attention**



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