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RegaVir platform: Case discussions antiviral resistance testing

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Rega Institute for Medical Research

Laboratory of Virology & Chemotherapy

48-years old women

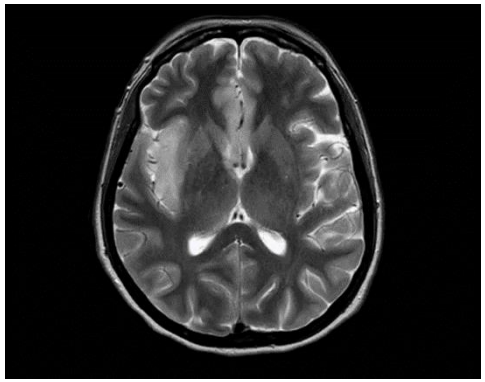
- **Past history:**
  - grade B gastritis and esophagitis treated with PPI
  - right breast mastectomy (benign cyst), hormonal implant right arm.

- Belgian-Belgian, married, one daughter. Teacher.

- **Family history:**
  - Father died of cirrhosis of unknown origin.
  - Sister died of idiopathic pulmonary fibrosis.
  - Sister died of sepsis in Staphylococcus aureus.

Development of encephalitis

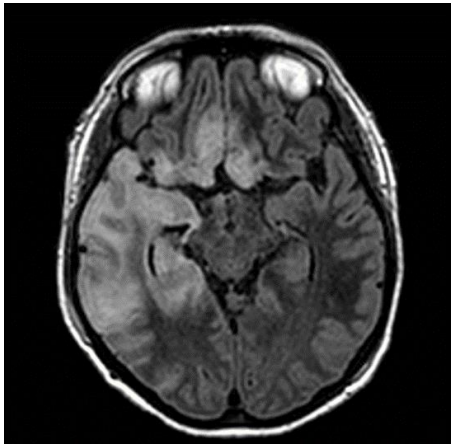
02/02/2022



Mainly temporal right, but also bilateral internal frontal involvement, with massive cortical and hypersignal thickening.

Radiological aspect quite compatible with herpetic encephalitis.

11/02/2022



- **Suggestive of herpetic encephalitis (HSV-1)**.
- Alternative diagnoses may be other viral encephalitis including HHV-6, VZV or EBV; the hypothesis of limbic encephalitis seems unlikely in the absence of antecedents

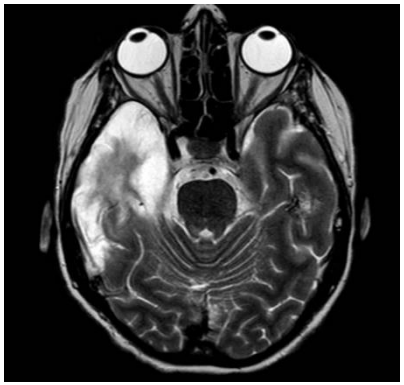
ICU Erasme 18/02-12/04

- **Persistence neurological impairment without a clear explanation.**
- MRI 01/03: Necrotic conversion of right temporal involvement. Appearance of a right parietal and temporal ischemic cortical involvement
- **High-dose corticosteroid therapy (01-03/3) for suspected post-viral autoimmune encephalitis (at HSV1)**

Blood EDTA 03/03 : quantitative PCR HHV6 > 10x10^6 UI/mL

Little neurological response & new state of epilepticus after the start of treatment.

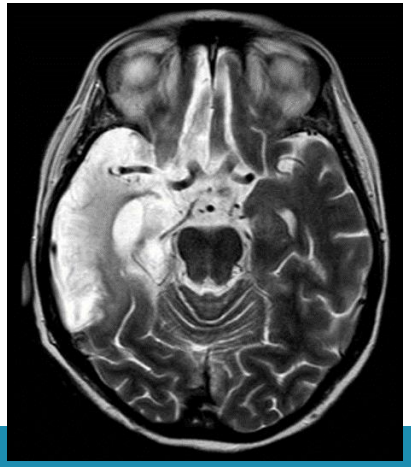
Encephalitis not responding to antiviral therapy



09/03/2022

Increased encephalitis lesions in the corpus callosum. Extensive necrosis of the right temporal parenchyma and diffuse hemorrhagic cortical laminar necrosis, better visible compared to the previous examination.

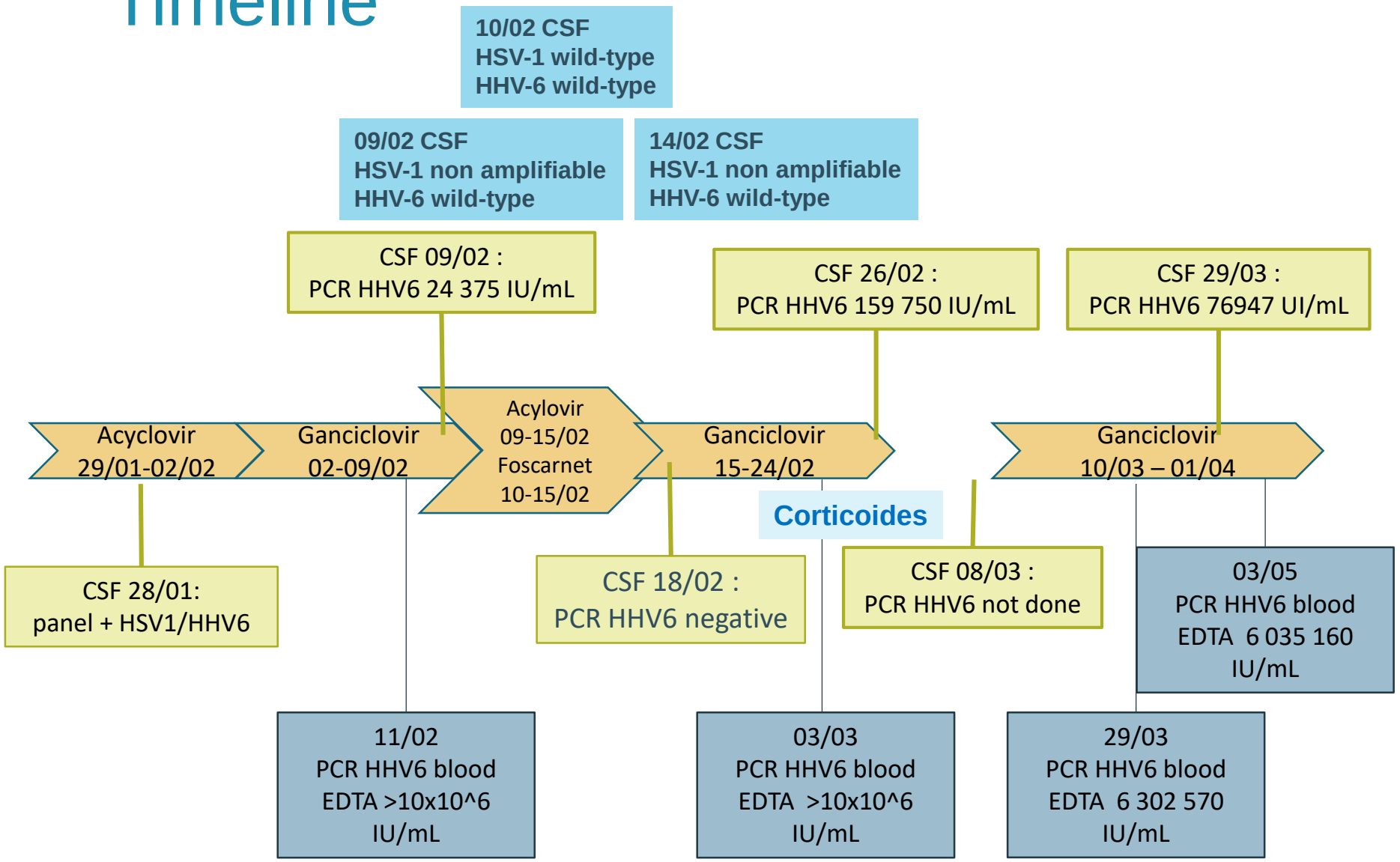
Persistence comatose state and management of complications
(epilepsy, iatrogenic pneumothorax, ionogram disorders)



28/03/2022

Increased extent of known encephalic lesions and associated necrosis of the right temporal parenchyma.

Timeline


10/02 CSF
HSV-1 wild-type
HHV-6 wild-type
09/02 CSF
HSV-1 non amplifiable
HHV-6 wild-type
14/02 CSF
HSV-1 non amplifiable
HHV-6 wild-type
CSF 09/02 :
PCR HHV6 24 375 IU/mL
CSF 26/02 :
PCR HHV6 159 750 IU/mL
CSF 29/03 :
PCR HHV6 76947 UI/mL
Acyclovir
29/01-02/02
Ganciclovir
02-09/02
Acylovir
09-15/02
Foscarnet
10-15/02
Ganciclovir
15-24/02
Ganciclovir
10/03 – 01/04
Corticoides
CSF 28/01:
panel + HSV1/HHV6
CSF 18/02 :
PCR HHV6 negative
CSF 08/03 :
PCR HHV6 not done
03/05
PCR HHV6 blood
EDTA 6 035 160
IU/mL
11/02
PCR HHV6 blood
EDTA >10x10^6
IU/mL
03/03
PCR HHV6 blood
EDTA >10x10^6
IU/mL
29/03
PCR HHV6 blood
EDTA 6 302 570
IU/mL

Case study

Date of sample arrival	Original ID sample	RegaVir ID	Date of sample collection	Type of sample	Herpesvirus genotyping
10/02/2022	SXC323	**RV-2436**	09 February 2022	CSF	**HHV-6B: wild-type** HSV-1, HSV-2, VZV, HCMV: not amplifiable
14/02/2022	59774397-9100	**RV-2439**	10 February 2022	CSF	**HHV-6B: wild-type** **HSV-1: TK wild-type & DNA pol R1019W (novel mutation)***
16/02/2022	SXC36951	**RV-2443**	14 February 2022	Blood EDTA	**HHV-6B: wild-type** HSV-1 & HCMV: not amplifiable

**** R2019W most likely linked to a genetic polymorphism since R1019G is a known genetic polymorphism***

Evolution of the patient

12/04

Favorable evolution from a neurological point of view and goes back to neurology

20/05

- **Awakened spontaneously**
- No verbal response, Blink as a sign of "yes"
- Smooth eye chase, blink at the threat of the 2 sides
- Left central facial paralysis
- Severe tetraparesis with 2-3/5 at MSD and 1/5 for the rest of the segments
- Unvaluable sensitivity

Case study summary

HSV1 herpetic meningoencephalitis

- in a 48-year-old
- **non-immunocompromised patient**
- **HHV6 persistent high viral load in blood EDTA**
- complicated by a **coma on epilepticus state**
- having been treated with **appropriate antivirals**
- but **secondary neurological degradation on possible autoimmune encephalitis** that justified corticosteroid therapy but without clinical response

Persistent HHV6 viral load in blood

- Blood EDTA 03/05 : PCR HHV6 quantitative : 6035160 UI/mL.

Hair follicles sent on 25/03/22 to the CHU Limoges (France) to perform HHV6 PCR

→ results obtained on 19/05/22: positive (Ct = 27.25 on hair follicle 1 & Ct = 29.06 on hair follicle 2)

→ presence of integrated HHV6 DNA

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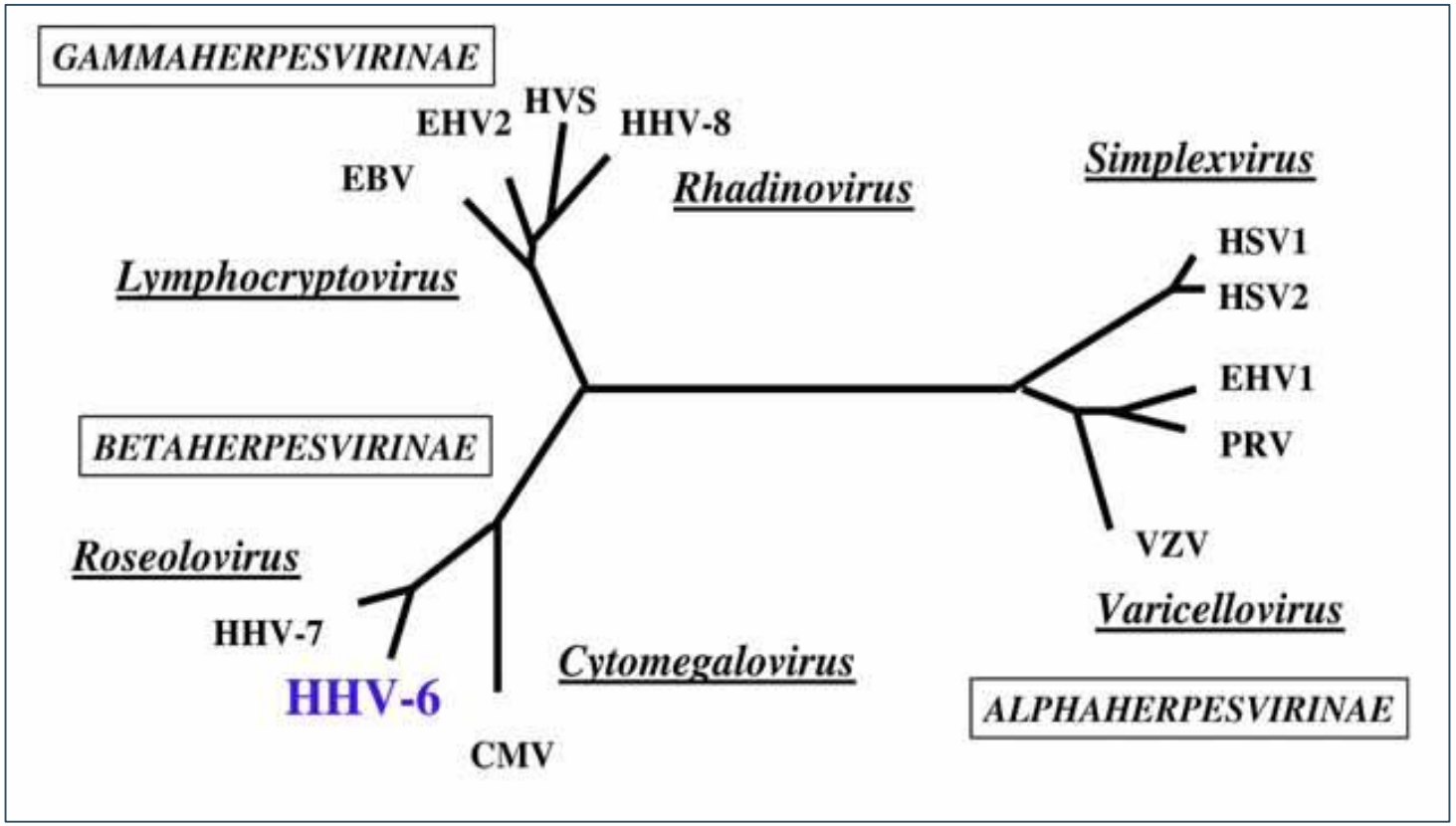
HHV-6

HHV-6A & HHV-6B

- Isolated by accident from T-cell blood cultures from patients with lymphoproliferative disorders:
  - ✓ 1986 (HHV-6A)
  - ✓ 1988 (HHV-6B)
- Initially considered to variants from a single virus
- In 2014, classified as 2 separate virus species

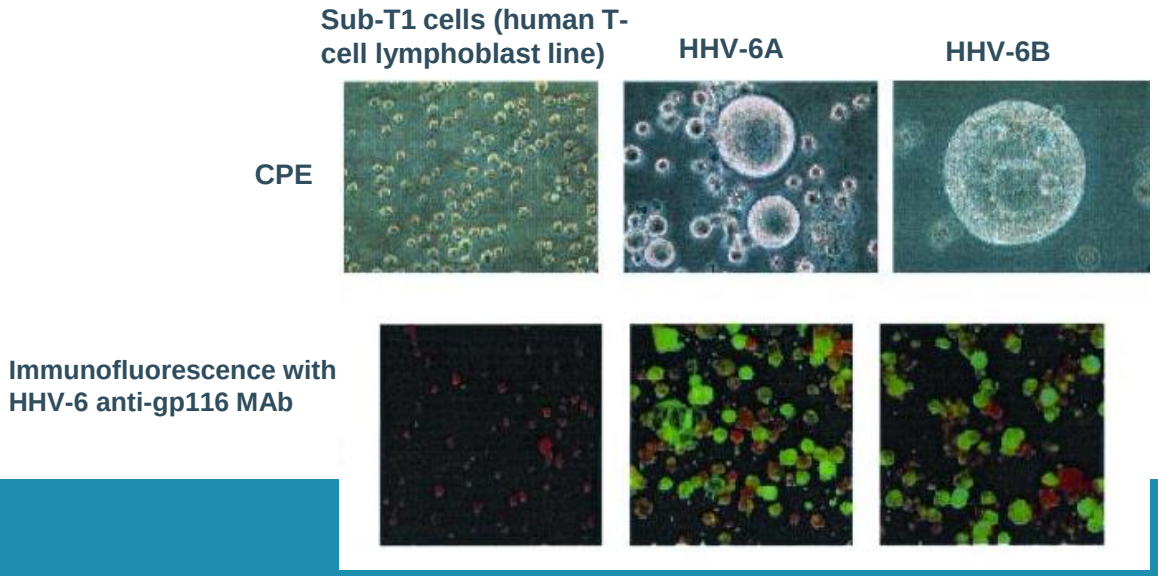
- Closely related to HHV-7 (discovered in 1990)
- HHV-6A, HHV-6B & HHV-7 are members of the *Roseolovirus* genus, β-herpesvirus subfamily

Herpesvirus classification



HHV-6A & HHV-6B

- Classified as two distinct herpesvirus species based on their distinctive biological properties, genome sequences, epidemiology, growth properties, antigenic properties, and restriction endonuclease profiles
- The nucleotide sequence identity between the two variants ranges from 75 to 95 percent depending upon which gene is compared



Mayne et al, 2001

HHV-6A & HHV-6B tropism

- **HHV-6A & HHV-6B infect many cells in vivo:**
  - ✓ CD4+ T cells but also CD8+ T cells (HHV-6A)
  - ✓ Monocytes-macrophages
  - ✓ Hematopoietic cells of the bone marrow
  - ✓ Epithelial cells of the kidney and salivary glands
  - ✓ Endothelial cells
  - ✓ Neural cells: oligodendrocytes, astrocytes, and microglial cells

HHV-6A & HHV-6B cell receptor

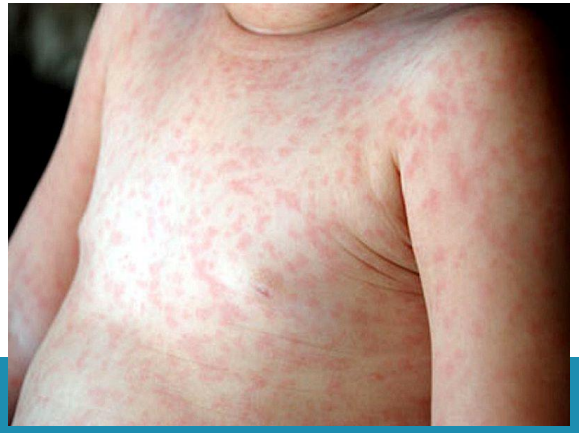
- **Cell receptor:**
  - ✓ **HHV-6A: CD46 molecule** (a regulator of complement activation expressed on all nucleated cells)
  - ✓ **HHV-6B: CD 134 molecule** (a member of the tumor necrosis factor (TNF) receptor superfamily resent only on activated T lymphocytes

HHV-6 epidemiology

- **Infection**
  - ✓ Ubiquitous virus
  - ✓ Highly prevalent: >90% in adult population
- **Transmission**
  - ✓ **Horizontal** through close contacts: **saliva**
  - ✓ Transplacental route
  - ✓ Vaginal delivery
  - ✓ Organ transplantation
  - ✓ Not demonstrated with breast feeding
  - ✓ Not demonstrated with blood transfusion
  - ✓ **Vertical** through chromosomally integrated HHV-6 genome (rare event)

HHV-6 pathophysiology

- **Primary infections** early in life (between 6 months and 2 years of age) following the loss of maternal antibodies
- **HHV-6B** primary infections are **often asymptomatic**, although the virus ***Roseola Infantum*** **(exanthema subitem or 6th disease)** in ~30% of children, presenting with high-grade fever followed by a characteristic rash along the trunk, neck, and face
- **Complications of exanthema subitem** include benign febrile convulsions and rarely status epilepticus



HHV-6 physiology

- **HHV-6A** less prevalent than HHV-6B
- **HHV-6A** casual role in disease and route of transmission remains poorly understood

- **Latent infections** in in CD34+ hematopoietic stem cells, monocytes, and macrophages
- **Persistent infection** in salivary glands
- **Reactivations:** presence of the virus in saliva & blood
- **Chromosomal integration** (<1% of infections)

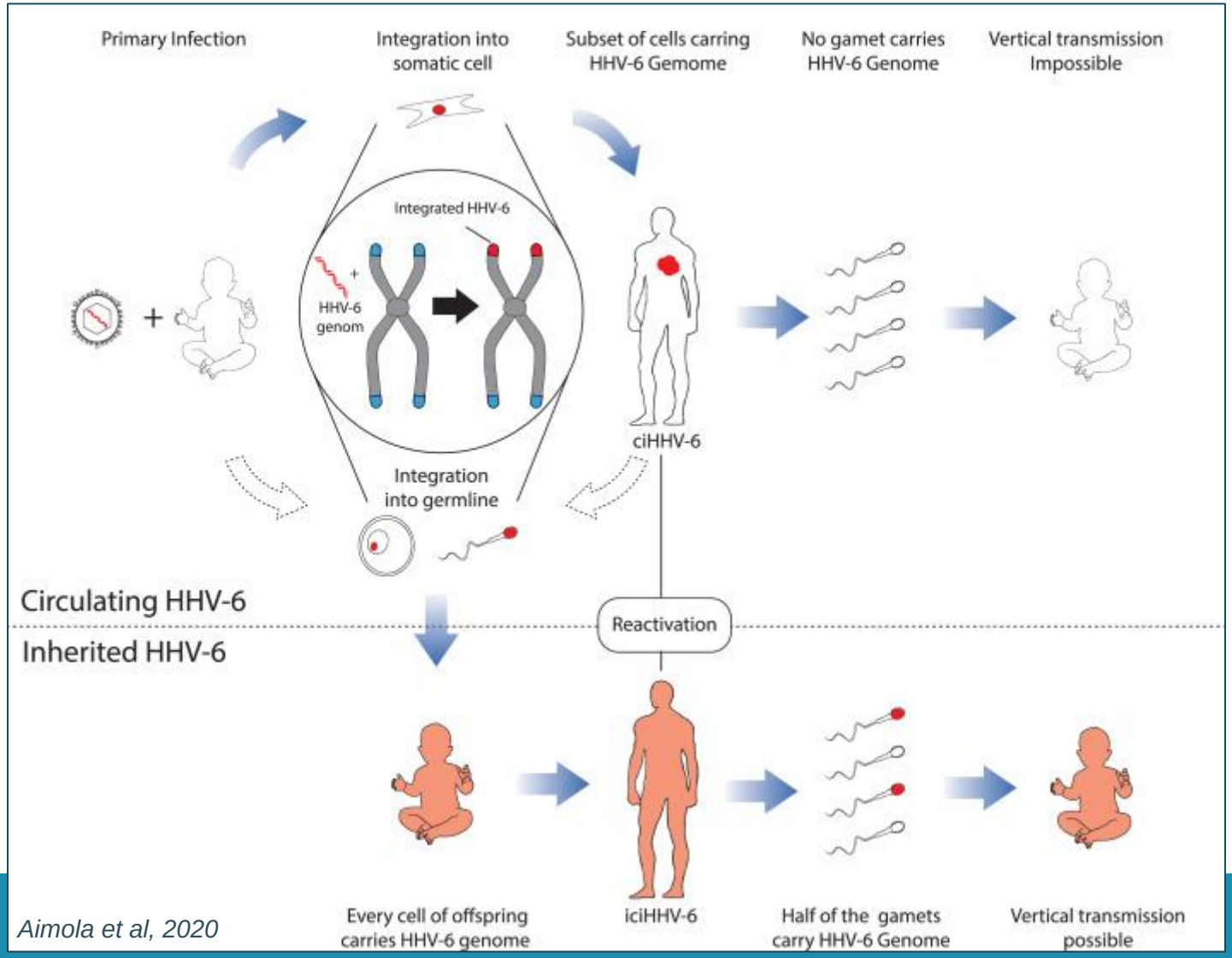
What is chromosomally integrated HHV-6 (ciHHV-6)?

- Most herpesviruses maintain their latent genome as a circular episome in the nucleus of latent cells

- However, HHV-6A or HHV-6B can integrate their genomes in latently infected cells without detection of a circular episome

- "Inherited chromosomally integrated HHV-6" (iciHHV-6) — sometimes referred to just as ciHHV-6 — is an inherited condition in which the complete HHV-6 genome is **integrated into the telomere of every chromosome** and is transmitted in a Mendelian manner

Chromosomally integrated human herpesvirus 6 (ciHHV-6)



Aimola et al, 2020

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Chromosomally integrated human herpesvirus 6 (ciHHV-6)

- **Found in <1% of controls in the USA and UK** (varies from 0.2% to 2.9%, depending on the population and region investigated)
- Transplant recipients with ciHHV-6 may be at increased risk for bacterial infection, graft rejection and developing GvHD

- HHV-6A/B carry telomeric repeat (TMR) arrays identical to the host telomere sequences at the ends of their genomes
- HHV-6A/6B TMR facilitate integration likely mediated by virus and host factors

- The integrated HHV-6 genome can reactivate resulting in virus replication

Why does ciHHV-6 matter?

- The high HHV-6 DNA load in patients with ciHHV-6 can lead to misdiagnosis due to the incorrect assumption that the patient is experiencing active HHV-6 infection for which antiviral treatment might be warranted

- A hypothetical risk is that clinical or environmental exposure to certain drugs and chemicals may inadvertently activate the virus in patients with ciHHV-6

When should chromosomally integrated human herpesvirus 6 screening take place?

- No pathology has been conclusively associated with ciHHV-6 → **routine screening is not recommended**

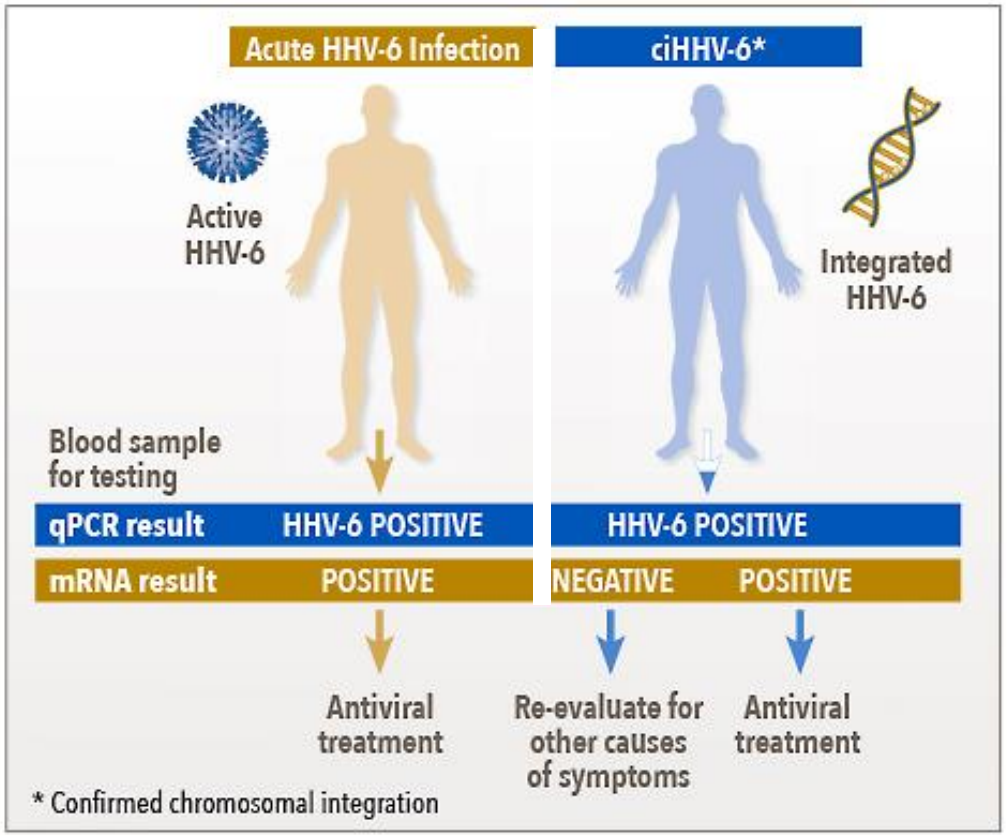
- However, **screening for ciHHV-6 should be considered:**
  - ✓ When there is clinical suspicion for HHV-6 reactivation and patient's ciHHV-6 status would influence treatment decisions
  - ✓ For patients who have had an adverse reaction to a drug previously shown to be associated with HHV-6 reactivation

ciHHV-6 Testing

- **Whole blood qPCR DNA test** (most practical method)
  - If >500,000 copies/ml in absence of acute illness → patient has ciHHV-6.
  - Attention! viral load of a non-ciHHV-6 individual can be >500,000 copies/ml when the patient is extremely ill (encephalitis or acute GVHD or extreme drug hypersensitivity (DIHS/DRESS).
- **Testing the patient's parents** (at least one of the two would have a high positive result) to confirm suspicious ciHHV-6
- **FISH (fluorescence in situ hybridization)** can confirm integration.
- **Fingernails or hair follicles qPCR test** - only ciHHV-6 individuals would have a positive PCR test on a fingernail or hair follicle.

ciHHV-6 risk for misdiagnosis

- The high HHV-6 DNA load in patients with ciHHV-6 can lead to misdiagnosis



HHV-6 reactivation

- Frequent in immunocompromised patients
- ~ 50% of HSCT and ~ 33% of SOT reactivate HHV-6 as defined by detection of viral DNA in the peripheral blood
- Reactivation usually occurs within the first months of transplant

- Reactivation is increased with:
  - **reduced cellular immunity**, particularly in patients receiving anti-CD3 antibody or corticosteroids
  - **in allogeneic or cord blood transplants**
- Reactivation occurs also in critically ill patients who are not otherwise immunocompromised (54% of the patients)

HHV-6 reactivation

- Clinical symptoms associated with HHV-6 reactivation in Tx recipients appear to occur in a minority of patients but are involved in a wide spectrum of syndromes:
  - ✓ Non-specific:
    - Fever
    - Skin rash
    - Thrombocytopenia, leukopenia, anemia
  - ✓ Specific
    - Subacute limbic encephalitis
    - Bone marrow suppression, which may evolve to secondary graft failure

Typical opportunistic diseases due to HHV-6 reactivation in HSCT recipients

HHV-6 pathogenicity: diseases related to reactivation in IC patients

- Fever with skin rash and cytopenia
- Pneumonitis (HSCT patients, liver patients)
- Encephalitis (HSCT patients)
- Myelosuppression, delayed engraftment (HSCT patients)
- Retinitis (AIDS patients)
- Acute GVHD (HSCT patients)
- Graft rejection (kidney and liver transplant recipients)
- Drug induced hypersensitivity syndrome (DRESS)

Neurological conditions associated with HHV-6

- HHV-6 establishes latent infections in the CNS, especially in the hippocampus and amygdala & induces neurologic diseases
- HHV-6 was shown to invade the brain by the route of olfactory pathway, which is connected to the limbic lobe (Harberts et al, 2011)
- HHV-6 & Cognitive dysfunction
  - ✓ HHV-6 reactivation is the most common cause of mental confusion among post-transplant patients (Zerr 2011).
  - ✓ HHV-6 limbic encephalitis (occurring in ~ 1-4% of all transplant patients), often results in intermittent confusion, poor coordination, flat affect and somnolence.

Neurological conditions associated with HHV-6

- HHV-6 & epilepsy
  - ✓ Temporal lobe epilepsy (TLE) patients with HHV-6B in their brains suffer from reiterative attacks of febrile seizures and hippocampal sclerosis (Wang et al, 2021).
  - ✓ HHV-6B virus infection may play an important role in the pathogenesis of febrile seizures (FS), but the specific mechanism is unknown (Wang et al, 2021)
  - ✓ Neuroinflammation is increasingly recognized as a key component underlying epileptic disease pathogenesis (Vezzani 2014).

    - increased inflammatory pathway expression in HHV-6B-positive patients with mesial temporal lobe epilepsy (MTLE)

Neurological conditions associated with HHV-6

- HHV-6 and Status Epilepticus
  - ✓ Prolonged febrile seizures, or febrile status epilepticus (FSE), are associated with an increased risk of temporal lobe epilepsy (TLE) (Shinnar 2003, Leibovitch 2015).
  - ✓ HHV-6 encephalitis can present as status epilepticus, even in immunocompetent patients → **HHV-6 encephalitis should be considered among immunocompetent patients presenting with encephalitis and having signs of temporal lobe involvement** (Shahani 2014).
  - ✓ HHV-6 should be considered in the differential diagnosis of non-convulsive status epilepticus after allo-HSCT. Empirical antiviral therapy targeting HHV-6 should be administered to these patients (de Souza Franceschi 2014).

HHV-6 encephalitis

- **Primary HHV-6 infection** is occasionally accompanied by **diverse clinical forms of meningoencephalitis**.
- Some **encephalitis** cases associated with **HHV-6B primary infection have a poor outcome:** mortality or significant neurological sequelae.
- Encephalitis due to primary HHV-6B infection in children is **commonly reported from Japan**, but very rarely elsewhere in the world, suggesting a genetic predisposition.

- HHV-6B is the most common cause of encephalitis after allo-transplantation

HHV-6 & multiple sclerosis (MS)

- Conflicting evidence of a possible role of HHV-6 in MS
- MS is characterized by inflammation and demyelination of neurons
- MS has long been thought to have a viral etiology

- **HHV-6A (but not HHV-6B) linked to increased risk of multiple sclerosis**

  *Karolinska Institute researchers, in collaboration with a group from Heidelberg, developed a **novel serological assay to determine if individuals with antibodies to HHV-6A early proteins are more likely to develop multiple sclerosis**. HHV-6A antibodies were highest in the presence of elevated **EBV antibodies**, suggesting that the two viruses could jointly contribute to the development of multiple sclerosis.*

- **Latent HHV-6A may impair myelin repair in multiple sclerosis**

  *A group at University of Rochester demonstrated that the **HHV-6A latency gene, U94**, **inhibits migration of cells involved in myelin repair.** Inefficient myelin repair is associated with progression MS. The ability of HHV-6A to impede this process suggests that it could be involved in the progression of MS and raises questions about the virus's role in other chronic demyelinating diseases.*

HHV-6 & Alzheimer's disease

- **HHV-6 and HSV-1 dramatically accelerate beta-amyloid (Aß) plaque production in Alzheimer's model** (Eimer et al 2018, Neuron)
- Overactivated Aß deposition may result in heightened neuroinflammation, neuropathology, and neuronal death. Consequently, it is possible that amyloidosis triggered or exacerbated by herpesvirus infection may contribute to AD.

- **Increased HHV-6A and HHV-7 from subjects with AD compared with controls** (Readhead et al, 2018)

Strategies to manage HHV-6A & HHV-6B infections

- Routine screening of HHV-6 DNA in blood post-transplantation is not recommended
- Anti-HHV-6 prophylaxis or pre-emptive therapy is not recommended for prevention of HHV-6 reactivation
- No drugs are approved to treat HHV-6 infections

- HHV-6 can cause **morbidity and mortality among transplant patients**
- **HHV-6 in CNS diseases (Komaroff et al, 2021)**

Need to develop anti-HHV-6 drugs

Licensed anti-herpesvirus drugs

	DNA polymerase inhibitors					Terminase inhibitor
	Acyclovir Valacyclovir	Penciclovir Famciclovir	Ganciclovir Valganciclovir	Cidofovir	Foscarnet	Letermovir
HSV-1 (HHV-1)	1st line	approved		resistance	resistance	
HSV-2 (HHV-2)	1st line	approved		resistance	resistance	
VZV (HHV-3)	1st line	approved		resistance	resistance	
EBV (HHV-4)			off-label	off-label	off-label	
HCMV (HHV-5)			1st line	approved	approved	Approved for prophylaxis
HHV-6A			off-label	off-label	off-label	
HHV-6B			off-label	off-label	off-label	
HHV-7			off-label	off-label	off-label	
HSHV (HHV-8)			off-label	off-label	off-label	

Treatment of HHV-6 encephalitis

- IV foscarnet (90 mg/kg b.d. for foscarnet) or ganciclovir (5 mg/kg b.d.) are recommended. Drug selection should be dictated by the drug's side effects and the patient's comorbidities
- Antiviral therapy for at least 3 weeks and until clearance of HHV-6 DNA from blood and CSF
- Ganciclovir and foscarnet combination therapy should be considered
  - ✓ Retrospective case analysis of antiviral therapies for HHV-6 encephalitis after HSCT (Toomet et al): combination therapy with foscarnet and ganciclovir may reduce sequelae, but not mortality, secondary to HHV-6 encephalitis.
- Reduction of immunosuppressive medications if possible
- There are insufficient data on the use of cidofovir to make a recommendation.

Perspectives

- The development of specific therapeutics for HHV-6 has been hampered by the lack of a causative role of HHV-6 in diseases with high significant impact or morbidity

- The increasing interest on the pathological role attributed to HHV-6 in diseases of the CNS may boost the development of anti-HHV-6 agents

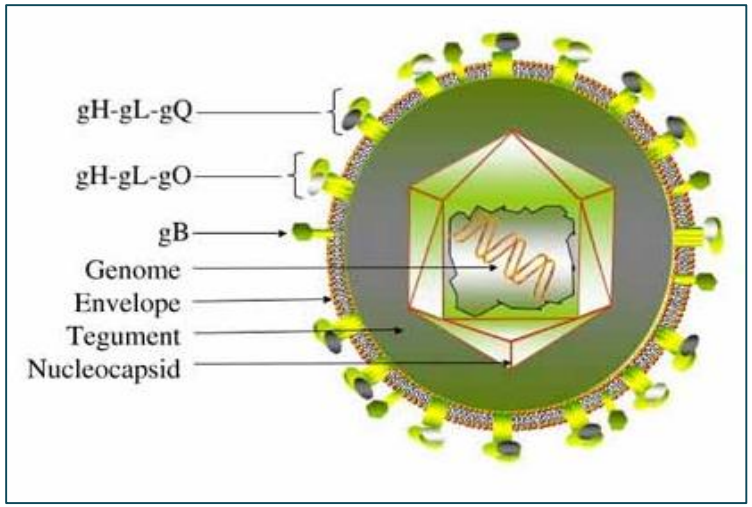
- **ciHHV-6 should be considered when high viral loads are present in blood and/or CSF to avoid misdiagnosis**

Human Herpesviruses

Subfamily	Biological properties
α-Herpesvirinae (HSV-1, HSV-2, VZV)	Fast growth and spread in cell cultures , short replication cycle, lytic infection in fibroblasts and epithelial cells Latent in sensory neuronal ganglia
β-Herpesvirinae (CMV, HHV-6A, HHV6B, HHV-7)	Slow grow in cell culture, long replication cycle Form enlarged (cytomegalic) cells Latent in myeloid lineage hematopoietic cells
γ-Herpesvirinae (EBV, HHV-8)	Lymphoproliferative Latent in B cells

HHV-6A & HHV-6B

- They share with other herpesviruses:
  - ✓ a similar virion structure
  - ✓ genomic architecture
  - ✓ ability to establish latency & reactivate



HHV-6 virion structure

Why does ciHHV-6 matter?

- Individuals with ciHHV-6 harbor the complete HHV-6 genome covalently linked to a human chromosome

  → whole blood, leukocytes, plasma, and tissue specimens have **high levels of HHV-6 DNA when tested by qPCR**

- Peripheral blood contains between 4 and 7 million leukocytes/ml (and a corresponding number of viral genomes/ml)

  → qPCR results: **>1x10^6 HHV-6 genomes/ml of whole blood** in ciHHV-6 patients

- **Body fluids harbor only small numbers of cells (serum, plasma, and CSF) & are often positive for HHV-6 DNA** as they contain DNA released by cell lysis

HHV-6 reactivation

- In HSCT recipients:
  - ✓ > 95% of HHV-6 reactivations are due to HHV-6B
  - ✓ HHV-6 reactivation is associated with high risk for developing life-threatening illnesses concerning the CNS and/or bone marrow
- In SOT recipients HHV-6 reactivates at different rates according to:
  - Transplanted organ
  - Nature of immunosuppressive therapy
  - Administration of prophylactic anti-HCMV treatment

- **Encephalitis** develops in a small proportion of patients experiencing HHV-6 reactivation

HHV-6 encephalitis in transplant recipients

- **HHV-6B reactivation** is well established as **causing limbic encephalitis after HSCT**, particularly after receipt of **cord blood** - outcome is poor and preventive strategies are ineffective.

Disease onset	**Usually 2-6 weeks post HSCT, but can be later**
Symptoms / signs	Confusion, encephalopathy, short-term memory loss, SIADH (syndrome of inappropriate antidiuretic hormone secretion), seizures, insomnia
Brain MRI	Often normal. Typically, but not exclusively, circumscribed, non-enhancing, hyperintense lesions in the medial temporal lobes (especially hippocampus and amygdala)
CSF	HHV-6 DNA, +/- mild protein elevation, +/- mild lymphocytosis pleocytosis
Prognosis	Memory defects and neuropsychological sequelae in 20-60%. Death due to progressive encephalitis is up to 25% of all HSCT and up to 50% of cord blood recipients

Therapies for HHV-6 are needed

- Therapeutic options in transplant recipients: **anti-CMV drugs**

	Antiviral activity: mean EC_{50} (range)		Adverse effects	Administration
	HHV-6A (µM)	**HHV-6B (µM)**		
Ganciclovir (GCV)	17 (2-25)*	4.5	Bone marrow suppression	IV Valganciclovir (VGCV): approved oral prodrug
Cidofovir (CDV)	4.7 (0.33-14)	6.5 (2.3-13)	Nephrotoxicity	IV Brincidofovir (BCV): investigational oral prodrug
Foscarnet (PFA)	23.4 (6.7-53)	50 (22-86)	Nephrotoxicity	IV

Values from Prichard and Whitley, Current Opinion in Virology, 2014

- **Viral DNA polymerase** – common target

- **Drug-resistance** – can be an issue

MoA and resistance mechanisms