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RegaVir platform: Case discussions antiviral resistance testing

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Clinical syndromes associated with human herpesviruses

	HSV-1	HSV-2	VZV	CMV	EBV	HHV-6	HHV-7	KSHV
Gingivostomatitis	+	+	-	-	-	-	-	-
Genital lesions	+	+	-	-	-	-	-	-
Keratoconjuntivitis	+	+	+	-	-	-	-	-
Cutaneous lesions	+	+	+	-	-	-	-	+
Neonatal infection	+	+	+	+	-	-	-	-
Retinitis	+	+	+	+	-	-	-	-
Esophagitis	+	+	+	+	-	-	-	-
Pneumonitis	+	+	+	+	+	+	-	-
Hepatitis	+	+	+	+	+	+	-	-
Meningitis	-	+	+	-	-	+	-	-
Encephalitis	+	+	+	+	+	+	-	-
Myelitis	+	+	+	+	+	+	-	-
Mononucleosis	-	-	-	+	+	+	-	+?
Hemolytic anemia	-	-	+	+	+	-	-	-
Leukopenia	-	-	+	+	+	+	-	-
Trombocytopenia	-	-	+	+	+	+	-	-

Herpes simplex encephalitis (HSE)

- Caused by HSV-1 or HSV-2
  - **HSV-1:** commonly causes HSE **in children beyond the neonatal period and in adults**
  - **HSV-2:** commonly causes encephalitis in **neonates and the immunocompromised (IC) host**.
- Significant morbidity and mortality in adults and children despite antiviral treatment.
- Devastating disease in infants and children, irrespective of treatment.

Herpes simplex encephalitis (HSE)

- HSE: is an acute or subacute illness associated with focal or global cerebral dysfunction.

- **HSV-1 >>>HSV-2 (~90% versus 10%)**
- **HSV-1:**
  - causes almost all HSE beyond the neonatal period
  - **most common cause of fatal encephalitis**
  - HSE occurs in a **sporadic and non-seasonal pattern** across the globe

- **HSV-2**
  - causes most of the HSE in the neonate
  - causes encephalitis in the immunocompromised (IC) host

Herpes simplex encephalitis (HSE)

- **HSE in children:** mostly due to primary infection

- **Neonatal encephalitis** could be either due to:
  - primary infection - almost always acquired due to perinatal exposure during labor or post-delivery
  - secondary infection due to viremia and multisystem involvement

Herpes simplex encephalitis (HSE) – pathogenesis & pathology

- ~30% of HSE cases are related to **primary HSV infection** (more commonly in children and adolescents)
- ~70% of cases are attributed to **HSV reactivation**.
- Clinical features do not distinguish between HSE caused by primary infection or reactivation.
- **Reactivation of HSV in peripheral ganglia with axonal transport to the temporal lobe *versus* reactivation of virus latent in the brain has both been proposed.**

Herpes simplex encephalitis (HSE) – potential routes of infection

- Primary HSV-1 infection of the oropharynx with CNS invasion **via the trigeminal or olfactory tracts.**
- Primary HSV-1 infection with CNS infection resulting from **hematogenous spread.**
- CNS invasion after an episode of **recurrent orofacial HSV-1 infection**, representing peripheral viral reactivation with subsequent axonal spread.
- CNS infection without defined peripheral HSV-1 infection, possibly representing **reactivation of latent virus within the CNS.**
- **More than one mechanism may be responsible for HSV entry into the brain (at least half of the cases of HSE are caused by a different viral strain from the one responsible for cold sores in the same patient).**

Herpes simplex encephalitis (HSE)

- Studies in both animal models and humans indicate that both:
  - **cytolytic viral replication**
  - **immune-mediated responses (including cytotoxic T lymphocytes and immune mechanisms mediated by TLR 2)**

contribute to the pathology of HSE

Possible new therapeutic approaches

- Selective immunosuppressive therapy, coupled with potent antiviral drugs, may eventually play a role in the therapeutic management of HSV.

Herpes simplex encephalitis (HSE)

- **HSE may result from a novel group of single-gene inborn errors of interferon (IFN)-mediated immunity.**
  - Defects in the TLR3-IFN and IFN-responsive pathways
  - Autosomal recessive STAT-1 deficiency
  - X-linked NEMO deficiency
  - Autosomal recessive UNC-93B deficiency
  - Autosomal dominant TLR3 deficiency

Predisposition to HSE in children

- **The TLR3–UNC-93B-dependent production of IFN-α/β and IFN-λ is essential to confer protective immunity to HSV-1 in the CNS during primary infection in childhood.**

Herpes simplex encephalitis (HSE)

- **Increased susceptibility in people with:**
  - defective molecular signaling in **Toll-like receptors-3 (TLR-3) pathways**
  - alteration in MHC class 1 allotype, and the high-affinity receptor/ligand pair KIR2DL2/HLA-C1 and the CD16A-158V/F dimorphism.

- **People taking certain immunosuppressive drugs:**
  - **natalizumab** (mAb against alpha-4 (α4) integrin FDA-approved monoclonal antibody approved for the treatment of multiple sclerosis and Crohn's disease.
  - **anti-inflammatory agents (TNFα inhibitors)**

Herpes simplex encephalitis (HSE)

- HSV-1 is the **most common cause of life-threatening sporadic encephalitis across the globe** and does not exhibit any seasonal variation.
- About 60 to 90% of older adults worldwide are seropositive for HSV-1.
- The annual incidence of HSE is 1 in 250,000 to 1 in 500,000.
- HSV-1 encephalitis constitutes 10 to 20% of the 20,000 annual viral encephalitis patients in the USA.
- The incidence is most common and severe in children and the elderly.

Herpes simplex encephalitis (HSE)

- Both sexes are equally affected.
- There is no evidence of increased incidence of HSE in IC hosts though the mortality and morbidity are significantly higher.
- The virulence and invasiveness depend on **viral as well as host immune factors.**
- **Common symptoms of HSE:** changes in mental status, abnormal behavior, fever, headache, and seizures but clinical presentation differs among patients, making diagnosis difficult.
- **Diagnostic method of choice** is detecting HSV DNA in the cerebrospinal fluid (CSF).

Presenting signs and symptoms in patients with herpes simplex encephalitis

Finding	Percentage of patients	Reported range
Fever	80%	70–97%
Confusion/disorientation	72%	54–81%
Personality changes/behavioral disturbances	59%	42–92%
Headache	58%	42–70%
Altered mental status/impaired consciousness	58%	54–100%
Seizures	54%	35–65%
Focal neurological deficits	41%	26–79%
Nausea and vomiting	40%	19–46%
Aphasia/altered speech	40%	12–65%
Coma	33%	4–48%
Meningismus	28%	13–38%

HSE diagnosis

- Typical cerebrospinal fluid (CSF) abnormalities include:
  - **elevated protein** (50–200 mg/dL or more; median about 80 mg/dL).
  - **leukocytosis** (5–500 cells/mm^3 or more, median about 70 cells/mm^3) **with predominance of lymphocytes** (60–98%, median about 80%), although HSE cases without CSF leukocytes have been described.
  - Red blood cells (RBCs) in CSF occur more often in HSE patients with significant tissue necrosis (although the presence of RBCs is nonspecific and can also result from lumbar puncture trauma).
  - Glucose values are usually normal, although cases of hypoglycorrhachia (an abnormally low glucose concentration within the CSF) have been described.

Laboratory test	Typical finding
Leukocytes	25–75/mm^3 (range 0–>500)
Percent lymphocytes	75–90% (range 60–98%)
Glucose	60–75 mg/dL **(about 25% of patients will have CSF glucose <50% of serum glucose level)**
Protein	65–85 mg/dL **(about 60–70% of patients will have elevated CSF protein)**

HSE treatment

- **Immunocompetent adults:** IV acyclovir needs to be started in all patients with suspected or confirmed cases of HSE at the dose of 10 mg/kg body weight every 8 hours for 14 to 21 days

- **Children up to 11 years and neonates:** higher doses IV acyclovir (15-20 mg/kg body weight) for 21 days.

- **IC persons:** may require a higher dose with a longer duration.

- Most of the patients with HSE are older than 50 years.

- **Oral acyclovir prophylaxis** has been shown to reduce relapse in children after the initial treatment with IV acyclovir.

HSE treatment

- Without treatment, the mortality rate of HSE is ~70%.
- Despite acyclovir therapy, mortality remains high (~20%).
- Most survivors suffer from **severe neurologic sequelae.**
- Neurologic sequelae remain common despite antiviral treatment, especially when treatment is delayed.
- A longer duration between onset of symptoms and initiation of effective antiviral therapy correlates directly with less favorable clinical outcome.
- **ACV-resistance HSV infections of the CNS** have been described in several case reports

Characterization of HSV-1 isolates recovered from the CSF of patients suffering from herpetic encephalitis

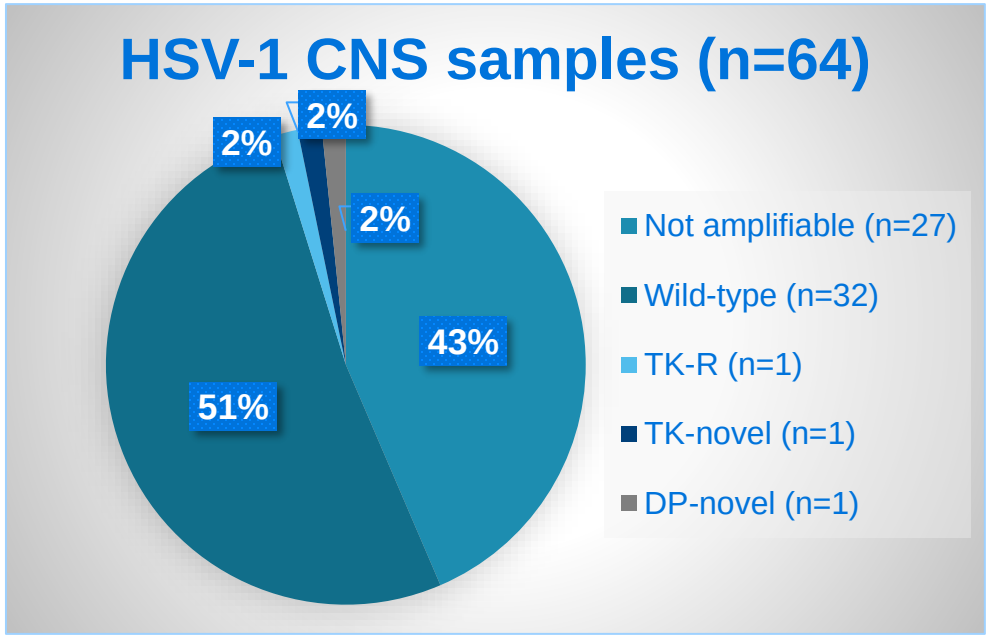
- **Patient 1 (immunocompetent):** 72 years old man with HSV-1 encephalitis under acyclovir therapy. He died under ACV treatment.

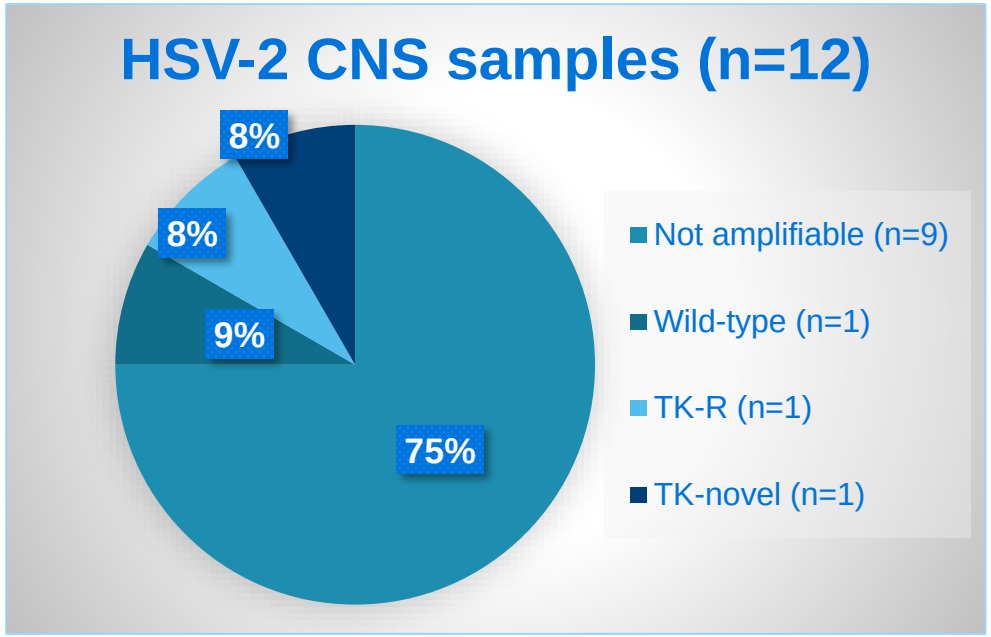
  - CSF → DNA isolation → sequencing HSV TK → G59W mutation

- **Patient 2 (immunocompetent):** 45 years old man with HSV-1 encephalitis under acyclovir therapy. His treatment was changed to foscarnet and he survived.

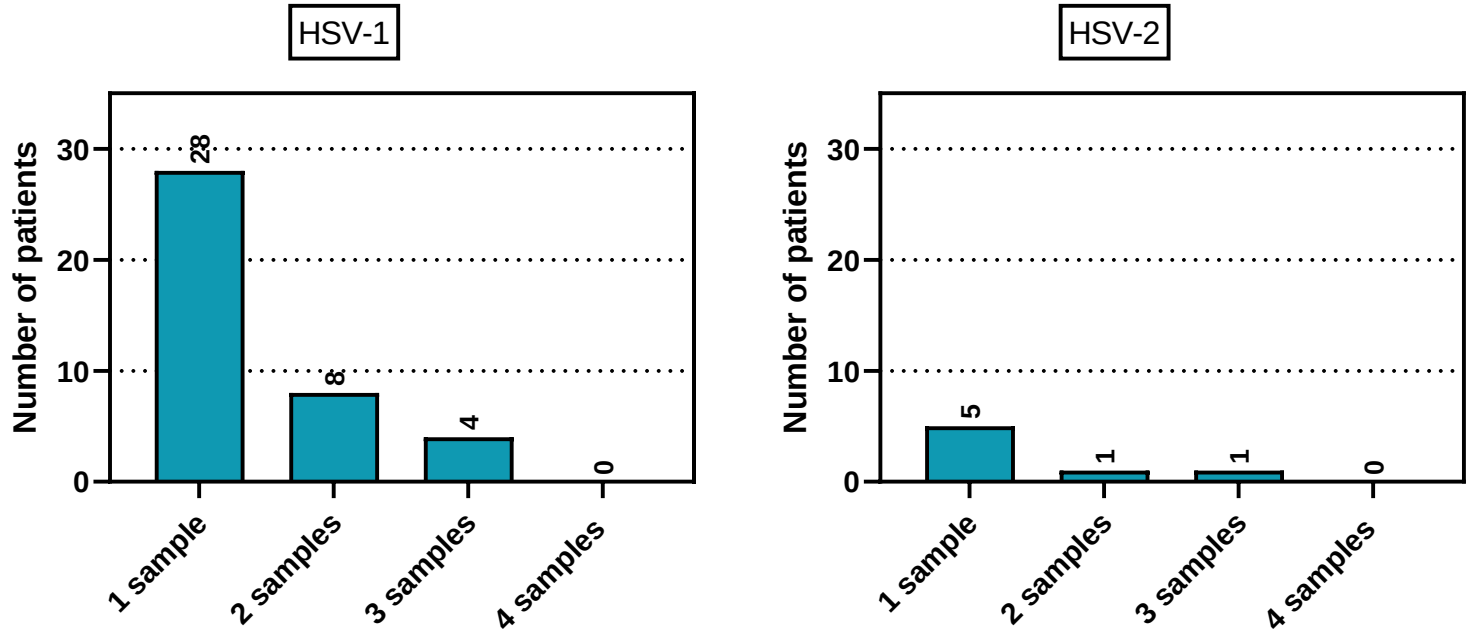
  - CSF → DNA isolation → sequencing HSV TK → G59W mutation

CNS Samples analyzed by RegaVir

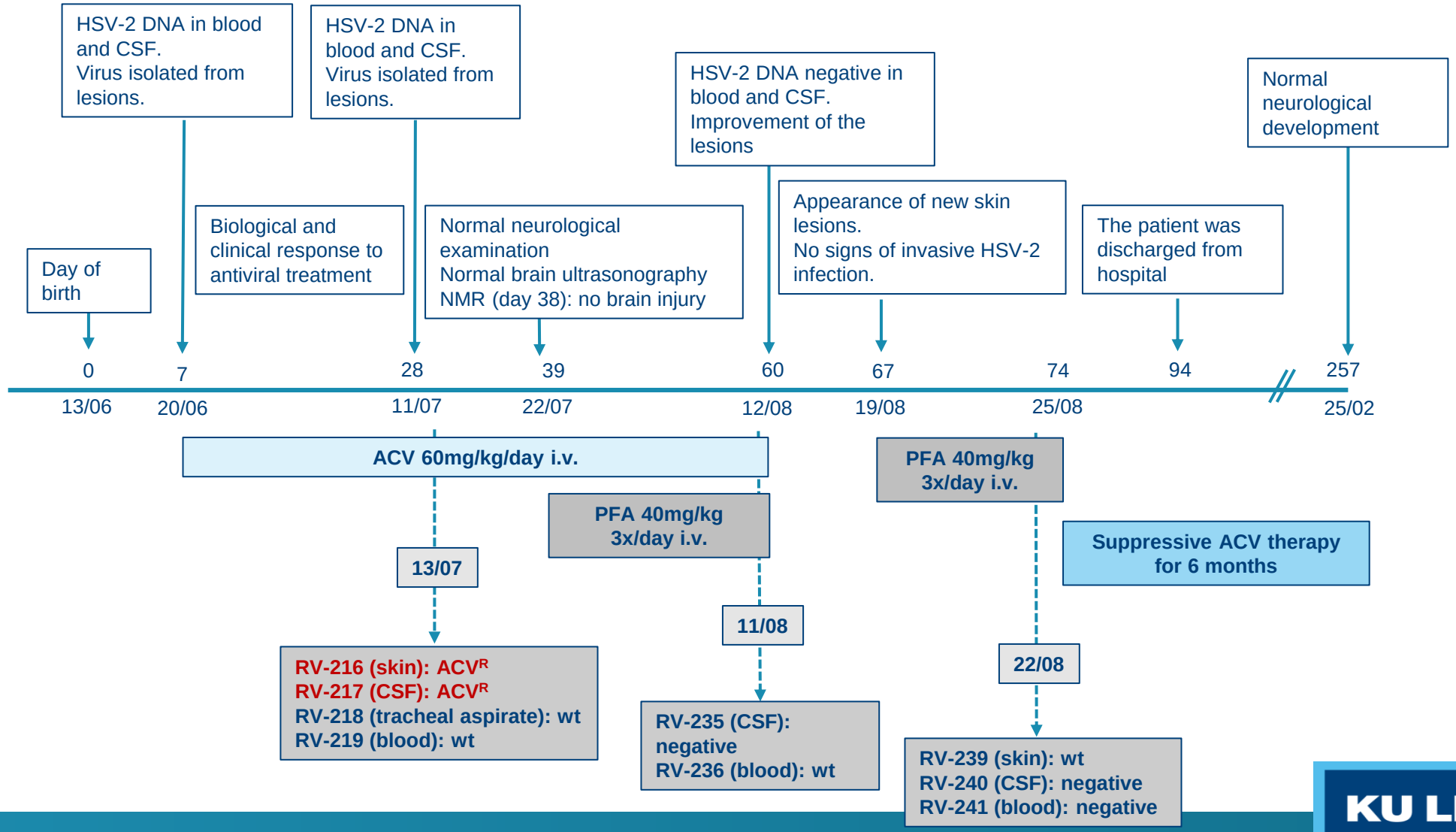




CSF samples per patient



Neonatal HSV-2 encephalitis



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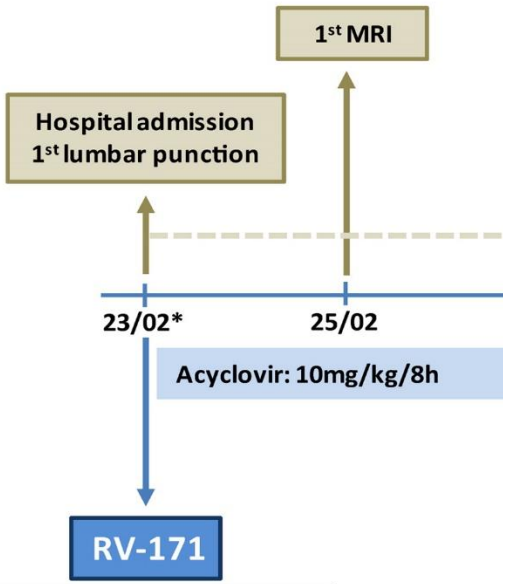
Case study

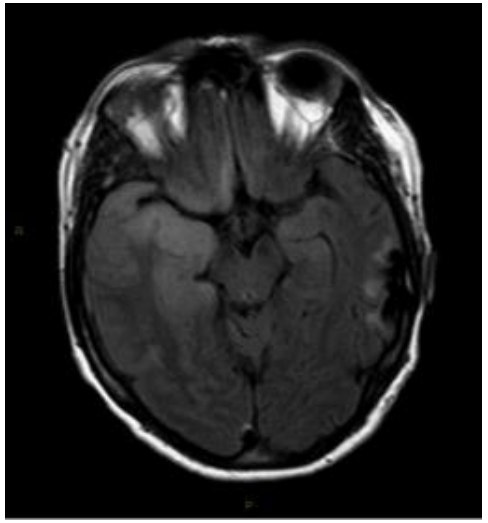
- A 50-year-old woman presented to emergency for an **inaugural generalized epileptic seizure.**
- Her medical history:
  - severe plaque psoriasis without rheumatologic complaints treated with **adalimumab (monoclonal anti-TNFα Ab)** for 24 months.
  - **frequent recurrent untreated herpes labialis**.
- On admission
  - febrile (38.5 °C)
  - bradypsychic
  - presented herpes labialis lesions
  - CSF: increased leukocytes (mainly lymphocytes)
  - EEG: periodic lateralized epileptiform discharges in right temporal localization

Case study I



- Treatment consisted of IV:
  - levetiracetam
  - ceftriaxone (2 g q12h)
  - ampicillin (2 g q4h)
  - **ACV (10 mg/kg/8 h)**

- Cerebral magnetic resonance imaging (MRI) performed two days later.
  - Right medial temporal lobe, right insular cortex, and subcortical white matter-hyper-intensities.



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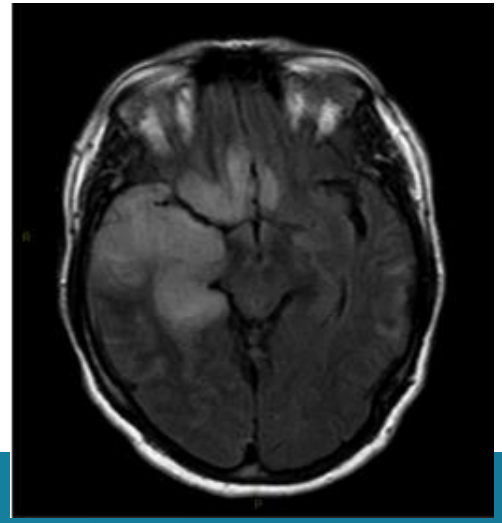
Case Study I

- HSV-1 PCR of CSF specimen was positive.
- Antibacterial treatment was discontinued
- IV ACV treatment was continued.

- During the following days: the **patient's neurologic symptoms worsened** with increasing confusion and behavioral changes.
- A new **MRI** revealed an injury progression in both hemispheric sides with slight worsening of the right temporal lobe involvement.



Case study I

- A new lumbar puncture was performed and showed increased WBC and RBC counts, augmented protein levels and a persistent positive HSV-1 PCR.

			Normal values
White blood cell (WBC) count	236/mm^3	660/mm^3	<3/mm^3
Formula	PMN: 8% Lymphocytes: 83%	PMN: 13% Lymphocytes: 81%	≤5/mm^3 Lymphocytes or mononuclear cells. No PMN
Red blood cell (RBC) count	36/mm^3	730/mm^3	No RBC
Protein level	61mg/dl	242mg/dl	18-45mg/dl
Glucose level Synchronous serum level	75mg/dl 96mg/dl	51mg/dl 86mg/dl	40-70mg/dl 70-110mg/dl
Glucose CSF :Serum ratio	0.78	0.59	0.6
Lactate	2.5nmol/L	3.1nmol/L	<2.4nmol/L
IgG/albumin Index	0.47	N.A.	<0.7
Herpes Index	N.A.	N.A.	<1.9

PMN: polymorphonuclear cells.
Herpes index: (CSF IgG_{HSV} x serum albumin) / (serum IgG_{HSV} x CSF albumin).
N.A.: not available.

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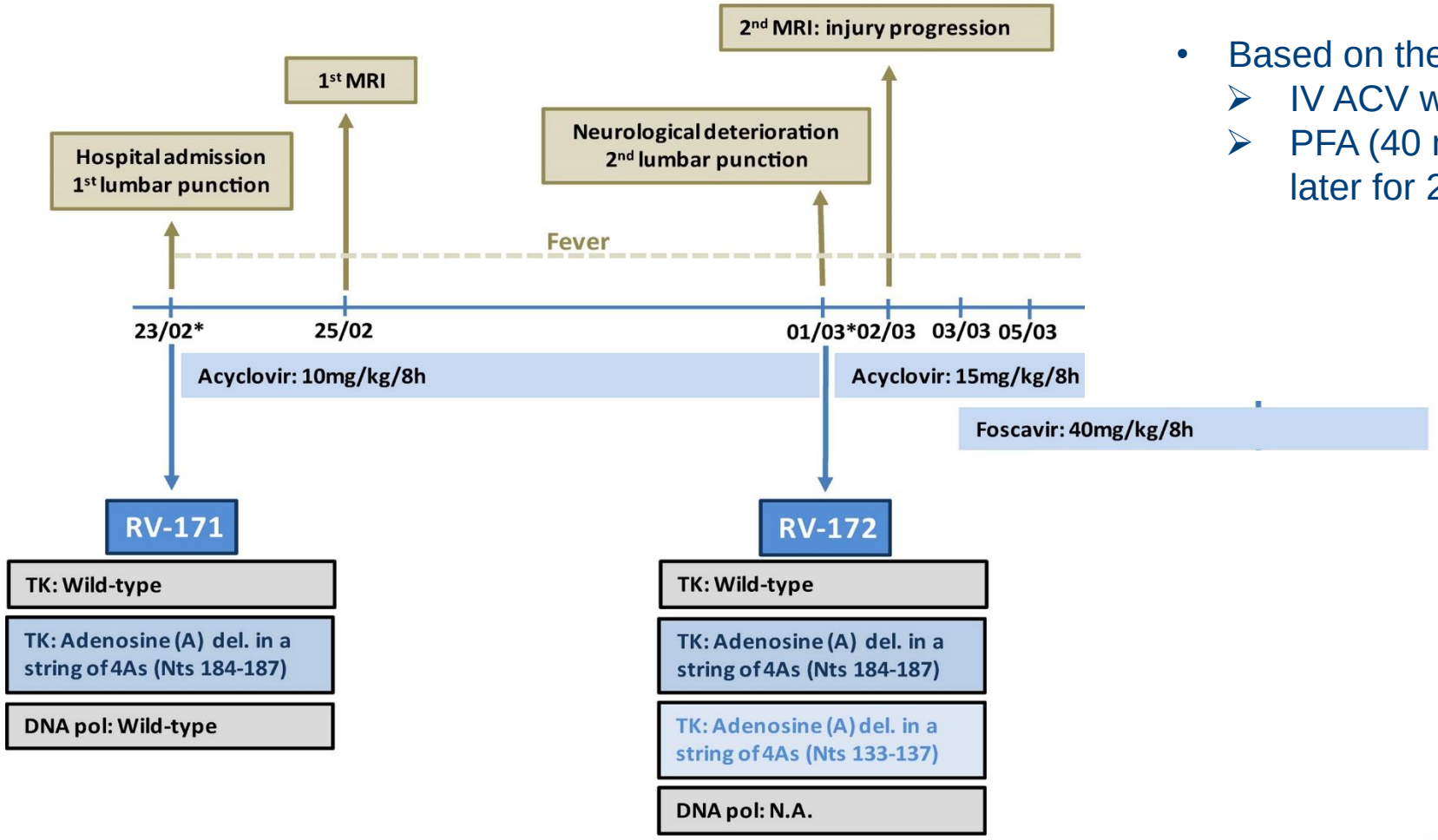
Case study I



- Based on the genotyping data:
  - IV ACV was increased to 15 mg/kg/8 h.
  - PFA (40 mg/kg/8 h) was added two days later for 21 days.

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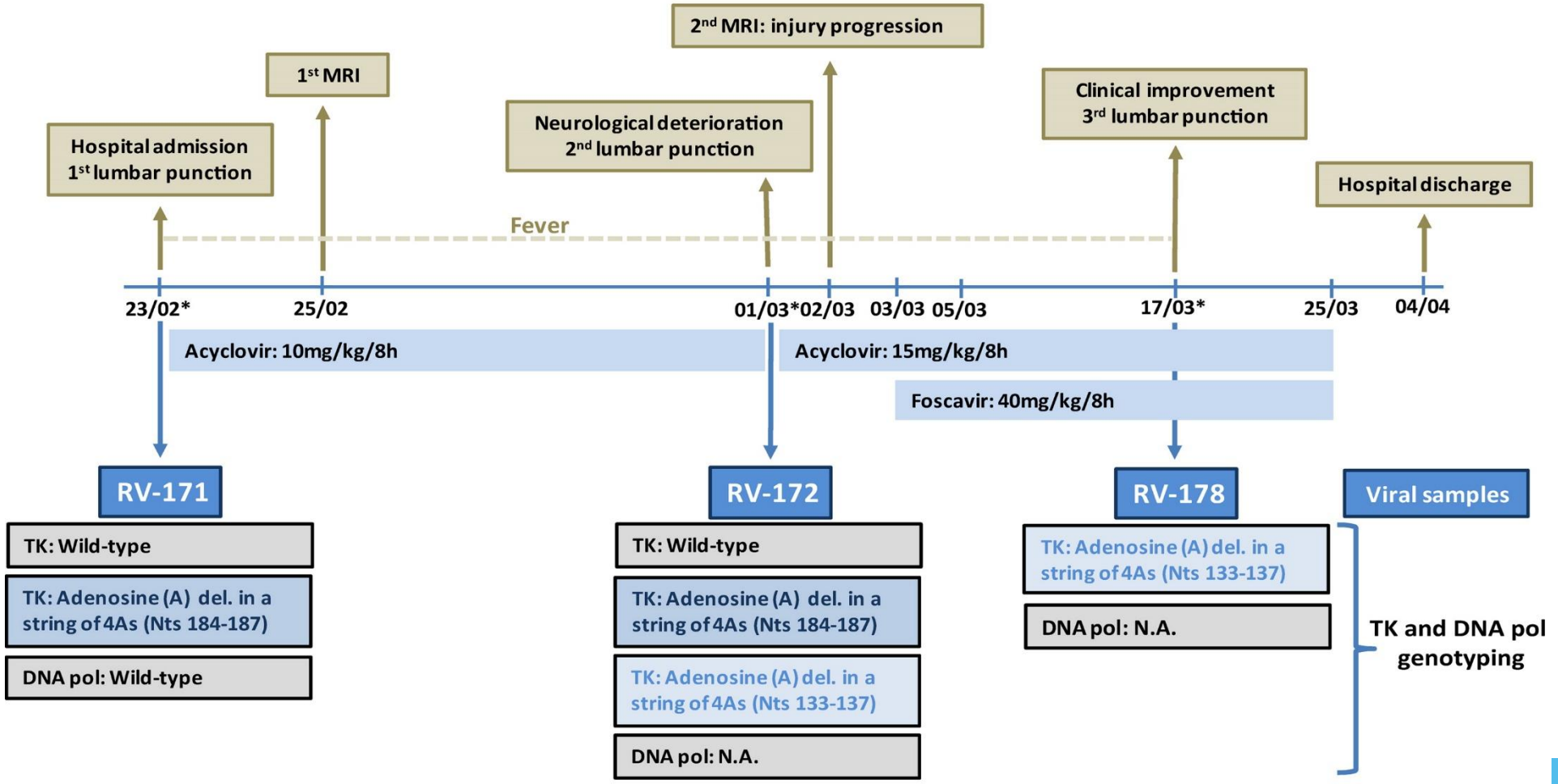
Case study I

- Patient's clinical condition improved but also the CSF parameters after 14 days of combined antiviral therapy (17/03).
- Neurological evolution remained favorable for a follow up period of one year.
- No further adalimumab treatment was given despite relapse of her psoriasic disease.



Case study I

- Improved CSF parameters after 14 days of combined antiviral therapy (17/03).

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Red blood cell (RBC) count	36/mm^3	730/mm^3	0mm^3	No RBC
Protein level	61mg/dl	242mg/dl	112mg/dl	18-45mg/dl
Glucose level Synchronous serum level	75mg/dl 96mg/dl	51mg/dl 86mg/dl	52mg/dl 110mg/dl	40-70mg/dl 70-110mg/dl
Glucose CSF :Serum ratio	0.78	0.59	0.47	0.6
Lactate	2.5nmol/L	3.1nmol/L	2.5nmol/L	<2.4nmol/L
IgG/albumin Index	0.47	N.A.	2.23	<0.7
Herpes Index	N.A.	N.A.	4.6	<1.9

PMN: polymorphonuclear cells.
Herpes index: (CSF IgG_{HSV} x serum albumin) / (serum IgG_{HSV} x CSF albumin).
N.A.: not available.

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HSE in patients treated with TNF-α inhibitors

- In a patient under **infliximab therapy**, IV ACV therapy for 21 days resulted in normalization of the patient's mental status and subtle neuropsychiatric changes resolved within one year.

- **Two patients under adalimumab and corticosteroids:** progressive neurologic and radiologic disease while receiving standard ACV dose and increased ACV dose resulted in improved patient's conditions but subtle neuropsychiatric changes persisted.

HSE in a 75 years old woman

- HSE diagnosis made on 05.12.2016
- Acyclovir treatment (10 mg/kg/8 h) started on 05.12.2016

RegaVir Identification	Date collected	Type	HSV-1 TK genotyping	DNA pol genotyping
RV-1122	14/12/2016	CSF	G6C, P42L, R89Q, G240E, C251G, S321P **M85V (novel)**	T566A
RV-1123	05/12/2016	CSF	G6C, P42L, R89Q, G240E, C251G, S321P	

Advisable to shift the therapy to foscavir considering that the M85V substitution might be linked to acyclovir-resistance.

ACV-R in HSE

- Should be considered if the patient's condition deteriorates and CSF viral load does not drop despite ACV treatment, even following a short treatment.
- **May emerge relatively fast in the CNS**, ‘immune-privileged site’.
- **Primary ACV-R** can be seldom found.
- Decisions to alter therapy without laboratory confirmation of resistance should be based on clinical emergency.
- Increasing ACV dosage can be encouraged while resistance is being evaluated but this is of little benefit in cases of clinical resistance and **shift to or addition of PFA** should be envisaged.

Prevalence of ACV-R HSV infections

Immunocompetent (21 studies)		**< 1% (0% - 6.2%)**
Immune privileged sites (6 studies)	Eye infections	0% - 34.6%
	Herpetic keratitis	6.4% - 34.6%
	CNS	0% (1 study)
Immunocompromised (13 studies)		**> 3%** (0% - 28.8%)
	SOT (7 studies)	**< 3.5%** (0% -10%) (> lung & heart Tx)
	HIV infected (9 studies)	**3.4 – 7.3%** (0% - 25%) HSV-2 > HSV-1
	HSCT (16 studies)	**0% - 100%** HSV-1 > HSV-2