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RegaVir platform: Case discussions antiviral resistance testing

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Leuven, April 26, 2022

Cytomegalovirus (CMV) infection in hematopoietic stem cell transplantation (HSCT) patients

- **Prophylactic or preemptive therapy against CMV infection** has successfully reduced the incidence of CMV disease in transplant recipients.
- However, **CMV still remains one of the major infectious complications** and causes of morbidity and mortality among HSCT recipients.

- **Risk factors for CMV infection in HSCT patients:**
  - **CMV seropositivity** of donor and recipient.
  - **Allogeneic transplantation** (especially with T cell-depleted unrelated or HLA-mismatched donors).
  - **Cell source:** cord blood recipients have both longer and higher cumulative incidences of CMV infection compared to peripheral blood or allogeneic bone marrow recipients from related or unrelated donor origin.

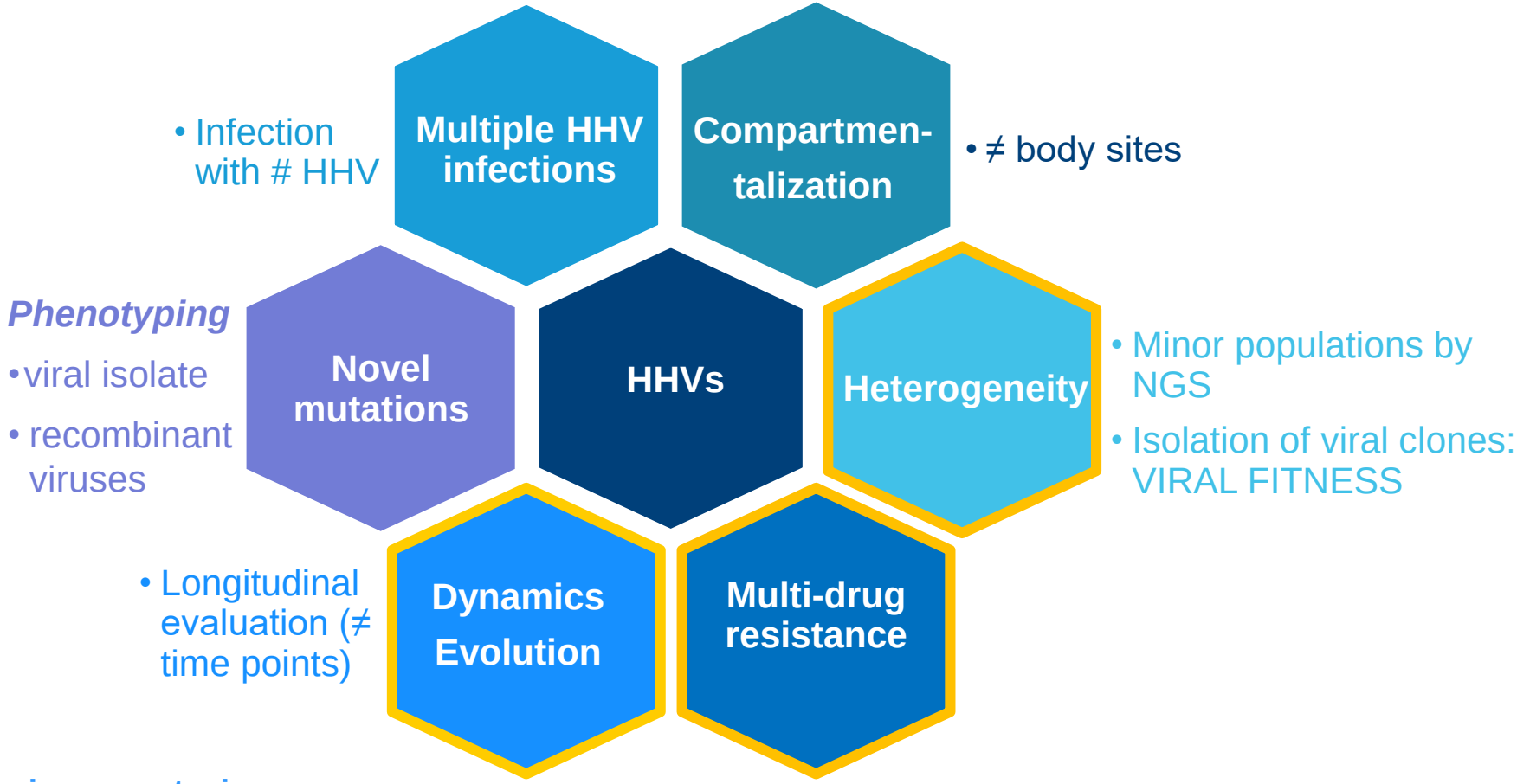
Cytomegalovirus (CMV) infection in hematopoietic stem cell transplantation (HSCT) patients

- In HSCT patients, cellular immunity is mainly significantly impaired during the **first 100 days post-transplant.**
- CMV infection in HSCT patients manifests usually as **interstitial pneumonia and gastrointestinal disease**.

- Although less frequently, **late CMV reactivation (>100 days posttransplant) can occur**.

- A few reports have highlighted the **importance of CMV resistance in pediatric HSCT populations**.
  - GCV-R associated with the UL97 protein kinase (Ohta et al., 2001, Gohring et al., 2009, Kim et al., 2012, Erice et al., 1989).
  - GCV-R & PFA-R conferred by multiple UL97 and UL54 (DNA polymerase) mutations (Choi et al., 2014)
  - GCV-R, PFA-R & CDV-R conferred by DNA polymerase mutations (Blackman et al., 2004, Drouot et al., 2014, Springer et al., 2005).

Case study I: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient



Herpesvirus genotyping

- **Prospectively:** capillary (Sanger sequencing)
- **Retrospectively:** next-generation sequencing (NGS)

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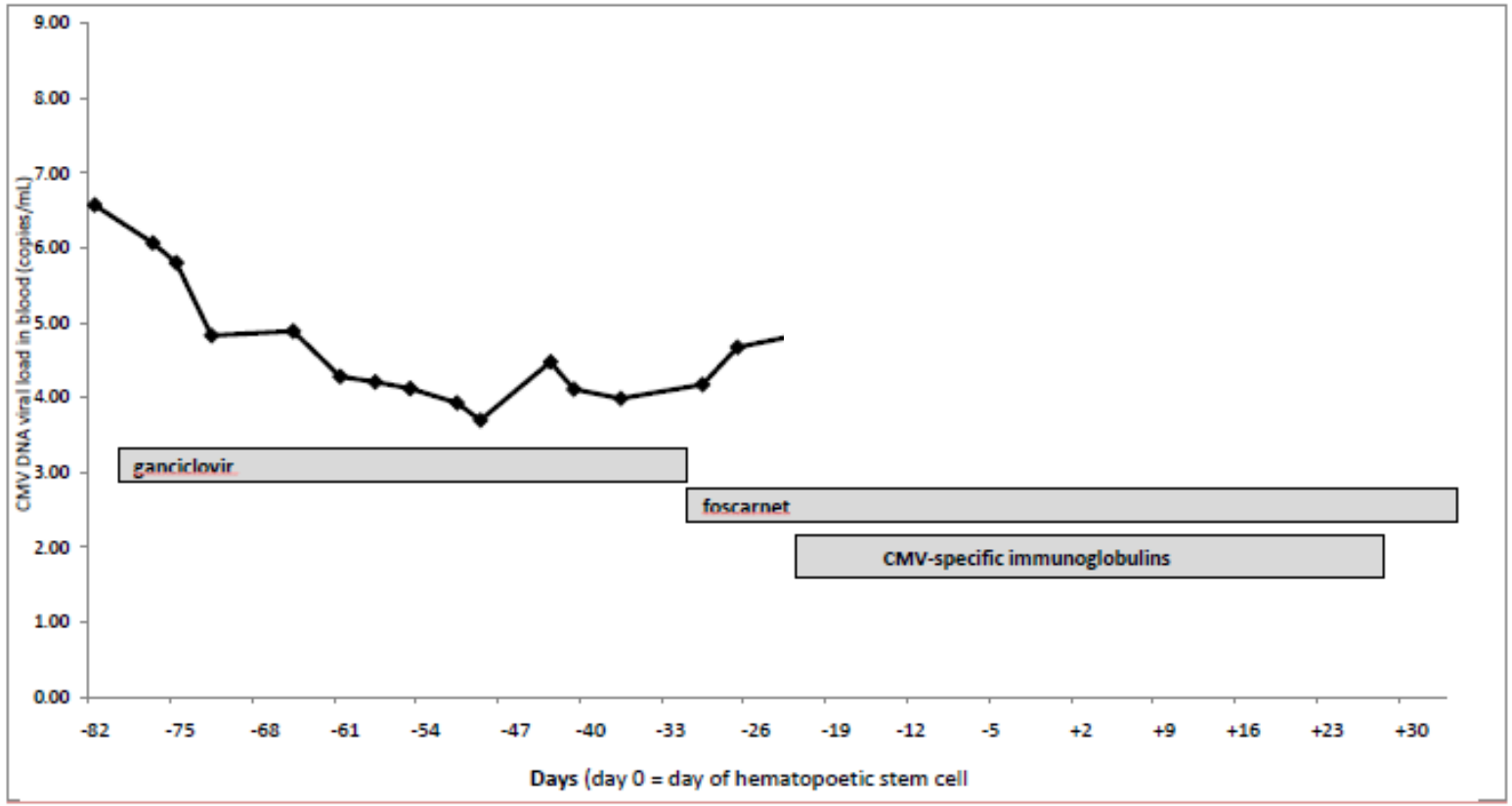
Case study I: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient

- A **4-month-old girl** (weight 5.8 kg) was referred for fever, failure to thrive and bloody diarrhea.
- She was the first child of **consanguineous parents**, so **severe immunodeficiency** was presumed.
- **CMV was detected in the blood**: viral load of 3.77 10E6 copies/ml → GCV (5 mg/kg, 2x/d) started.
- **MHC class II deficiency** was diagnosed and the **search for a stem cell donor** was initiated.

Case study I: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient



- Four weeks later the dose frequency of GCV was reduced to 1x/d.
- However, 1 week later, the CMV viral load started to increase → GCV was switched from 1x/d to 2x/d.
- As the **CMV viral load still increased**, GCV administration was stopped, and therapy was switched to PFA.
- **No HLA identical donor nor cord blood** was found so haploidentical HSCT was planned.
- For more than one month, the patient received **PFA (2 × 350 mg) and simultaneously CMV-specific immunoglobulins.**

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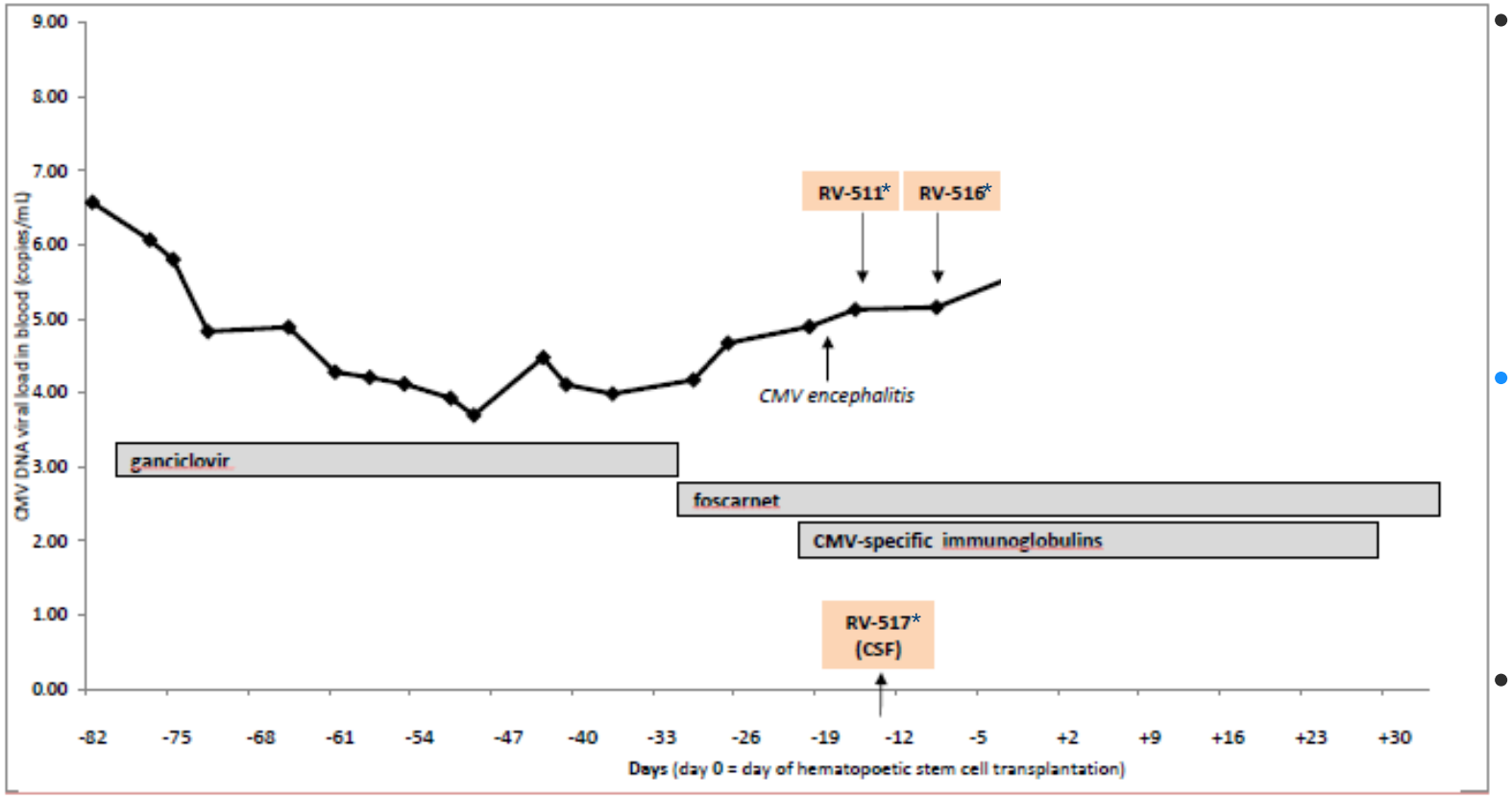
Case study I: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient

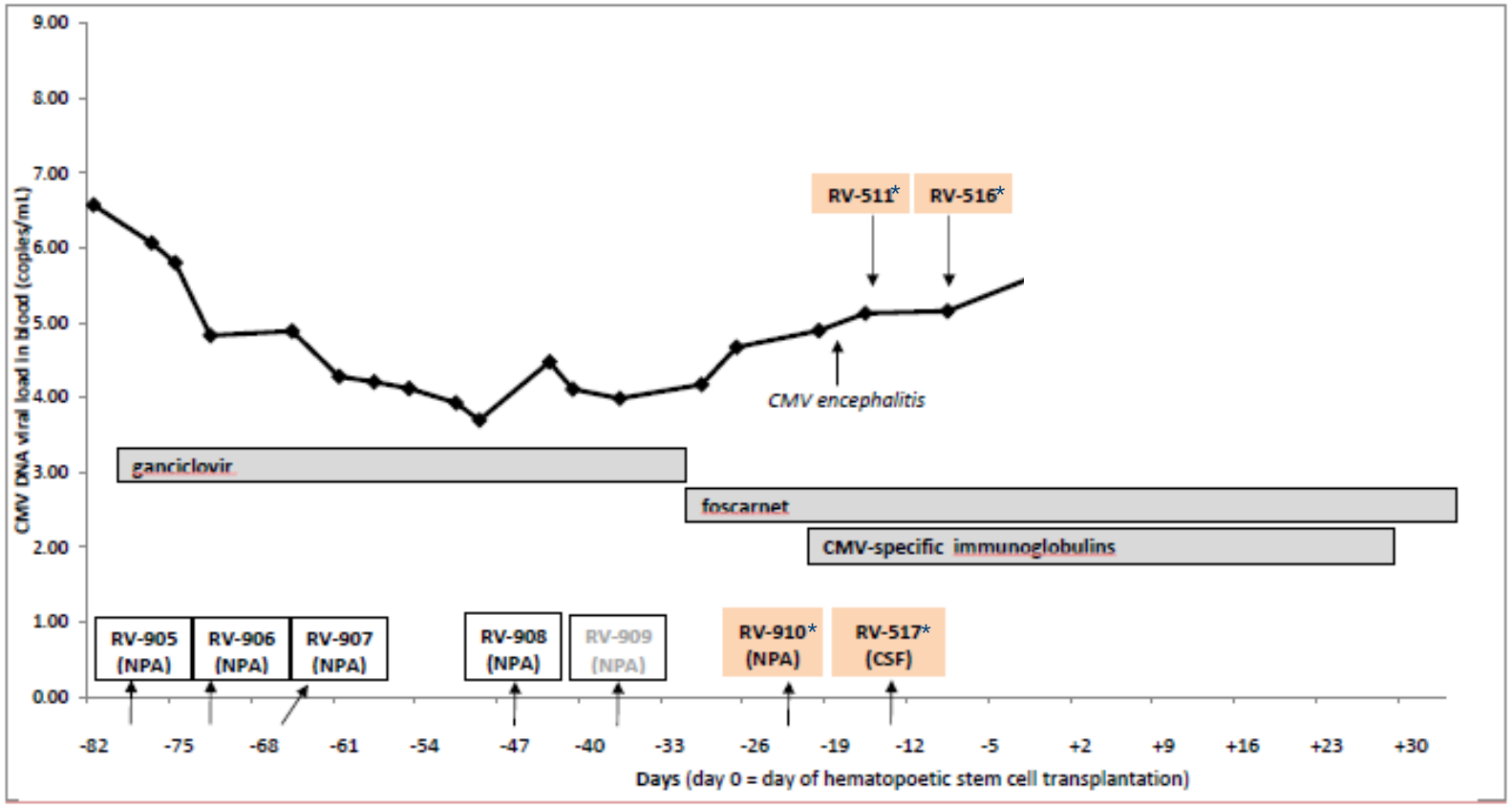


Abbreviations: NPA (nasopharyngeal aspirate), CSF (cerebrospinal fluid) * mixed population

GCV-R: UL97 (M460V) + UL54 (wt)

- Antiviral resistance testing in a **blood** sample **(RV-511)** was performed and revealed the presence of a mixed population of **M460V-mutant** and wild-type virus in the UL97 protein kinase.
- **M460V UL97 mutation** is known to confer **GCV-R** with susceptibility to foscarnet and cidofovir (Lurain and Chou, 2010).
- Despite antiviral treatment the patient deteriorated and developed **CMV encephalitis** confirmed by positivity of CMV PCR on **CSF** taken on day −14 (**RV-517)**, which showed a similar resistance profile as the blood samples **(RV-511 and RV-516)**

Case study I: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient



Abbreviations: NPA (nasopharyngeal aspirate), CSF (cerebrospinal fluid) * mixed population

Wild-type | Not amplifiable | GCV-R: UL97 (M460V) + UL54 (wt)

- Retrospective analysis of **NPA samples (RV-905 to RV-910) was performed.**
- ➢ The **NPA** sample taken during the same time period **(RV-910**) had a mixed population of **M460V-mutant** and wild-type virus in the UL97 protein kinase
- ➢ NPA samples recovered at earlier time points (RV-905 to RV-908) showed a **wild-type CMV genotype**, indicating that GCV-R emerged at the time the patient developed encephalitis.

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Specimens recovered before transplantation (day 0 = day of HSCT)

	Day	Samples code	CMV DNA	Type of sample	Amino acids changes related to resistance in:		Resistance to:	Amino acid changes related to genetic polymorphisms	
					UL97 (protein kinase)	UL54 (DNA pol)		UL97 (protein kinase)	UL54 (DNA polymerase)
Retrospective analysis)	−82	*Not available*	3.77 × 10e6	Blood	Not available	Not available	Not available	Not available	Not available
	−79	*RV-905*	Positive	NPA	None	None	None	None	S655L, F669L, N685S, A885T & N898D
	−72	*RV-906*	Positive	NPA	None	None	None	None	S655L, F669L, N685S, A885T & N898D
	−65	*RV-907*	Positive	NPA	None	None	None	None	S655L, F669L, N685S, A885T & N898D
	−43	*RV-908*	Positive	NPA	None	None	None	None	S655L, F669L, N685S, A885T & N898D
	−37	*RV-909*	Detection limit	NPA	Not available	Not available	–	Not available	Not available
	−19	*RV-910*	Positive	NPA	M460V*	None	GCV	None	S655L, F669L, N685S, A885T & N898D
Prospective analysis)	−16	**RV-511**	1.34 × 10e5	Blood	M460V*	none	GCV	None	S655L, F669L, N685S, A885T & N898D
	−14	**RV-517**	Positive	CSF	M460V*	none	GCV	None	S655L, F669L, N685S, A885T & N898D
	−9	**RV-516**	1.44 × 10e5	Blood	M460V*	none	GCV	None	S655L, F669L, N685S, A885T & N898D

NA (not amplifiable), NPA (nasopharyngeal aspirate), CSF (cerebrospinal fluid), GCV (ganciclovir), CDV (cidofovir), FOS (foscarnet), del (deletion), DL (detection limit), ND (not detected).
Samples marked in bold were analyzed when they were recovered from the patient in order to adapt antiviral treatment while those indicated in italic were evaluated retrospectively.

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Case study I: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient

- Despite CMV infection, an **allogeneic transplantation** was performed **(day 0 = day of HSCT)** with **peripheral blood stem cells from a haploidentical donor**, after selection of CD34 cells from the graft.

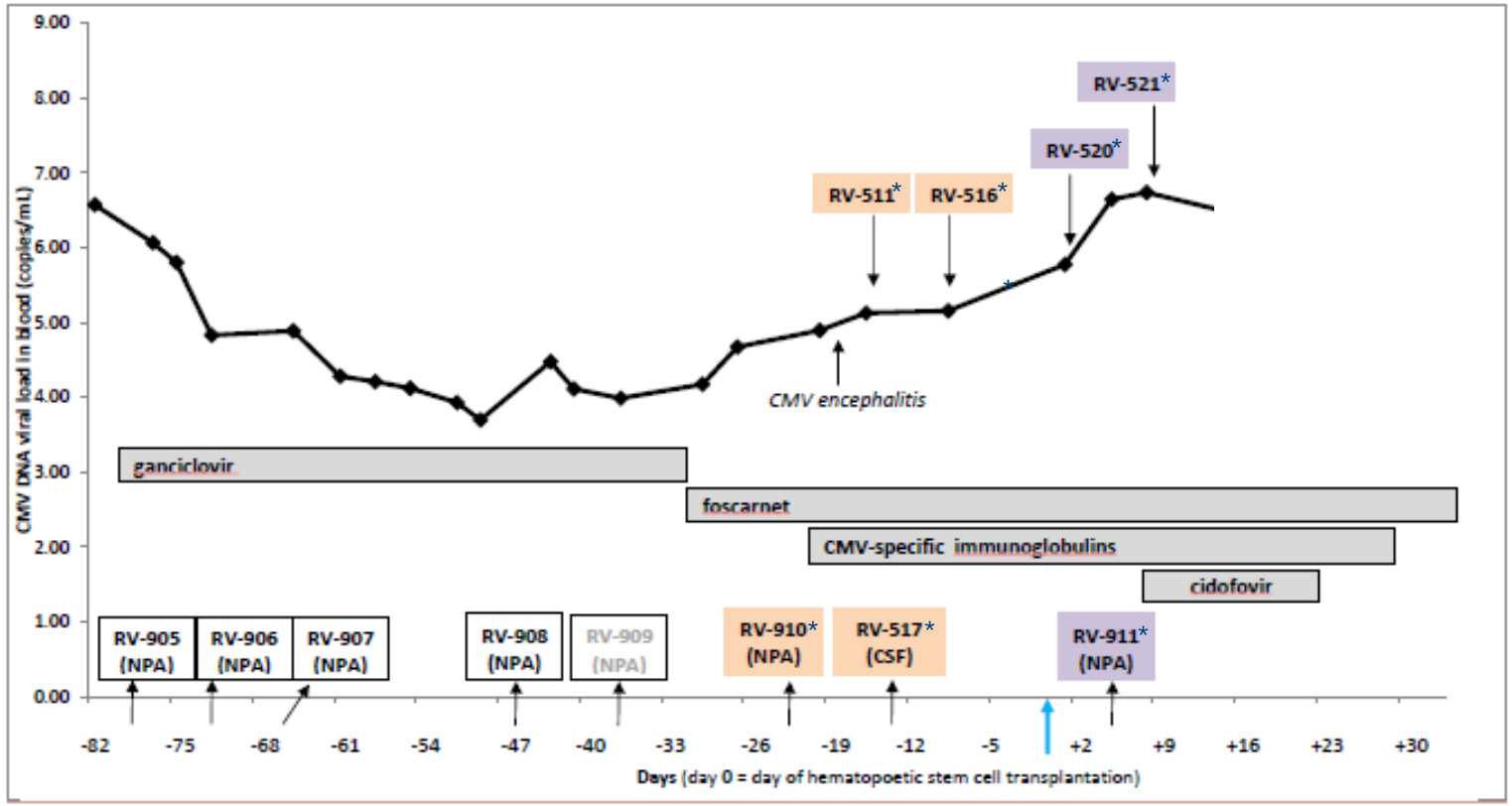
- **Both the donor and receptor were CMV IgG seropositive (D+/R+).**

- The **conditioning regimen** consisted of intravenous busulfan (16 × 1mg/kg) and fludarabine (4 × 35 mg/m2).

- Three days after HSCT, the patient developed fever for which **antibiotics were started**.

- Because of progression of **neurologic encephalitic disturbances suggestive of central CMV infection**, **prophylactic anti-epileptics** were started (phenobarbital) and **cidofovir was added empirically to the foscarnet antiviral treatment.**

Case study I: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient



- Antiviral resistance testing of a **blood sample on day +1 (RV-520)**:
  - mixed population of **M460V-mutant** and wild-type virus at the UL97 protein kinase.
  - mixed population of **981-982 deletion** mutant and wild-type virus in the DNA polymerase.

- **DNA pol 981-982 del.** is known to confer **resistance to ganciclovir, foscarnet and cidofovir** (Lurain and Chou, 2010).

- The same viral populations were detected at the same time in the NPA (**RV-911**) on day +8 in blood. (**RV-521**).

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Specimens recoverted after day 0 (the day of transplantation)

Day	Samples code	CMV DNA (copies/ml)	Type of sample	Amino acids changes related to resistance in:		Resistance to:	Amino acid changes related to genetic polymorphisms	
				UL97 (protein kinase)	UL54 (DNA pol)		UL97 (protein kinase)	UL54 (DNA polymerase)
+1	**RV-520**	5.98 × 10e5	Blood	M460V*	981-982 del.*	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+5	*RV-911*	Positive	NPA	M460V*	981-982 del. *	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+8	**RV-521**	5.52 × 10e6	Blood	M460V*	981-982 del. *	GCV, CDV, FOS	N68D, I244V	S655L, F669L, N685S, A885T & N898D

NA (not amplifiable), NPA (nasopharyngeal aspirate), CSF (cerebrospinal fluid), GCV (ganciclovir), CDV (cidofovir), FOS (foscarnet), del (deletion), DL (detection limit), ND (not detected).
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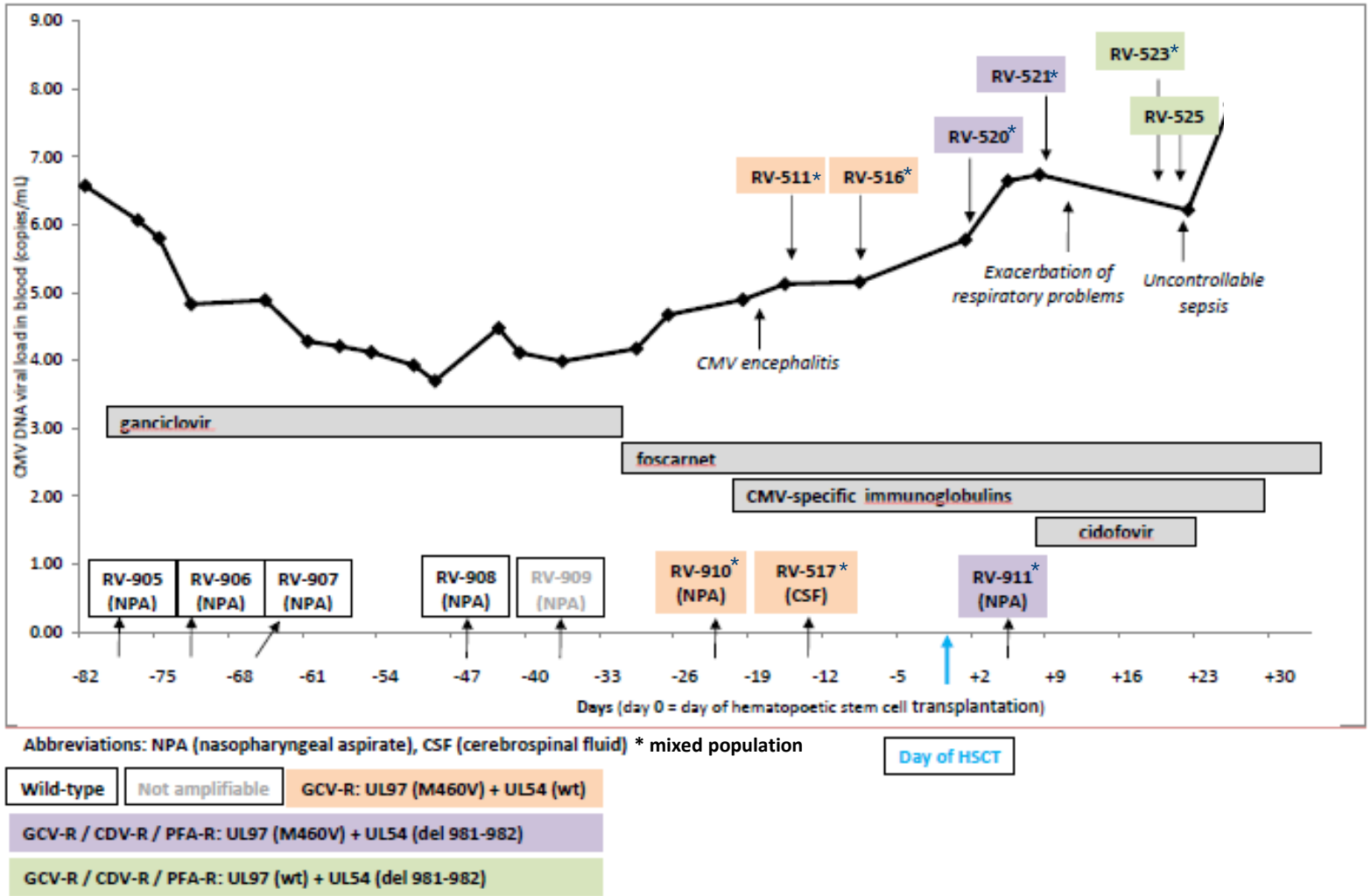
Case study I: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient



- Day +13, the patient was referred to the **intensive care unit** because of exacerbation of respiratory problems.

- **Cidofovir was stopped because of severe cytopenia and increase of the CMV viral load.**
- Day +19, in the **blood sample** (**RV-523**)
  - UL97 M460V-mutant population was not identified anymore.
  - A mixed population of DNA polymerase mutant virus bearing the 981-982 deletion was present.

- Day +22, in only 3 days, the mutant virus totally replaced the wild-type virus (**RV-525**) and presented a **pure population of DNA polymerase mutant virus with the 981-982 deletion**.

Specimens recoverted after day 0 (the day of transplantation)

Day	Samples code	CMV DNA (copies/ml)	Type of sample	Amino acids changes related to resistance in:		Resistance to:	Amino acid changes related to genetic polymorphisms	
				UL97 (protein kinase)	UL54 (DNA pol)		UL97 (protein kinase)	UL54 (DNA polymerase)
+1	**RV-520**	5.98 × 10e5	Blood	M460V*	981-982 del.*	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+5	*RV-911*	Positive	NPA	M460V*	981-982 del. *	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+8	**RV-521**	5.52 × 10e6	Blood	M460V*	981-982 del. *	GCV, CDV, FOS	N68D, I244V	S655L, F669L, N685S, A885T & N898D
+19	**RV-523**	1.06 × 10e7	Blood	none	981-982 del. *	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+22	**RV-525**	1.65 × 10e6	Blood	none	981-982 del.	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D

NA (not amplifiable), NPA (nasopharyngeal aspirate), CSF (cerebrospinal fluid), GCV (ganciclovir), CDV (cidofovir), FOS (foscarnet), del (deletion), DL (detection limit), ND (not detected).
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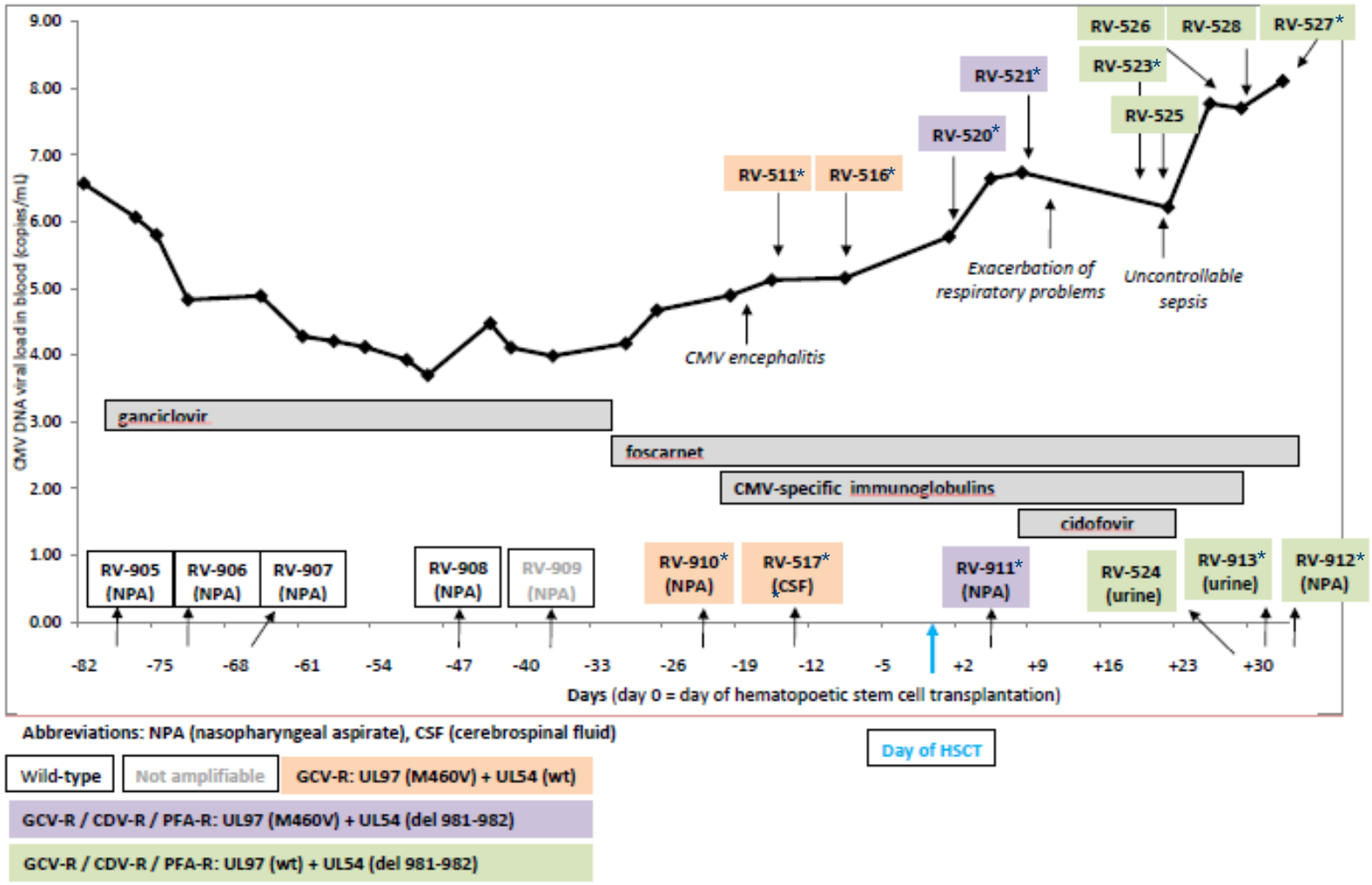
Case study I: **Multidrug-resistant CMV** infection in a pediatric stem cell transplantation patient



- During the following days there was a **further clinical deterioration of respiratory and liver function and uncontrollable sepsis.**
- **No access to compassionate use of novel anti-CMV drugs under clinical development.**
- It was decided to **stop antiviral treatment and to start palliative care**.
- Multidrug-resistant CMV infection [due to the presence of the **981-982 deletion** in the viral DNA polymerase not only in the blood (**RV-526**, **RV-527**, and **RV-528**) but also in urine (**RV-524** and **RV-913**) and NPA (**RV-912**)] together with **encephalitis**, were all cumulating in a **respiratory arrest with cardiac arrest and death of the patient at day +36.**

Specimens recoverted after day 0 (the day of transplantation)

Day	Samples code	CMV DNA (copies/ml)	Type of sample	Amino acids changes related to resistance in:		Resistance to:	Amino acid changes related to genetic polymorphisms	
				UL97 (protein kinase)	UL54 (DNA pol)		UL97 (protein kinase)	UL54 (DNA polymerase)
+1	**RV-520**	5.98 × 10e5	Blood	M460V*	981-982 del.*	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+5	*RV-911*	Positive	NPA	M460V*	981-982 del. *	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+8	**RV-521**	5.52 × 10e6	Blood	M460V*	981-982 del. *	GCV, CDV, FOS	N68D, I244V	S655L, F669L, N685S, A885T & N898D
+19	**RV-523**	1.06 × 10e7	Blood	none	981-982 del. *	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+22	**RV-525**	1.65 × 10e6	Blood	none	981-982 del.	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+26	**RV-526**	5.94 × 10e7	Blood	none	981-982 del.	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+28	**RV-524**	Positive	Urine	none	981-982 del.	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+29	**RV-528**	5.09 × 10e7	Blood	none	981-982 del.	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+30	*RV-913*	Positive	Urine	none	981-982 del. *	GCV, CDV,FOS	None	S655L, F669L, N685S, A885T & N898D
+31	*RV-912*	ND	NPA	none	981-982 del. *	GCV, CDV, FOS	None	S655L, F669L, N685S, A885T & N898D
+33	**RV-527**	1.29 × 10e8	Blood	none	981-982 del. *	GCV, CDV, FOS	Non	S655L, F669L, N685S, A885T & N898D

NA (not amplifiable), NPA (nasopharyngeal aspirate), CSF (cerebrospinal fluid), GCV (ganciclovir), CDV (cidofovir), FOS (foscarnet), del (deletion), DL (detection limit), ND (not detected).
Samples marked in bold were analyzed when they were recovered from the patient in order to adapt antiviral treatment while those indicated in italic were evaluated retrospectively.

CMV disease of the CNS

- Has occurred mostly in patients with advanced AIDS, rarely reported in HSCT patients.

- Is a rare complication after HSCT or solid-organ transplantation.

- In our patient, CMV encephalitis was associated with:
  - **recurrent CMV viremia** following **prolonged administration of ganciclovir and foscarnet.**
  - **emergence of drug-resistance.**

CMV central nervous system (CNS) disease after allogeneic HSCT

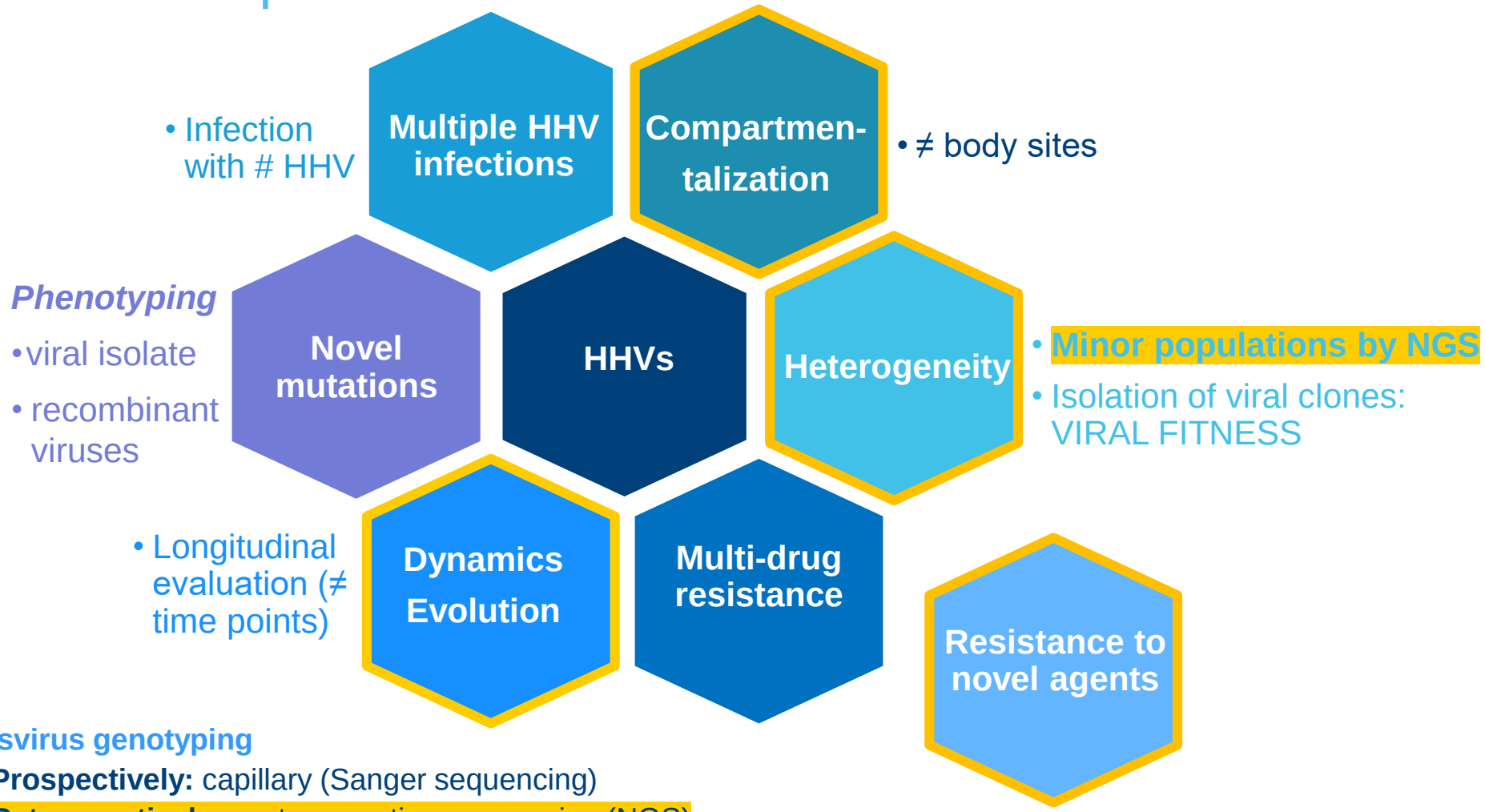
- Is a late-onset disease (median time of onset, 210 days) and is usually manifested as encephalitis (**Reddy et al, 2010**)

- **Associated with:**
  - **T-cell depletion, anti-thymocyte globulin, umbilical cord blood transplantation** that cause severe and extended T-cell immunodeficiency (8 of 11 cases)
  - a history of **recurrent CMV viremia** treated with multiple courses of preemptive ganciclovir or foscarnet therapy (11 of 11 cases)
  - **drug-resistance** (11 of 11 cases)
  - **high mortality** despite therapy with a combination of anti-CMV drugs (ganciclovir, foscarnet and cidofovir) (10 of 11 cases)

CMV central nervous system (CNS) disease after allogeneic HSCT

- **Suboptimal ganciclovir dose** may promote the emergence of drug-resistance since adequate drug levels are crucial to control viral replication.
- High drug levels are known to delay or prevent development of resistance while **suboptimal drug doses encourage viral replication and emergence of drug-resistance**.
- Rapid availability of **CMV viral load measurements** as well as **sensitive detection of CMV in CSF** are crucial for adequate antiviral chemotherapy.
- In the presented case report, **close monitoring of genotypic resistance** gave an opportunity to modulate the antiviral treatment in function of the identified mutant viruses.
- In our case, the same CMV strain in different body compartments was found.

Case study II: Multidrug-resistant CMV infection in a pediatric stem cell transplantation patient



Herpesvirus genotyping

- **Prospectively:** capillary (Sanger sequencing)
- **Retrospectively:** next-generation sequencing (NGS)

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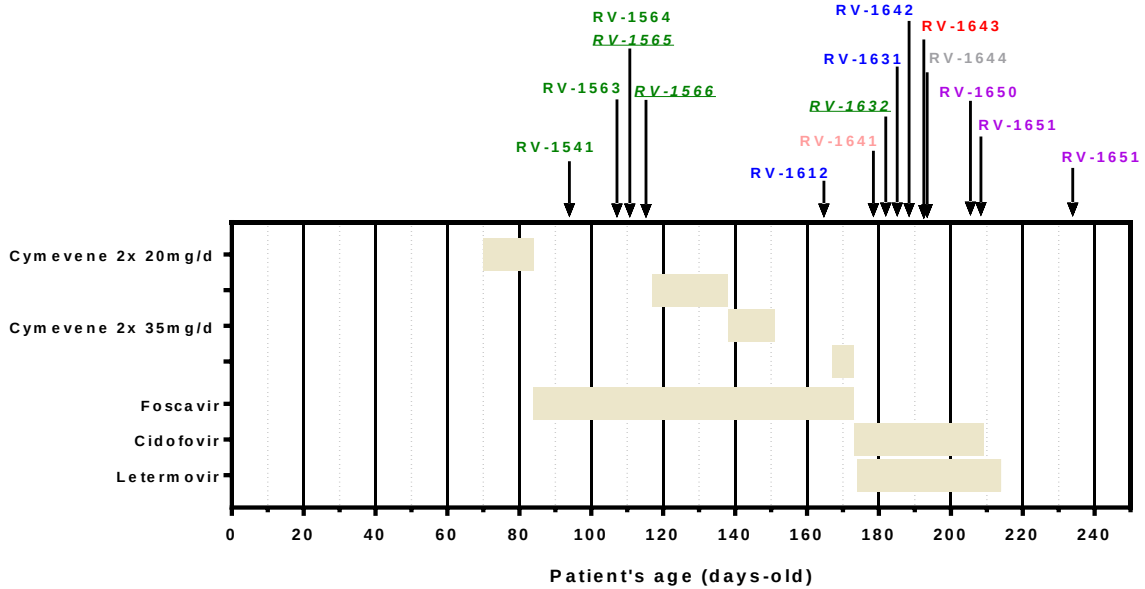
Emergence of resistance to new anti-HCMV agents in an HSCT pediatric patient



Blood samples except for the CSF specimens RV-1565, RV-1566, RV-1632 and RV-1644, which are underlined and in italics

** Heterogeneous populations*

Abbreviations: GCV: ganciclovir, PFA: foscavir, LMV: letermovir, R: resistance

RegaVir ID	Sanger sequencing		NGS	
	UL54	UL56	UL54	UL56
RV-1541	Wild-type	ND	Wild-type	Wild-type
RV-1563	Wild-type	ND	Wild-type	Wild-type
RV-1564	Wild-type	ND	Wild-type	Wild-type
RV-1565	Wild-type	ND	ND	ND
RV-1566	Wild-type	ND	ND	ND
RV-1612	V715M* A809V*	ND	V715M: 20.5% V781I: 11.1% A809V: 23.3%	Wild-type
RV-1631	V715M* A809V*	Wild-type	V715M: 20.4% V781I: 7.9% A809V: 57.6%	Wild-type
RV-1632	Wild type	Wild-type	ND	ND
RV-1641	V715M* V781I* A809V*	Wild-type	V715M: 12.2% V781I: 12.8% A809V: 58.1%	Wild-type
RV-1642	V715M* A809V*	Wild-type	V715M: 64.2% V781I: 6.9% A809V: 20.5%	Wild-type
RV-1643	V715M* V781I* A809V*	C325F*	V715M: 19.5% V781I: 12.7% A809V: 41.8%	C325F: 26.1%
RV-1644	Not amplifiable	ND	ND	ND
RV-1650	V781I	C325F	V781I: 92.7%	C325F: 90.3%
RV-1651	V781I	C325F	V781I: 98.5%	C325F: 98.3%
RV-1694	V781I	C325F	V781I: 97.1%	C325F: 95.9%

- **Heterogeinicity**
- **NGS advantage**
- **Compartmentalization**

➢ **Letermovir resistance**

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