Refractory and resistant HSV infections:

the virologist point of view

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Herpes simplex virus (HSV) infection

- **Global prevalence**
  - One of the most prevalent infection worldwide
  - Seroprevalence of **HSV-1** in the population aged 15-49 years: **66.6%** (3,752 million people)
  - Seroprevalence of **HSV-2** in the population aged 0-49 years: **13.2%** (492 million people)

- **Shift in HSV-1 epidemiology**
  - Decline of oral acquisition in childhood
  - Increase of genital acquisition during adolescence and adulthood

- **Clinical manifestations in immunocompetent individuals**
  - Immunocompetent individuals:
    - Relatively short duration and generally self-limited
    - More severe (even life-threatening) pathologies may occur
  - Immunocompromised patients (transplantation, HIV infection, chemotherapy/radiotherapy, immunosuppressive drugs for auto-immune diseases):
    - More invasive diseases (aggressive and extensive) with a risk of dissemination
    - Prolonged viral shedding and slower healing (persistent infection)
    - Severe morbidity and mortality

James et al., 2020; Schalkwijk et al., 2022; Burrel and Boutolleau, 2022

Clinical manifestations of HSV infections

- **Type of HSV infections in transplant recipients**
  - Rare primary infections: from the allograft in SOT (quite severe)
  - HSV reactivations: frequency of reactivation in HSCT recipients = 80%
  - Need for prophylactic antiviral treatments

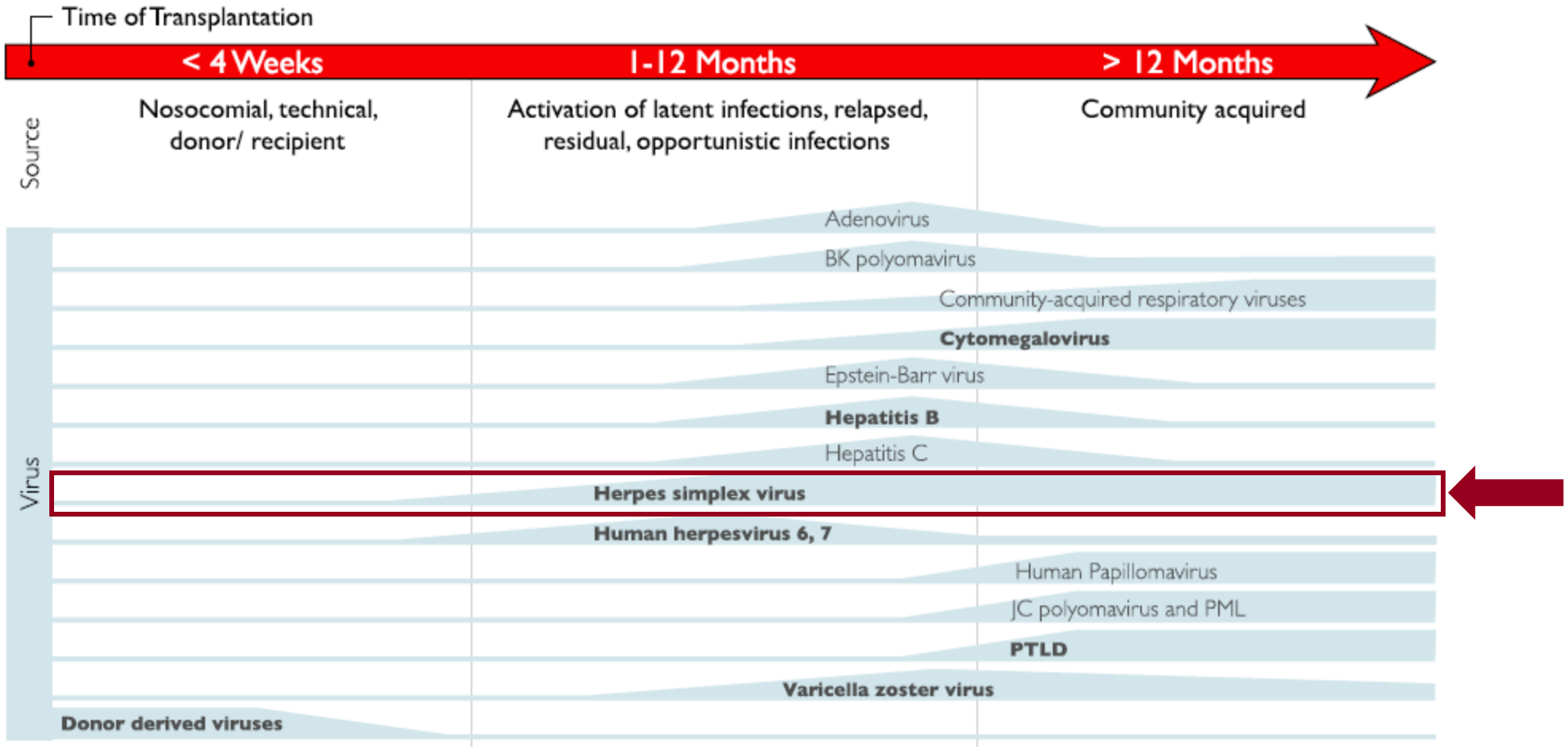
- **Clinical manifestations**

Virus	Immunocompetent	Immunocompromised
HSV-1	Gingivostomatis (primary infection) Orolabial herpes Mucocutaneous herpes Anogenital herpes Neonatal herpes Herpetic keratitis Herpectic encephalitis	Disseminated mucocutaneous infection (orolabial, anogenital herpes) Disseminated visceral infection (hepatitis, œsophagitis, colitis...) Herpetic keratitis Herpectic encephalitis
HSV-2	Anogenital herpes Neonatal herpes Herpetic meningitis	Disseminated mucocutaneous infection (anogenital herpes)

Schalkwijk et al., 2022

Burden of HSV infections in transplantation

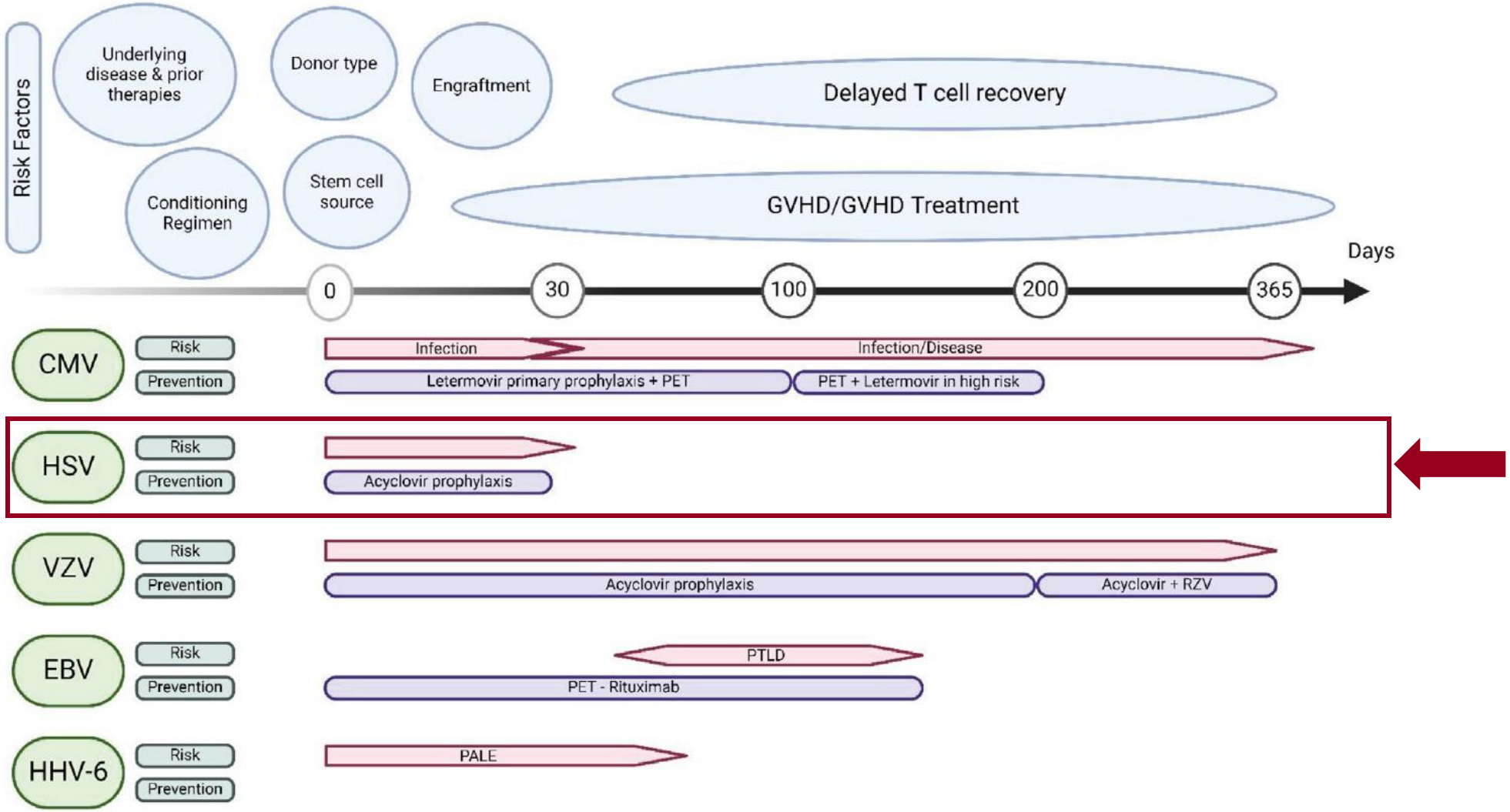
Solid organ transplantation



Fishman, 2017

Burden of HSV infections

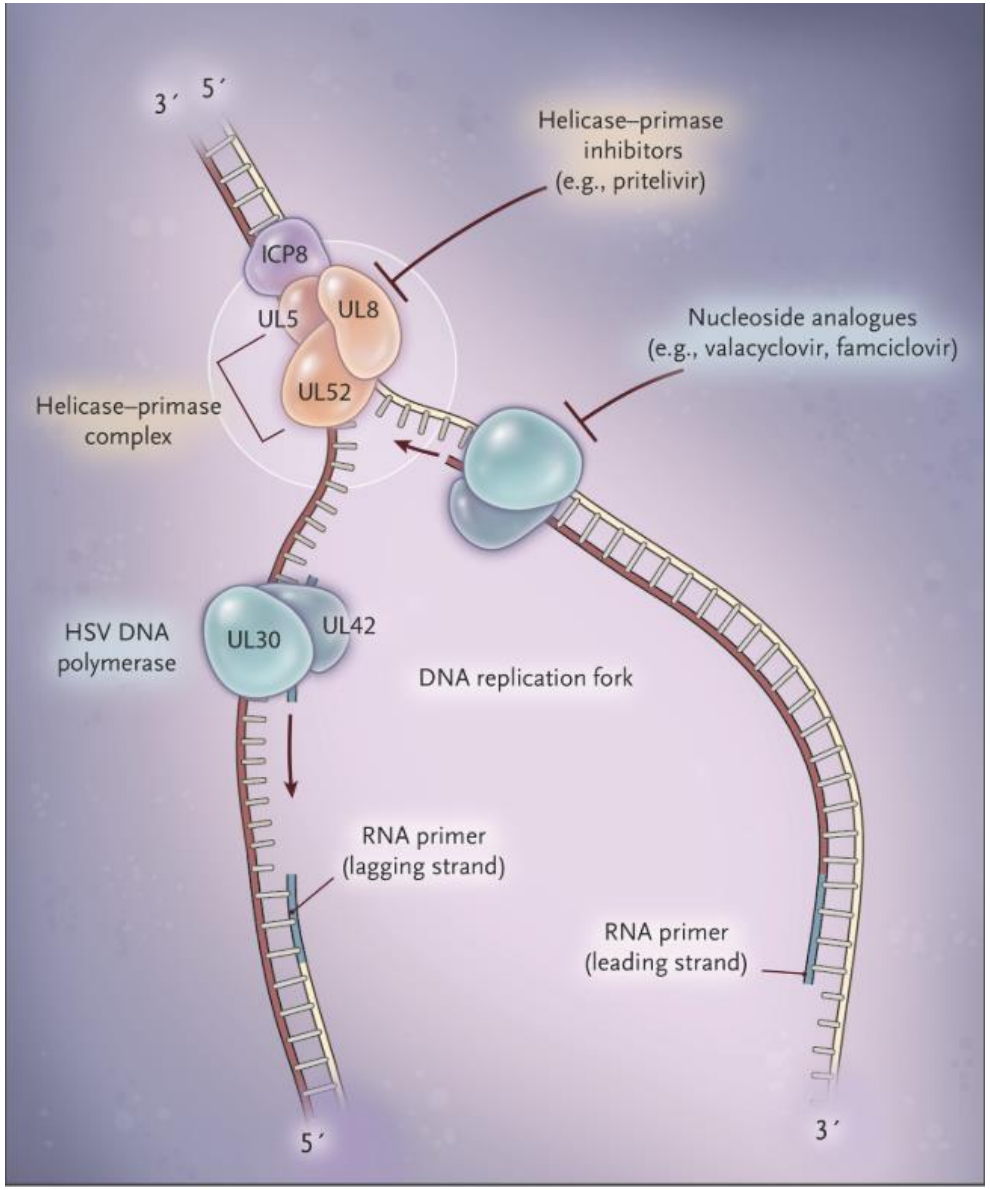
Hematopoietic stem cell transplantation



Sassine et al., 2024

Mechanism of action of antivirals

Inhibition of viral DNA replication



Two viral targets:

- **DNA polymerase** (UL30 gene)
- **Helicase-primase complex** (UL5/UL52 genes)

Whitley and Prichard, 2014

Antivirals for prophylactic or curative treatments of HSV infections

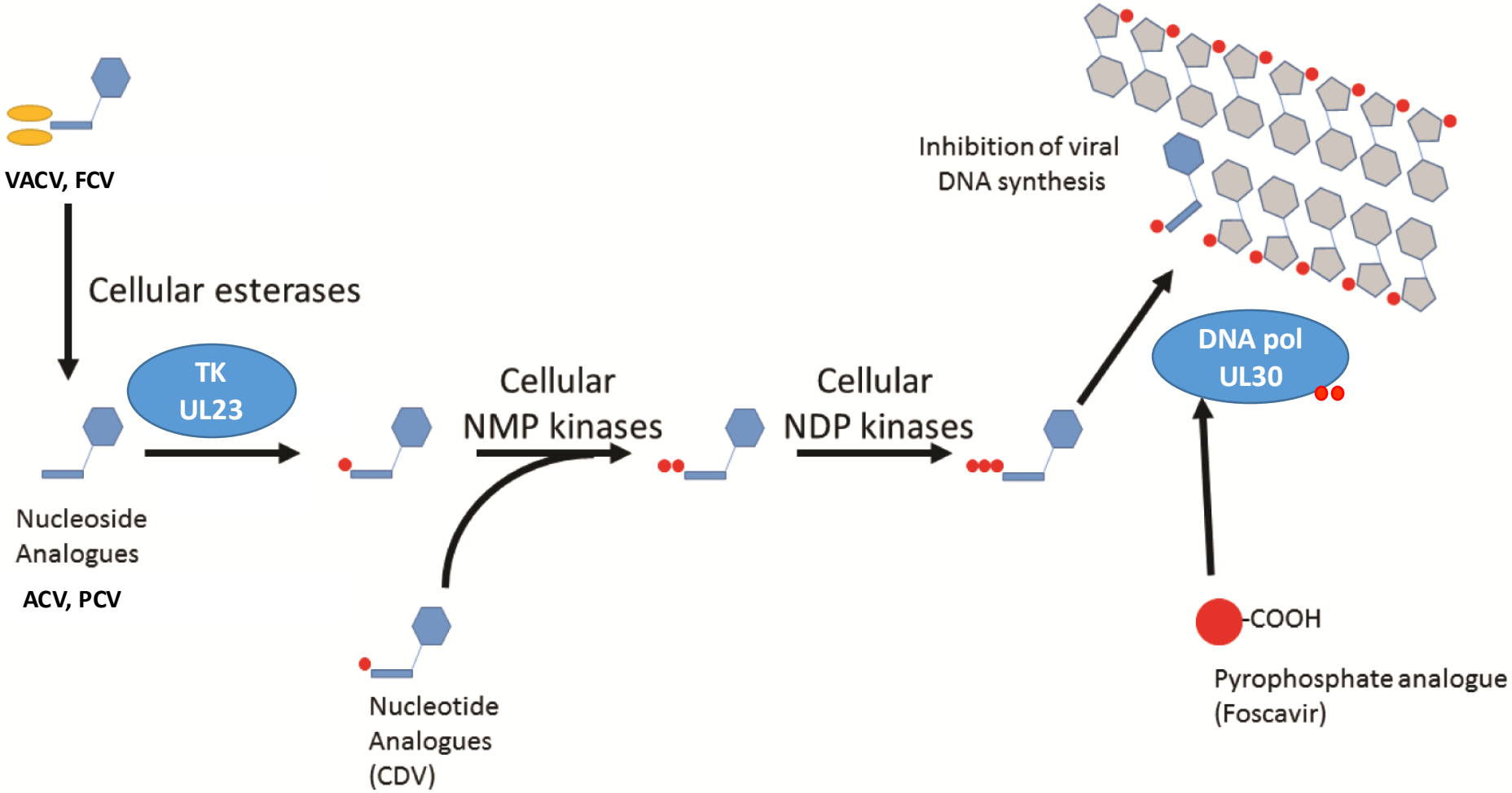
HSV target	Type	Antiviral	Administration	
DNA polymerase	Nucleoside analogues	Acyclovir (ACV	IV, topical (oral)	*1st line*
		Valacyclovir (VACV)	oral	
		Famciclovir (FCV)	oral	
		Ganciclovir (GCV)	Topical (keratitis)	
		Trifluridine (TFT)	Topical (keratitis)	
	Nucleotide analogue	Cidofovir (CDV)	IV	*Off-label : compassionate use*
	Pyrophosphate analogue	Foscarnet (FOS)	IV	*2nd line*
Helicase-primase	Phenyloxadiazole derivative	Amenamevir (AMNV)	oral	*Compassionate use*
	Thiazolylamide derivative	Pritelivir (PTV)	oral	*Compassionate use*

- **HSV prophylaxis after solid organ transplantation**
  - CMV prophylaxis (valganciclovir)
  - Prophylaxis with VACV (500 mgx2/day) for at least one month
- **HSV prophylaxis after hematopoietic stem cell transplantation**
  - Prophylaxis with VACV (500 mgx2/day) at least until neutrophil engraftment

Lee and Zuckerman, 2019; Lewalle et al., 2019 ; Sallée and Boutolleau, 2024

Mechanism of action of antivirals

Antivirals targeting the DNA polymerase



Burrel et al, 2020

Refractory/resistant HSV infections

- **Definitions (proposal)**
  - Refractory infection: failure to improve lesions or to prevent new lesions after 7 days of an appropriate route and dosage of antiviral therapy
  - Resistant infection: refractory infection with virologically proven HSV mutation of resistance to antiviral(s)

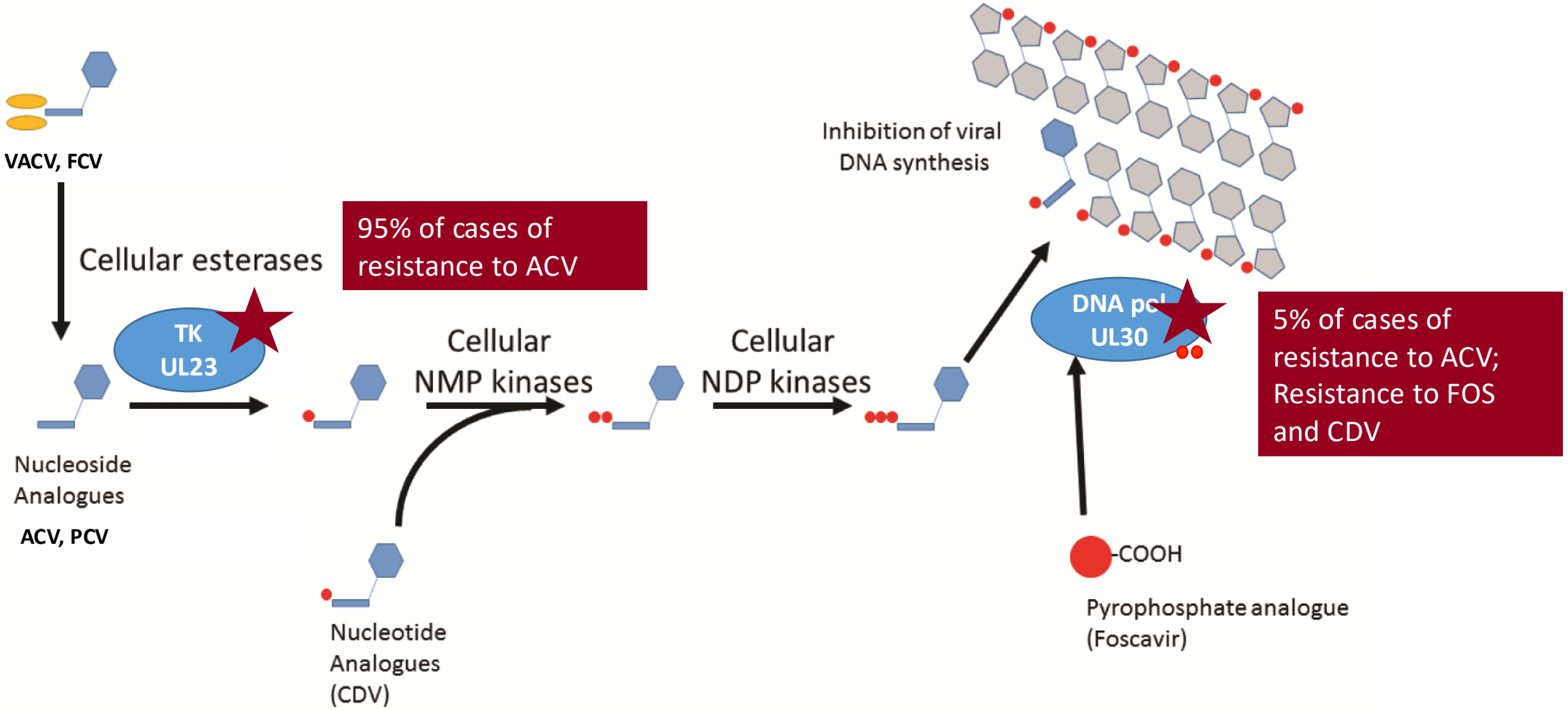
- **Frequency of HSV resistance to antivirals**
  - Immunocompromised patients: **3.5%** to **14%**
    - HSCT recipients: up to **30%** to **40%**
  - Immunocompetent patients: **<1%**
    - Recurrent herpetic keratitis: **6.5%**

- **Risk factors for emergence of HSV resistance to antivirals**
  - High immunosuppression (HLA mismatch, GvHD or graft rejection [corticosteroids]…)
  - Recurrent infections, ongoing viral replication
  - Prolonged antiviral treatments, intermittent treatments, inappropriate dose of antiviral (malabsorption) -> antiviral dosage

Piret and Boivin, 2014; Shafat and Chemaly, TAVI Forum 2024; Boutolleau and Sallée, 2024

HSV resistance to antivirals

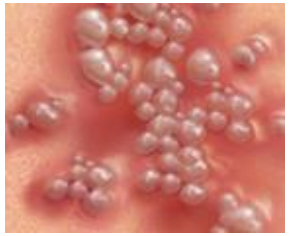
Molecular mechanism: mutations in viral targets involved in the mechanism of action of antivirals



Burrel et al, 2020

HSV antiviral resistance testing

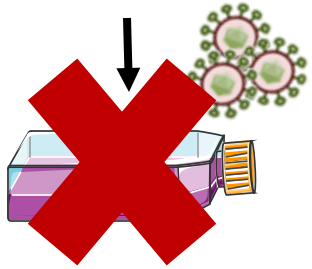
NRC for Herpesviruses: two-step procedure



HSV-1 / HSV-2

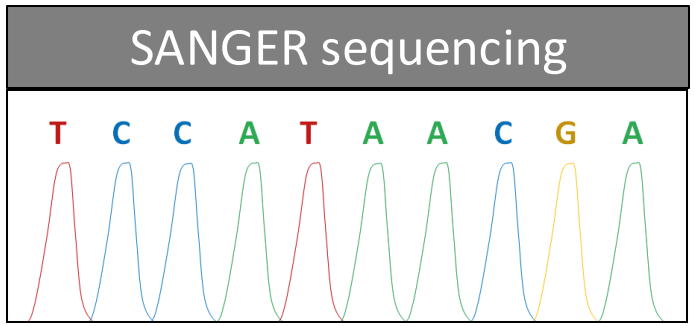
↓

Cell culture



1) Genotypic test

TK and Pol gene sequencing



Interpretable profile

Uninterpretable profile *(unknown mutation)*

2) Phenotypic test

(acyclovir and foscarnet EC_{50} values)

Increasing dilutions of antivirals

Plaque reduction assay (PRA) in Vero cells

Interpretable profile

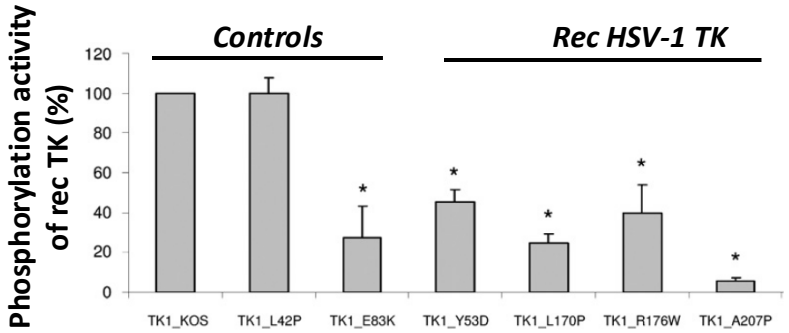
3) Role of unpreviously reported mutations in HSV TK

- Functional enzyme assays *in vitro*
- Recombinant viral assays

Burrel et al., AVR, 2013

Role of HSV unpreviously reported mutations

- **Non-radioactive phosphorylation activity assay of recombinant TK *in vitro***



  - Novel HSV-1 TK mutations: Y53D, L170P, R176W, A207P
  - Novel HSV-2 TK mutations: I101S, M183I

- **Phenotypic assay with recombinant virus (BAC technology)**
  - HSV BAC vector (kind gift from J. Cohen, NIH/NIAID, US)
  - HSV-2 TK : S66P -> acyclovir resistance ; A72S -> natural polymorphism
  - HSV-1 TK : L340R -> acyclovir resistance

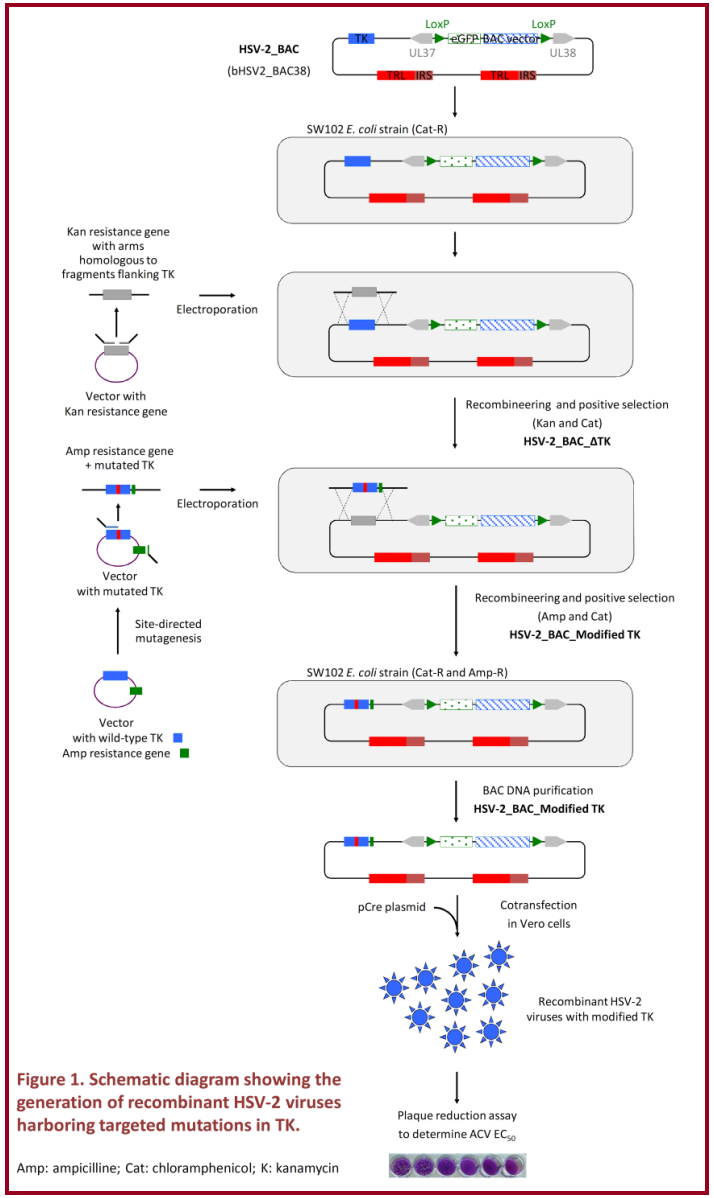
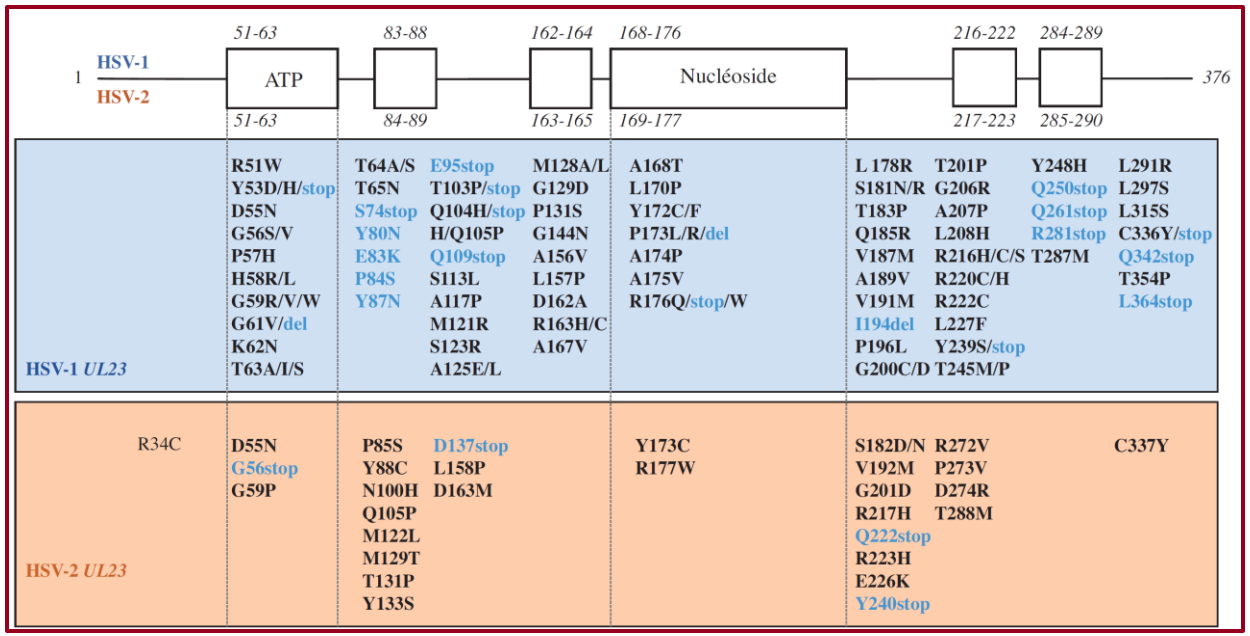


Figure 1. Schematic diagram showing the generation of recombinant HSV-2 viruses harboring targeted mutations in TK.

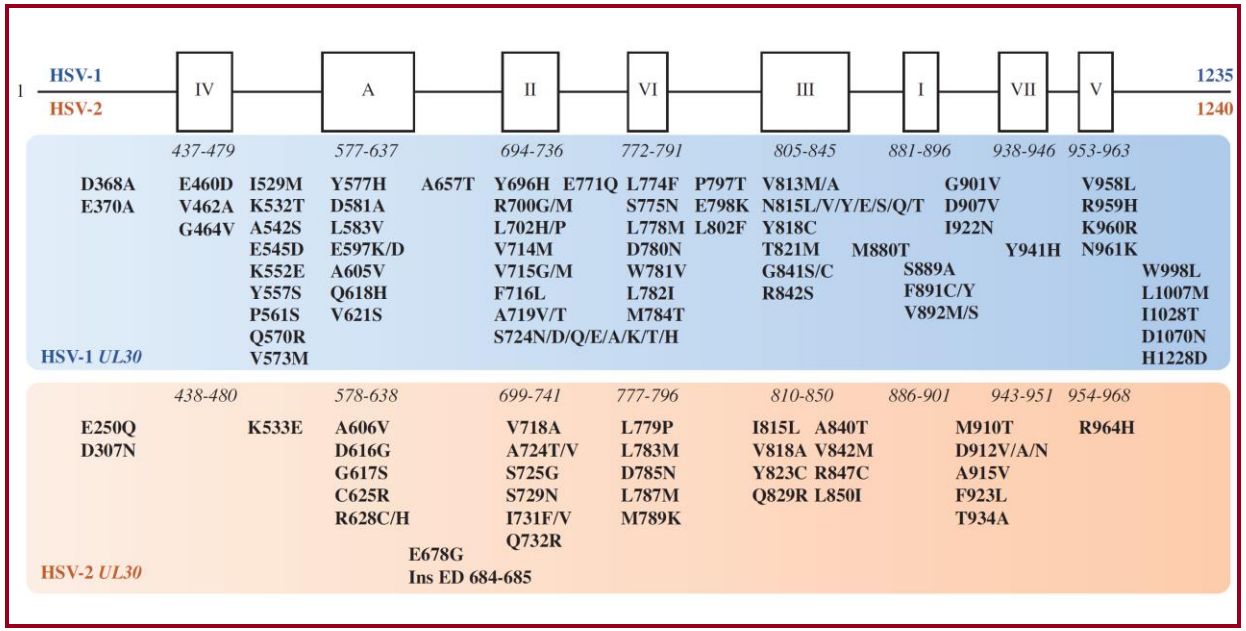
Burrel et al., 2012; Boutolleau et al., 2017; Robinet-Perrin et al., 2019

Mutations of HSV resistance to antivirals

Thymidine kinase UL23



DNA polymerase UL30



➔ **Need for an updated European database for all HSV resistance mutations**

- Ongoing in 2025: collaboration of Dr D. Boutolleau (NRC Herpesviruses, Paris) and Dr E. Frobert (Lyon)
- Other teams: Leuven (H. Schalkwijk, G. Andrei), London (D. Biby, T. Mbisa), Freiburg (L. Jaki, M. Panning)

Burrel et al., 2020

Results from the National Reference Center

HSV resistance to antivirals among transplant recipients with refractory HSV infection (at least 7 days of antiviral treatment): 2008-2024

59 SOT recipients (91 clinical samples)
28 males, 31 females, median age: 56y

- **36 patients with HSV-1 infection**
  - **21 patients (58%) with HSV-1 resistance to antivirals**
- **23 patients with HSV-2 infection**
  - **11 patients (48%) with HSV-2 resistance to antivirals**

Resistance to acyclovir: 100%

223 HSCT recipients (340 clinical samples)
110 males, 113 females, median age: 52y

- **197 patients with HSV-1 infection**
  - **152 patients (77%) with HSV-1 resistance to antivirals**
- **26 patients with HSV-2 infection**
  - **18 patients (69%) with HSV-2 resistance to antivirals**

Resistance to acyclovir: 88%

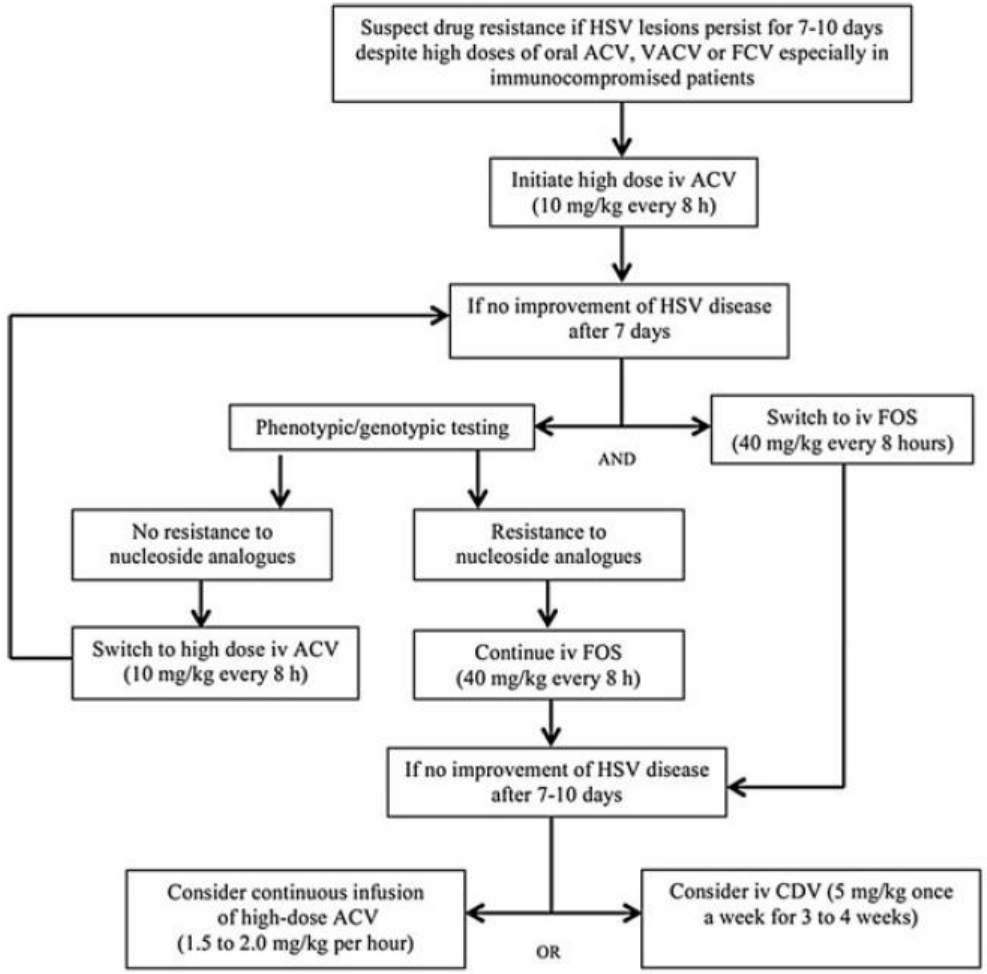
Resistance to acyclovir + foscarnet: 9%

Resistance acyclovir + foscarnet + cidofovir : 3%

Data from the National Reference Center for Herpesviruses, Pitié-Salpêtrière Hospital, Paris

Management of refractory/resistant HSV infections

Suggested algorithm for the management of suspected nucleoside analogues-resistant HSV infections



Dosing of antiviral in blood

Consider investigational therapy -> helicase-primase inhibitors

A new algorithm is coming soon (TAVI Forum 2024)

High doses oral
ACV 800 mg 5 times a day
VACV 1000 mg TID
FCV 500 mg TID

Piret and Boivin, 2014

VIRALLO study

Refractory/resistant HSV infections to antivirals among adult allogeneic hematopoietic stem cell transplant recipients: a retrospective national French study (2015-2023)

NRC for Herpesviruses (Paris)
Data regarding diagnosis of HSV resistance to antivirals in patients with HSV refractory HSV infection in France (Pitié-Salpêtrière, Saint Louis, Lyon, Limoges)

French Society for Bone Marrow Transplantation (SFGM-TC)
Patients' characteristics (demographics, clinic, biologic)
Patients with HSV resistance
Patients without HSV resistance

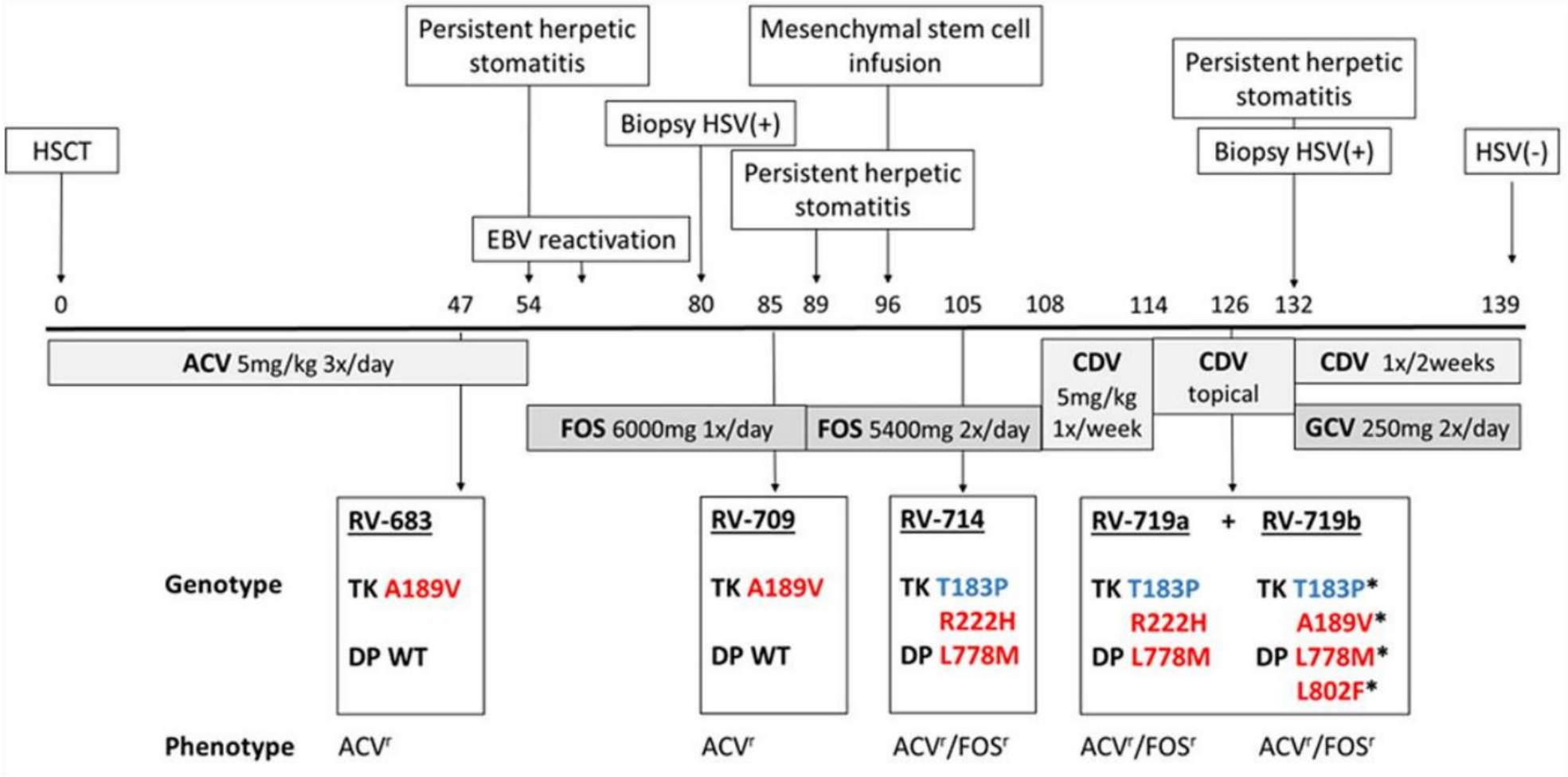
Merging of both databases

National frequency of HSV resistance to antivirals
Risk factors for HSV resistance to antivirals
Prognostic impact of HSV resistance to antivirals

Waiting for the approval by the French National Commission for Data Protection and Liberties (CNIL)

Case report [1]

41-year-old man with HSCTx
Persistent HSV-1 stomatitis

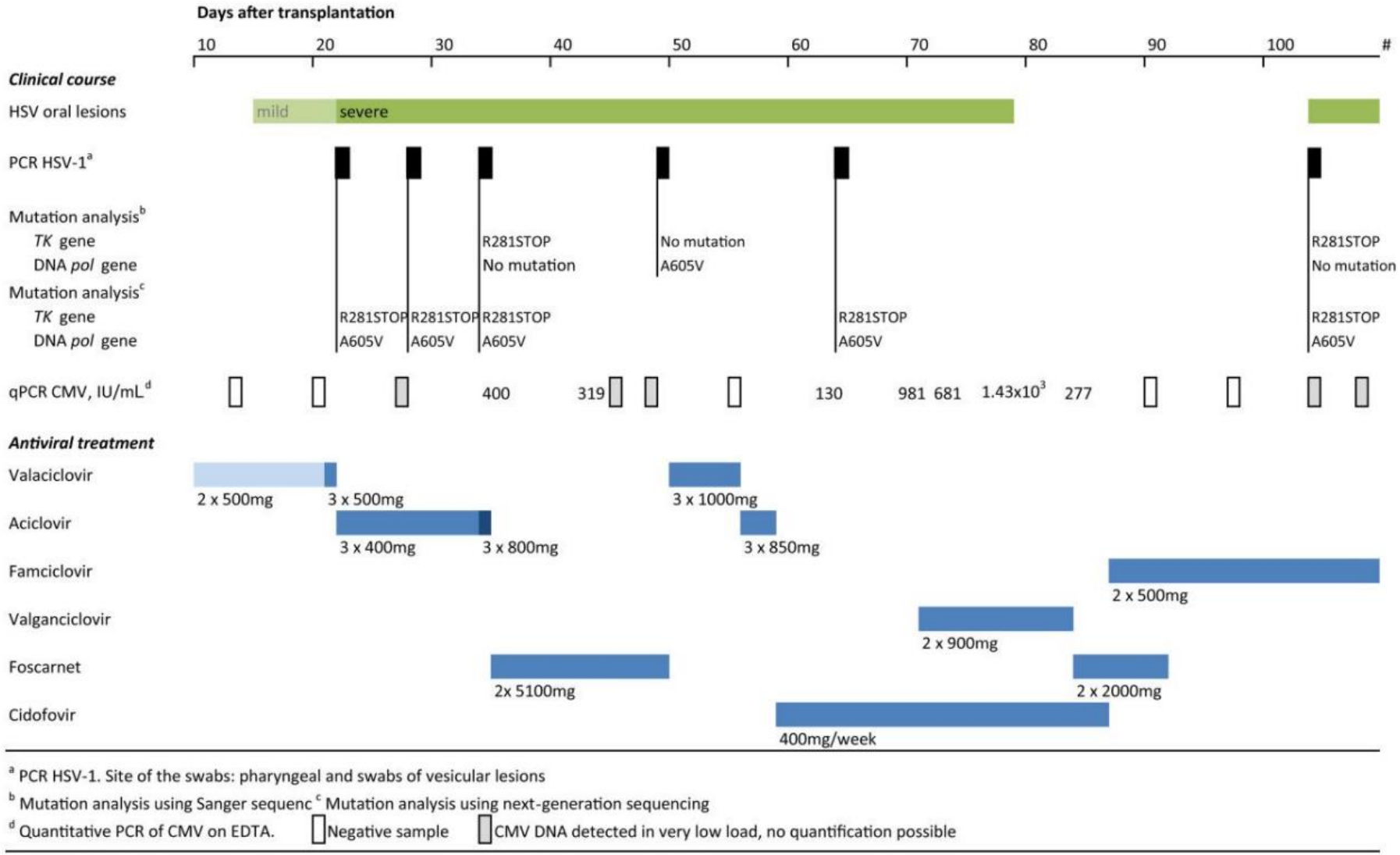


Mutation conferring drug resistance

Schalkwijk et al., 2022

Case report [2]

61-year-old man with HSCTx
Persistent HSV-1 stomatitis (†)



[a] PCR HSV-1. Site of the swabs: pharyngeal and swabs of vesicular lesions

[b] Mutation analysis using Sanger sequenc [c] Mutation analysis using next-generation sequencing

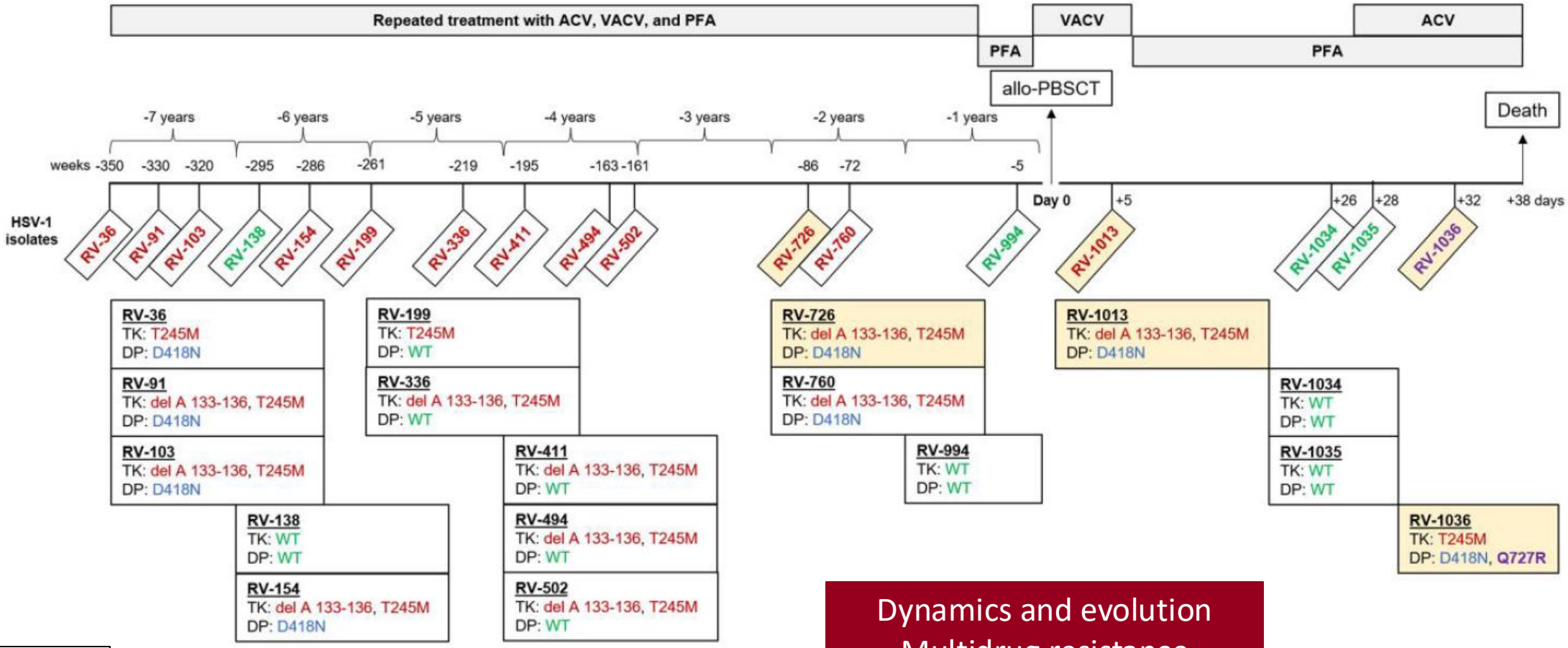
[d] Quantitative PCR of CMV on EDTA. Negative sample CMV DNA detected in very low load, no quantification possible

Resistance to ACV and FOS

Zheng et al., 2024

Case report [3]

21-year-old man with primary immunodeficiency and HSCTx
Recurrent orofacial and genital HSV-1 lesions (†)



Orofacial samples

Anogenital samples

Mutation conferring drug resistance

Dynamics and evolution
Multidrug resistance
Compartmentalization

Schalkwijk et al., 2022

Case report [4]

76-year-old woman with kidney tranplantation
Fatal disseminated HSV-2 infection (†)

		Donor	Recipient											
		10/31/21	11/01/21	12/06/21	02/02/22	02/14/22	02/21/22	03/01/22	03/03/22	03/07/22	03/08/22	03/11/22	03/16/22	03/21/22	03/23/22
Clinical event			Kidney transplant		Cutaneous herpetic lesions		Esophagitis; renal failure; hospitalization in nephrology	Neurological symptoms	Transfer to intensive care unit						Death
Immunosuppressive treatment			Mycophenolic acid (500 mg bid), tacrolimus (target concentration 5–7 ng/mL), corticoids (5 mg)					Corticoids (5 mg)		None					
Renal function (mL/min/1.73 m²)			4	43	37		35	76	64	40	41	54	61	53	52
Antiviral treatment			Valganciclovir (450 mg/48 h)			Acyclovir (200 mg tid) IV		Acyclovir (600 mg tid) IV					Foscarnet (4.5 g bid) IV		
Antiviral concentration (mg/L)					Valganciclovir <0.2				Cmin: 3.6 Cmax: 18		Cmin: 4.3 Cmax: 13		Cmin: 1.7 Cmax: 13		
IgG HSV-1/2 serology	Serum	HSV-1-/HSV-2+	HSV-1-/HSV-2-		HSV-1-/HSV-2- No HSV DNA		HSV-1-/HSV-2-		HSV-1-/HSV-2+				HSV-1-/HSV-2+		
HSV qPCR (viral load, copies/mL) and sequencing (resistance)	Kidney biopsy														
	Spleen biopsy	No HSV DNA													
	BAL									HSV-2 (6.2) [R]				HSV-2 (5.6) [NP]	
	CSF							HSV-2 (4.3) [R][a]		HSV-2 (3) [NP]					No HSV DNA
	Skin lesion/oral mucosa					HSV-2 (7.9) [R]	HSV-2 (>8)					HSV-2 (>8) [R]			
	Serum/whole blood	No HSV DNA		HSV-2 (5.3) [S]	HSV-2 (8.6) [NP]		HSV-2 (7.0) [R]		HSV-2 (>8) [R]	HSV-2 (7.7) [NP]	HSV-2 (7.7) [R]		HSV-2 (6.2) [NP]		HSV-2 (4.7) [NP]

➔ Transmission of HSV-2 by kidney graft from the donor to the recipient

➔ Selection of HSV-2 resistance to acyclovir during valganciclovir prophylaxis for CMV

Truffot et al., 2023

Donor-derived HSV infections after SOTx

Two case-reports and review of literature

Two cases of donor-derived fulminant HSV hepatitis after liver transplantation

- F, 58 y and M, 66 y
- Negative HSV serostatus for both recipients
- No anti-HSV or anti-CMV prophylaxis
- Fatal fulminant HSV hepatitis

Author, year of publication	Age, gender	Type of transplant	Donor HSV serology profile	Receptor HSV serology profile	HSV prophylaxis	Time of onset of symptoms following transplant	Diagnosis (PCR, blood)	Outcome
Koneru, 1998	21, M	Kidney	HSV 1/2 positive	HSV 1/2 negative	No	10 days	HSV 2 positive	Died
	30, M	Kidney	HSV 1/2 positive	HSV 1/2 negative	No	10 days	HSV 2 positive	Died
Gabel, 1988	48, M	Kidney		HSV 1/2 negative	No	21 days	HSV 1 positive	Survived
Goodman, 1989	30, F	Pancreas	HSV 1/2 positive	HSV 1/2 negative	No	8 days	HSV 2 positive	Died
	64, F	Heart	HSV 1/2 positive	HSV 1/2 negative	No	14 days	HSV 2 positive	Survived
Kusne, 1991	26, F	Liver	HSV 1/2 positive	HSV 1 negative HSV 2 positive	No	18 days	HSV 1 positive	Died
Nebbia, 2006	44, F	Liver	HSV 1 negative HSV 2 positive	HSV 1/2 negative	No	10 days	HSV 2 positive	Died
Basse, 2008	58, M	Liver	ND	HSV 1/2 negative	ND	12 days	HSV 2 positive	Survived
Al Midani, 2011	26, F	Kidney	HSV 1 positive HSV 2 negative	HSV 1/2 negative	No	14 days	HSV 1 positive	Survived
	59, M	Kidney	HSV 1/2 negative	HSV 1/2 negative	No	12 days	HSV 1 positive	Died
Côté D, 2014	64, M	Liver	HSV 1 positive HSV 2 negative	HSV 1/2 negative	No	9 days	HSV 1 positive	Died
Pietrucha-Dilanchian, 2016	24, F	Heart	ND	HSV 1/2 positive	No	3 years	HSV 2 positive	Died
Feugeas, 2016	41, F	Kidney–Pancreas	HSV 1/2 negative	HSV 1/2 negative	No	23 days	HSV 1 positive	Survived
Macesic, 2017	30, M	Kidney–Pancreas	HSV 1 negative HSV 2 positive	HSV 1/2 negative	No	7 days	HSV 2 positive	Died
	20, F	Liver	HSV 1 negative HSV 2 positive	HSV 1/2 positive	No	13 days	HSV 2 positive	Survived
Shaw, 2018	31, F	Kidney	HSV 1 negative HSV 2 positive	HSV 1/2 serology negative	6 months val-ganciclovir	7 months	HSV 2 positive	Survived
Zeidan, 2021	43, F	Kidney	HSV 1 negative HSV 2 positive	HSV 1 positive HSV 2 negative	No	9 days	HSV 2 positive	Survived
Arana, 2022	66, F	Liver	ND	HSV 1/2 negative	No	14 days	HSV 1 positive	Died
	69, M	Liver	ND	HSV 1/2 negative	No	13 days	HSV 1 positive	Died
	45, F	Kidney	ND	HSV 1/2 negative	No	21 days	HSV 1 positive	Died

Reinhold et al., 2023

Helicase-primase inhibitors

Amenamevir (AMNV)

Amenamevir, a novel helicase–primase inhibitor, for treatment of herpes zoster: A randomized, double-blind, valaciclovir-controlled phase 3 study

Makoto KAWASHIMA,[1] Osamu NEMOTO,[2] Mariko HONDA,[3] Daisuke WATANABE,[4] Juichiro NAKAYAMA,[5] Shinichi IMAFUKU,[6] Toshiyuki KATO,[7] Tsuneo KATSURAMAKI,[7] for the study investigators*

Single-Dose, Patient-Initiated Amenamevir Therapy for Recurrent Genital Herpes: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study

Makoto Kawashima,[1] Shinichi Imafuku,[2] Kosuke Fujio,[3] and Hiroshi Komazaki[3]

A phase 3, randomized, double-blind, placebo-controlled study evaluating a single, patient-initiated dose of amenamevir for recurrent herpes labialis

Makoto Kawashima[1] | Daisuke Watanabe[2] | Kosuke Fujio[3] | Hiroshi Komazaki[3]

- **Amenamevir**
  - Efficacy for herpes zoster treatment
  - Efficacy for orolabial herpes treatment
  - Efficacy for genital herpes treatment
- **Japan**
  - AMNV approved for treatment of herpes zoster and recurrent labial or genital herpes
- **France**
  - Authorization of compassionate use for refractory/resistant HSV or VZV infections (200 to 400 mg/day)
- **Good tolerance** (thrombocytopenia)
- **No HSV resistance to AMNV described to date in patients**

Kawashima et al., 2017, 2022a and 2022b

Helicase-primase inhibitors

Pritelivir (PTV)

Helicase–Primase Inhibitor Pritelivir for HSV-2 Infection

Anna Wald, M.D., M.P.H., Lawrence Corey, M.D., Burkhard Timmler, M.D., Amalia Magaret, Ph.D., Terri Warren, M.N., Stephen Tyring, M.D., Ph.D., Christine Johnston, M.D., M.P.H., John Kriesel, M.D., Kenneth Fife, M.D., Ph.D., Lawrence Galitz, M.D., Susanne Stoelben, M.D., M.P.H., Meei-Li Huang, Ph.D., Stacy Selke, M.A., Hans-Peter Stobernack, D.V.M., Helga Ruebsamen-Schaeff, Ph.D., and Alexander Birkmann, Ph.D.

Effect of Pritelivir Compared With Valacyclovir on Genital HSV-2 Shedding in Patients With Frequent Recurrences
A Randomized Clinical Trial

Anna Wald, MD, MPH; Burkhard Timmler, MD; Amalia Magaret, PhD; Terri Warren, ANP; Stephen Tyring, MD, PhD; Christine Johnston, MD, MPH; Kenneth Fife, MD, PhD; Stacy Selke, MA; Meei-Li Huang, PhD; Hans-Peter Stobernack, PhD; Holger Zimmermann, PhD; Lawrence Corey, MD; Alexander Birkmann, PhD; Helga Ruebsamen-Schaeff, PhD

- **Pritelivir**
  - Efficacy for genital herpes treatment
  - PTV > VACV for viral shedding

Phase 3 clinical trial PRIOH-1 (NCT03073967) : Efficacy and safety of **Pritelivir** tablets for treatment of acyclovir-resistant mucocutaneous HSV infections in immunocompromised subjects (*vs* foscarnet) *(ongoing)*

Helicase-primase inhibitors

Use of HPIs in transplant recipients

Orolabial herpes (HSV-1) in HSCTx (PTV)

Salvage Treatment of Refractory HSV Oral Lesions with Pritelivir in Allogeneic Hematopoietic Cell Transplant Recipients

Davide Bosetti,[a] Chiara Bernardi,[b] Marie Maulini,[b] Federica Giannotti,[b] Anne-Claire Mamez,[b] Stavroula Masouridi-Levrat,[b] Yves Chalandon,[b] Dionysios Neofytos[a]

Orolabial herpes (HSV-1) in HSCTx (AMNV)

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/ijantimicag

Letter to the Editor

Amenamevir for treating acyclovir-resistant or refractory herpes simplex virus infection in allogeneic hematopoietic stem cell transplantation recipients: Two case reports ☆

Sallée L, Souchet L, Boutolleau D, Nguyen S

Genital herpes (HSV-2) in HSCTx (PTV)

Pritelivir for recurrent aciclovir-resistant herpes simplex virus 2 infections in immunocompromised patients

Alexandra Serris[1], Anne Pouvaret[1], Clémence Loiseau[2], Hanene Abid[3], Sonia Burrel[4,5], Jacques Fourgeaud[3,6,7], Claire Rouzaud[1], Fanny Lanternier[1], David Boutolleau[4,5] and Pierre Frange [3,7]*

Herpetic keratitis (HSV-1) (AMNV)

Efficacy and Safety of Amenamevir, a Helicase-Primase Inhibitor for the Treatment of Acyclovir-Resistant Herpes Simplex Virus 1 Keratitis

Rafael Boucher, MD,† David Boutolleau, PharmD, PhD,‡§ Sonia Burrel, PharmD, PhD,‡¶ Oscar Haigh, PhD,† José Fernandez,‡ Christelle Vauloup-Fellous, MD, PhD,‖ Emmanuel Barreau, MD,* Antoine Rousseau, MD, PhD,*†**†† and Marc Labetoulle, MD, PhD*†**††*

Sallée and Boutolleau, 2024

Combination therapy

- **Use of combination therapy**
  - Common strategy for HIV and HCV infections
  - Sporadic use for HSV infections
  - Treatment of HSV infections with multiple viral strains with dissimilar resistance pattern (compartmentalization)
- **Synergistic or additive activity of anti-HSV drugs in vitro**
  - Synergistic activity of TFT + GCV
  - Synergistic activity of AMNV + ACV or AMNV + PCV
  - Additive effect of AMNV + ACV + CDV
  - Use of ACV + PTV or FOS + PTV prevent the emergence of HSV-1 resistance mutations to PTV
- **Among patients**
  - Case-reports : effective use of ACV+ FOS for ACV-refractory HSV infections
  - 1st case-report of this presentation: effective use of CDV + GCV for herpetic stomatitis
  - Use of topical CDV + systemic antiviral therapy (ACV, FOS)

Schalkwijk et al., 2022; Sallée and Boutolleau, 2024; Schalkwijk et al., 2024

Conclusion

- HSV infections in transplant recipients (SOT or HSCT) are common, potentially serious, and sometimes life-threatening
- Frequency of HSV resistance to antivirals varies from 3.5% to 14% (up to 30% to 40% in HSCT recipients)
- Significant increase of frequency of HSV resistance to antivirals in recent years
- Definition of HSV refractory infection: failure to improve lesions or to prevent new lesions after 7 days of an appropriate route and dosage of antiviral therapy
- Diagnosis of HSV resistance to antivirals (genotypic method)
- Therapeutic management of antiviral-resistant HSV infection may be very complicated
- Novel IHPs: amenamevir and pritelivir
- Possibilities of combination therapies


Thank you for your attention
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What about vaccination?

- **Three candidate vaccines in phase 1 or phase 1/2 clinical trials**
  - Safety, efficacy, immune response
  - Prevention of genital herpes (primary infection or recurrences)
  - mRNA for 2 vaccines
  - Recombinant protein for 1 vaccine

Overview of vaccine candidates in clinical trials.

Candidates currently in active phase clinical trials						
Candidate	**Antigen platform**	**Developer/ manufacturer**	**Phase of development**	**Route of administration, no. of doses, schedule**	**Vaccine approach**	**Registration ID**
BNT163	mRNA vaccine containing gD2, gE2, gC2	BioNTech	Phase 1	IM (schedule TBD)	Prophylactic	NCT05432583
GSK3943104A	Recombinant Protein-Adjuvanted	GlaxoSmithKline	Phase 1/2	IM, Day 1 and Day 29	Therapeutic	NCT05298254/EUCTR2021-003586–35-BE
mrRNA-1608	mRNA	Moderna	Phase 1/2	IM, Day 1 and Day 57	Therapeutic	NCT06033261

Johnston et al., 2024