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RegaVir platform: Case discussions antiviral resistance testing

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Herpes simplex virus (HSV) infections

HSV-1: Orofacial

Global prevalence: 67%

3.7 billion people under age 50

- **Mainly transmitted by oral-to-oral contact**
- **Commonly causes cold sores (fever blisters), but it can also lead to genital herpes**
- It is also a cause of encephalitis (brain infection) & eye infections.

HSV-2: Anogenital

Global prevalence: 13%

491 million people under aged 50

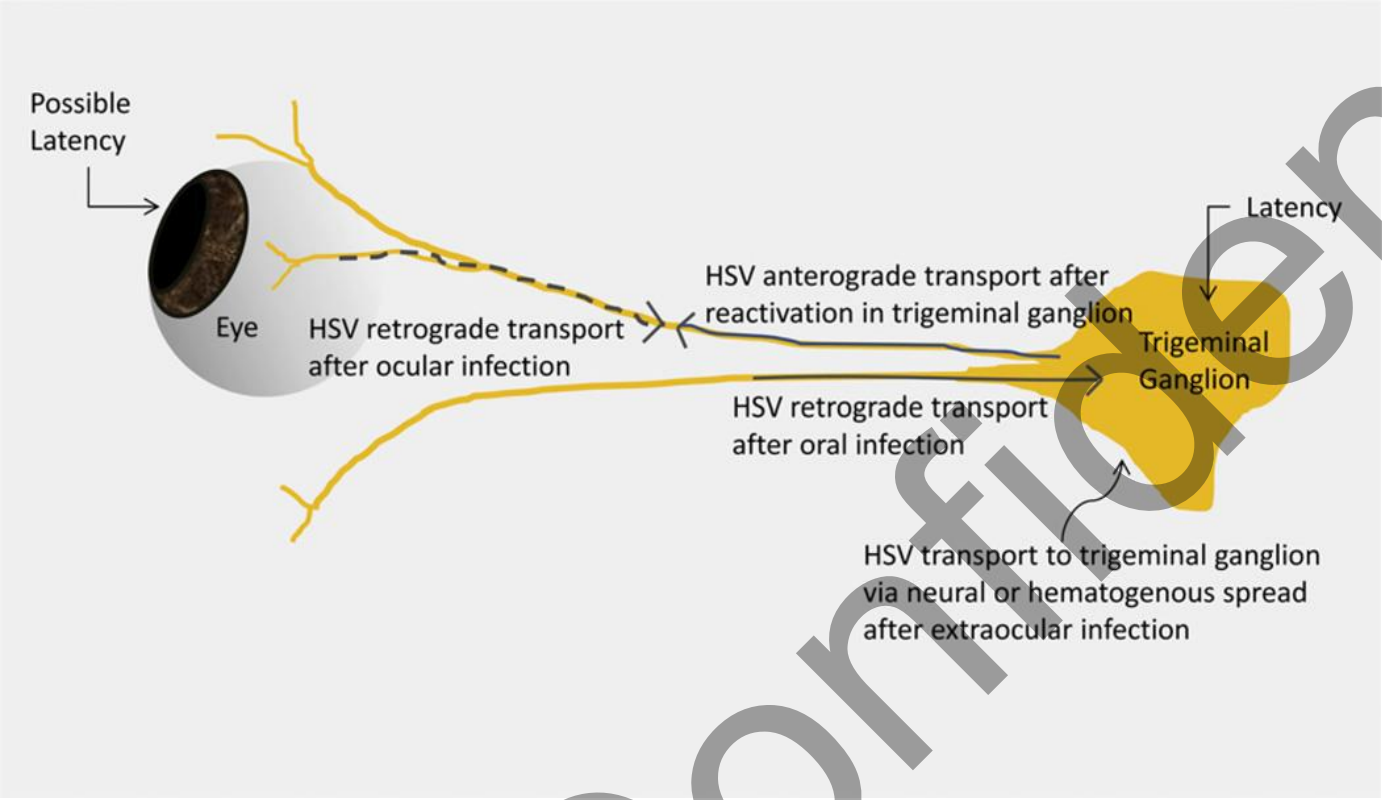
- **Mainly transmitted sexually or from mother to child**
- **Causes mainly genital herpes**
- It can also cause meningoencephalitis (brain infection) and neonatal herpes.

- **HSV is usually acquired in childhood or adolescence.**
- **After the initial infection, the virus can enter nerve cells in the dorsal ganglia and lie dormant, or latent.**

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 virus (HSV) ocular infections



HSV ocular infection, latency, and recurrence.

- HSV travels retrograde along the ophthalmic division of the fifth cranial nerve after ocular infection or via other routes after extraocular infection to develop **latency in trigeminal ganglia.**
- **It also may develop latency locally in the cornea.**
- Corneal latency of HSV may influence eye banking and corneal transplantation

HSV ocular pathology

- **HSV-1 >>> HSV-2**
- Ocular HSV manifests as:
  - Conjunctivitis
  - Iridocyclitis
  - Acute retinal necrosis
  - Keratitis: **an important cause of infectious blindness**, mainly resulting from stromal opacification.

- An estimated 500,000 people in the United States have ocular HSV.
- Treatment of new and recurrent cases costs the country US$ 17.7 million annually.
- The global impact of ocular HSV is difficult to ascertain because of a lack of surveillance-based epidemiologic studies.

HSV keratitis classification

- **The initial (primary) infection is usually nonspecific self-limiting conjunctivi**tis, often in early childhood and usually without corneal involvement.

- Recurrent ocular herpes affects the cornea.

- Herpes simplex keratitis usually affects the corneal surface but sometimes involves the corneal stroma (the deeper layers of the cornea) or the inner corneal surface (endothelium), anterior chamber, and iris. **Stromal involvement is probably an immunologic response to the virus.**

HSV CATEGORY	COMMON NOMENCLATURE	BASIC TREATMENT APPROACH
Epithelial keratitis	• Dendritic keratitis • Geographic keratitis	Antiviral (topical or oral)
Stromal keratitis without ulceration	• Interstitial keratitis • Immune stromal keratitis	Topical steroid + oral antiviral prophylaxis
Stromal keratitis with ulceration	• Necrotizing keratitis	Oral antiviral in therapeutic doses + topical steroid
Endothelial keratitis	• Disciform keratitis	Oral antiviral in therapeutic doses + topical steroid

Antivirals for HSV epithelial keratitis

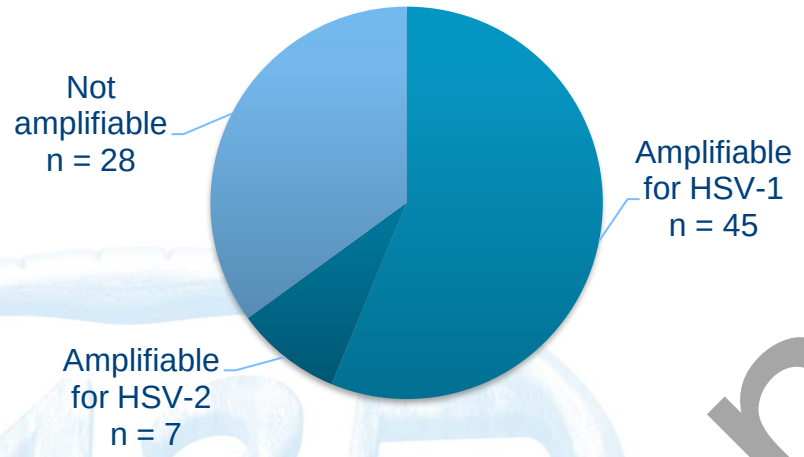
ORAL ANTIVIRALS		
Agent	**Treatment dose**	**Prophylactic dose**
Acyclovir	Adults: 400 mg 3x to 5x/day Children: 12 to 15 mg/kg/day in divided doses	400 mg 2x/day
Valacyclovir	500 mg 3x/day	500 mg 1x/day
Famciclovir	250 mg 3x/day	250 mg 1x/day or 125 mg 2x/day

TOPICAL ANTIVIRALS	
Agent	**Treatment Dose (for Short-Term Use Only)**
Trifluridine 1 percent	One drop every two hours, reduced to 5x/day after 3 to 7 days
Ganciclovir gel 0.15 percent	5x/day (better dosing and surface toxicity profile vs. trifluridine, but more expensive)

Immunocompromised patients may require IV antivirals (e.g., acyclovir 5mg/kg IV every 8 hours for 7 days)

RegaVir ocular samples for HSV

Ocular samples requested for HSV drug-resistance analysis (n = 80)



- Samples not amplifiable: 35% (28/80)
  - 21 patients with negative samples
  - 3 patients with positive & negative samples
  - *Why so many negative samples?*
    - Too old (> 5 days) and/or badly stored
    - Low viral load HSV Ct >36
- HSV positive samples: 65% (52/80)
  - HSV-1: 45 samples
  - HSV-2: 7 samples (2 patients)

RegaVir ocular samples for HSV

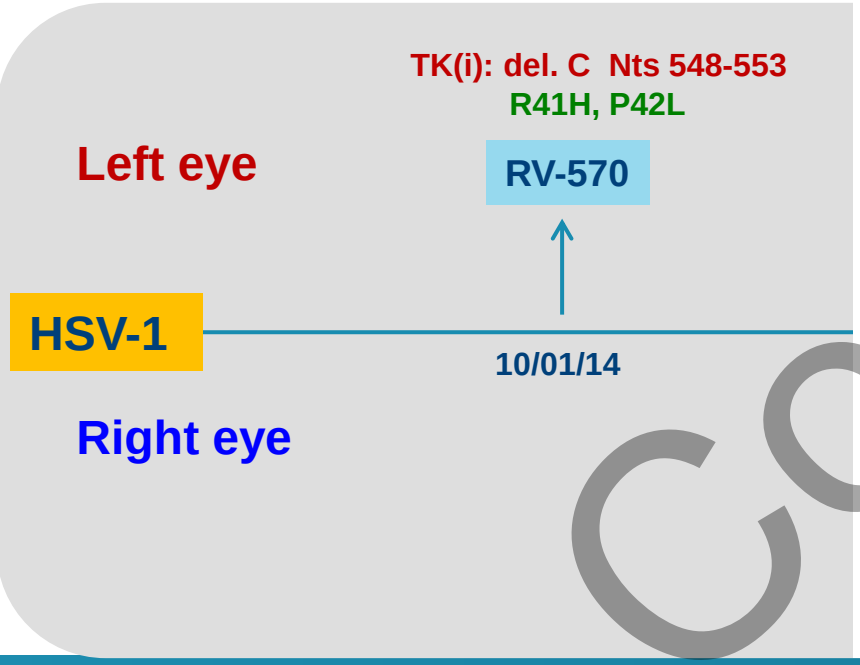
	Number of samples analyzed per patient					
	1	**2**	**3**	**4**	**5**	**6**
N° of patients	**56**	**4**	**2**	**1**		**1**
	55 HSV-1	HSV-1	HSV-1	HSV-1		HSV-2
	1 HSV-2					

Total number of patients analyzed: 65

- 63 patients analyzed for HSV-1 ocular infections
- 2 Patients analyzed for HSV-2 ocular infections

Ophthalmic herpes in a renal transplant after 6 months valacyclovir therapy - UZ Leuven

- The patient (62-years old woman) was reported to have ocular VZV infection in the left eye for several months.
- **Persistent complaints despite valaciclovir therapy (>6 months).**
- A recent tear sample showed HSV by PCR.
- RegaVir results HSV-1: positive (only TK, DNA pol not amplifiable) - VZV not amplifiable



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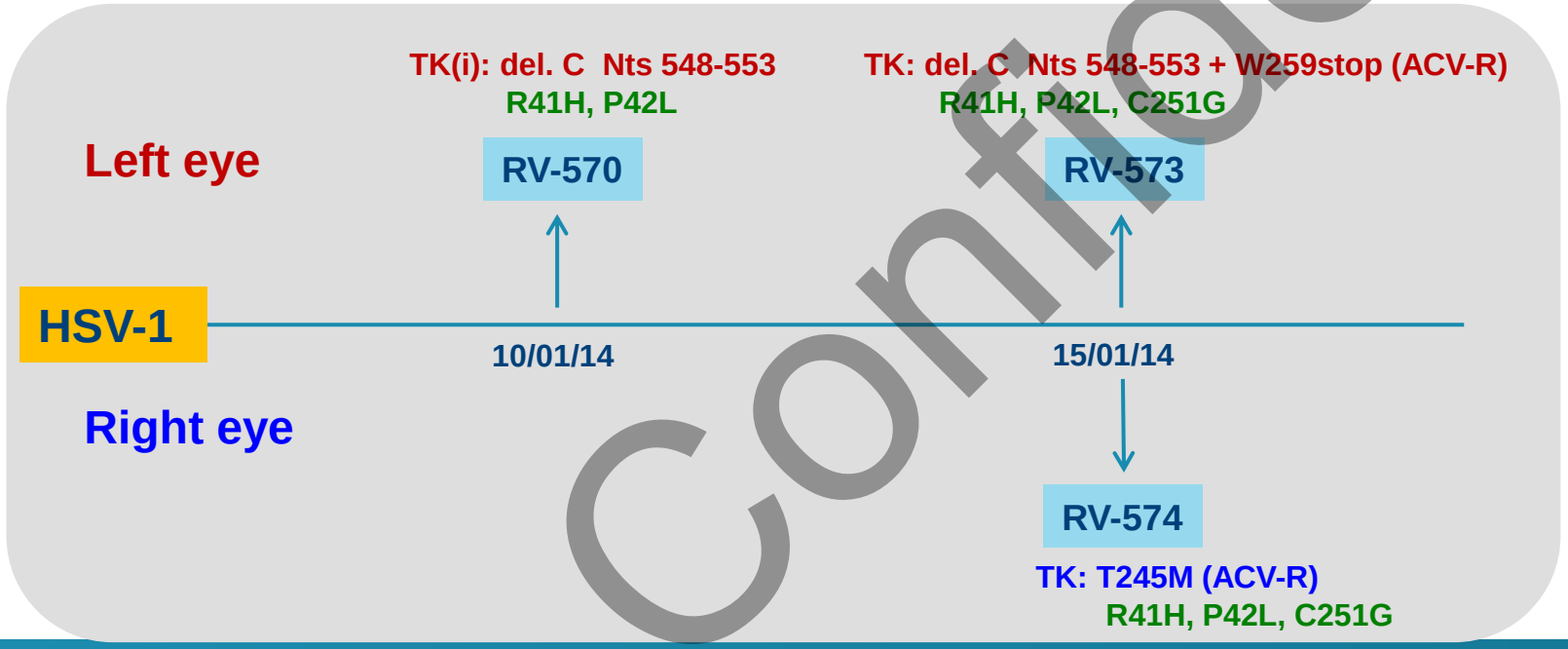

- **Rapid evolution**
- **Compartmentalization**

Extensive stomatitis and bilateral herpetic keratitis in an AML 60-years old female with persistent neutropenia – UZ Leuven

- Therapy related AML
- Persistent neutropenia
- **Extensive stomatitis and bilateral herpetic keratitis**
- Immunosuppressive treatment: Ruxolitinib

RegaVir ID	Antiviral therapy	Sample type	Sample date	Virus	Genotyping
RV-2672	Therapeutic acyclovir 3x 900 mg	**Skin lesion swab**	21.01.2023	HSV-1	**TK: deletion G Nts 430-436** G6C, D14E, P42L, R89Q,G240E, C251G, S321P, S345P **DNA pol: wild-type** TK: T566A, K700R, H1124P

Extensive stomatitis and bilateral herpetic keratitis in an AML 60-years old female with persistent neutropenia – UZ Leuven

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RV-2677	Therapeutic acyclovir 3x 900 mg	**Conjunctival swab**	09.02.2023	HSV-1	**TK: deletion G Nts 430-436** G6C, D14E, P42L, R89Q,G240E, C251G **DNA pol: wild-type** TK: T566A, K700R, H1124P

80-years old immunocompromised female (Jan Yperman Ziekenhuis)

- Acute HSV disease
- HSV ophthalmologic disease: keratitis, uveitis, acute retinitis – risk of blindness
- Neurological symptoms of encephalitis, meningoencephalitis, meningitis, myelitis
- Immunocompromised with esophageal, intestinal, and respiratory track lesions

	Antiviral therapy	Sample type	Sample date	Virus	Genotyping
RV-1186	Acyclovir oral suppression therapy	Eye swab	10.03.2017	HSV-1 (positive PCR)	**TK : Del. nucleotides 476-610, resulting in del. of 11 amino acids (ACV-R)** DNA pol: wild-type
RV-1260	BVDU, TFT **Foscavir (recently)**	Eye swab	05.07.2017	HSV-1	**Not amplifiable**

54-years old HSCT female patient – UZ Leuven

- 06./2017 - 07.08.18: HSCT
- 09/2019: refractory DLBCL; CAR-T treatment
- November 2019, recurrent herpetic keratitis (left eye)
- December 2022: **recurrent herpetic keratitis under ACV therapy** – dendritically corneal lesions (inferior cornea left eye).

	Viral disease	Immuno-suppressive therapy	Antiviral therapy	Sample type	Sample date	Virus	Genotyping
RV-1635 (EX-181217-16)	**Varicella-zoster Th4**	Medrol	Acyclovir 3x 500mg iv started (11.12.18)	Skin lesion swab	17.12.18	VZV	wild-type
RV-1890 (EX-191022-32)	Genital and ocular herpetic infection (01.10.2019)	/	Therapeutic acyclovir 5x 800 mg + cream (01.10.2019)	Eye swab	22.10.19	HSV-1	Not amplifiable
RV-1919 (0485614599)	Recurrent herpetic keratitis (12.11.19)	/	Therapeutic acyclovir 5x 400mg (12.11.2019)	Corneal swab	12.11.19	HSV-1	Not amplifiable
RV-2652 (65915290)	Recurrent herpetic keratitis under acyclovir prophylaxis (20.12.2022)	Hydrocortisone Currently no chemotherapy	Prophylactic acyclovir 2x 400mg → started Virgan (0.15% ganciclovir aqueous gel) cream 5x/d & acyclovir maintained	Corneal swab	20.12.22	HSV-1	**Novel DNA pol G355D** (linked to natural genetic polymorphism by phenotyping) - Wild-type
						VZV	Not amplifiable

Genotyping RV-2652

	Amino acid changes in UL23 (thymidine kinase): 376 amino acids (Complete sequence)	
	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with drug-resistance
RV-2652	G6C P42L R89Q G240E C251G S321P	None

	Amino acid changes in UL30 (DNA polymerase): 1235 amino acids (Partial sequence: amino acids 308-1132)		
	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with drug-resistance	Novel of unknown significance
RV-2652	T566A K700R H1124P	None (all positions verified)	G355D

Novel HSV-1 DNA pol **G355D linked to natural genetic polymorphism** by phenotyping

Strain	EC_{50} (µg/ml)								
	Acyclovir	Penciclovir	Brivudin	Ganciclovir	Foscavir	Cidofovir	Adefovir	Trifluridine	Pritelivir
Experiment 1									
RV-2652	0.021 0.084 0.088	0.068 0.086 0.21	0.012 0.032 0.039	0.0078 0.021 0.027	14.21 21.87 27.59	1.27 1.59 1.68	16.95 14.36 25.26	1.53 2.62 4.09	0.0043 0.0043 0.0072
Reference HSV-1 Kos strain)	0.082 0.088	0.21 0.16	0.025 0.036	0.016 0.010	12.67 25.26	1.64 2.53	16.36 25.26	0.73 0.89	0.0072 0.010
Experiment 2									
RV-2652	0.050 0.076	0.072 0.27	0.0087 0.0072	0.0077 0.024	12.41 16.81	0.80 0.93	17.89 8.0	1.09 2.39	0.0048 0.0072
Reference HSV-1 Kos strain)	0.10	0.27	0.027	0.032	40.0	1.87	23.39	2.0	0.036

EC_{50} : Concentration required to reduce virus induced cytopathicity by 50%

74-years old male patient with keratouveitis from EMC Rotterdam

- **Recurrent virus reactivations** in previous years responding to acyclovir (topical) & valacyclovir (*per os*).
- No information available of underlying disease.
- November 2017: Keratouveitis – right eye not responding to treatment → virus isolate from corneal swab sent to RegaVir.

Original identification	RegaVir identification	Date collected	Type	Additional information
19002284 k 009 (K19-2284)	**RV-1927**	13.09.2019	Vero cell culture (corneal swab)	HSV-1

74-years old male patient with keratouveitis from EMC Rotterdam

	Amino acid changes in UL23 (thymidine kinase): 376 amino acids (Complete sequence)		
	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with drug-resistance	Novel of unknown significance
RV-1927	G6C P42l D78N G240E C251G R281Q S321P	none	F289S

	Amino acid changes in UL30 (DNA polymerase): 1235 amino acids (Partial sequence: amino acids 301-1141)	
	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with drug-resistance
RV-1927	T566A A646T K700R M905V S1123L	None (all positions verified)

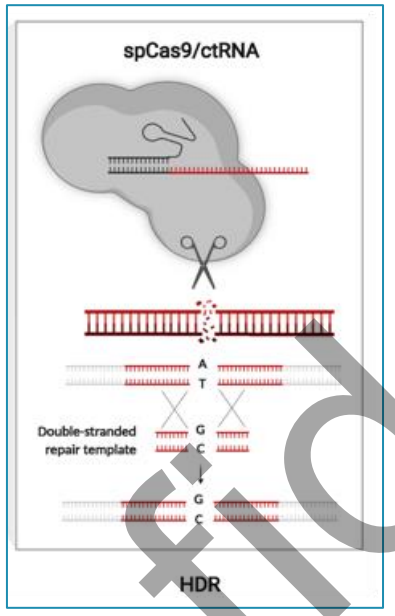
74-years old male patient with keratouveitis from EMC Rotterdam

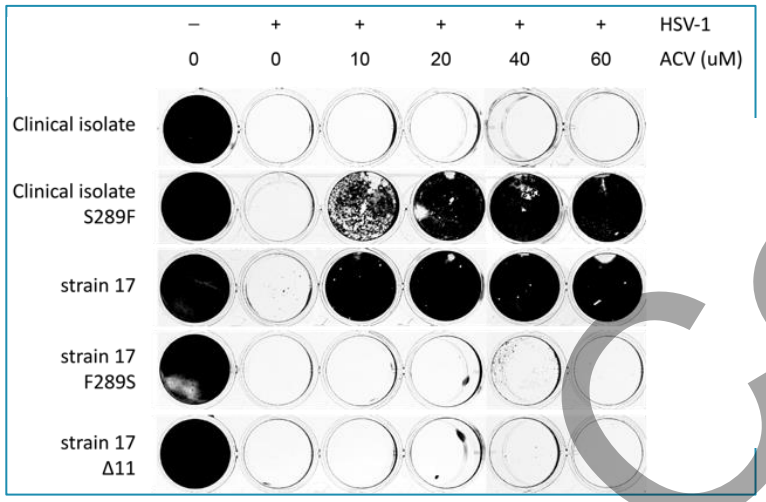
Strain	EC_{50} (µg/ml) (Fold-resistance: ratio mean EC_{50} clinical specimen / EC_{50} reference Kos strain)								
	Acyclovir	Penciclovir	Brivudin	Ganciclovir	Foscavir	Cidofovir	Adefovir	Trifluridine	Pritelivir
RV-1927 **(TK F289S)**	**13.37** **20.0** **(1043)**	**4.0** **6.84** **(169)**	**0.4** **0.89** **(202)**	**0.4** **1.05** **(806)**	11.96 13.68 (0.32)	0.36 0.42 (3)	5.35 17.79 (1.9)	0.23 0.27 (0.3)	0.0094 0.0084 (3)
Reference HSV-1 Kos strain)	0.016	0.032	0.0032	0.0009	40.0	0.12	6.12	0.73	0.003

EC_{50} : Concentration required to reduce virus induced cytopathicity by 50%

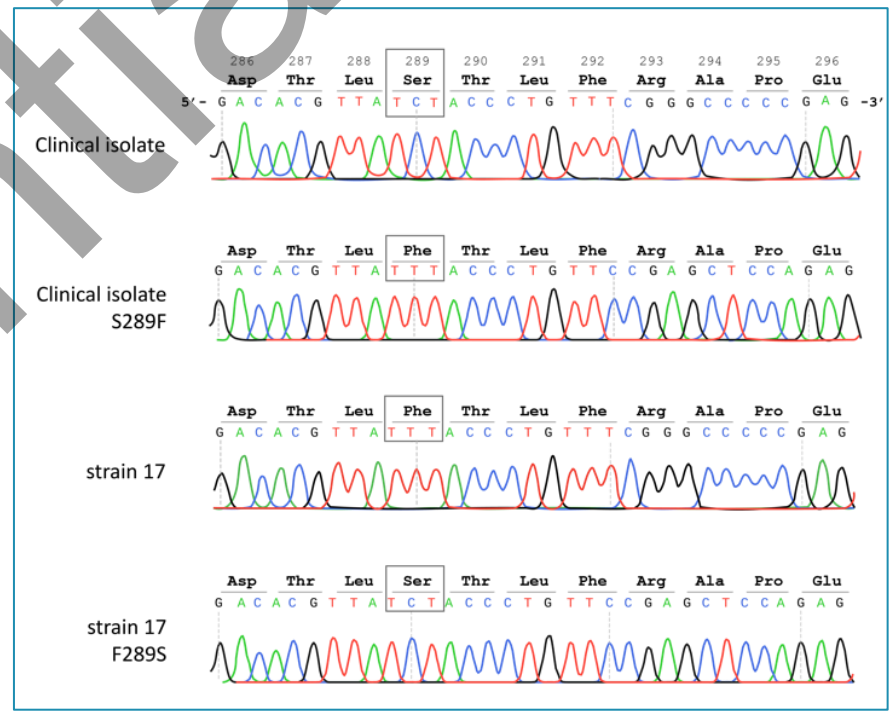
Validation of the TK **F289S** as an ACV-R substitution by CRISPR/Cas-9-mediated genome editing

Schematic overview of the CRISPR/Cas9-mediated genome editing procedure of the clinical HSV-1 strain. *CRISPR/Cas9 RNPs are introduced in Vero E6 cells together with dsDNA repair templates carrying the desired mutations. Upon infection by HSV-1, Cas9 cleaves the viral TK gene close at the F289S locus, triggering homologous recombination thereby incorporating the dsDNA repair template at the lesion.*





Sanger sequencing traces of the parental and CRISPR/Cas9-aided generated HSV-1 strains. *The amino-acid changes at position 289 are indicated; additional silent mutations were engineered in the viral genomes to prevent CRISPR/Cas9-mediated genome cleavage upon successful recombination.*



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Jeroen J.A. van Kampen (Department of Viroscience, Erasmus Medical Center, Rotterdam)
Robert Jan Lebbink (Department of Medical Microbiology, University Medical Center Utrecht)

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Herpetic keratitis with perforated conjunctiva in an 87-years old male (UMC Amsterdam)

- Treatment with doxocycline 100mg & Ofloxacine 3mg/ml eye drops
- HSV-1 Ct = 32.95 / 8-days old sample

	Viral disease	Immuno-suppressive therapy	Antiviral therapy	Sample type	Sample date	Virus	Genotyping
RV-2490	Herpetic keratitis with perforated conjunctiva	??	Famciclovir 500 mg *per os*	Eye fluid	18.04.22	HSV-1	**TK: C insertion Nt 548-553 F289C novel mutation, most likely linked to drug-resistance** DNA pol: wild-type

Dendritic herpetic keratitis in a 57-years old female patient - UMC Amsterdam

- Dendritic epithelial keratitis since 11.01.2017
- HSV-1 Ct = 32.95 / 8-days old sample

	Immuno-suppressive therapy	Antiviral therapy	Sample type	Sample date	Virus	Genotyping
RV-2329	??	Long term treatment with acyclovir & valacyclovir without success	Eye fluid	10.08.2021	HSV-1 (Ct = 25)	**TK: Y248S? (Y248H known) F289L?_mix** DNA pol: wild-type

Herpetic keratitis in a 69-years old male patient – UZ Leuven

- **Myasthenia gravis**
- Herpetic keratitis left eye emerged on 06.05.2019

- Antiviral treatment:
  - Therapeutic valacyclovir 3x 1000 mg *per os* since 04.06.2019
  - BVDU cream 8x since 14.05.2019

- Immunosuppressive treatment: Medrol 16 mg
- Lesions increasing despite therapy; initially limited response

	Immuno-suppressive therapy	Antiviral therapy	Sample type	Sample date	Virus	Genotyping
RV-1794	Medrol	Valacyclovir per os BVDU topical	Eye swab, cornea scraping	25.06.2019	HSV-1	**TK: N202K** DNA pol: wild-type

Herpetic keratitis in a 69-years old male patient – UZ Leuven

- **Myasthenia gravis**
- Herpetic keratitis left eye emerged on 06.05.2019

- Antiviral treatment:
  - Therapeutic valacyclovir 3x 1000 mg *per os* since 04.06.2019
  - BVDU cream 8x since 14.05.2019

- Immunosuppressive treatment: Medrol 16 mg
- Lesions increasing despite therapy; initially limited response

Strain	EC_{50} (µg/ml)						
	Acyclovir	Penciclovir	Brivudin	Ganciclovir	Foscavir	Cidofovir	Adefovir
RV-1794	**>20**	**>20**	**>10**	**>2**	20.1	0.47	17.9
	20	**20**	**>10**	**>2**	20.1	0.4	25.3
TK N202K	**>20**	**>20**	**>10**	**>2**	17.9	0.44	23.4
	>20	**>20**	**>10**	**>2**	30.6	0.8	30.6
Reference HSV-1 (Kos strain)	0.055	0.088	0.13	0.0094	40	1.79	30.6

EC_{50} : Concentration required to reduce virus induced cytopathicity by 50%

Dendritic herpetic keratitis in a 74-years old female patient – UZ Leuven

- Dendritic herpetic defect on left cornea (relapse/ emergence of lesions on cornea left eye on 16.06.2022).
- **Antiviral treatment:** therapeutic BVDU eye drops 6-7x/ day since 16.06.2022 → switched to 10x/day

Original Identification0	RegaVir Identification	Sample date	Sample type	HSV-1 genotyping
EX-220831-3	RV-2593	30.08.2022	Eye swab	**TK. A168T (altered TK phenotype)** DNA pol: wild-type

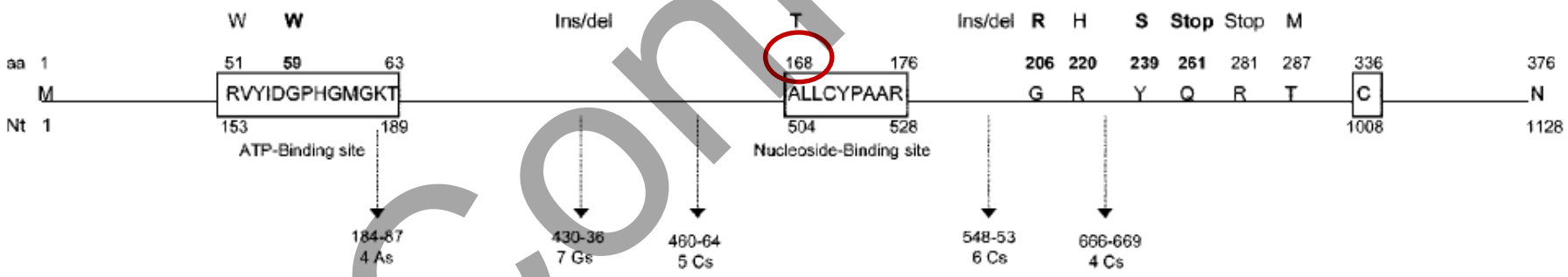

FIG. 1. Mutations identified in the *TK* gene of HSV-1 mutants resistant to BVDU (not to scale). ATP-binding site, nucleoside-binding site, and cysteine-336 are indicated by open boxes. The codon (aa) and nucleotide (Nt) numbers of the wild-type HSV-1 sequence are indicated. Mutational homopolymer runs and the nucleotides involved in drug resistance are indicated by vertical arrows. Amino acid changes and the relative positions of stop codons (Stop), insertions (Ins), and deletions (del) are indicated above the diagram of the protein and the gene. Novel mutations described in the present study (G59W, G206R, Y239S, and G261stop) are indicated in bold letters.

Andrei et al, J Virol 2005

TABLE 1. Drug susceptibility profile[a] of selected BVDU^r clones and comparative neurovirulence in NMRI mice

Clone or wild type	Mutation	*n*-Fold increase in IC_{50}[b]										Neurovirulence[d] log (PFU/LD_{50})
		BVDU	BVaraU	ACV	GCV	PCV	PFA	PMEA	PMEDAP	HPMPC	HPMPA	
CI-1	Ala 168 to Thr	≥2,420	>3,450	0.9	1.0	0.92	1.3	1.5	1.7	0.7	1.3	0.05
CI-5	Ala 168 to Thr	≥2,260	≥3,450	1.0	0.9	0.73	1.2	1.0	1.1	0.8	0.9	0.03
CI-24	Arg 51 to Trp	565	>3,450	1,000	3,400	500	0.9	1.1	1.2	1.2	1.0	2.92
CI-28	Arg 51 to Trp	500	>3,450	984	10,670	450	1.1	1.5	1.8	1.4	1.6	2.92
CI-3	Gly 59 to Trp	≥2,550	>3,450	605	1,400	73	0.6	0.7	0.6	2.0	0.9	2.54
CI-27	Gly 206 to Arg	≥3,230	>3,450	≥979	20,670	>769	1.2	1.5	2.2	1.1	1.6	3.30
CI-2	Arg 220 to His	>3,230	>3,450	147	57	5.4	0.7	1.2	1.0	0.7	1.1	0.94
CI-22	Tyr 239 to Ser	113	931	247	180	20	1.0	1.5	1.5	0.8	1.1	ND[e]
CI-23	Tyr 239 to Ser	23	1,400	126	63	24	1.1	1.2	1.4	1.0	1.9	1.40
CI-29	Gln 261 to stop	≥3,230	>3,450	468	667	≥769	1.5	1.5	2.5	1.8	2.5	3.68
CI-25	Arg 281 to stop	≥1,770	>3,450	316	333	≥438	1.1	1.2	1.3	0.9	1.0	4.22
CI-34	Thr 287 to Met	>3,230	>3,450	≥1,050	≥33,330	>769	1.0	0.8	1.5	1.3	2.3	2.07
CI-4	146 frameshift	≥2,020	>3,450	789	4,600	>769	0.5	1.1	0.9	1.1	1.7	3.43
CI-15	185 frameshift	≥3,230	>3,450	≥947	6,270	≥627	1.1	1.5	1.0	1.3	0.7	2.5
CI-30	185 frameshift	>3,230	ND	>1,050	14,000	≥769	1.3	1.5	1.6	0.9	1.1	ND
Wild type		0.0062[c]	0.0058	0.019	0.00015	0.026	19.6	7.4	1.91	0.114	0.035	0.05

[a] Drug susceptibility profiles of BVDU^r HSV-1 clones were determined in HEL cells.
[b] The increase in IC_{50}s was calculated from at least two independent experiments.
[c] Mean IC_{50}s were from seven independent experiments.
[d] Neurovirulence of the different mutants was evaluated by intracerebral inoculation of the viruses into mice. The log (PFU/LD_{50}) was determined as a reciprocal parameter of neurovirulence.
[e] ND, not determined.

- The affinity for BVDU, but not for dThd, was severely affected in the TKs of the mutant A168T virus clones, resulting in an at least 40- to 100-fold **decreased affinity for BVDU**.
- The **catalytic efficacy of the mutant enzymes** were quite comparable to that of wild-type enzyme with dThd as the substrate, but heavily compromised (> 100-fold) with BVDU as substrate.

Andrei et al, J Virol 2005

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Recurrent herpetic keratitis in a 27-years old patient – EMC Rotterdam

- 27 years old medical student, who has a history with recurrent herpetic keratitis over the past ten years (unknown which treatments he had over time).

- He presented in August with a keratouveitis of the right eye and was treated with acyclovir locally and systemically without success even after elevating the dose.

- Rotterdam results:
  - Eye swab of 17.09.2019 showed HSV-1.
  - Sequencing analysis showed the **T354P mutation in the viral thymidine kinase**, which confers too low-level resistance according to Duan *et al* (Journal of Infectious Diseases 2009).
  - However, phenotypical resistance tests could not confirm resistance.

Recurrent herpetic keratitis in a 27-years old patient – EMC Rotterdam

- RegaVir results - genotyping

Strain	Amino acid changes in UL23 (thymidine kinase) : 376 amino acids (complete sequence: amino acids 1-376)	
	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral agents
RV-1902	G6C P42L R89Q A192V K219E (K219T/Q known polymorphisms) G240E C251G S321P	**T354P**

Strain	Amino acid changes in UL30 (DNA polymerase): 1235 amino acids (partial sequence: amino acids 301-1141)	
	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral agents
RV-1902	T566A A646T K700R M905V S1123L	None (all positions verified)

Recurrent herpetic keratitis in a 27-years old patient – EMC Rotterdam

- **RegaVir results**

Strain	EC_{50} (µg/ml) (Fold-resistance: ratio mean EC_{50} clinical specimen / EC_{50} reference Kos strain)								
	Acyclovir	Penciclovir	Brivudin	Ganciclovir	Foscavir	Cidofovir	Adefovir	Trifluridine	Pritelivir*
RV-1902 (experiment 1)	1.52	0.30	0.15	0.036	52.31	0.80	21.01	0.89	0.025
	0.47	0.061	0.08	0.016	25.26	0.094	5.05	0.76	0.0069
(experiment 2)	0.27	0.072	0.025	0.0062	40.0	0.36	14.63	0.40	0.027
Mean	**0.75**	**0.144**	**0.085**	**0.019**	**39.1**	**0.418**	**13.56**	**0.683**	**0.20**
Fold-resistance	**16.6**	**2.6**	**8.9**	**9.0**	0.8	1.4	1.7	1.0	0.9
Reference HSV-1 (Kos strain) (experiment 1)	0.072	0.061	0.016	0.003	40.0	0.47	8.0	0.89	0.016
(experiment 2)	0.019	0.051	0.0032	0.0013	52.31	0.12	8.0	0.52	0.027
Mean	0.0455	0.056	0.0096	0.00215	46.155	0.295	8.0	0.705	0.0215

EC_{50} : Concentration required to reduce virus induced cytopathicity by 50%
*Helicase-primase inhibitor

Ocular HSV-2 infections

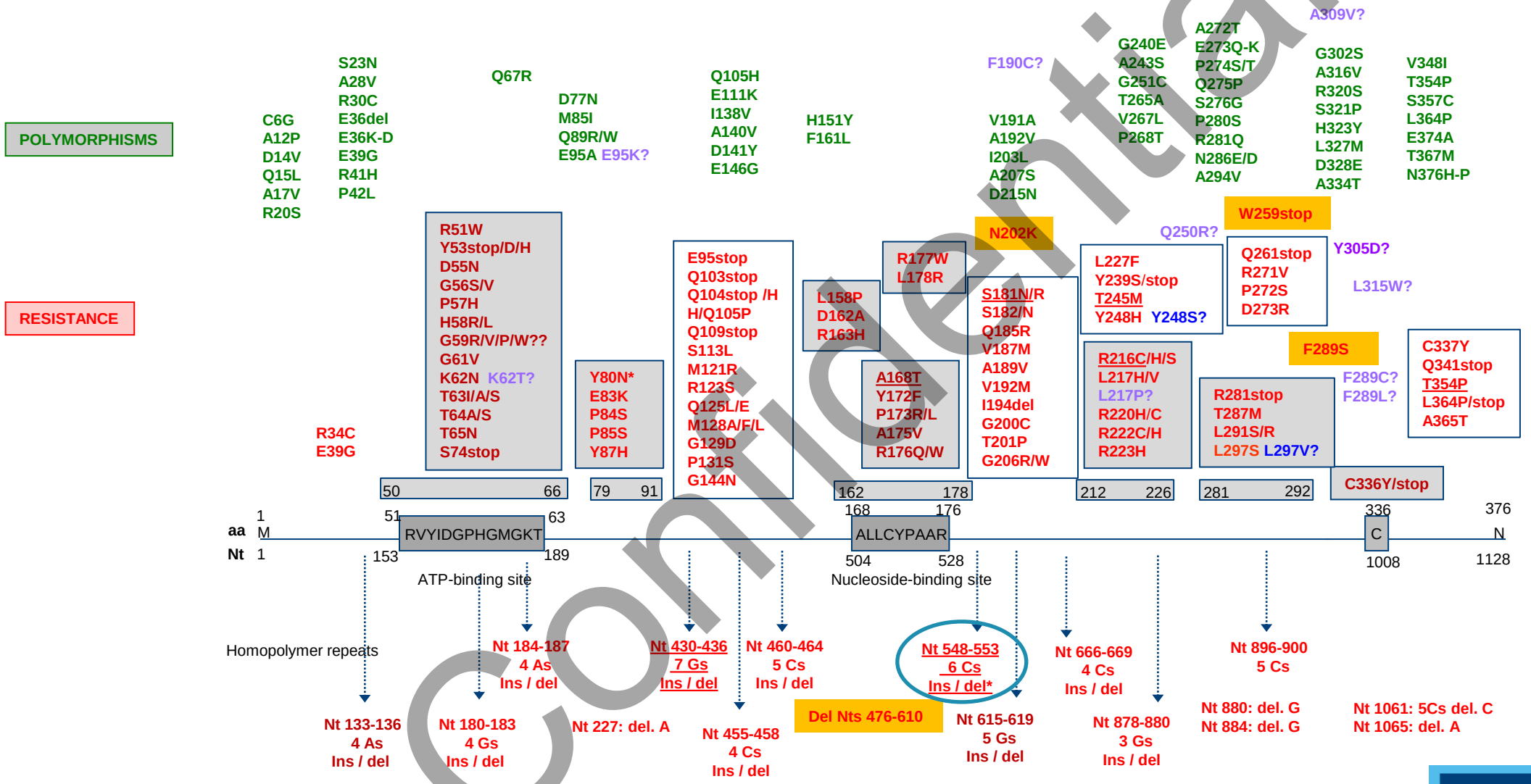
Patient	Hospital	Immune suppression	Antiviral treatment	RegaVir identification (date)	Sample type	HSV-2 genotyping
46-years old female with acute retinal necrosis	UZ Gent	No predisposition / immunosuppression	* Prophylactic – therapeutic (in case of acute infection) acyclovir (08.12. 21 10mg/kg 3x/day IV) (18.12. 21 PO 5x 800 mg/day) * Foscarnet intravitreal 2x/week (2.4mg)	RV-2408 (17.12.21) RV-2409 (27.12.21) RV-2421 (21.01.22) RV-2432 (24.01.22) RV-2433 (27.01.22)	DNA extract – eye fluid	Wild-type
24-years old female patient with HSV ocular infection	CHU Saint-Luc / UCL	Ocular HSV infection	The patient did not received treatment during the last 10 days	RV-2500 (10.05.22)	Eye fluid right eye	Wild-type

Challenges in controlling ocular herpes

- Ocular herpes is currently an infection for life.
- Currently approved therapies provide limited efficacy and often must be combined with steroids to reduce symptoms especially during the recurrent cases of HSK.
- In general, current treatment modalities reduce the symptoms only by a few days.
- Long-term use of steroids has its own serious side effects: increase in intraocular pressure and possible onset of secondary glaucoma.
- Clinically approved drugs are clearly beneficial but suffer from their share of pitfalls.
  - development of resistance
  - long-term toxicity

⇒ great need to develop new antiviral therapies for HSV ocular infections

HSV-1 thymidine kinase



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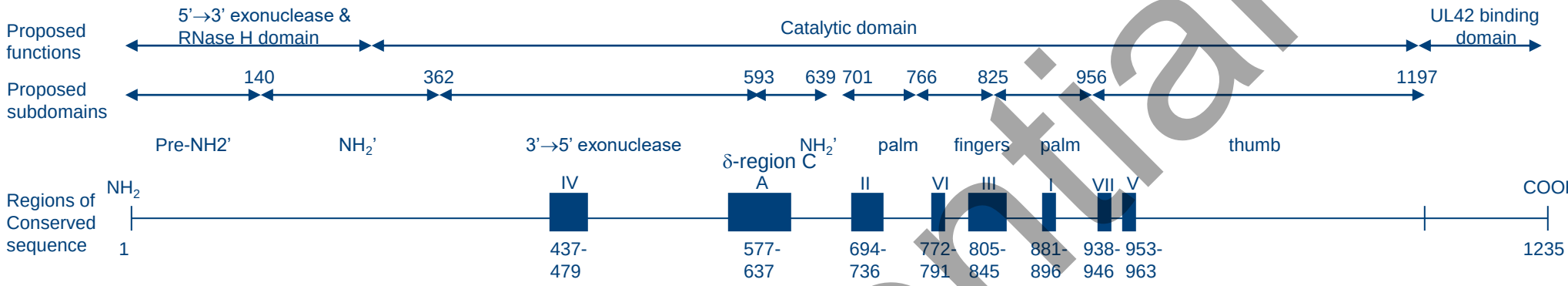
* Found in 5 ocular samples in 4 patients

HSV-1 DNA polymerase



Mutations known to confer altered sensitivity to ACV, PFA and/or aphidilcolin. (*hypersensitivity to ACV)

D368A E370A V462A K532T Y557S Q570R L583V Y577H* D581A* E597K/D A605V Y696H R700G V715G/M A719V/T S724N Q727R L774F L778M D780N L782I P797T V813M N815S/T N815L/V/Y/E Y818C T821M G841C/S R842S Y941H N961K S889A F891C/Y V892S

Mutations lethal to the virus

E460D G464V K522E P561S R500C?

Novel mutations selected *in vitro* under PFA or PME derivatives and characterized in our Laboratory

Q618H V714M L802F T821M A857V? Y878H? R959H D1070N

Mutations selected *in vitro* under HPMP derivatives and characterized in our Laboratory

V573M R700M K960R W998L L1007M I1028T

Mutations found in clinical isolates associated with drug-resistance found in our previous studies.

H386Y A542S Del E667? E798K E798Q? I922T

Genetic polymorphisms

G12E A20V F23I A27T S33G E70D D72N A78V/D A102T K104Q D114A R116H G132C P137Q A138E F171S A229T A232T F248L R330A S338L I352V G355D E421D N425T T434M D418N N522S A566T T639I A646T D660E E684D D672N E675A D703N R700K V743M N711K G749D L753M P875S A899V V904M V905M M906V K908R P920S A995F E1005K I1026S E1082K A1099T D1103H E1104D S1123L-C P1124H A1128V G1129V L1166W E1194D P1199Q A1203T A1204T T1208A A1209T T1219M

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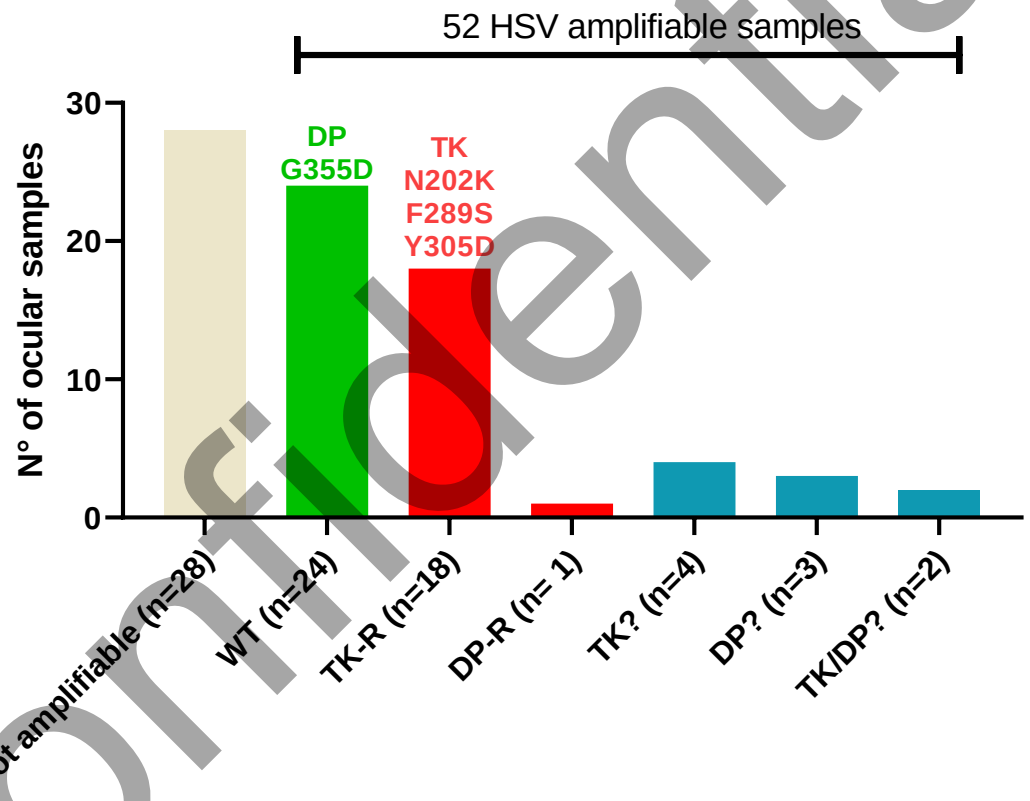
RegaVir ocular samples for HSV



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