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RegaVir platform: Case discussions antiviral resistance testing

Robert Snoeck & Graciela Andrei

Leuven, November 22, 2022

Case study

- 30 years old woman suffering from **autoimmune medullar aplasia** since the age of 15.
  - Anti-thymocyte globulin
  - G-CSF
  - Cyclosporine → stop in March 2011 because of digestive intolerance
  - Corticosteroids
  - Allo-transplantation not yet planned (no matched sibling donor – no 10/10 HLA-matched unrelated donor)

- Danatrol (Danazol)
- Folic acid
- Seroplex
- Excision of a fibroadenoma of the left breast in 2007
- Cervical HPV diagnosed in January 2010 (conization)

Case study

- From 16/02/2011until 22/02/2011: patient hospitalized
  - Recurrence of the aplasia
  - Fever
  - No acute bacterial or viral infection
  - Suspicion of whooping cough (no biological documentation) → Tazocilline (Pipéracilline, Tazobactam) & Erythromycin

- Back-to-work (technician at a pharmacy, suspicion of contact with patients suffering from whooping cough)

Case study

- **11.04.2011: appearance of sudden symptoms → emergency**
  - Very high fever (40°)
  - Painful swallowing
  - Cervical adenopathy's
  - Sensitive to pressure in right hypochondrium
  - Muco-cutaneous disseminated vesicular lesions (mainly in thorax, arms, and lower limbs)
  - Ulcerative-necrotic angina
  - Urinary functional signs
  - No cough
  - No digestive troubles

Case study

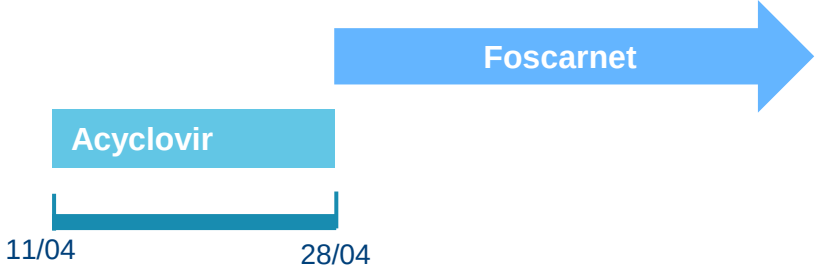
- **11.04.2011: appearance of brutal symptoms**
  - **Abdominal echography:** no cholecystitis, no angiocholitis
  - **Abdominal, pelvic and thoracic CT-Scan:** small homogeneous hepatomegaly
  - Pancytopenia (mainly of platelets & leukocytes) – low hemoglobin, elevated CRP, elevated creatinine, elevated transaminases

    → **Patient transferred to ICU**

  - Antiviral treatment started: acyclovir 10 mg/kg/8h
  - Antibiotic treatment maintained: Tazocilline (Pipéracilline, Tazobactam) & Amiklin (which was started at Emergency Department)
  - Danazol halted
  - G-CSF maintained

Symptoms at ICU

- Vesicular-crusty rash in the face, thorax, abdomen, an inner thighs
- Hyperkeratotic warts in left hallux, and in the thumb and middle finger of the left hand
- Right cervical adenopathy's
- No cardio-vascular abnormalities
- Pain on the right hypochondrium
- Painful urination
- Biology
  - **HSV-2 serology positive (3.4 IgM & 2.4 IgG) (it was negative in February)**
  - Confirmation of cytopenia on 12.04.2011
  - Cholestatic hepatitis without jaundice

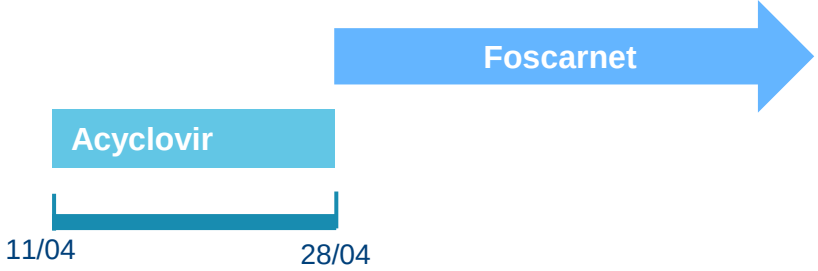


- The eruption was initially very extended, with a modification of the topography:
  - ✓ Lesions of the face, the trunk, and the roots of the limbs quickly regressed
  - ✓ Despite the antiviral treatment, **new lesions continued to appear** predominantly on:

    - palm and fingertips of the left hand, in particular the index finger (where there was a hyper keratinized lesion suggestive of HPV warts)

    - left foot (there was also a hyper keratinized lesion of the hallux, which also evolved in the form of hyper keratinized necrotic patches)

    - mouth: two vesicles on the left hemi-tongue, as well as ulcerated areas on the level anterior palate
  - ✓ During evolution, the lesions reached the extremities of the right hemi-body with a relatively similar topography (palm and plant)
  - ✓ Lesions also in the genital-anal area throughout the evolution



- Lesions were very painful and of hyper tense aspect → lesions were incised gradually to relieve the patient and to take samples for bacteriological, mycological and virological purposes
- Skin biopsies were also performed to ensure the absence of bullous dermatosis

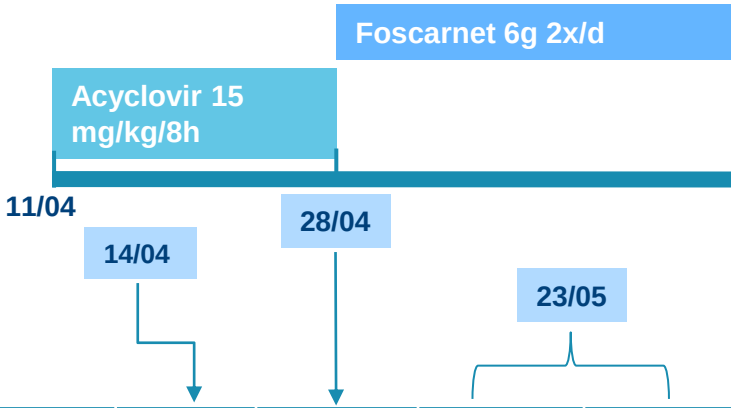
  → result: herpetic eruption without superinfection or superimposed dermatosis

- The most worrying lesion was at the level of the left hallux with pain radiating to the pre-tibial area

  → ultrasound: no sign of superinfection on the back of the foot

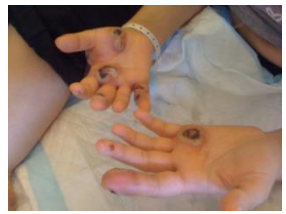
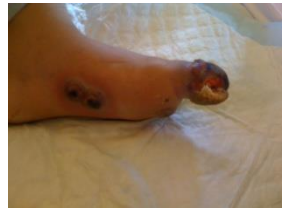
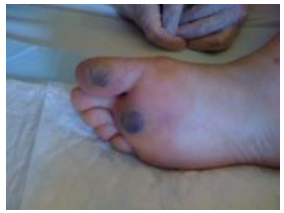
  → X-rays: no osteitis

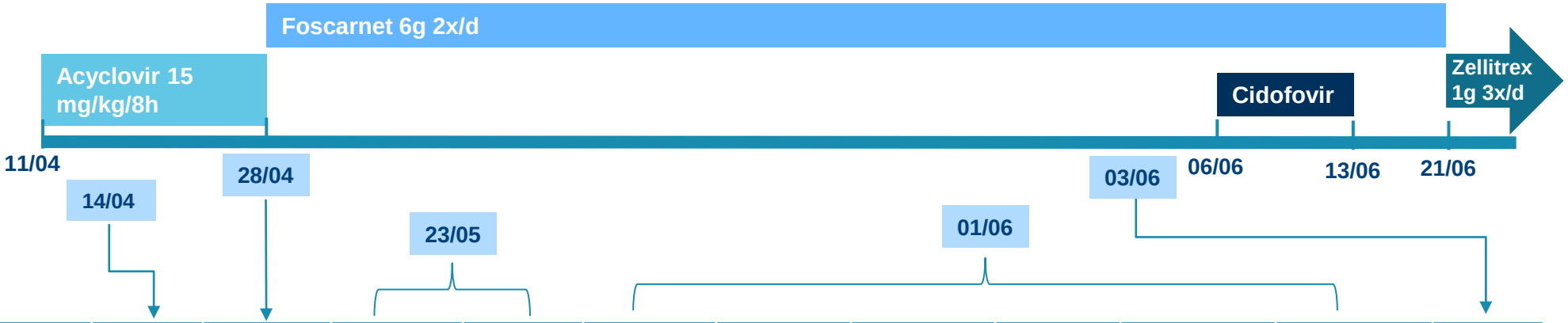
- On 27/05/2011, the first samples were sent to RegaVir



	RV-194	RV-195	RV-196	RV-197
	Buccal swab	Skin swab	Skin swab	Skin swab
TK mutations	wt	R221H	wt	wt
DNA pol mutations	wt	wt	A606V* T934A*	V842M* R964H*
Pheno-typing	wt	ACV^R PFA^S	ACV^r PFA^R	ACV^r PFA^R

*Heterogeneous populations of wt and mutant virus.
- Novel mutations are underlined.
- Mutations known to confer drug-resistance in HSV-2 are highlighted in bold
- Mutations deduced to be linked to drug-resistance because of homology to known mutations in other herpesviruses are shown in red.
- Mutations most probably linked to drug resistance because of their location in conserved regions of the viral enzyme are indicated in blue.
- “R”: highly resistant, “r” weakly resistant, “S”: sensitive



	RV-194	RV-195	RV-196	RV-197	RV-200	RV-201	RV-202	RV-203	RV-204	RV-205	RV-206
	Buccal swab	**Skin swab**	**Skin swab**	**Skin swab**	**Right hand**	**Left foot**	**Sole of the left foot**	**Left hallux**	**Palm of the left hand**	**Left index**	**Buccal swab**
TK mutations	wt	R221H	wt	wt	wt	wt	wt	wt	wt	**C del. Nts 246-249**	wt
DNA pol mutations	wt	wt	A606V* T934A*	V842M* R964H*	R964H*	I731F	Q732R	A724V* S725G	A606V	Q732R* R847C*	**S729N** M789T*
Pheno-typing	wt	ACV^R PFA^S	ACV^r PFA^R	ACV^r PFA^R	ACV^r PFA^R	ACV^r PFA^R	ACV^r PFA^R	ACV^r PFA^R	ACV^r PFA^R	ACV^R PFA^R	ACV^r PFA^R

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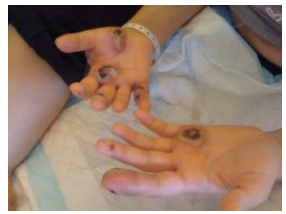
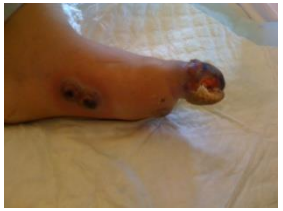
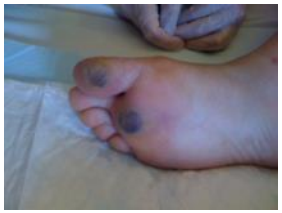

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Drug-susceptibility profile of the different isolates

	RV-194	RV-195	RV-196	RV-197	RV-200	RV-201	RV-202	RV-203	RV-204	RV-205	RV-206
Acyclovir	0,08 ± 0,06	**4,0 ± 0**	0,40 ± 0,30	0,33 ± 0,34	0,44 ± 0,05	0,16 ± 0	0,80 ± 0	0,71 ± 0,30	0,38 ± 0,15	**63,1 ± 28,6**	0,35 ± 0,27
Foscavir	30,8 ± 5,5	13,1 ±4,2	**166,2 ± 48,7**	**159,2 ± 31,1**	**121,8 ± 59,1**	**121,8 ± 59,1**	**132,1 ± 38,8**	**143,3 ± 37,2**	**93,2 ± 22,9**	**129,5 ± 15,6**	**136,5± 13,9**
Cidofovir	0,83 ± 0,94	0,18 ± 0,09	0,53 ± 0,29	0,56 ± 0,29	1,60 ± 0,28	1,60 ± 0,28	1,0 ± 0,28	1,35 ± 0,35	1,03 ± 1,07	1,07 ± 0,26	**2,40 ± 0,42**

The data represent the mean EC_{50} values ± STDEV of at least two independent experiments. EC_{50}: 50% effective concentration or drug concentration required to reduce viral CPE by 50%.

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Evolution of the patient

- Healing of the mucocutaneous lesions from mid-June
- Hepatitis regressed after stop of Danazol
- Persistent pain on the right hypochondrium - Cholecystitis due to the presence of a lithiasis
- Patient presented eating disorders (malnutrition)
- Anxio-depressive syndrome
- External otitis media
- HPV61 & mainly HPV39 in the left hallux
- Serology negative for Coxsackie, PCR negative for parvovirus & cytomegalovirus
- For all cutaneous lesions, hemocultures were negative

HSV-2 clinical specimens

Isolate	DNA pol mutant variants (Sanger sequencing)			
RV-196	A606V*			
RV-197				
	V842M*			
	R964H*			

*** DNA pol mutations detected by Sanger sequencing in original samples are in blue color.**

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HSV-2 clinical specimens

Isolate	DNA pol mutant variants (Sanger sequencing)	Number of clones isolated bearing DNA pol mutations		
RV-196	A606V*	0/21		
	T934A	**21/21 (100%)**		
RV-197	K533E	2/42 (4,8%)		
	G617S	1/42 (2,4%)		
	C625R	5/42 (11,9%)		
	R628C	6/42 (14,3%)		
	S725G	4/42 (9,5%)		
	V842M*	3/42 (7,1%)		
	R964H*	**14/42 (33,3%)**		

*** DNA pol mutations detected by Sanger sequencing in original samples are in blue color.**

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HSV-2 clinical specimens

Isolate	DNA pol mutant variants (Sanger sequencing)	Number of clones isolated bearing DNA pol mutations	% DNA pol mutant variants (NGS)	
RV-196	A606V*	0/21	41,60	
	Y823C	0/21	35,20	
	V842M	0/21	1,00	
	T934A	**21/21 (100%)**	**12,60**	
RV-197	K533E	2/42 (4,8%)	3,37	
	A606V	0/42	6,00	
	G617S	1/42 (2,4%)	1,68	
	C625R	5/42 (11,9%)	8,00	
	R628C	6/42 (14,3%)	1,83	
	S725G	4/42 (9,5%)	1,14	
	A840T	0/42	1,50	
	V842M*	3/42 (7,1%)	42,90	
	I950L	0/42	1,40	
	R964H*	**14/42 (33,3%)**	**27,10**	

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HSV-2 clinical specimens

Isolate	DNA pol mutant variants (Sanger sequencing)	Number of clones isolated bearing DNA pol mutations	% DNA pol mutant variants (NGS)	% DNA pol mutant variants (NGS) after 5 passages without drugs	
RV-196	A606V*	0/21	41,60	/	T934A
	Y823C	0/21	35,20	1,53	
	V842M	0/21	1,00	/	
	T934A	**21/21 (100%)**	**12,60**	**97,46**	
RV-197	K533E	2/42 (4,8%)	3,37	/	R964H
	A606V	0/42	6,00	/	
	G617S	1/42 (2,4%)	1,68	/	
	C625R	5/42 (11,9%)	8,00	/	
	R628C	6/42 (14,3%)	1,83	/	
	S725G	4/42 (9,5%)	1,14	/	
	A840T	0/42	1,50	/	
	V842M*	3/42 (7,1%)	42,90	/	
	I950L	0/42	1,40	/	
	R964H*	**14/42 (33,3%)**	**27,10**	**97,97**	

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HSV-2 clinical specimens

Isolate	DNA pol mutant variants (Sanger sequencing)	Number of clones isolated bearing DNA pol mutations	% DNA pol mutant variants (NGS)	% DNA pol mutant variants (NGS) after 5 passages without drugs	
RV-200	D616G	0/14	3.03	/	F923L
	R628C	3/14 (21,4%)	16.20	/	
	S725G	0/14	2.36	/	
	A840T	0/14	11.90	/	
	F923L	**7/14 (50,0%)**	**32.10**	**99.78**	
	I950L	1/14 (7,1%)	12.60	/	
	R964H*	2/14 (14,3%)	25.95	/	
RV-201	C625R	1/10 (10,0%)	<1%	/	I731F
	A724V	0/10	3.52	/	
	I731F*	**9/10 (90,0%)**	**94.01**	**99.80**	
RV-202	G607D	0/11	2.07	/	Q732R
	Q732R*	**10/11 (90,9%)**	**95.41**	**83.81**	
	L779P	0/11	1.03	/	
	M789T	1/11 (9,1%)	8.68	11.49	

*** DNA pol mutations detected by Sanger sequencing in original samples are in blue color.**

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HSV-2 clinical specimens

Isolate	DNA pol mutant variants (Sanger sequencing)	Number of clones isolated bearing DNA pol mutations	% DNA pol mutant variants (NGS)	% DNA pol mutant variants (NGS) after 5 passages without drugs	
RV-203	A724V*	3/18 (16,7%)	27,64	28,30	A724V ~ S725G
	S725G*	15/18 (83,3%)	71,66	71,41	
RV-204	A606V*	4/8 (50,0%)	85,03	/	Y832C
	Y823C	**4/8 (50,0%)**	**10,27**	**98,59**	
	T934A	0/8	1,00	/	
RV-205	**Q732R***	**20/21 (95,2%)**	**54,66**	**88,49**	Q732R
	R847C*	1/21 (4,8%)	30,40	/	
RV-206	R628H	0/25	3,11	/	S729N
	S729N*	**4/25 (16,0%)**	**64,81**	**99,36**	
	M789T*	21/25 (84,0%)	57,07	/	

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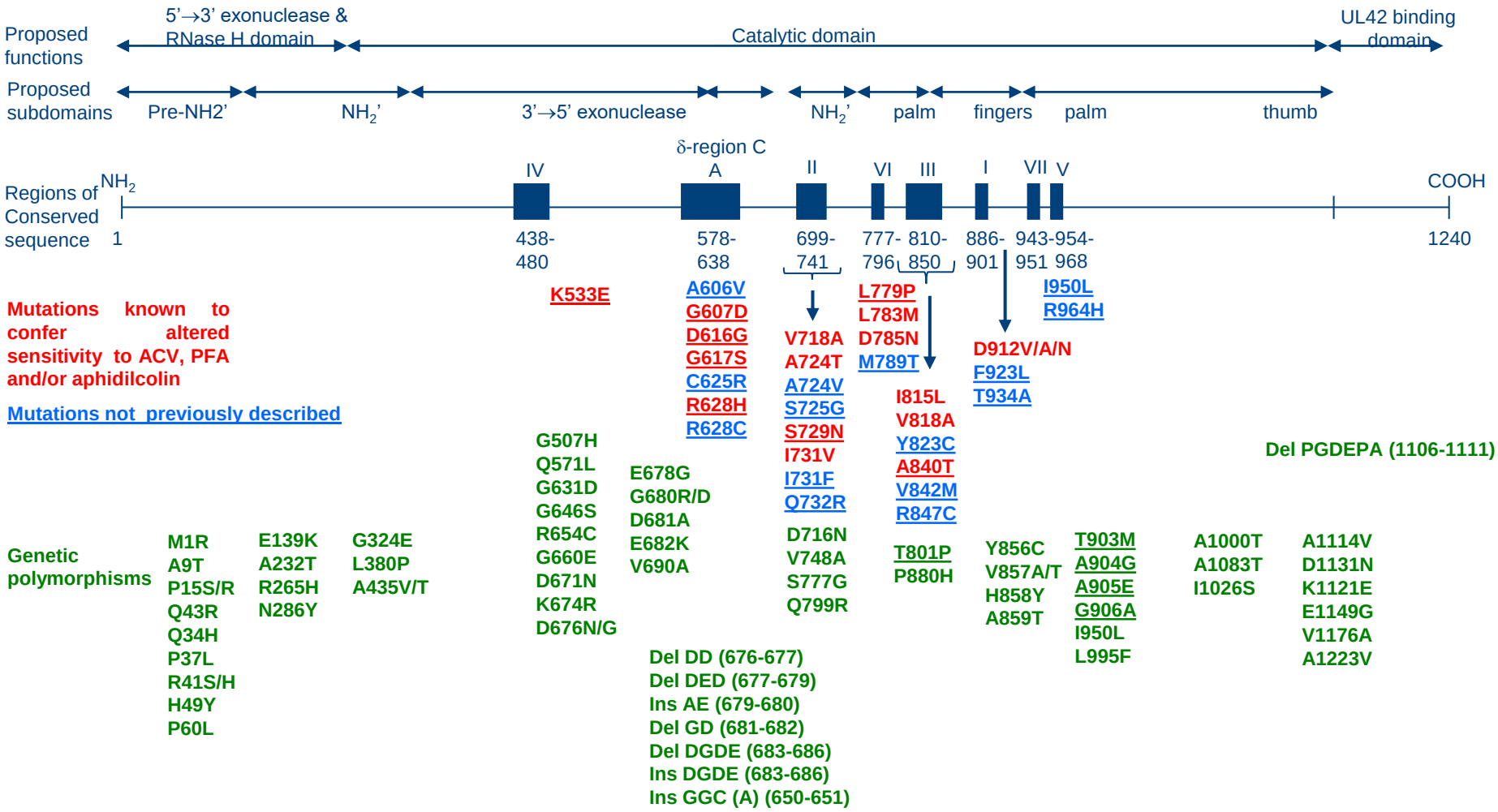
HSV-2 DNA polymerase



Mutations found in the patient are underlined: 23 ≠ mutations (15 new muations)

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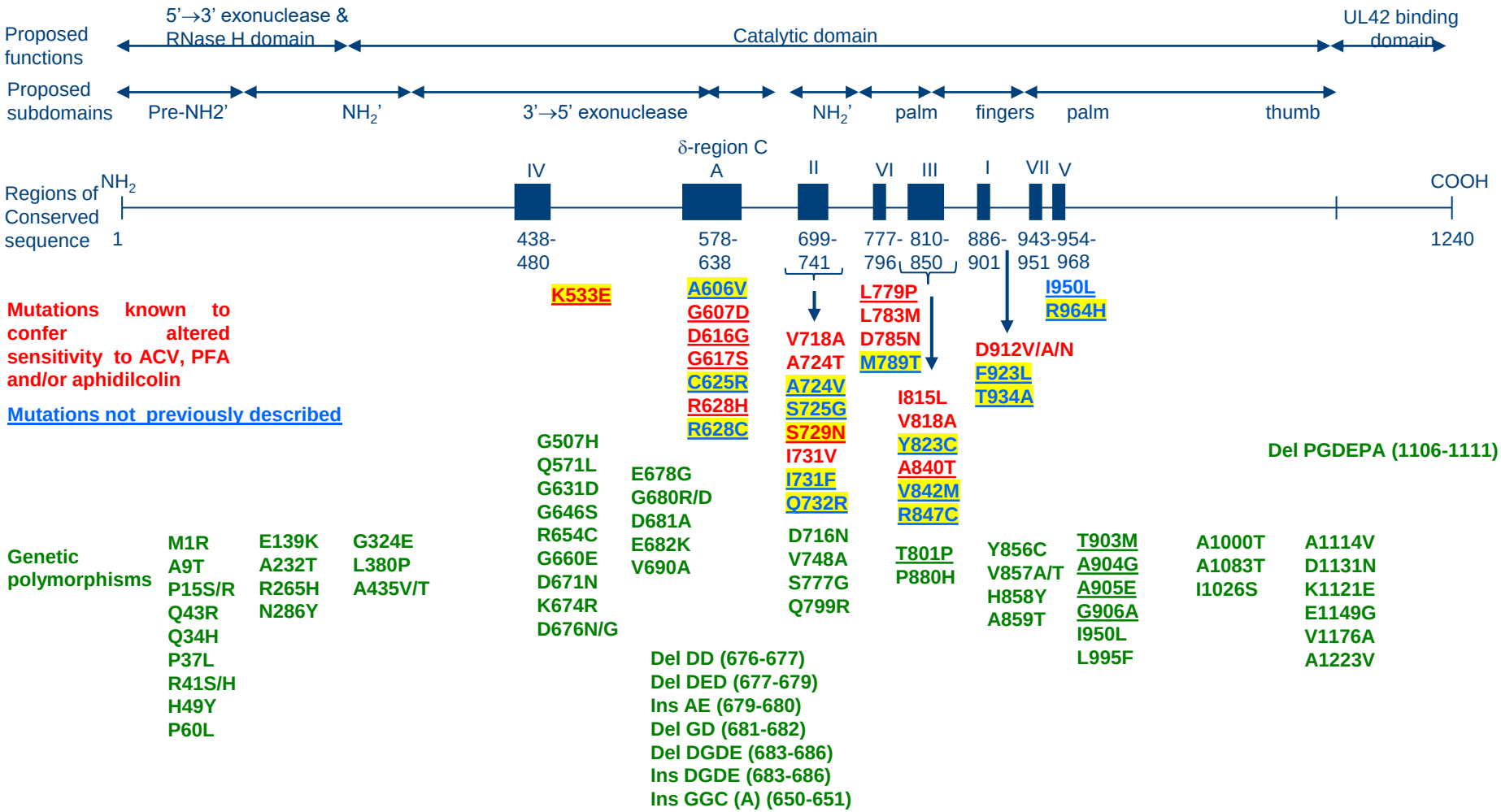
HSV-2 DNA polymerase



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Phenotyping performed

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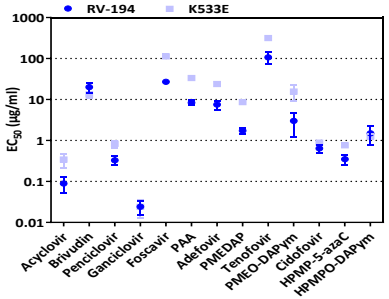
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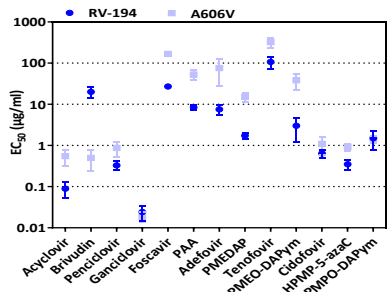
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym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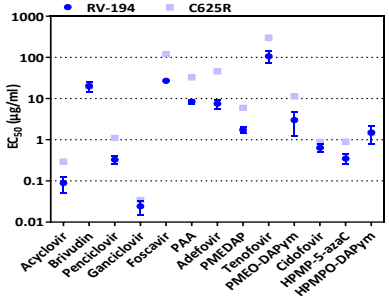
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C625R
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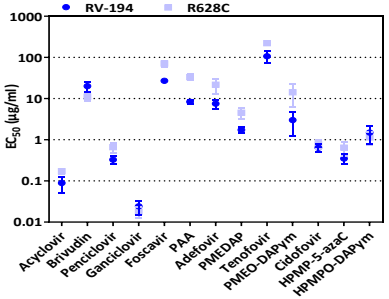
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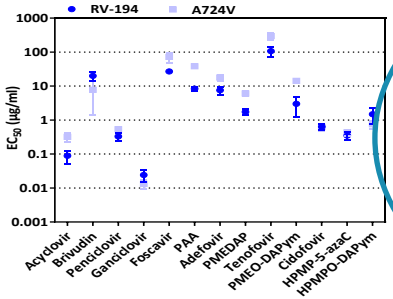
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EC50 (µg/ml)
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Brivudin
Penciclovir
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PMEO-DAPym
Cidofovir
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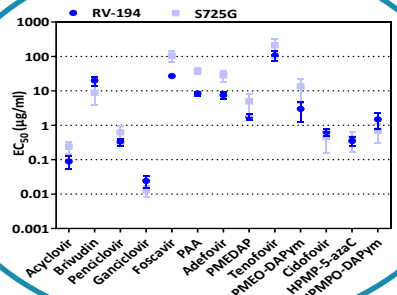
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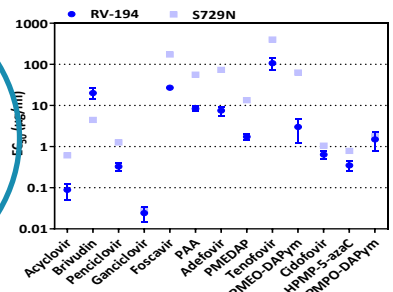
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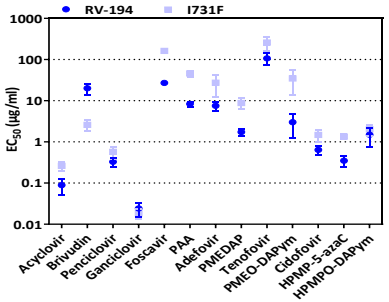
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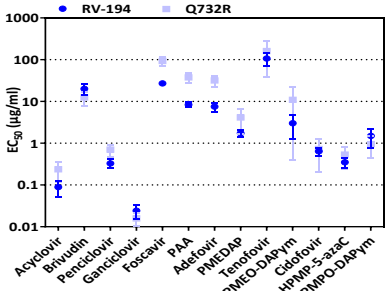
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Q732R
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
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PAA
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PMEDAP
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PMEO-DAPym
Cidofovir
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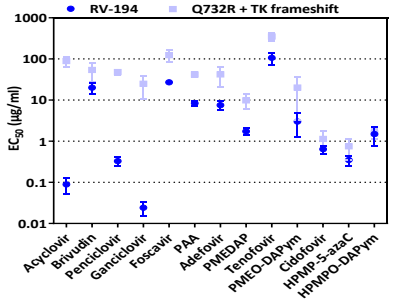
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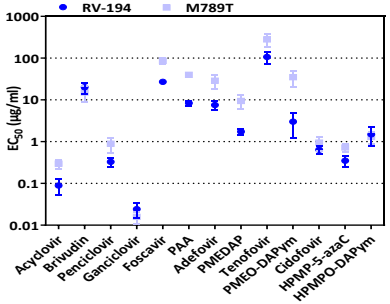
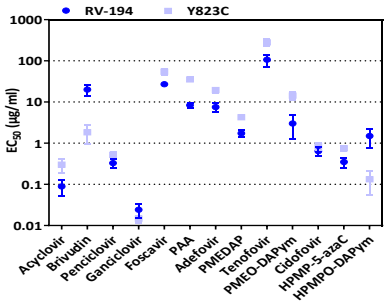
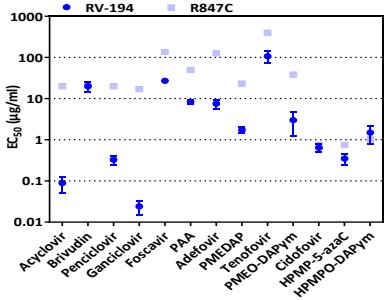
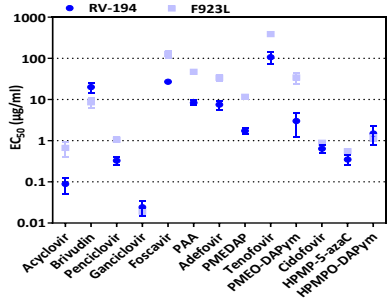
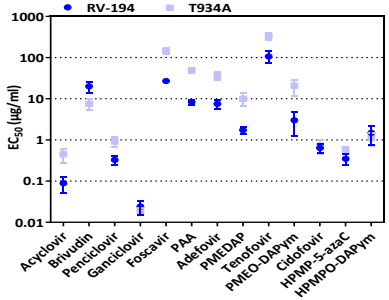
Q732R + TK frameshift
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym

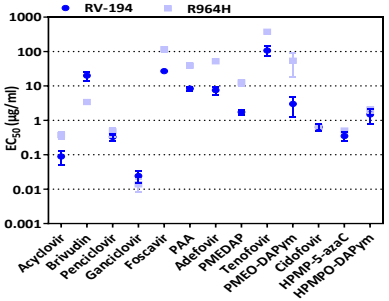

RV-194
M789T
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym

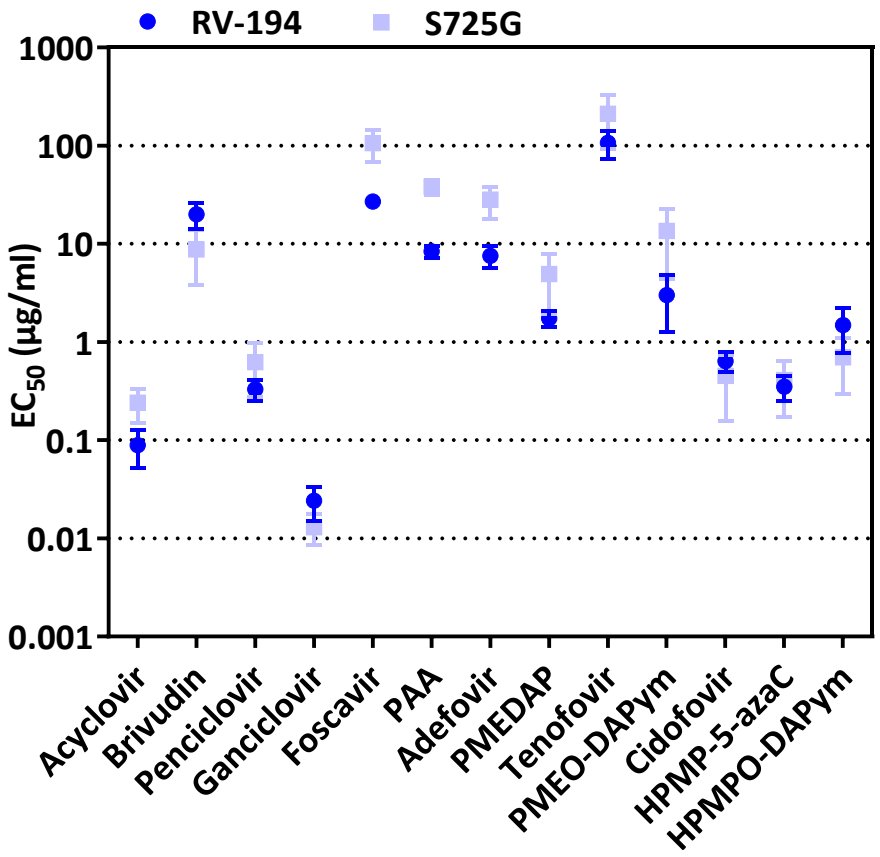

RV-194
Y823C
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym


RV-194
R847C
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym


RV-194
F923L
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym


RV-194
T934A
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym


RV-194
R964H
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym


RV-194
S725G
1000
100
10
1
0.1
0.01
0.001
EC50 (µg/ml)
Acyclovir
Brivudin
Penciclovir
Ganciclovir
Foscavir
PAA
Adefovir
PMEDAP
Tenofovir
PMEO-DAPym
Cidofovir
HPMP-5-azaC
HPMPO-DAPym

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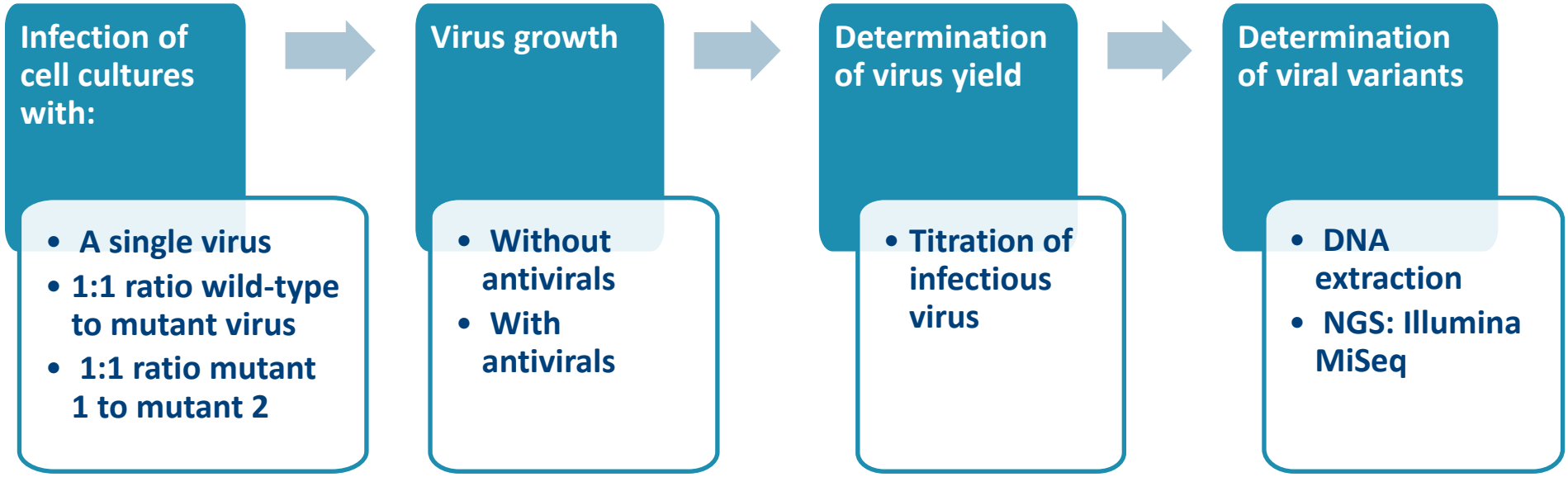
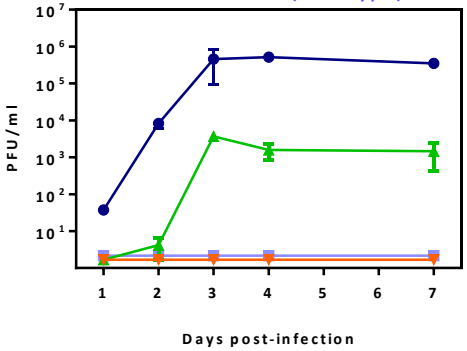
In vitro competitive fitness studies



Drug susceptibility – virus growth – variants detection

Virus growth

RV-194 clone 2 (wild-type)



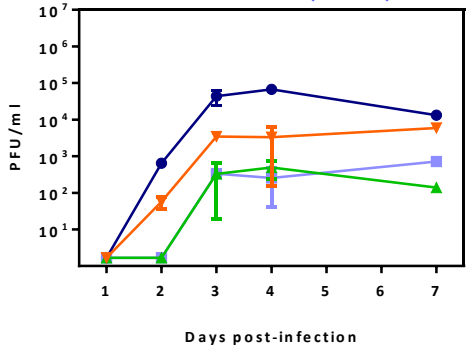
NGS

Treatment	Variants (%)
	Wild-type
None	99.78
ACV	ND
CDV	99.78
PFA	ND

Untreated
ACV 1 µg/ml
CDV 1 µg/ml
PFA 100 µg/ml

Virus growth

RV-204 clone 1 (A606V)



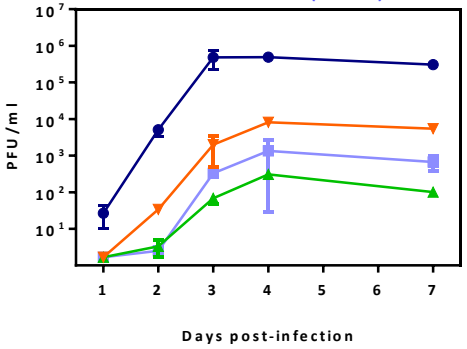
NGS

Treatment	Variants (%)
	A606V
None	99.83
ACV	99.79
CDV	99.85
PFA	99.84

Untreated
ACV
CDV
PFA

Virus growth

RV-200 clone 1 (F923L)



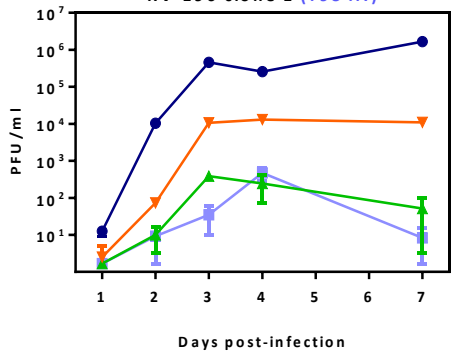
NGS

Treatment	Variants (%)
	F923L
None	99.88
ACV	99.83
CDV	99.86
PFA	99.88

Untreated
ACV 1 µg/ml
CDV 1 µg/ml
PFA 100 µg/ml

Virus growth

RV-196 clone 1 (T934A)



NGS

Treatment	Variants (%)
	T934A
None	99.88
ACV	99.95
CDV	99.91
PFA	99.95

Untreated
ACV 1 µg/ml
CDV 1 µg/ml
PFA 100 µg/ml

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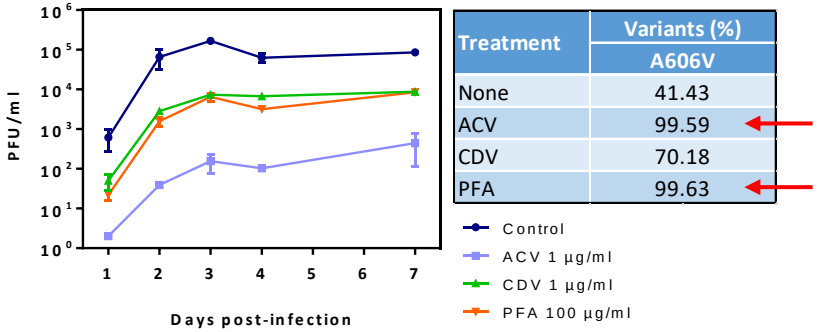
Competitive fitness

1:1 ratio wt to mutant

Virus growth

RV-194 clone 2 (wild-type) + RV-204 clone 1 (A606V)

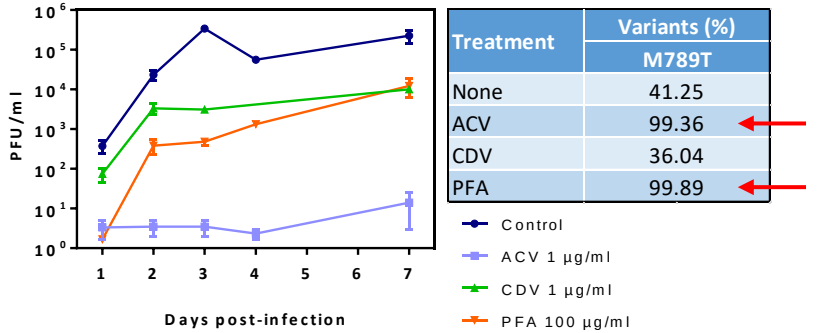


NGS

Treatment	Variants (%) A606V
None	41.43
ACV	99.59
CDV	70.18
PFA	99.63

Virus growth

RV-194 clone 2 (wild-type) + RV-206 clone 2 (M789T)



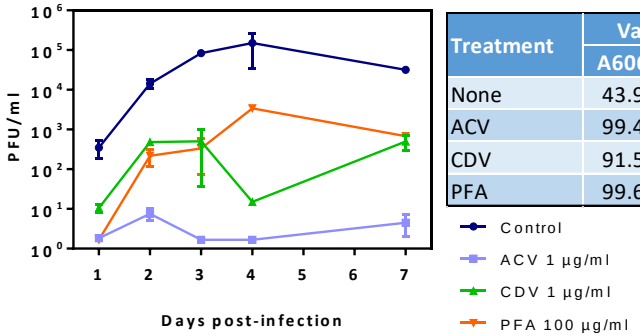
NGS

Treatment	Variants (%) M789T
None	41.25
ACV	99.36
CDV	36.04
PFA	99.89

1:1 ratio mutant A to mutant B

Virus growth

RV-204 clone 1 (A606V) + RV-206 clone 2 (M789T)



NGS

Treatment	Variants (%) A606V	M789T
None	43.99	53.55
ACV	99.44	<1
CDV	91.56	7.56
PFA	99.65	<1

A606V more fit than M789T under selective drug pressure

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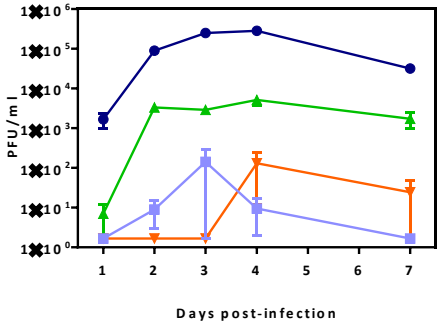
Competitive fitness

1:1 ratio wt to mutant

Virus growth

RV-194 clone 2 (wild-type) + RV-203 clone 5 (S725G)



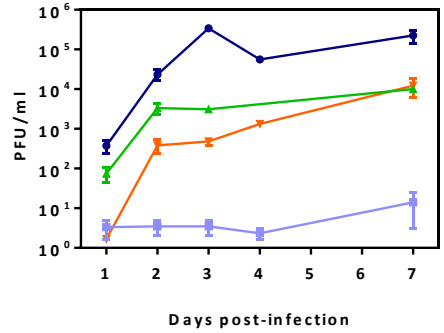
NGS

Treatment	Variants (%)
	S725G
None	<1%
ACV	Not done
CDV	<1%
PFA	99.61

Control
ACV 1 µg/ml
CDV 1 µg/ml
PFA 100 µg/ml

Virus growth

RV-194 clone 2 (wild-type) + RV-206 clone 2 (M789T)



NGS

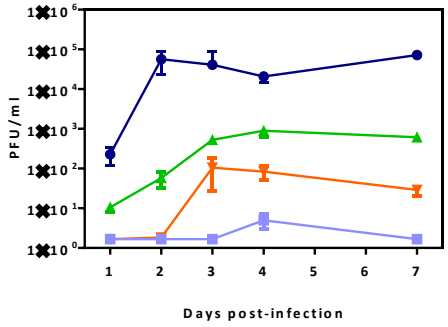
Treatment	Variants (%)
	M789T
None	41.25
ACV	99.36
CDV	36.04
PFA	99.89

Control
ACV 1 µg/ml
CDV 1 µg/ml
PFA 100 µg/ml

1:1 ratio mutant A to mutant B

Virus growth

RV-203 clone 5 (S725G) + RV-206 clone 2 (M789T)



NGS

Treatment	Variants (%)	
	S725G	M789T
None	7.83	90.57
ACV	Not done	Not done
CDV	1.02	98.94
PFA	<1	99.46

Control
ACV 1 µg/ml
CDV 1 µg/ml
PFA 100 µg/ml

M789T more fit than S725G with or without selective drug pressure

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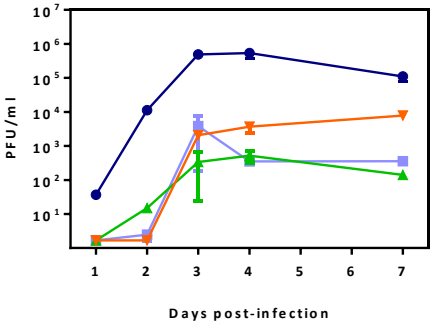
Competitive fitness

1:1 ratio wt to mutant

Virus growth

RV-194 clone 2 (wild-type) + RV-200 clone 1 (F923L)



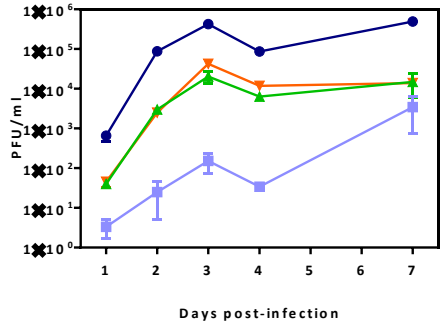
NGS

Treatment	Variants (%)
	F923L
None	56.91
ACV	99.85
CDV	37.03
PFA	99.91

Control
ACV
CDV
PFA

Virus growth

RV-194 clone 2 (wild-type) + RV-196 clone 1 (T934A)



NGS

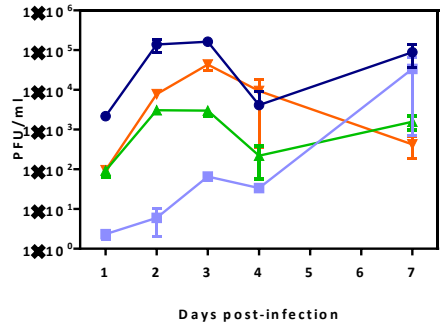
Treatment	Variants (%)
	T934A
None	71.16
ACV	99.74
CDV	53.28
PFA	99.79

Control
ACV 1 µg/ml
CDV 1 µg/ml
PFA 100 µg/ml

1:1 ratio mutant A to mutant B

Virus growth

RV-200 clone 1 (F923L) + RV-196 clone 1 (T934A)



NGS

Treatment	Variants (%)	
	F923L	T934A
None	34.67	62.08
ACV	73.14	17.86
CDV	31.19	65.93
PFA	21.23	75.24

Control
ACV 1 µg/ml
CDV 1 µg/ml
PFA 100 µg/ml

F923L more or less fit than T934A depending on selective drug pressure

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Relapse of HSV-2

- Allo-HSCT in October 2011
- HSV-2 PCR positive (cutaneous lesion) on 07/10/2011
- Lesions in genital area, ear, nose and hands not responding to foscarnet
- Cidofovir IV is given 1x/week

Relapse of HSV-2 on 31/10/2011

RegaVir identification	Date collected	Type	TK genotype	DNA polymerase genotype	Phenotyping
RV-274	31/10/2011	Genital swab	**G insertion Nts 433-439 (frameshift at amino acid 147)**	None	Not available
RV-271	07/11/2011	Ear swab (#0)	**T288M**	**Q732R**	Not available
RV-272	07/11/2011	Ear swab (#1)	**T288M**	**Q732R**	ACV^R/PFA^R
RV-273	07/11/2011	Plasma	**T288M**	**Q732R**	Not available
RV-269	09/11/2011	Right ear swab	**T288M**	**Q732R**	Not available
RV-265	10/11/2011	Vaginal swab	**G insertion Nts 433-439 (frameshift at amino acid 147)**	None	Not available
RV-266	10/11/2011	Right ear swab	**T288M**	**Q732R**	ACV^R/PFA^R
RV-268	10/11/2011	Left ear swab	Not available	**L480Q**	Not available
RV-282	15/11/2011	belly	None	None	Not done
RV-283	15/11/2011	vulva	**G insertion Nts 433-439 (frameshift at amino acid 147)**	None	Not done
RV-284	15/11/2011	anal	**C deletion Nts 551-556 mixed (frameshift at amino acid 186)**	**L788M***	Not done
RV-285	15/11/2011	chin	Not available	None	Not done
RV-286	15/11/2011	middle forehead	None	None	Not done
RV-287	15/11/2011	nose	None	None	Not done

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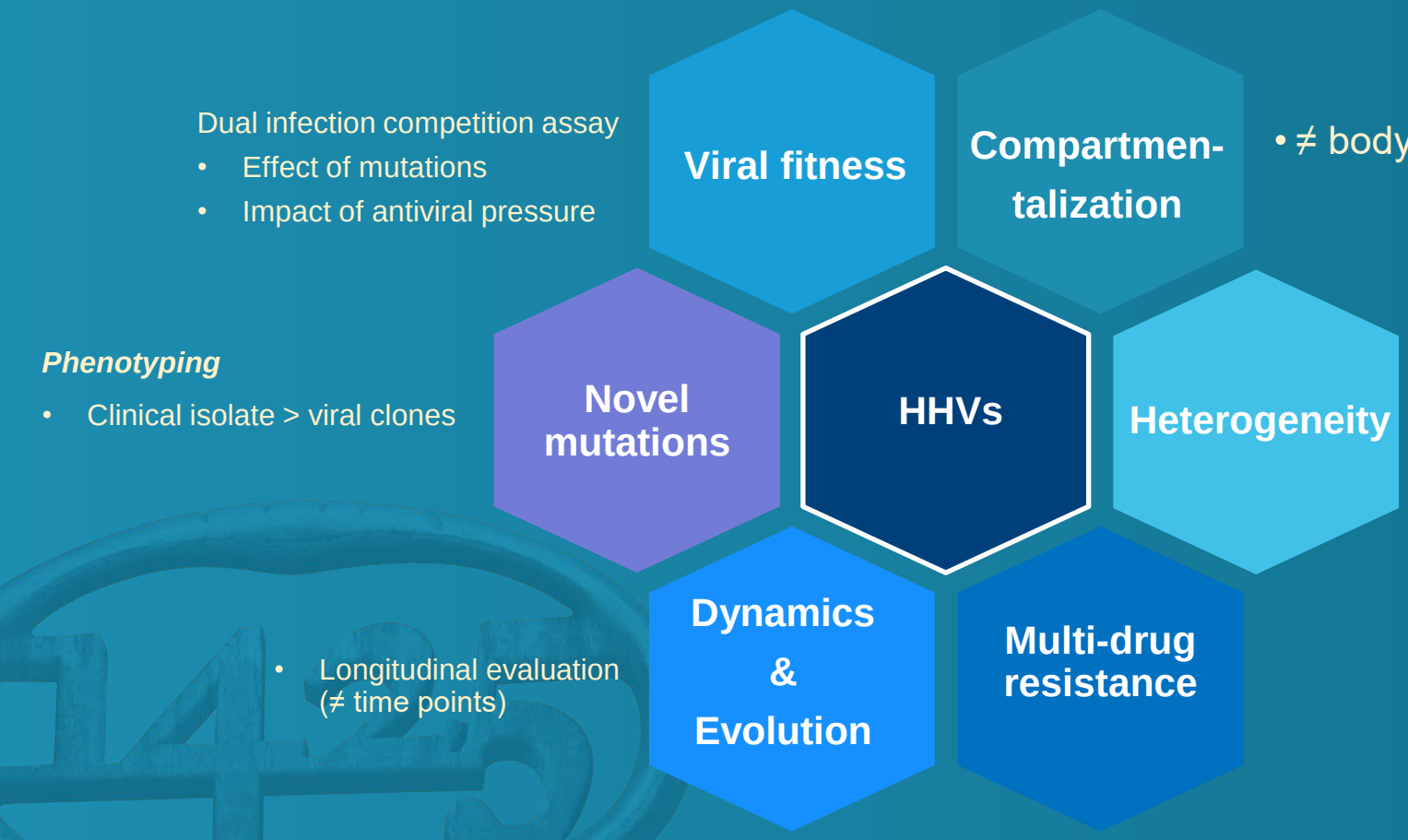
SUMMARY

Primary herpes infection with herpes type 2 complicated by viremia with probably viral hepatitis, nephritis of still doubtful etiology, in a patient with autoimmune aplasia treated with Cyclosporine, with favorable outcome under treatment.

Conclusions



Dual infection competition assay
- Effect of mutations
- Impact of antiviral pressure

• ≠ body sites

Phenotyping
- Clinical isolate > viral clones

- Minor populations by NGS
- Isolation of viral clones: VIRAL FITNESS

- Longitudinal evaluation (≠ time points)

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Thank you for your attention

Any questions?

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