



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 16/02/2011 until 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

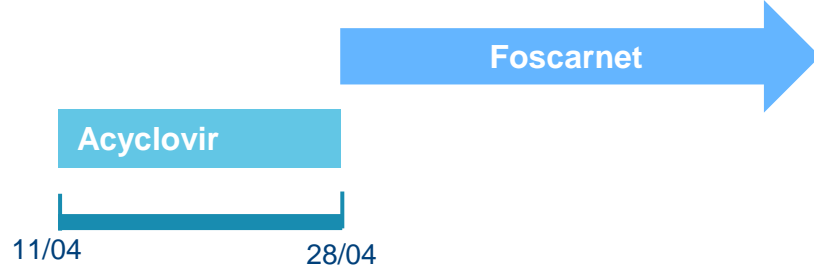
- **11.04.2011: appearance of sudden symptoms → emergency**
 - Very high fever (40°)
 - Painful swallowing
 - Cervical adenopathy's
 - Sensitive to pressure in right hypochondrium
 - Muco-cutaneous disseminated vesicular lesions (mainly in thorax, arms, and lower limbs)
 - Ulcerative-necrotic angina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

Case study

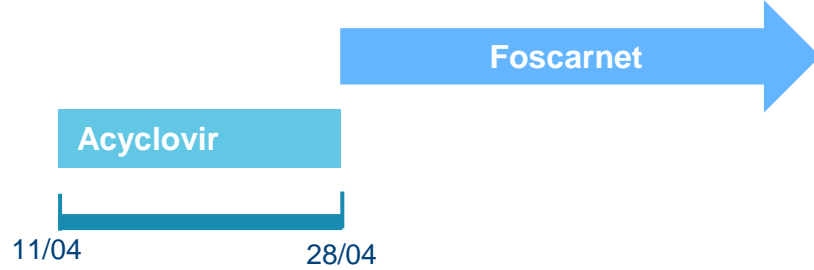
- **11.04.2011: appearance of brutal symptoms**
 - **Abdominal echography:** no cholecystitis, no angiocholitis
 - **Abdominal, pelvic and thoracic CT-Scan:** small homogeneous hepatomegaly
 - Pancytopenia (mainly of platelets & leukocytes) – low hemoglobin, elevated CRP, elevated creatinine, elevated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



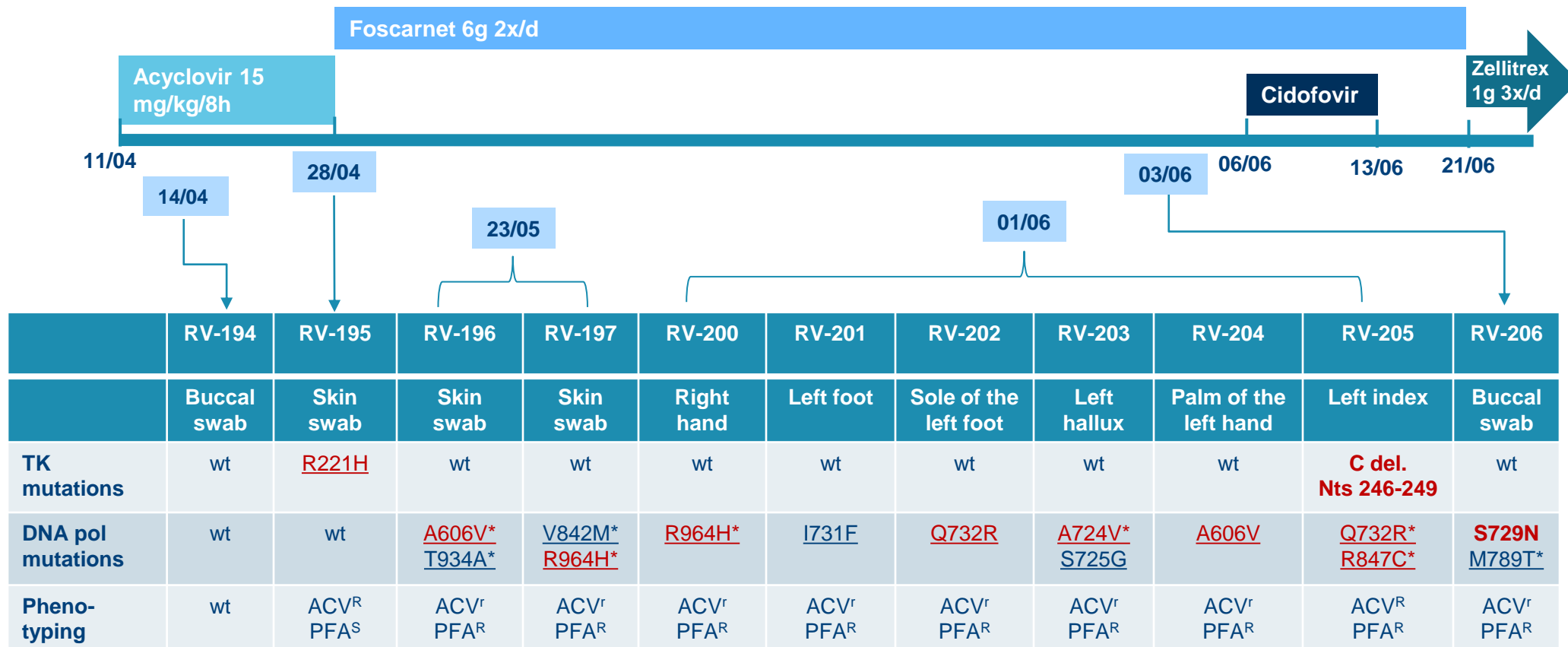
- Lesions were very painful and of hyper tense aspect → lesions were incised gradually to relieve the patient and to take samples for bacteriological, mycological and virological purposes
- Skin biopsies were also performed to ensure the absence of bullous dermatosis
→ result: herpetic eruption without 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

	Foscarnet 6g 2x/d			
	Acyclovir 15 mg/kg/8h			
	11/04	14/04	28/04	23/05
	RV-194	RV-195	RV-196	RV-197
	Buccal swab	Skin swab	Skin swab	Skin swab
TK mutations	wt	<u>R221H</u>	wt	wt
DNA pol mutations	wt	wt	<u>A606V*</u> <u>T934A*</u>	<u>V842M*</u> <u>R964H*</u>
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





*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



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 EC₅₀ values ± STDEV of at least two independent experiments. EC₅₀: 50% effective concentration or drug concentration required to reduce viral CPE by 50%.

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.

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.

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	

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

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	

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

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	/
	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%	/
	A724V	0/10	3.52	/
	I731F*	9/10 (90,0%)	94.01	99.80
RV-202	G607D	0/11	2.07	/
	Q732R*	10/11 (90,9%)	95.41	83.81
	L779P	0/11	1.03	/
	M789T	1/11 (9,1%)	8.68	11.49

F923L

I731F

Q732R

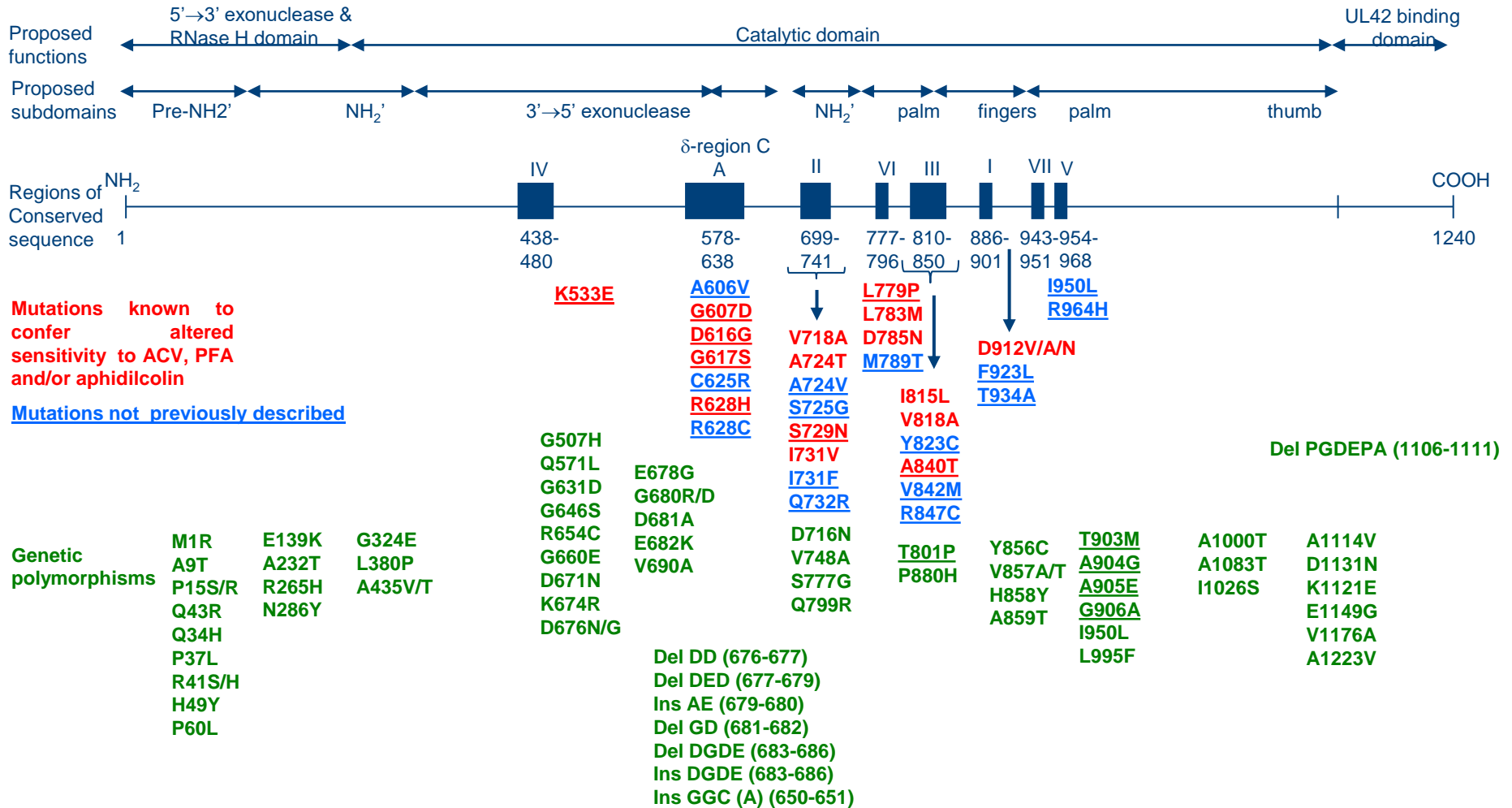
* DNA pol mutations detected by Sanger sequencing in original samples are in blue color.

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	/	

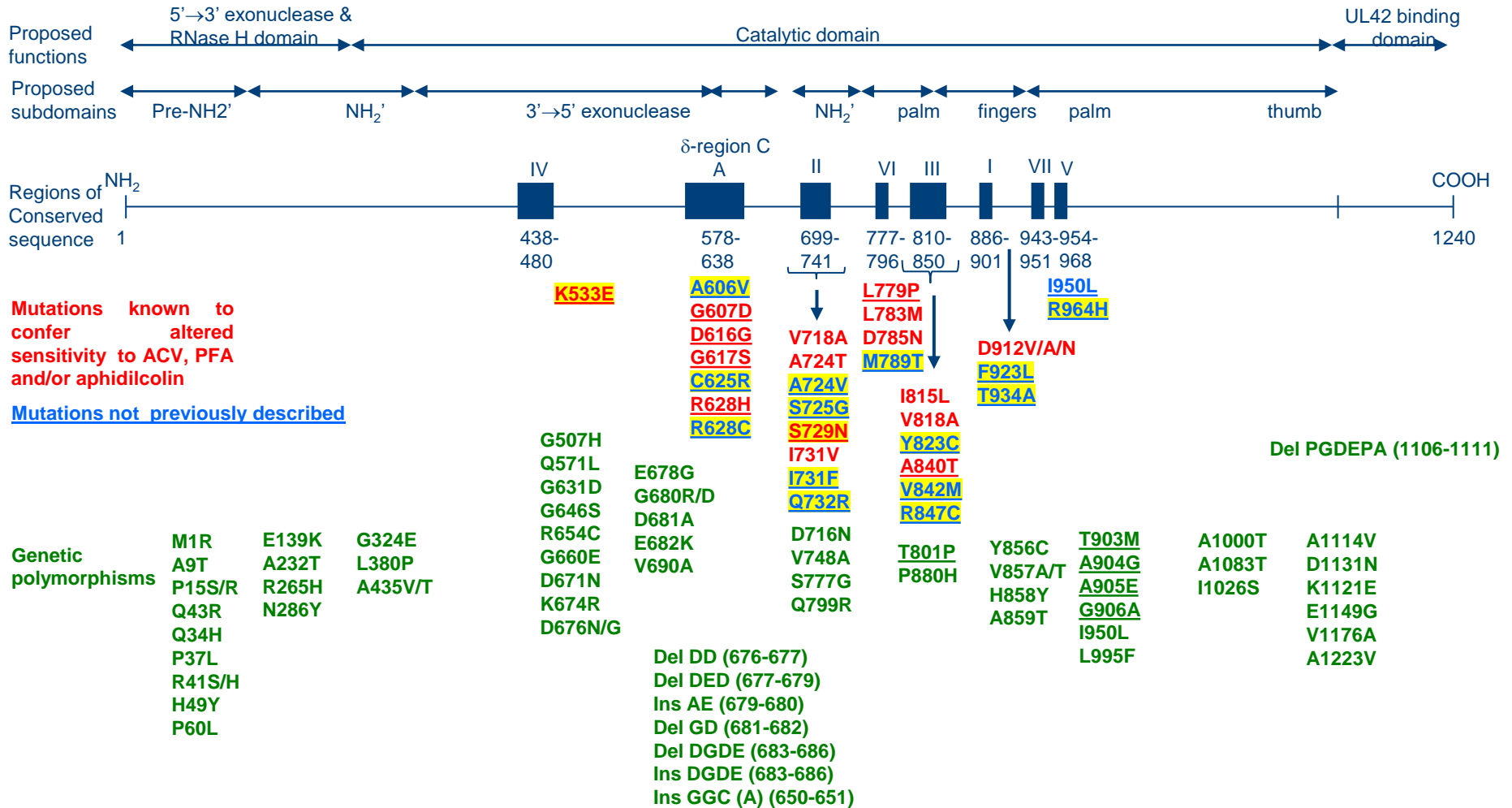
* DNA pol mutations detected by Sanger sequencing in original samples are in blue color.

HSV-2 DNA polymerase



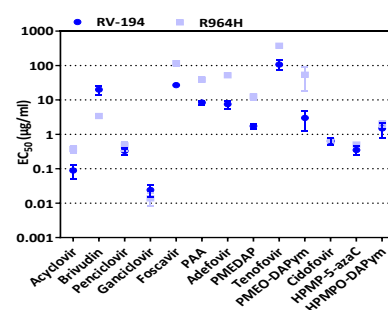
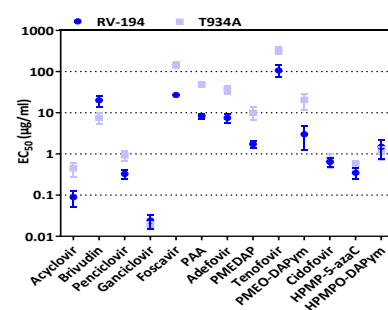
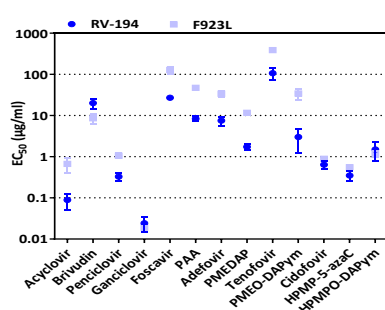
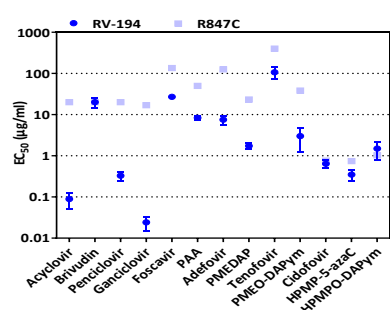
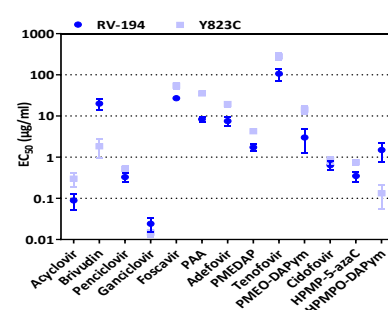
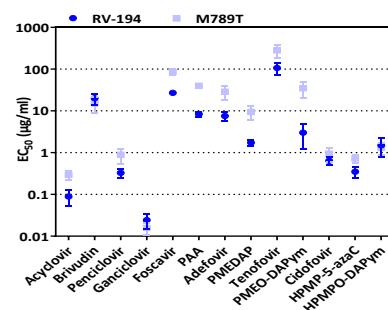
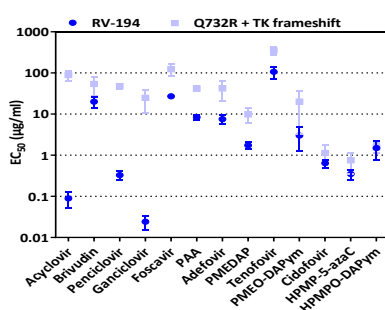
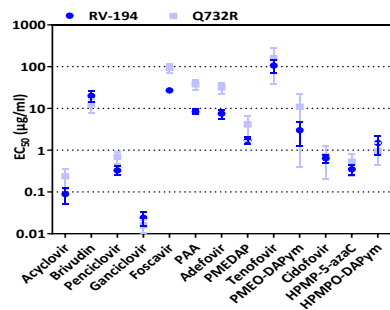
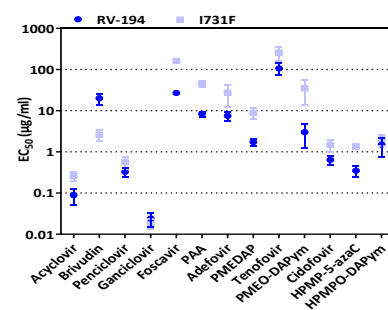
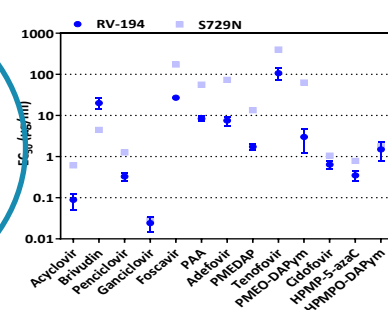
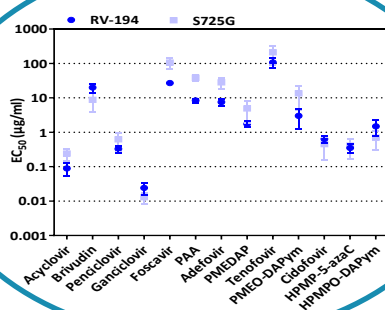
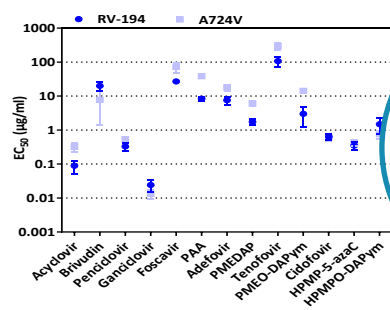
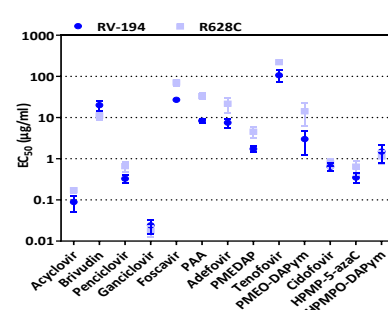
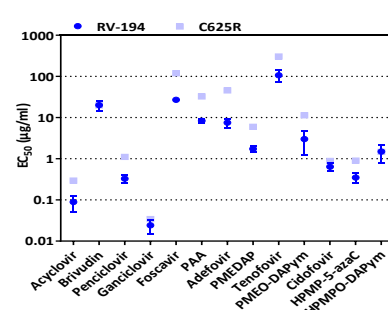
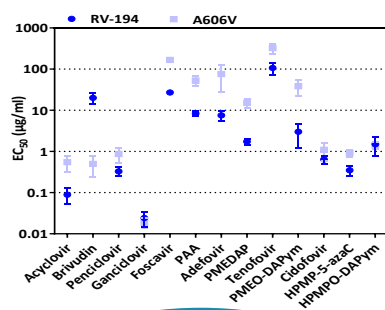
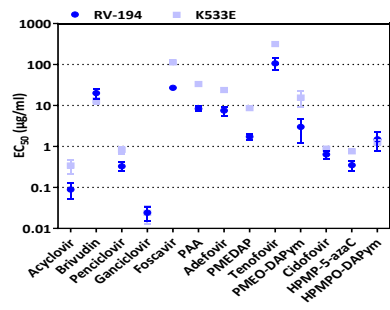
Mutations found in the patient are underlined: 23 ≠ mutations (15 new mutations)

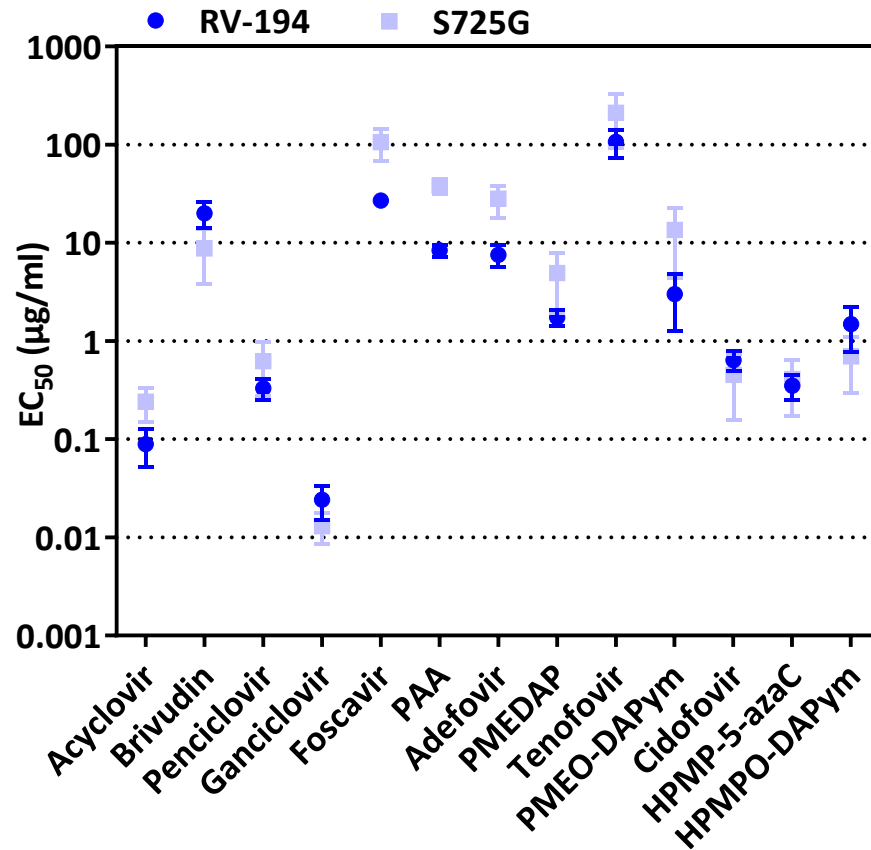
HSV-2 DNA polymerase



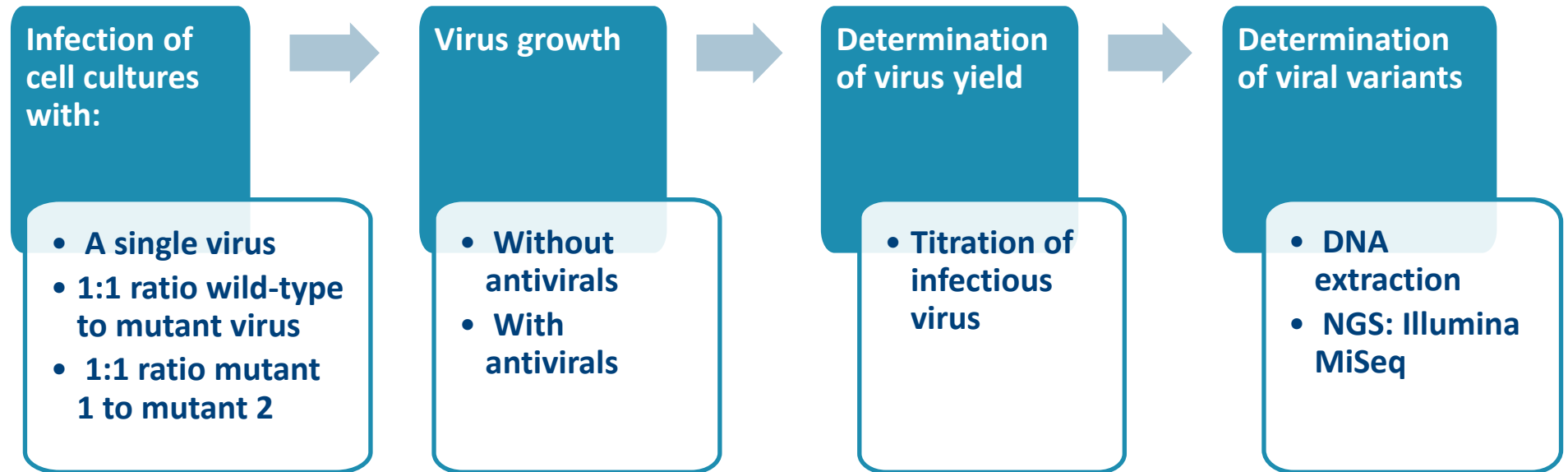
Mutations found in the patient are underlined: 23 ≠ mutations (15 new mutations)

Phenotyping performed





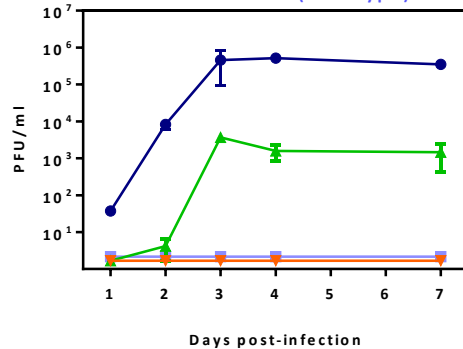
In vitro competitive fitness studies



Drug susceptibility – virus growth – variants detection

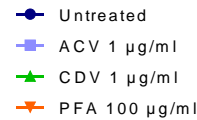
Virus growth

RV-194 clone 2 (wild-type)



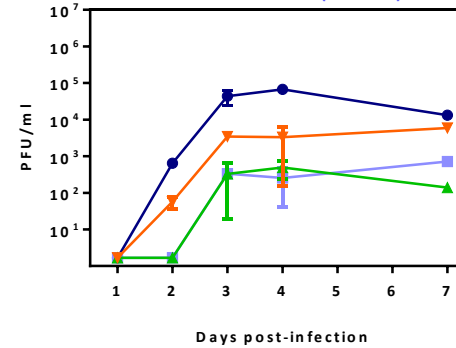
NGS

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



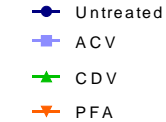
Virus growth

RV-204 clone 1 (A606V)



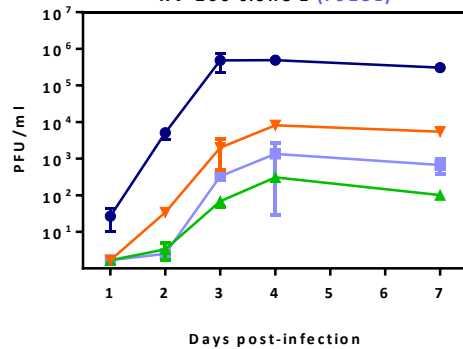
NGS

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



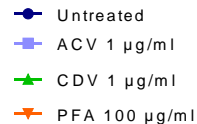
Virus growth

RV-200 clone 1 (F923L)



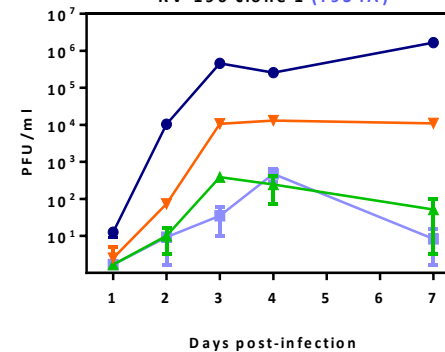
NGS

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



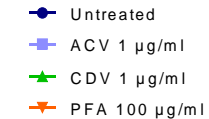
Virus growth

RV-196 clone 1 (T934A)



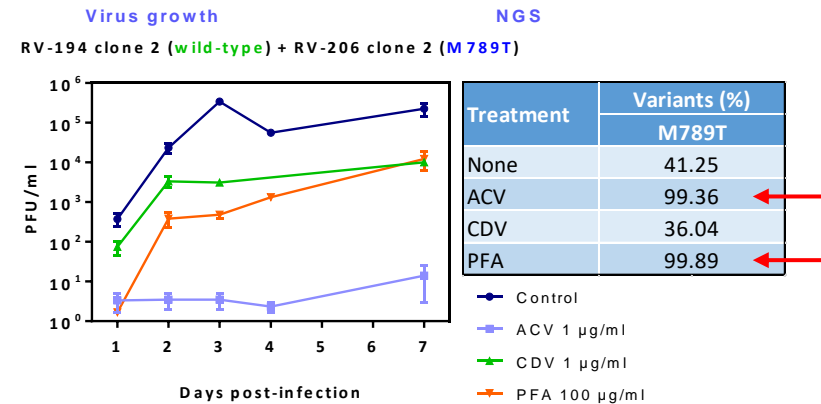
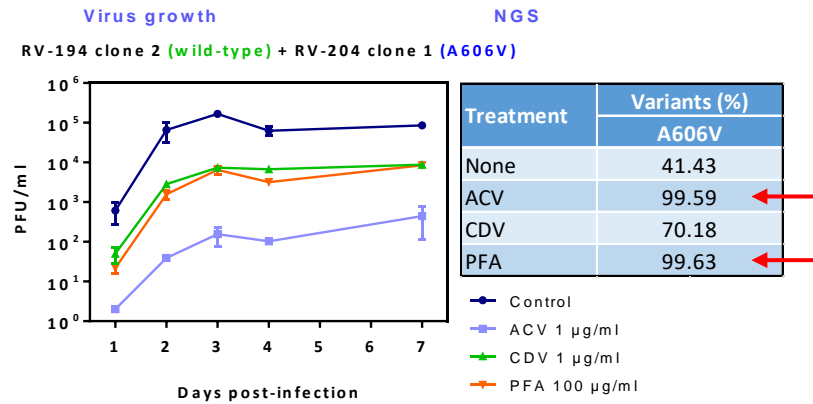
NGS

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

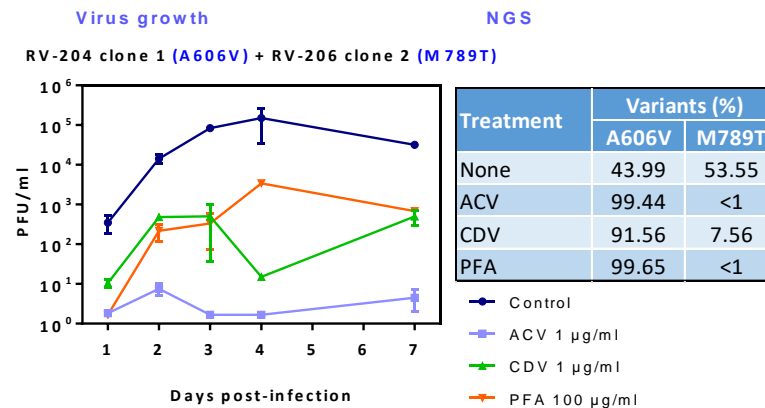


Competitive fitness

1:1 ratio wt to mutant



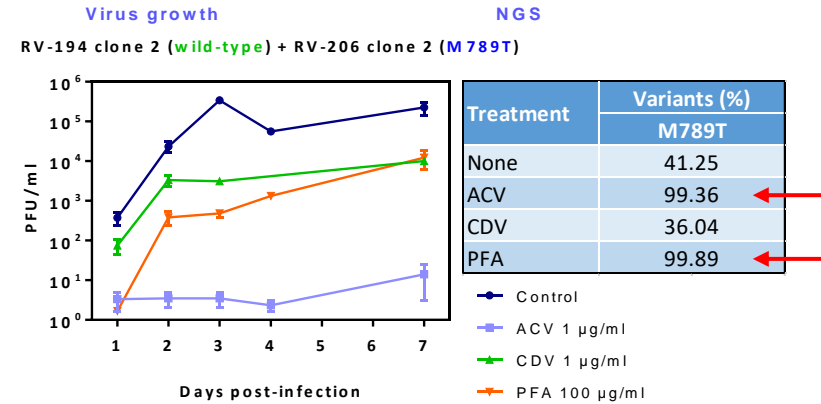
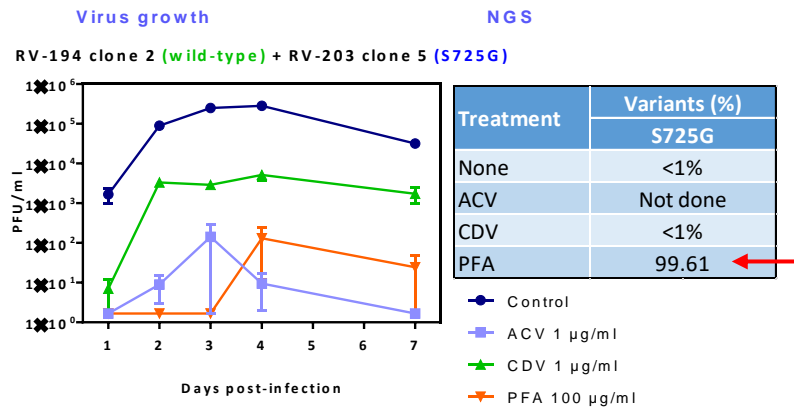
1:1 ratio mutant A to mutant B



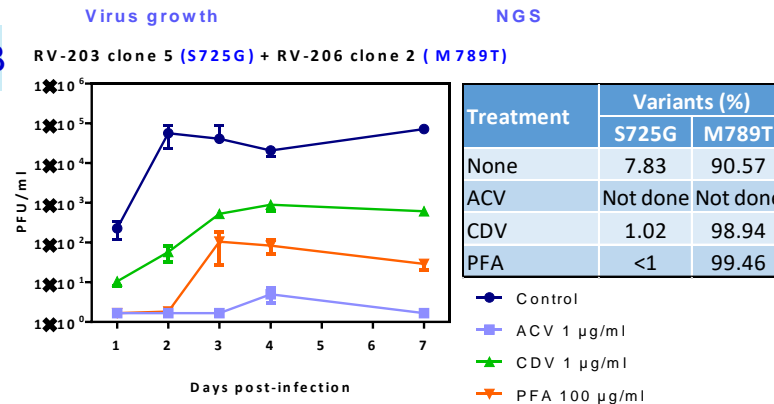
A606V more fit than M789T under selective drug pressure

Competitive fitness

1:1 ratio wt to mutant



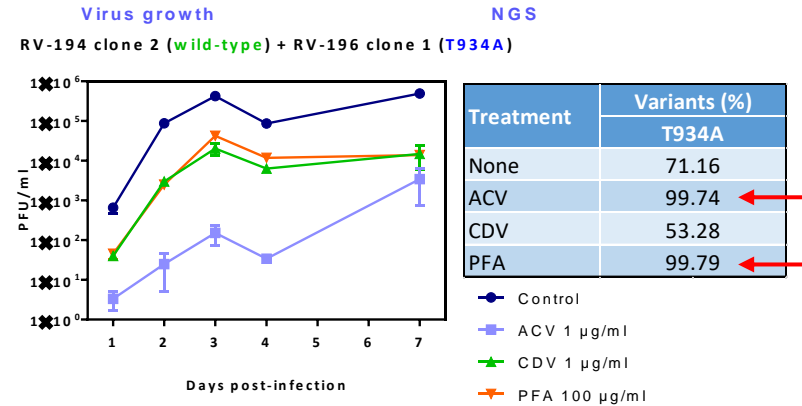
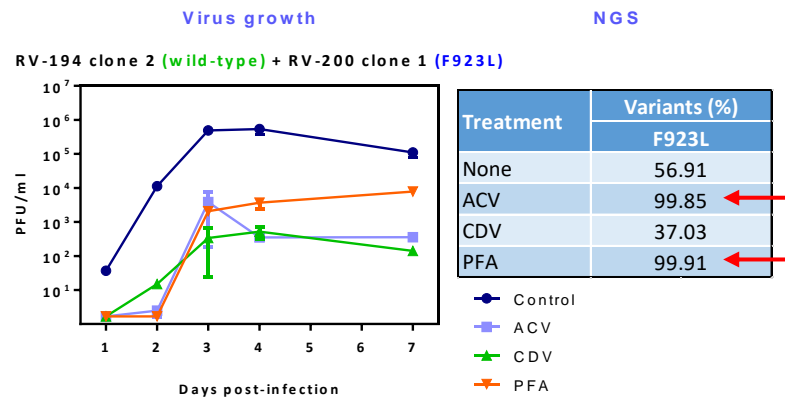
1:1 ratio mutant A to mutant B



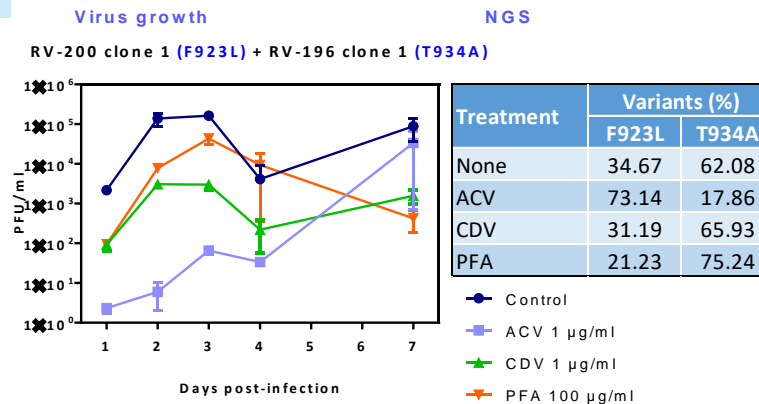
M789T more fit than S725G with or without selective drug pressure

Competitive fitness

1:1 ratio wt to mutant



1:1 ratio mutant A to mutant B



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

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

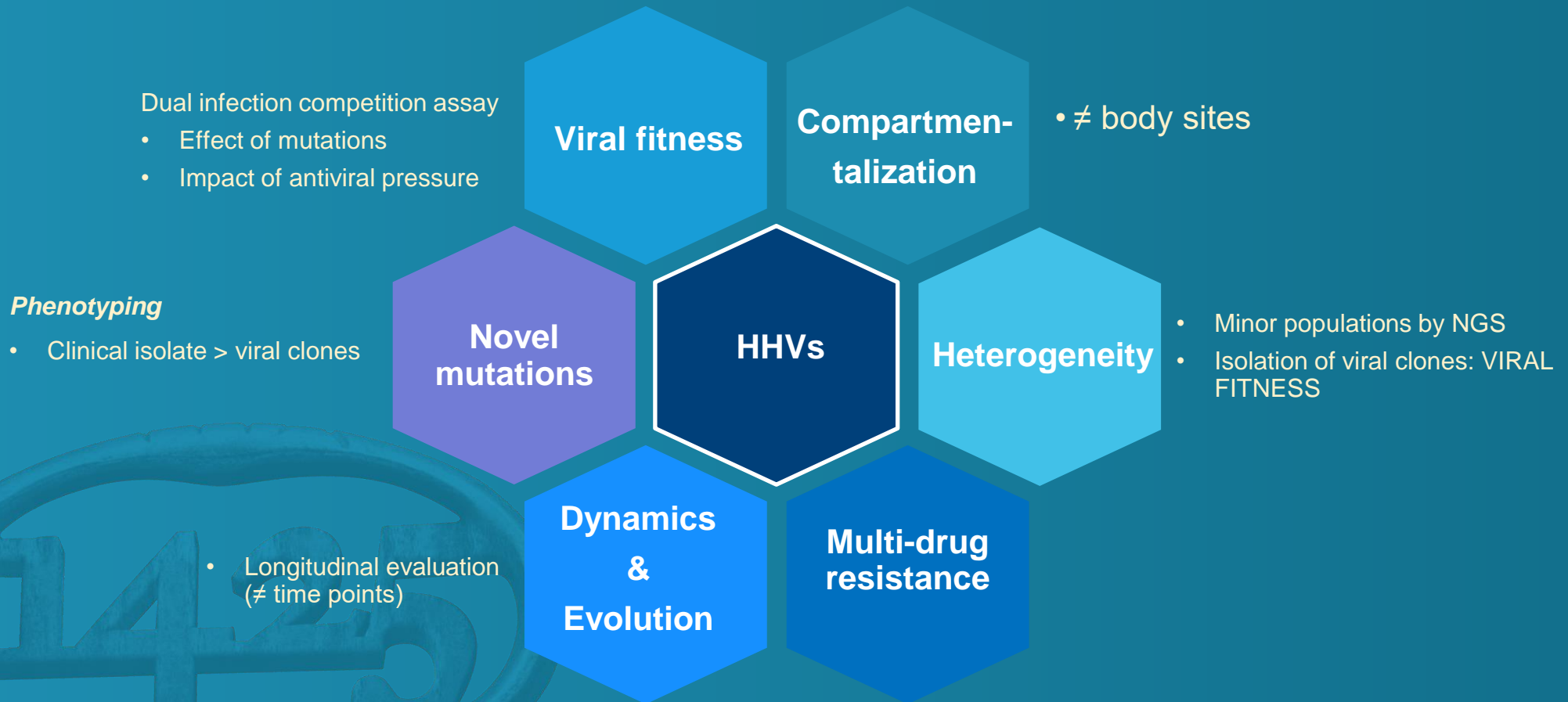
RegaVir identification	Date collected	Type	TK genotype	DNA polymerase genotype	Phenotyping
RV-274	31/10/2011	Genital swab	G insertion Nts 433-439 (frameshift at amino acid 147)	None	Not available
RV-271	07/11/2011	Ear swab (#0)	T288M	Q732R	Not available
RV-272	07/11/2011	Ear swab (#1)	T288M	Q732R	ACV ^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

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





Thank you for your
attention

Any questions?