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Introduction to the translational Research platform RegaVir

Robert Snoeck & Graciela Andrei

Leuven, February 22, 2022

RegaVir: Research Group for Antiviral Resistance

AIMS

- Provide rapid **genotyping and/or phenotyping** of clinical isolates of **herpesviruses** recovered from **immunocompromised patients** who **fail antiviral therapy** to:
  - determine viral drug-resistance as reason for failure of therapy
  - optimize antiviral therapy
  - avoid drug toxicity
  - improve patient care
  - reduce costs of antiviral treatment

- Get insights into **herpesvirus diversity & evolution** in the immunocompromised host

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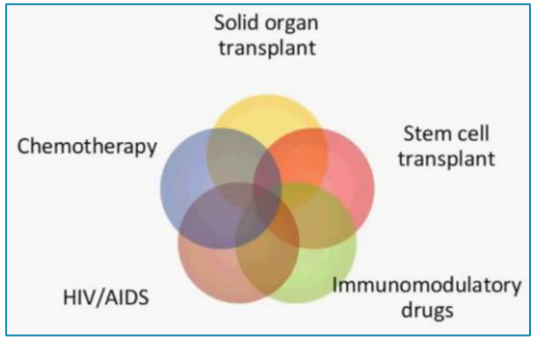
Immunocompromised (IC) patients

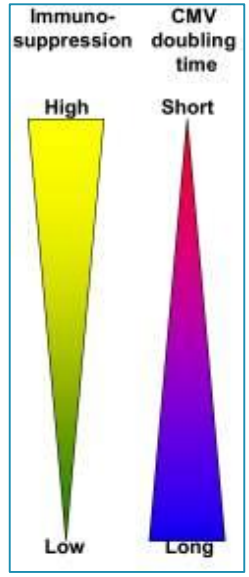
- **Increasing number**
  - HIV/AIDS
  - Solid organ transplant (SOT) recipients
  - Hematopoietic stem cell transplant (HSCT) recipients
  - Chemotherapy and/or radiotherapy
  - Immunomodulatory drugs
  - Congenital immunodeficiency's



The immunocompromised host

Immunocompromised (IC) patients

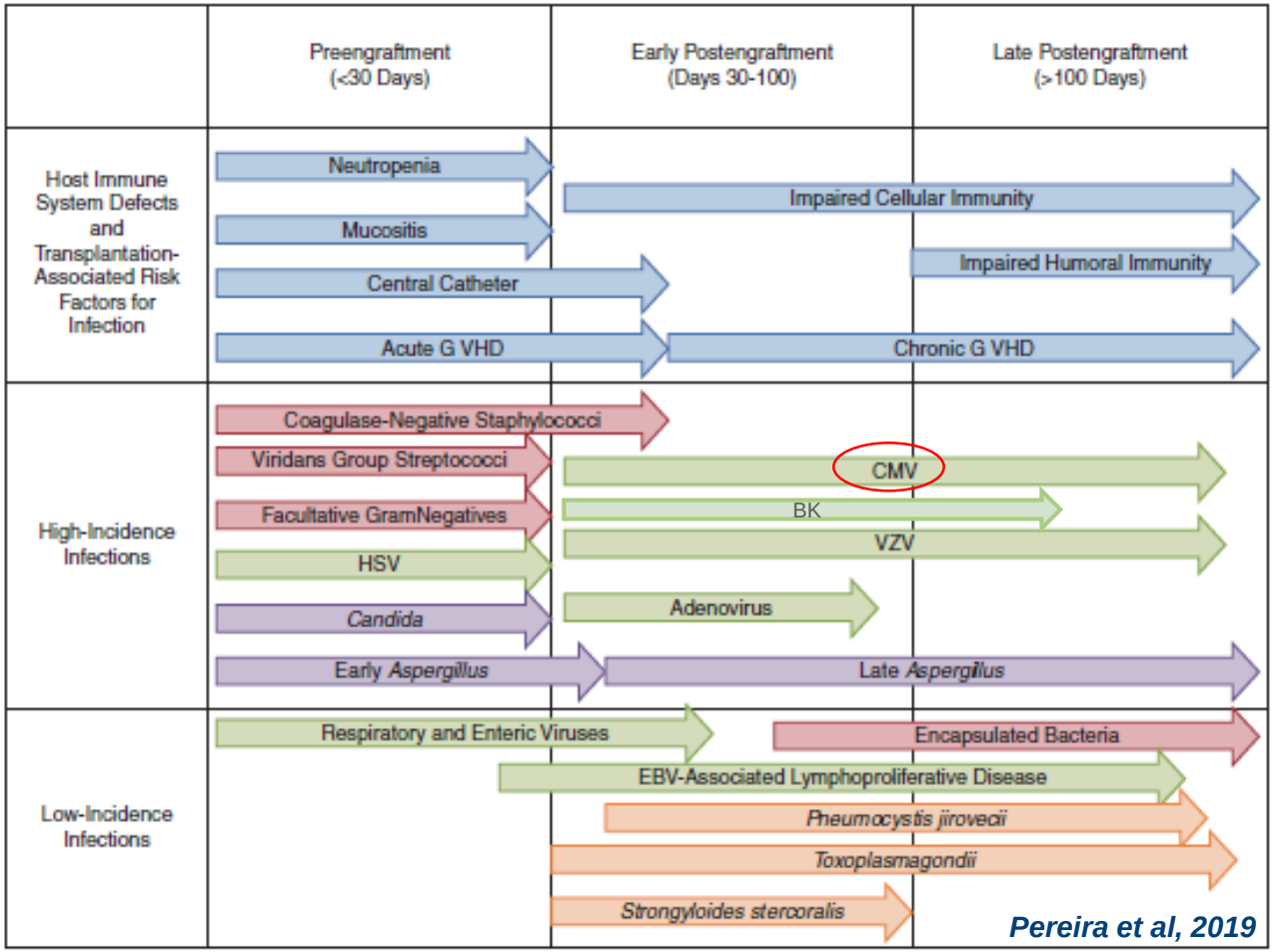
- More susceptible to viral infection and disease
- Higher risk to develop persistent infection
- More likely to have multiple infections
- May develop unusual clinical manifestations which are not seen in immunocompetent patients



Boeckh & Ljungman, 2009

- All human herpesviruses (HHV) can lead to severe disease among IC patients, due to primary infection, reactivation or re-infection

Timeline of common post-transplant infections



CMV: Most significant threat to patient and graft health

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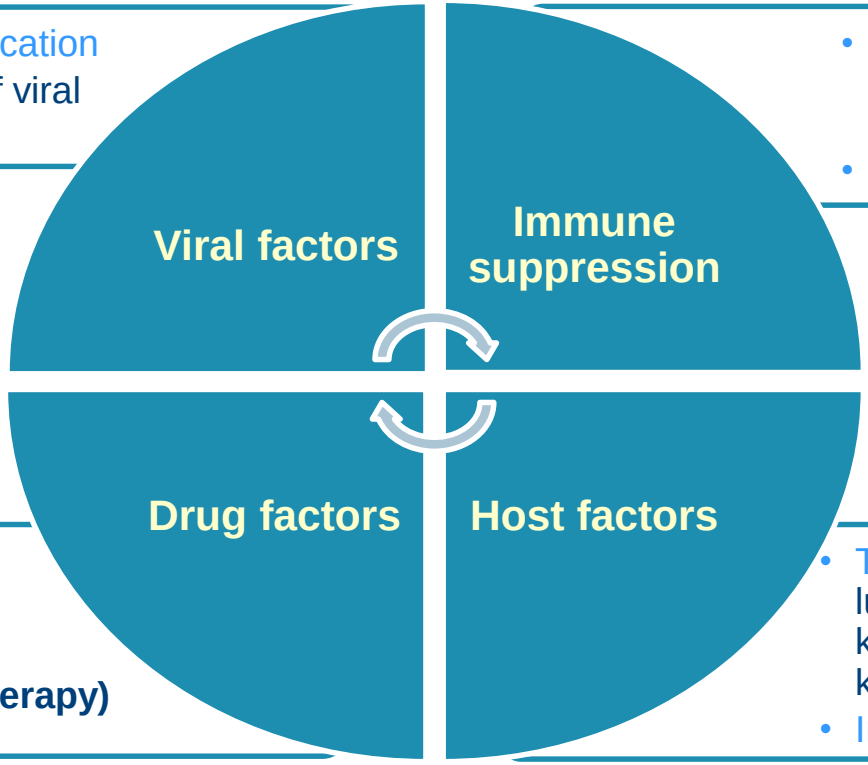
Impact of HCMV on transplant outcomes

Direct effects
CMV syndrome
Tissue-invasive CMV disease - Gastrointestinal disease - Pneumonitis - Hepatitis - CNS disease - Retinitis - Nephritis - Pancreatitis - Myocarditis
Mortality

Indirect effects
Acute allograft rejection
Chronic allograft rejection
Opportunistic and other infections - Fungal superinfection - Bacterial superinfection - EBV and PTLD - Hepatitis C recurrence - Other viruses (HHV-6, HHV-7)
New onset diabetes miellitus
Malignancies
Thrombosis
Mortality

Risk factors for herpesvirus drug-resistance



- High-level viral replication
- Multiple episodes of viral disease

- Potent immune suppression regimens (dose, duration, and overall intensity of drugs)
- T-cell depletion

- Suboptimal antiviral drug levels
- Prolonged antiviral drug administration

(prophylaxis > pre-emptive therapy)

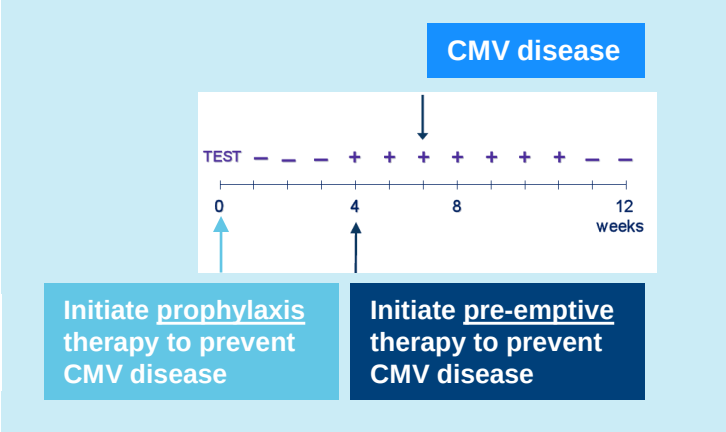
- Type of transplant: > HSCT, lung/small intestine > pancreas, kidney-pancreas, heart > liver, kidney
- Immune serostatus

Risk category	Type	Donor/Recipient Immune status
High	Primary infection	D+ /R-
Intermediate	Reactivation	D- /R+
Intermediate	Superinfection	D+ /R+
Low	Risk with exposure	D- /R-

Optimal care of transplant recipients is fundamental

- No universal agreement among transplant centers regarding:
  - Prophylaxis *versus* preemptive antiviral therapy

- < indirect effects
- Ease of administration



- < drug-exposure → < toxicity & costs
- < risk of resistance
- < late-onset CMV disease (may allow development of cell-mediated immune responses)

  - Optimal duration of antiviral treatment (may vary among subpopulations (e.g. D+/R- *versus* D-/R+)

Herpesvirus drug-resistance

- Associated with progressive disease and treatment failure: cause significant morbidity and mortality

- **Prevalence**
  - **Immunocompetent patients**

    Isolation of resistant isolates is <<1%

    EXCEPTION: infection of immune-privileged sites!

  - **Immunocompromised patients**

    HCMV: 1% to 13% among SOT recipients (> lung, small bowel transplants)

    HSV: 4.3 to 14 % among all immunocompromised groups (up to 36% in HSCT patients)

Antiviral agents for the management of HSV and VZV infections

Anti-herpesvirus drug	Trade name	Indication	Route of administration
Acyclovir	Zovirax®	HSV-1, HSV-2, VZV	Oral, iv, topical
Valacyclovir (VACV)	Valtrex®, Zelitrex®	HSV-1, HSV-2, VZV	Oral
Penciclovir (PCV)	Vectavir®, Denavir®	HSV-1, HSV-2, VZV	Topical
Famciclovir (FAM)	Famvir®	HSV-1, HSV-2, VZV	Oral
Brivudine (BVDU)	Zostex®, Zerpex®	HSV-1, VZV	Oral
Ganciclovir (GCV)	Cytovene® Cymevene®	HCMV	Oral, iv, intravitreal
Valganciclovir (VGCV)	Valacyte®	HCMV	Oral
Cidofovir	Vistide®	HCMV	iv
Foscarnet (PFA)	Foscavir®	HCMV, HSV-1, HSV-2, VZV	Iv, intravitreal

Common target: viral DNA polymerase

Licensed anti-herpesvirus drugs

	DNA polymerase inhibitors					Terminase inhibitor	UL97 PK inhibitor
	Acyclovir Valacyclovir	Penciclovir Famciclovir	Ganciclovir Valganciclovir	Cidofovir	Foscarnet	Letermovir	Maribavir
HSV-1 (HHV-1)	1st line	approved		resistance	resistance		
HSV-2 (HHV-2)	1st line	approved		resistance	resistance		
VZV (HHV-3)	1st line	approved		resistance	resistance		
EBV (HHV-4)			off-label	off-label	off-label		
HCMV (HHV-5)			1st line	approved	approved	approved for prophylaxis	orphan Drug Designation
HHV-6A			off-label	off-label	off-label		
HHV-6B			off-label	off-label	off-label		
HHV-7			off-label	off-label	off-label		
KSHV (HHV-8)			off-label	off-label	off-label		

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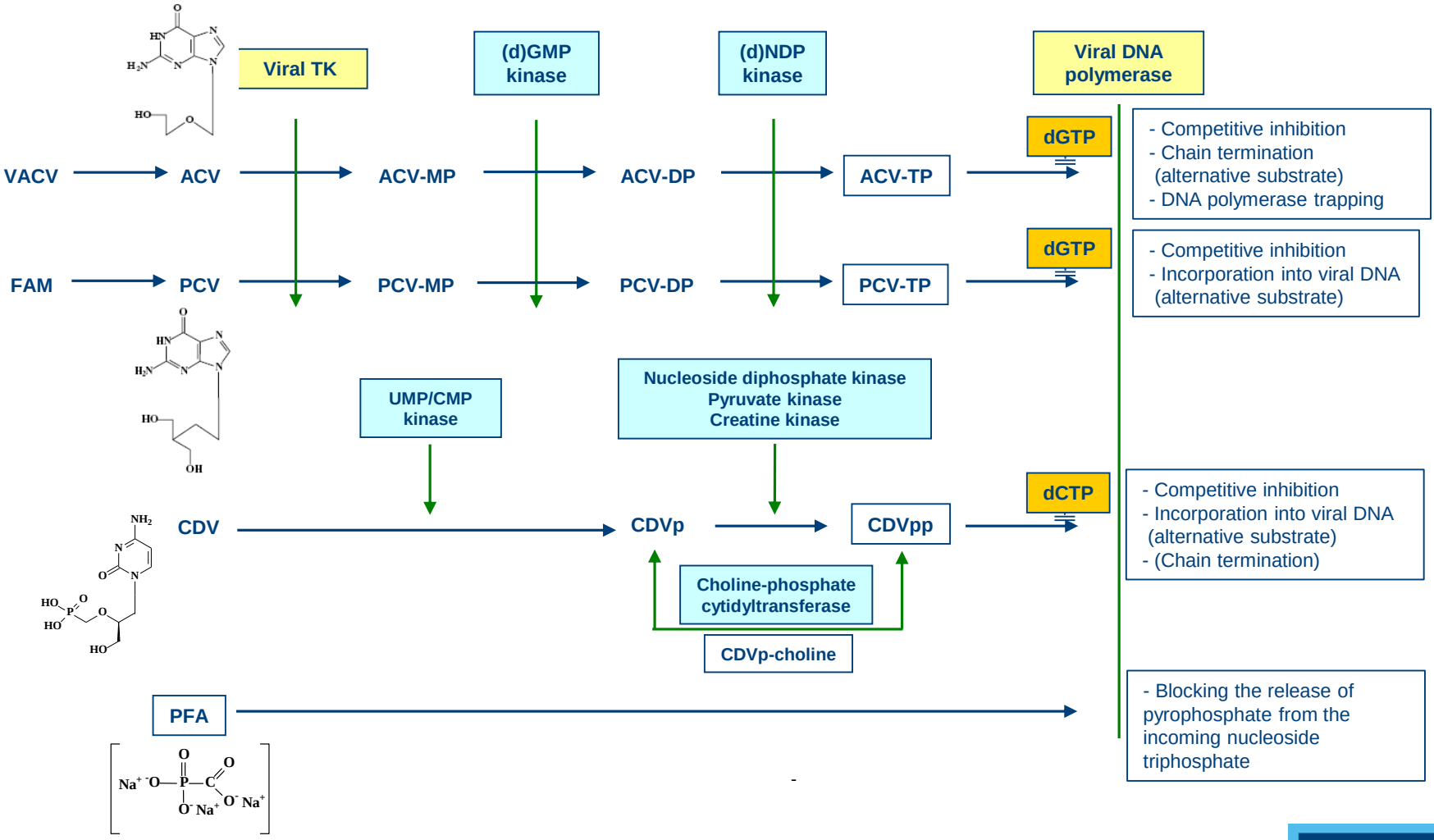
Mechanism of action of anti-HSV and anti-VZV agents



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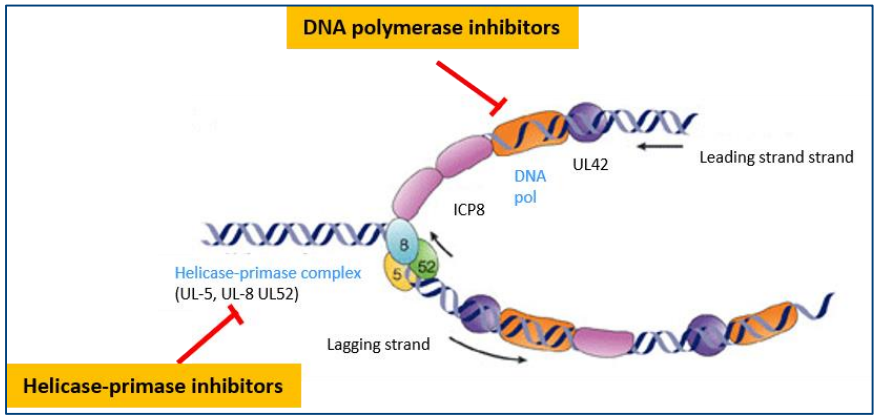
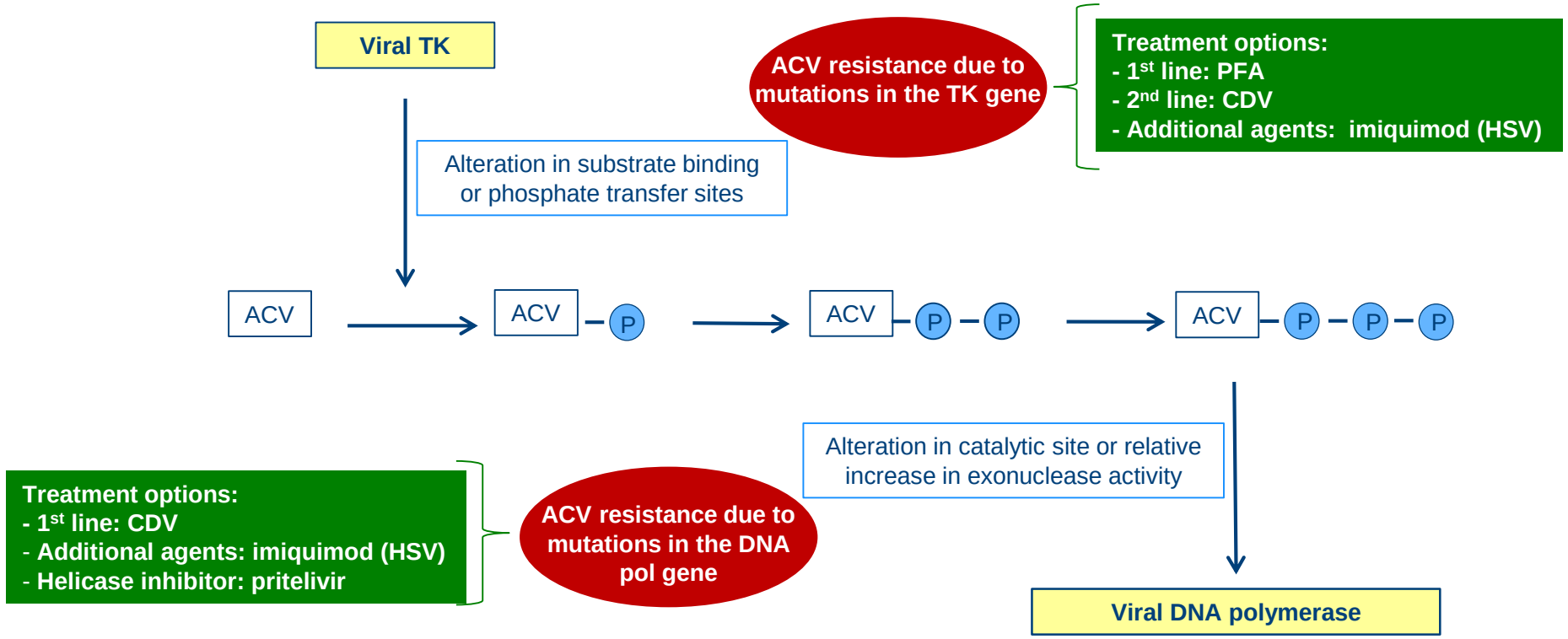
Therapy of HSV & VZV

- Novel anti-herpesvirus drugs
  - ➢ Helicase-primase inhibitors
    - Amenamevir (VZV)
    - Pritelivir (HSV)



Mechanisms of drug-resistance in HSV and VZV



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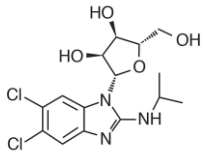
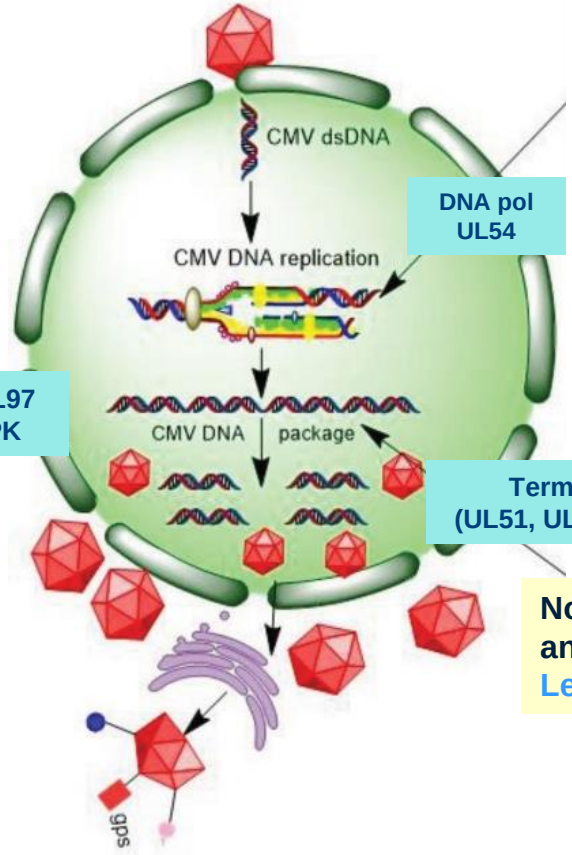
Anti-HCMV drugs



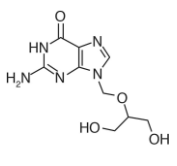
Benzimidazole riboside
Maribavir

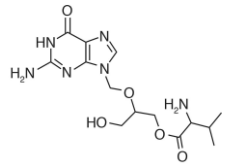


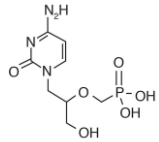
Nucleoside analogues
Ganciclovir (GCV) / valgancyclovir (VGCV)
Nucleotide analogues
Cidofovir (CDV)
Pyrophosphate analogues
Foscarnet (PFA)

DNA pol UL54

Viral genome replication


Ganciclovir


Valganciclovir


Cidofovir


Foscarnet

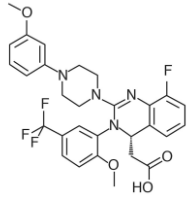
DNA elongation
DNA packaging
Capsid egress

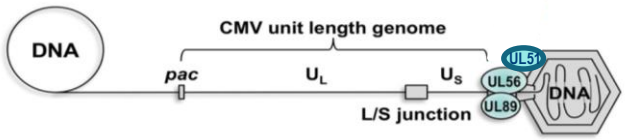
UL97
PK

Terminase (UL51, UL56, UL89)

Cleavage of viral DNA into unit length genomes

Non-nucleoside analogue
Letermovir





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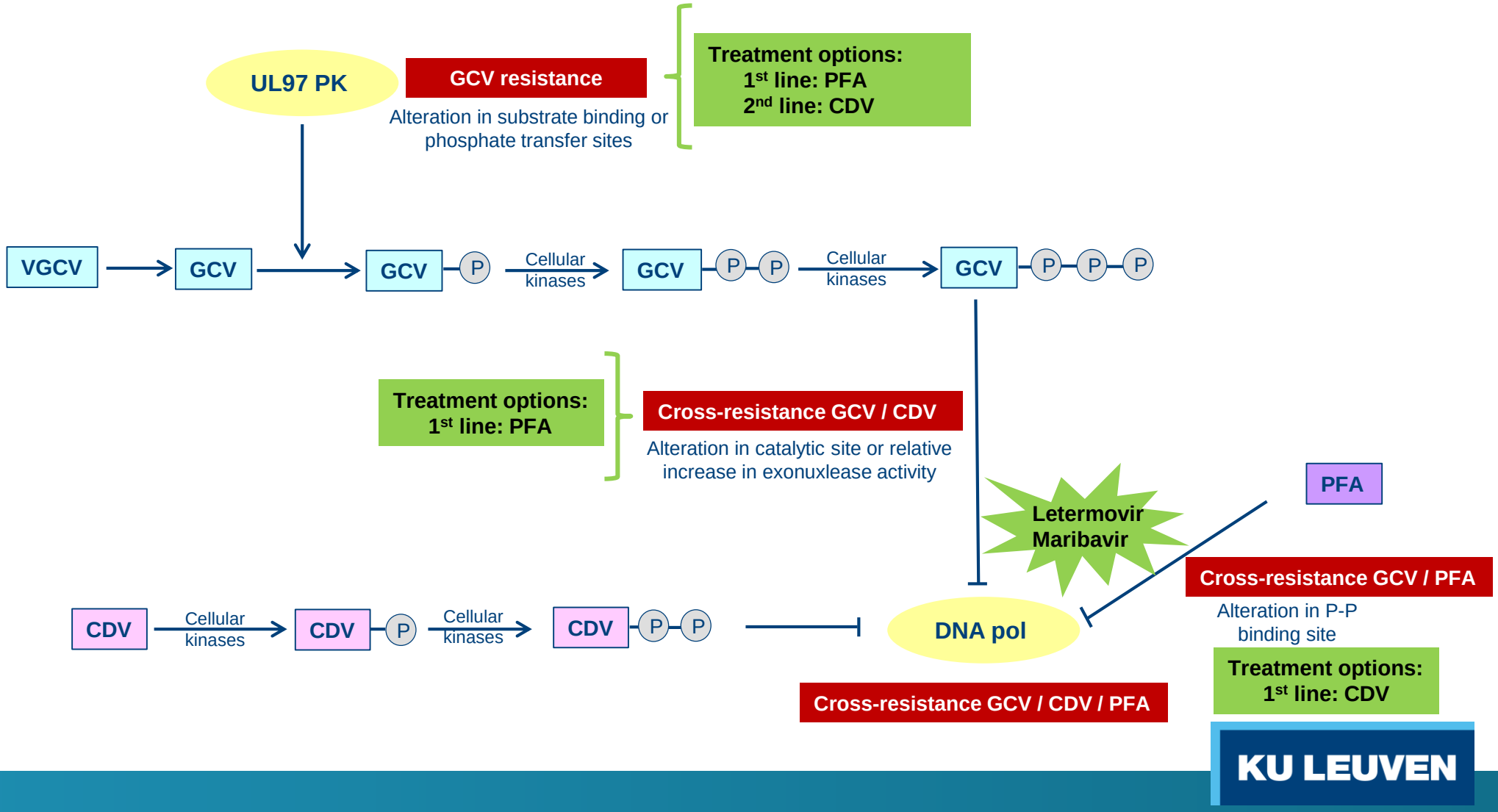
Mechanisms of drug-resistance in HCMV



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Why treatment with antiviral agents may lead to clinical failure?

- Poor drug-compliance
- Pharmacological factors:
  - poor drug absorption
  - incorrect dosage
- **Viral drug-resistance**

→ Clinical drug-resistance ≠ virological drug-resistance

Fundamental to evaluate virological drug-resistance

Herpesviruses drug-resistance

- Virologists have to provide clinicians with **fast and reproducible drug-resistance** diagnosis

- There is a **restricted number of active antivirals** against herpesviruses and a limited number of viral targets

  → limited options for alternative treatments in case of emergence of resistant viruses in IC patients

- In case of clinical evidence of resistance to the current available treatments, **monitoring of emergence of resistant viruses** is mandatory to **adjust antiviral therapy**

Antiviral resistance tests available at RegaVir

HSV-1
HSV-2

Genotyping
UL23 (TK)
UL30 (DNA pol)

Phenotyping

- *Helicase-primase complex*
  - ✓ *UL5 helicase*
  - ✓ *UL52 primase*
  - ✓ *UL8 accessory protein*

HCMV

Genotyping
UL97 (PK)
UL54 (DNA pol)

- *UL27 (maribavir)*
- *Terminase complex (letermovir)*
  - ✓*UL51*
  - ✓*UL56*
  - ✓*UL89*

VZV

Genotyping
ORF36 (TK)
ORF28 (DNA pol)

Phenotyping

- *Helicase-primase complex*
  - ✓ *ORF55 helicase*
  - ✓ *ORF6 primase*
  - ✓ *ORF52 accessory protein*

HHV-6

Genotyping
U69 (PK)
U38 (DNA pol)

- We ask to fill in a "Test request form"
- We provide Reports (Intermediate/Final) with:
  - Data (genotyping /phenotyping)
  - Interpretation of results
  - Conclusions
  - Optional therapies

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www.regavir.org

Translational Research Project

REsearch Group for AntiViral Resistance

Initially founded by the Belgium National Cancer Plan

TEST REQUEST FORM FOR EVALUATION OF HERPESVIRUS DRUG-RESISTANCE

Patient identification

Name (Last, First):	
Date of birth: ___ / ___ / ___	☐ ***Male*** ☐ ***Female***
Address:	

Patient clinical information

Underlying disease :	☐ Transplant ☐ HIV ☐ Cancer ☐ Other (specify):
Transplantation:	☐ HSCT ☐ kidney ☐ liver ☐ heart ☐ lung ☐ other (specify):
Transplantation date:	___ / ___ / ___
Immunosuppressive treatment:	
Additional information:	

Clinical manifestations of viral infection

Disease and/or type of lesion:
Date of relapse and/or emergence of lesions: ___ / ___ / ___
Localization:
Additional information:

Antiviral treatment

Type of treatment:	☐ Prophylactic ☐ Therapeutic
Antiviral drug:	☐ Acyclovir (*Zovirax*) ☐ Valacyclovir (*Zelitrex*) ☐ Ganciclovir (*Cymevene*) ☐ Valganclovir (*Valcyte*) ☐ Foscarnet (*Foscavir*) ☐ Cidofovir (*Vistide*) ☐ Other
Posology:	
Duration:	___ / ___ / ___
Additional information:	

Specimen information

Identification	
Date collected:	___ / ___ / ___
Type:	
Viral load:	
Additional information:	

Required pattern of antiviral resistance

Virus	☐ Human cytomegalovirus
	☐ Herpes simplex virus
	☐ Varicella-zoster virus
	☐ Human herpesvirus 6 (HHV-6)
	☐ Other (specify):

Requesting doctor(s) / Laboratory

Name (Last, First)	
e-mail	
Hospital	
Department	
Address	
Tel / Fax	***Tel:*** ***Fax:***
Date	___ / ___ / ___

Please, use only one form for each requested test and send the sample to the attention of Prof. Dr. Robert Snoeck to the address below:
For requests and complaints, please contact Prof. Dr. Robert Snoeck:

Prof. Dr Robert Snoeck
Rega Institute
Herestraat 49 – box 1030
3000 Leuven
Tel. 00-32-16-32.15.79
Fax. 00-32-16-33.00.26
Email: robert.snoeck@kuleuven.be
Rega Institute http://rega.kuleuven.be/
RegaVir http://www.regavir.org/

Type	*Amount and preservation for shipment*
Whole Blood	3 to 5 ml collected in EDTA tube, **do not freeze**
Bone Marrow	1 ml minimum, collected in EDTA tube, **do not freeze**
Bronchial Lavage / Bronchial Wash	1 to 3 ml, collected in sterile screw-cap tube
CSF	1 ml minimum, submitted in sterile screw-cap tube
Pleural Fluid	1 ml submitted in sterile screw-cap tube
Tissue	place fresh biopsy in a sterile screw-cap tube, add a small amount of saline to keep moist
Upper Respiratory Aspirate NP aspirate, nasal aspirate, tracheal aspirate, etc.	instill 1 to 2 ml sterile saline into desired location and gently aspirate contents, place collected fluid into sterile screw-cap tube
Swab NP swab, throat swab, etc.	swab the desired location with sterile flexible swab, preferably a flocked swab, place the swab into 1 to 2 ml sterile saline or viral transport media in sterile screw-cap tube **do not use calcium alginate swab or wood shafted swab**
Urine	5 to 10 ml sample collected in a sterile urinalysis container transfer to a 15 ml sterile screw-cap tube
Vitreous Fluid	place collected vitreous fluid into small sterile screw-cap tube
Viral Culture	culture supernatant or infected cells

Instructions for shipping samples

- Optimally, **pretreatment or early treatment samples** will enhance the diagnosis → provision of such samples is recommended
- All specimens must be labeled with the **patient's name and collection date.**
- A RegaVir **Test Request Form** must accompany each specimen.
- Please use a **separate Test Request Form** for each specimen when sending **multiple specimens.**
- The name and address of the **Requesting Doctor(s) / Laboratory** must be provided on the package.

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Instructions for shipping samples

- **IMPORTANT**
- After sample collection, please store the specimens a.s.a.p. cooled (max. 4°C) and **always ship refrigerated.**

- Longer storage on room temperature diminishes successful genotyping analysis.

- Please, send **recent and fresh samples.**

Results provided by RegaVir

- Results on **genotyping** are provided as gene mutations associated with antiviral resistance or genetic polymorphism.

- Results on **phenotyping** are expressed as EC_{50} values (50% effective concentration) for each compound not only for the patient sample but also for the corresponding reference laboratory strains.

- Additionally, an interpretation of the results, conclusions and guidelines for treatment are included in the reports.

www.regavir.org

Translational Research Project

REsearch Group for AntiViral Resistance

Initially founded by the Belgium National Cancer Plan

FINAL REPORT HCMV GENOTYPING RV-XXX

TEST REQUEST: Antiviral resistance for human cytomegalovirus (HCMV)

TEST PERFORMED AT: Rega Institute for Medical Research (Laboratory of Virology and Chemotherapy, and Laboratory of Immunobiology)

DATE SAMPLE RECEIVED BY REGAVIR:

REPORT DATE:

Patient identification	**Name (Last, First): XXXX**	
	Date of birth:	**□ Male □ Female**
	Identification number:	
	Address:	

Patient clinical information	**Disease:** •
	Antiviral treatment received previously •
	Immunosuppressive treatment:
	Additional information: Previous sample from this patient:

Original Identification	*RegaVir Identification*	*Date*	*Type*	*Additional information*	*HCMV genotyping*
xxx	*xxx*	*xxx*	*Blood EDTA*	*CMV Log 5.33 CMV IU/ml, 211,565 CMV IU/ml*	*UL97: A594V (GCV-R) - heterogeneous population UL54 wild-type*

Specimen information

Original Identification	RegaVir Identification	Date	Type	Additional information
EX-XXXX	RV-2438	xxx	xxx	

Doctor(s)	Katrien Lagrou / Claeys Eveline / Laurent Godinas
e-mail	katrien.lagrou@uz.kuleuven.be; lag.ua@uzleuven.be laboratoriumgeneeskunde@uzleuven.be; eveline.claeys@uzleuven.be;

Hospital	UZ Leuven
Department	Laboratoriumgeneeskunde - Pneumologie
Address	Herestraat 49, 3000 Leuven
Telephone Fax	Tel: 016 34 70 00 (Laboratoriumgeneeskunde) – 016 34 09 50 (Pneumologie) Fax: 016 34 70 10

TESTS

1) Genotyping of UL97 (protein kinase responsible for ganciclovir phosphorylation)
2) Genotyping of UL54 (viral DNA polymerase)
3) Genotyping of UL51, UL56 and UL89 (terminase complex)

PROTOCOL

1) Isolation of viral DNA from the sample
2) Amplification of the UL97 (protein kinase), UL54 (DNA polymerase), and UL51, UL56, and UL89 (terminase complex) genes by PCR
3) Direct sequencing of the UL97, UL54, UL51, UL56, and UL89 genes
4) Sequence alignment (derived sequences of patient isolate were aligned with the strain AD-169 reference sequence)

RESULTS HCMV genotyping: RV-xxxx (EX-XXX) – xxx

Sample	**Amino acid changes in UL97 protein kinase (707 amino acids)** Partial sequence (amino acids 337-707)	
	Related to genetic polymorphism (inter-strain variability)	Known to be associated with drug-resistance
RV-XXX	None	**A594V_mix population of wild-type and mutant virus Confirmed by two independent PCR's**

Sample	**Amino acid changes in UL54 (DNA polymerase) (1242 amino acids)** Partial sequence (amino acids 263-1091)	
	Related to genetic polymorphism (inter-strain variability)	Known to be associated with drug-resistance
RV-XXX	A885T P887S S897L N898D	None (all positions could be verified)

CONCLUSIONS HCMV genotyping: RV-xxxx (EX-XXX) – xxx

- **Similar to a previous sample from this patient (i.e. RV-XXX), the UL97 protein kinase A594V change was detected as a heterogeneous population in the RV-XXX sample. This mutation is known to confer (val)ganciclovir resistance.**
- **Mutations in the DNA polymerase gene known to be associated with drug-resistance were not found.**
- **Therefore, (val)ganciclovir therapy is not recommended for this patient but cidofovir and foscarnet (DNA polymerase inhibitors), maribavir (UL97 protein kinase inhibitor), and letermovir (terminase inhibitor) are alternative therapies.**

Prof. Dr Robert Snoeck
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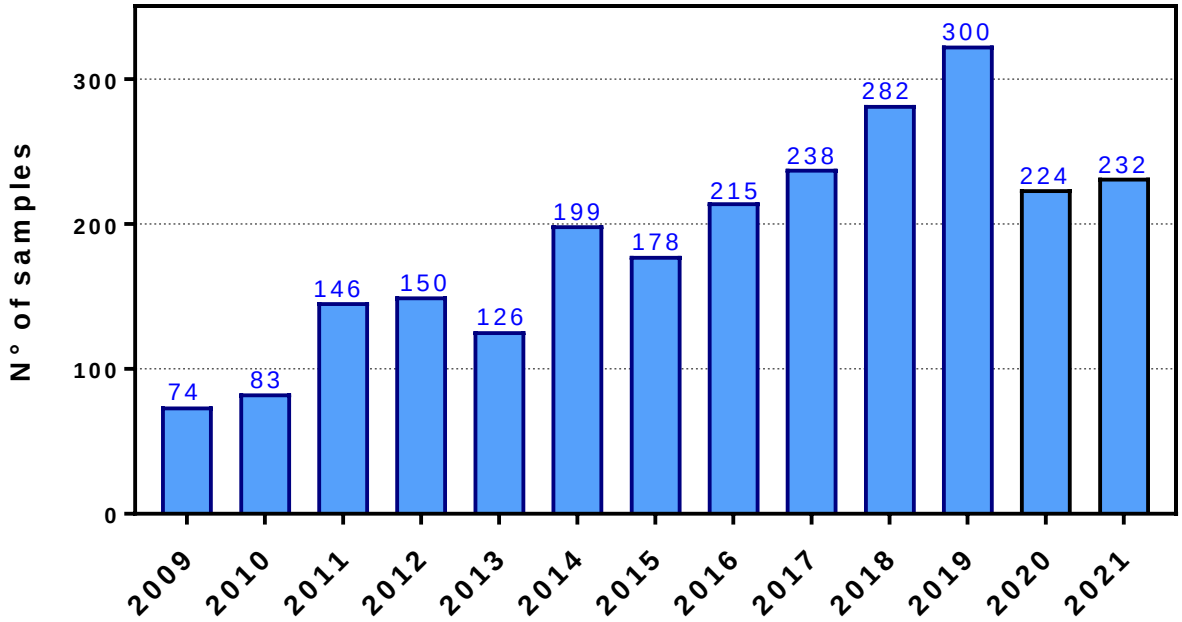
Rega Institute http://rega.kuleuven.be/
RegaVir http://www.regavir.org/

National collaborations	Number of samples
AZ Delta Roeselare-Menen	13
AZ Groeninge Kortrijk	11
AZ Nikolaas, Sint-Niklaas	5
AZ Sint Jan Brugge-Oostende	159
AZ Sint Lucas Brugge	3
AZ Sint Lucas Gent	4
AZ Turnhout	4
Clinique CHC MontLégia, Liège	8
Clinique Saint-Luc Bouge - Namur	1
Clinique St-Pierre Ottignies	1
Clinique Sud Luxembourg Arlon	3
Cliniques Universitaires Saint Luc, UCL, Brussels	68
Centre Hospitalier de Jolimont	10
CHIREC	11
CHR Citadelle Liège	30
CHU Ambroise Paré, Mons	6
CHU Charleroi	3
CHU Dinant Godinne - UCL Namur	31
CHU Liège, Sart Tilman, Liège	120
GasthuisZusters Antwerpen (GZA) ziekenhuizen	3
GHdC (Grand Hôpital de Charleroi)	8

National collaborations	Number of samples
Imeldaziekenhuis, Bonheiden	54
Institute of Tropical Medicine Antwerp	1
Jan Yperman ziekenuis, Ieper	5
Jessa ziekenhuis, Hasselt	57
LHUB (Laboratoire Hospitalier Universitaire de Bruxelles)-ULB - CHU Brugmann - CHU Saint-Pierre - Hôpitaux Iris Sud - Hôpital Universitaire des Enfants Reine Fabiola (HUDERF) - Institut Jules Bordet - Hôpital Erasme	803
LKO-LMC, Sint Truiden	5
Medisch Labo Medina Dendermonde	1
OLV Ziekenhuis Aalst Asse Ninove	32
UZA, Antwerpen	54
UZ Brussel	47
UZ Gent	182
UZ Leuven	510
ZNA Ziekenhuizen - ZNA Jan Palfijn - ZNA Middelheim - ZNA Stuivenberg	53
ZOL (Ziekenhuis Oost-Limburg) Genk	16

International	Number of samples
CBN-IAI Lab Virology, Hôpital de la Croix Rousse, Lyon, **France** (EQA)	48 + 3
UMC Amsterdam, **The Netherlands**	53
AZ Maastricht, **The Netherlands**	2
Benioff Children's Hospital, San Francisco, CA, **USA**	3
Centre Hospitalier de Luxembourg, **Luxembourg**	10
Centre Hospitalier Emile Mayrisch, Esch-sur Alzette, **Luxembourg**	1
CHU Lille, **France**	2
Erasmus MC Rotterdam, **The Netherlands**	12
Federal University of Minas Gerais, **Brazil**	1
Hôpital Foch, Paris, **France**	1
Hôpital Kirchberg, **Luxembourg**	1
Hôpital Necker – Enfants Malades, Paris, **France**	3
Hôpital Universitaire Pitié Salpêtrière, Paris, **France** (EQA)	6 + 6
Hôpitaux Universitaires Genève, Hôpital des Trois Chêne, **Switzeland**	1
Karolinska University Hospital, Huddinge, **Sweden**	3
Le Bonheur Children's Hospital University of Tennessee, Menphis, TN, **USA**	1
LNS Luxembourg Clinique Emile Mayrisch (ESCH/ ALZETTE), **Luxembourg**	1
National and Kapodistrian University of Athens Sotiria Hospital, **Greece**	3
Public Health England, Colindale, **United Kingdom** (EQA)	5
Uppsala University Hospital, **Sweden**	9
West Virginia University, Morgantown, WV, **USA**	3

N° of samples per year (Jan. 2009 – Dec. 2021)



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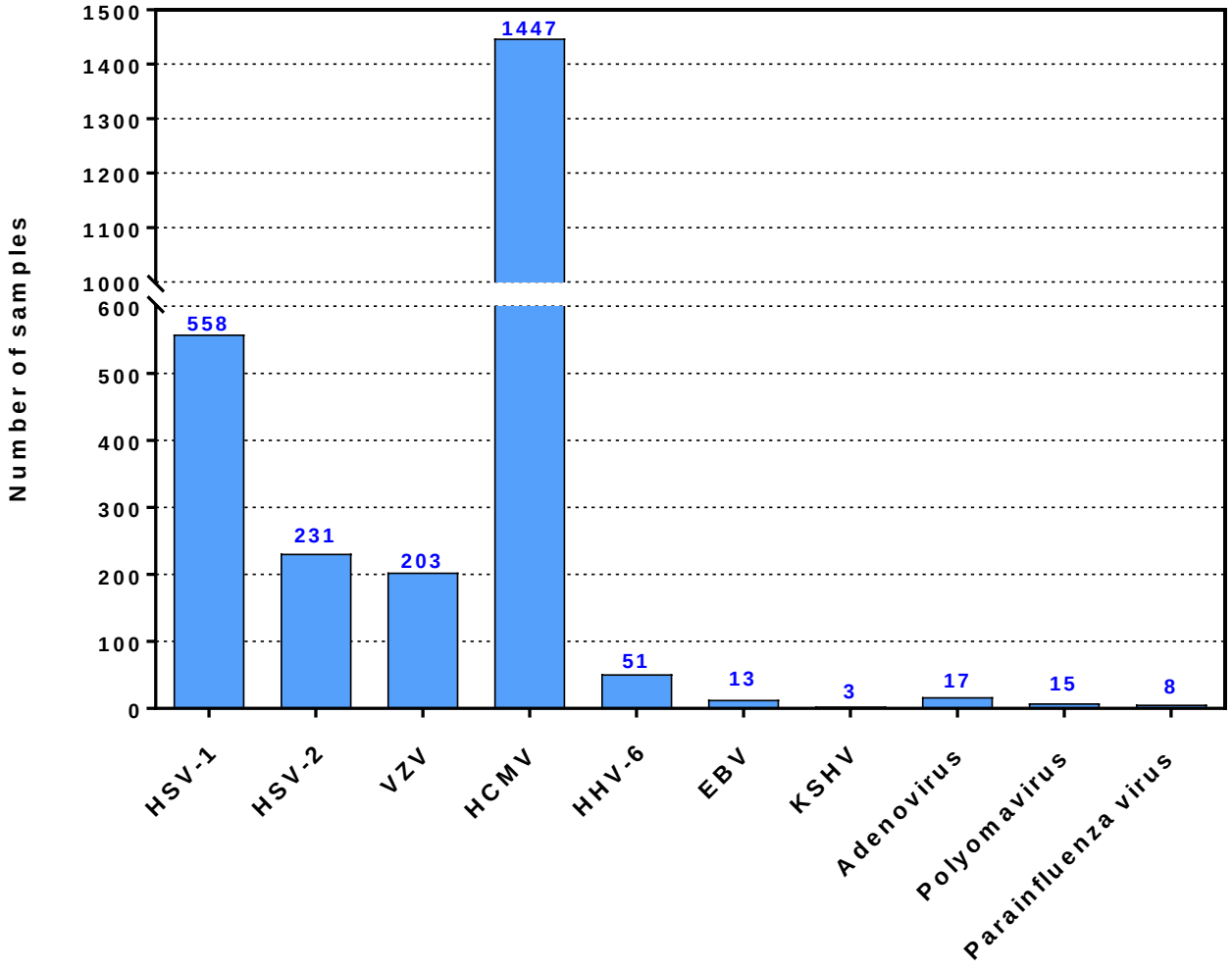
N° of samples per virus (Jan. 2009 – February 2022



Total samples encoded by RegaVir: 2447

Total number of tests performed by RegaVir: 2546

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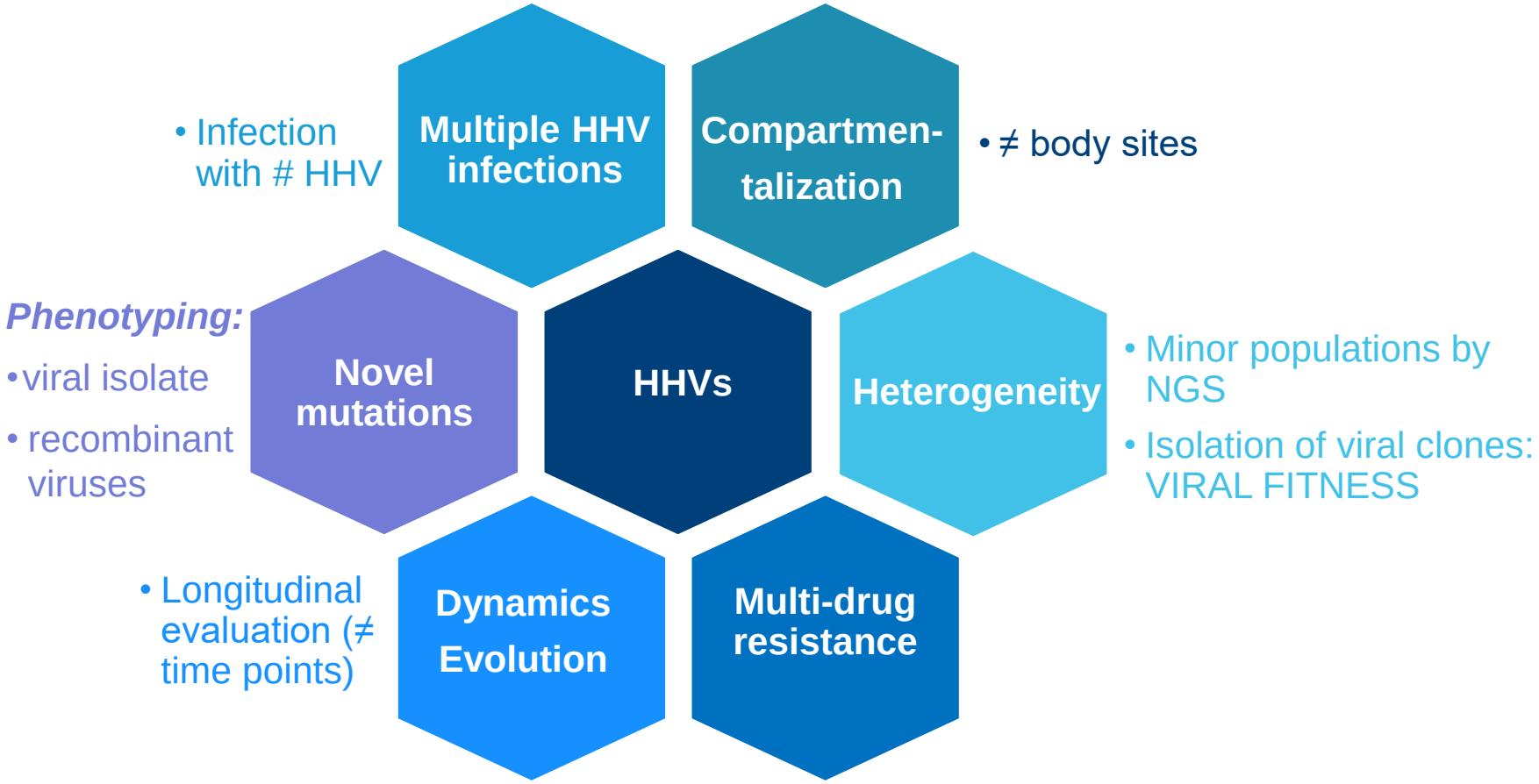
RegaVir platform for translational research



Herpesvirus genotyping

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

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Emergence of HCMV strains with a similar profile in ≠ transplant recipients

Patients (UZ Leuven)	RegaVir ID	Date (blood sample)	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
Bowel & pancreas transplantation on 19/03/2017	**RV-1366**	18.12.2017	Wild-type	A505G (GCV-R / CDV-R?)

Received by RegaVir 20.12.2017

A505V: GCV-R / CDV-R

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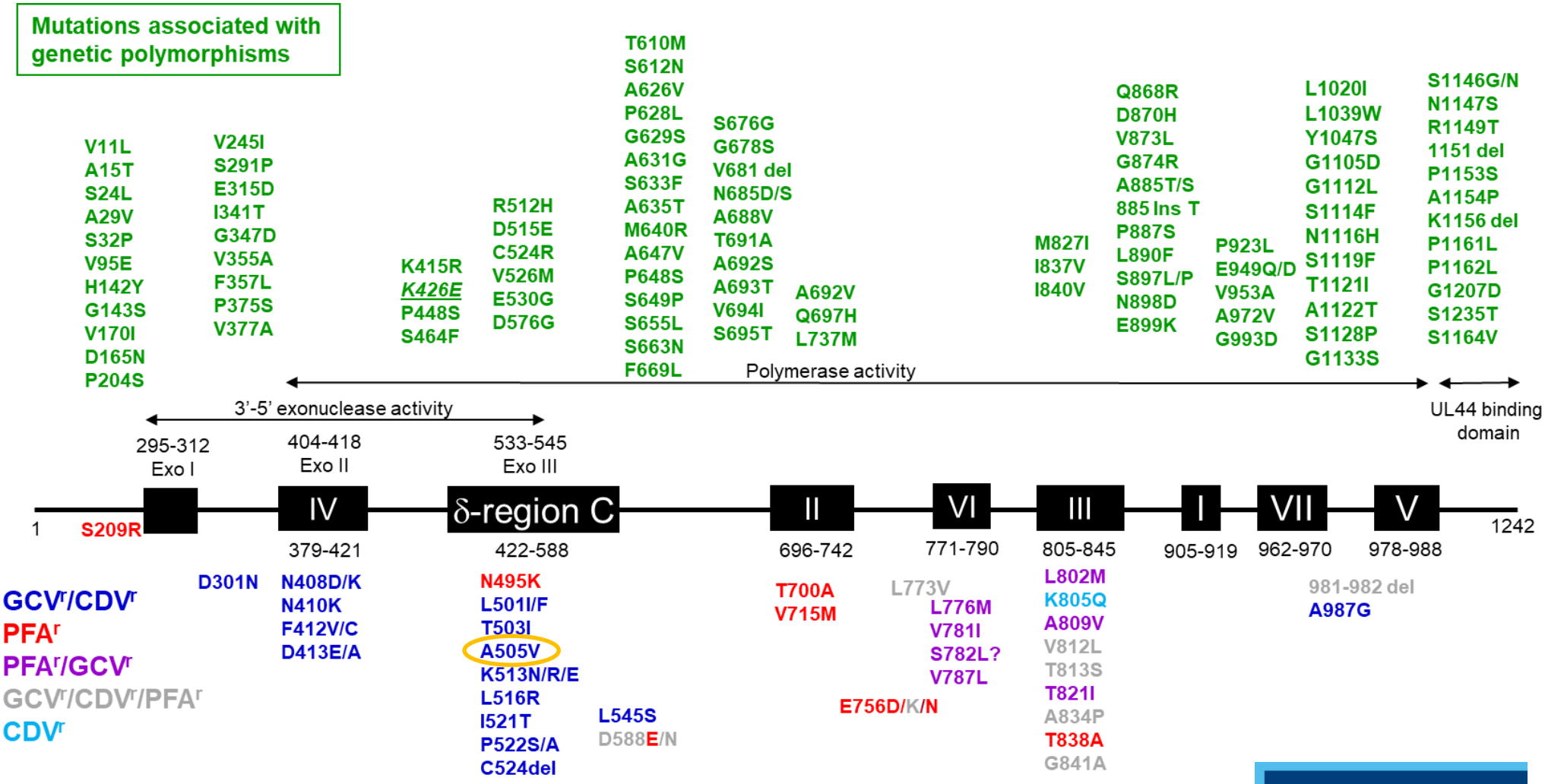
HCMV UL54 DNA polymerase mutations



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Emergence of HCMV strains with a similar profile in ≠ patients

Patients (UZ Leuven)	RegaVir ID	Date (blood sample)	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)	
Bowel & pancreas transplantation	**RV-1366**	18.12.2017	Wild-type	A505G (GCV-R / CDV-R?)	Received by RegaVir 20.12.2017
Kidney transplantation	**RV-1378**	12.01.2018	Wild-type	A505G (GCV-R / CDV-R?)	Received by RegaVir 12.01.2018

A505V: GCV-R / CDV-R

Same day of transplantation: 19/03/2017

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Emergence of HCMV strains with a similar profile in ≠ patients

Patients (UZ Leuven)	RegaVir ID	Date (blood sample)	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)	
Bowel & pancreas transplantation	**RV-1366**	18.12.2017	Wild-type	A505G (GCV-R / CDV-R?)	Received by RegaVir 20.12.2017
Kidney transplantation	**RV-1378**	12.01.2018	Wild-type	A505G (GCV-R / CDV-R?)	Received by RegaVir 12.01.2018
Lung transplantation	**RV-1389**	17.01.2018	**L595S* (GCV-R)**	A505G* (GCV-R / CDV-R?)	Received by RegaVir 30.01.2018

* Heterogeneous population

A505V: GCV-R / CDV-R

Same day of transplantation: 19/03/2017

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Emergence of HCMV strains with a similar profile in ≠ patients

Patients (UZ Leuven)	RegaVir ID	Date (blood sample)	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
Bowel & pancreas transplantation on 19/03/2017	**RV-1366**	18.12.2017	Wild-type	A505G (GCV-R / CDV-R?)
	RV-1395	02.02.2018	Wild-type	A505G (GCV-R / CDV-R?) **V781I (GCV-R / PFA-R)*** **E951Q (GCV-R / PFA-R)*** **V715M (PFA-R)***
Kidney transplantation on 19/03/2017 (D+/R-) Previous lung transplantation on 21/12/2004 (D-/R-)	**RV-1378**	12.01.2018	Wild-type	A505G (GCV-R / CDV-R?)
Lung transplantation on 19/03/2017	**RV-1389**	17.01.2018	**L595S* (GCV-R)**	A505G* (GCV-R / CDV-R?)
	RV-1445	17.04.2018	**T409M (MBV-R)**	A505G (GCV-R / CDV-R?)
	RV-1473	29.05.2018	**T409M (MBV-R)**	A505G (GCV-R / CDV-R?)

➢ **Heterogeneity**

➢ **Novel mutation → recombinant virus**

* Heterogeneous population
A505V: GCV-R / CDV-R

➢ **Maribavir-R mutations**

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HCMV UL97 protein kinase (pUL97) mutations

Mutations associated with GCV-R
* low levels GCV-R (2-4 fold)
**moderate levels GCV-R (5-15 fold)

Mutations associated with Maribavir-R

Mutations associated with Maribavir-R & ganciclovir-R

L405P*

M460V-I-T**
V466G

C518Y**
H520Q**
P521L

C592G*
C592F**
A594E/T*
A594P-V-G**
L595F-S-W**

E655K**

E596G*
E596Y**
K599T**

F342S/Y
V353A
K355del
V356G
K359E/Q

L397R
T409M
H411Y-N-L
F342S
D456N

C480R

C603S*
C603W-R**
C607F*
C607Y**
I610T*
A613V*
Y617del

Deletions of one or more amino acids in the region 590-603

del590-600**
del591-594**
del595**
del596*
del595-603**
del591-607**
del595-603**
del600*
del601-603**

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HCMV Pol A505V and A505G mutants – recombinant viruses

Strain	Drug	Genotype	Mean EC_{50} (µM)	St Dev EC_{50}	EC_{50} ratio	Published EC_{50} ratios
Control strains						
4198	CDV	Pol WT	0.27	0.06		
4376	CDV	**Pol del 981-982**	0.97	0.20	**3.6**	3.2, 3.7, 3.8
4198	GCV	Pol WT	1.26	0.22		
4376	GCV	**Pol del 981-982**	8.49	2.02	**6.7**	6.2, 7, 7.3
4198	PFA	Pol WT	38.33	6.22		
4376	PFA	**Pol del 981-982**	106.28	22.05	**2.8**	2.7, 3.1, 3.1
Newly constructed mutant strains						
4511	**CDV**	**Pol A505V**	0.67	0.1	**2.5**	2
4513	CDV	Pol A505G	0.37	0.07	1.4	
4511	**GCV**	**Pol A505V**	2.44	0.42	**1.9**	1.8
4513	GCV	Pol A505G	1.67	0.22	1.3	
4511	PFA	Pol A505V	35.77	6.05	0.9	1.1
4513	PFA	Pol A505G	45	6.51	1.2	

Data provided by Sunwen Chou

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Conclusions

- Usefulness of rapid HHV genotyping → adjustment of antiviral therapy.

- Heterogeneity and evolution of herpesvirus populations.
- Emergence of resistance to new anti-HCMV drugs.

- Appearance of novel mutations – utility of constructing recombinant viruses.

- Importance of providing the test request form.

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Thank you for your attention

Any questions?

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