KU LEUVEN

Why is it important to diagnose drug-resistance among DNA viruses?

Robert Snoeck
Rega Institute for Medical Research
KU Leuven, Leuven, Belgium

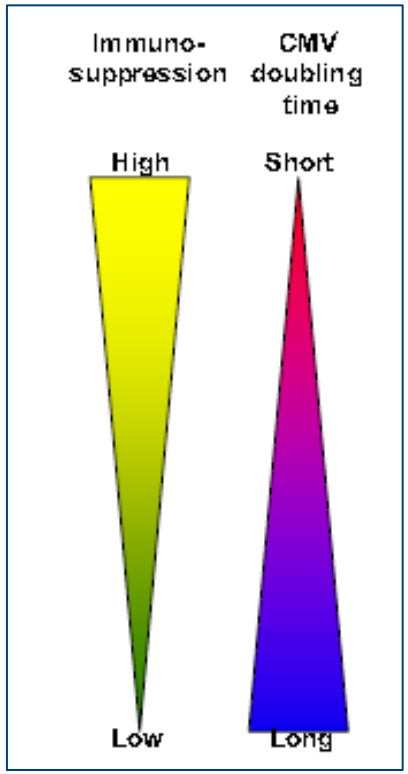
Life-long immunosuppression is the standard of care for transplant recipients

- Immunocompromised individuals are susceptible to infections from:
  - Common pathogens (e.g. influenza virus)
  - Opportunistic infection (e.g. Listeria)
  - Herpesviruses (e.g. CMV, VZV, HSV, EBV, HHV-6)

- Increasing number of immunocompromised populations

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, Blood 2009

Different immunocompromised (IC) populations

- AIDS patients
- Solid organ transplant (SOT) recipients
- Hematopoietic stem cell transplant (HSCT) recipients
- Patients with primary immune deficiency
- Patients undergoing chemotherapy and/or radiotherapy
- Patients under immunosuppressive therapy for autoimmune diseases

- All human herpesvirus (HHV) can result in severe disease among IC patients

- Due to primary infection or reactivation of latent virus

CMV infection in the transplant setting

- Most significant threat to patient and graft health

- Directly and indirect causes
  - allograft rejection
  - decreased graft and patient survival
  - Predisposition to opportunistic infections

Facts about CMV infection

- Seroprevalence: 50-90% of adults (geographic variation)
- Primary infection in immunocompetent individuals is generally asymptomatic (some may develop a mononucleosis-like illness)
- Life-long latency (feature of all herpesviruses) is well controlled by the immune system
- Reactivation rare in immunocompetent individuals but frequent under immune supppression

CMV risk in transplantation

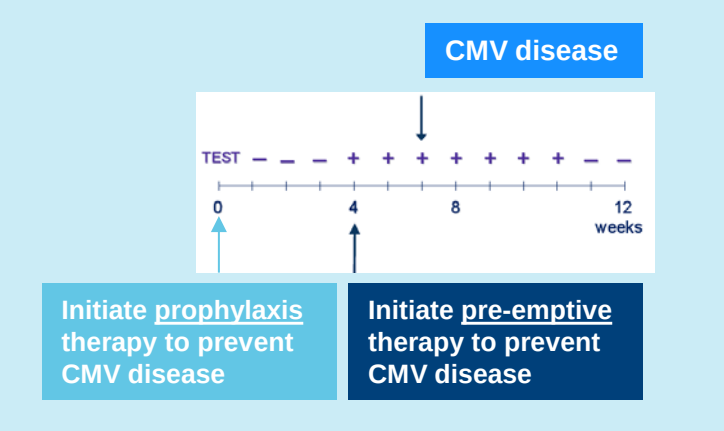
- Donor (D) / recipient (R) immune status

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

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+)

Risk of drug-resistance CMV in the transplant setting

- Type of organ: lung/small intestins > pancreas, heart >liver, kidney
- High-level viral replication
- Multiple episodes of viral disease
- Potent immunosuppression (dose, duration, and overall intensity of drugs)
- Suboptimal antiviral drug levels
- Prolonged antiviral drug administration (**prophylaxis** > **pre-emptive therapy)**

Herpesviruses drug-resistance

- **Prevalence**

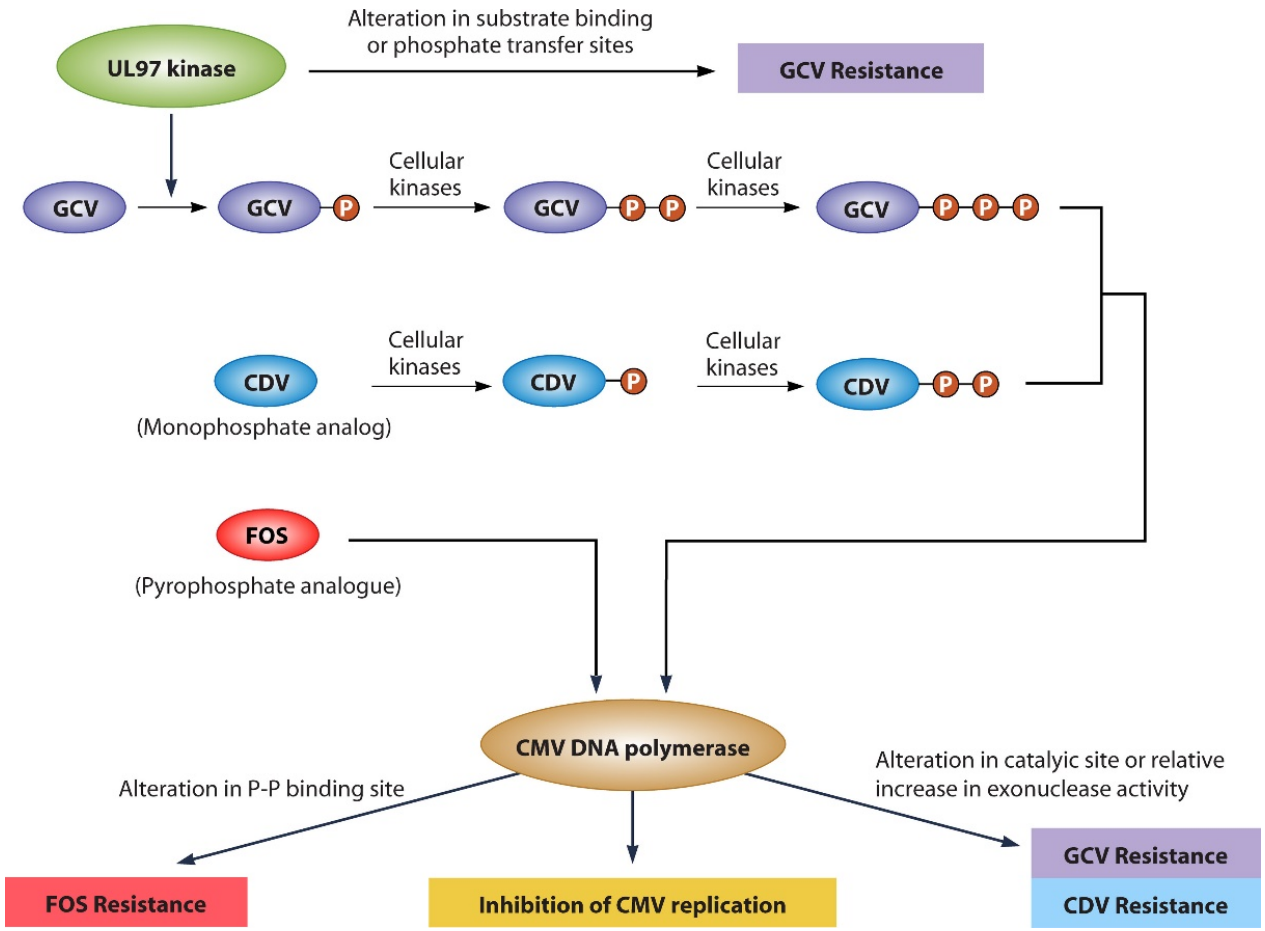
  in immunocompetent <<< in immunocompromised patients

  Exception: **“immune-privileged sites”**

  - ✓ **HSV :** 4.3 to 14 % among all immunocompromised groups (up to 36% in HSCT patients)
  - ✓ **CMV:** 5% to 10% among transplant recipients

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

Mechanism of drug-resistance in CMV

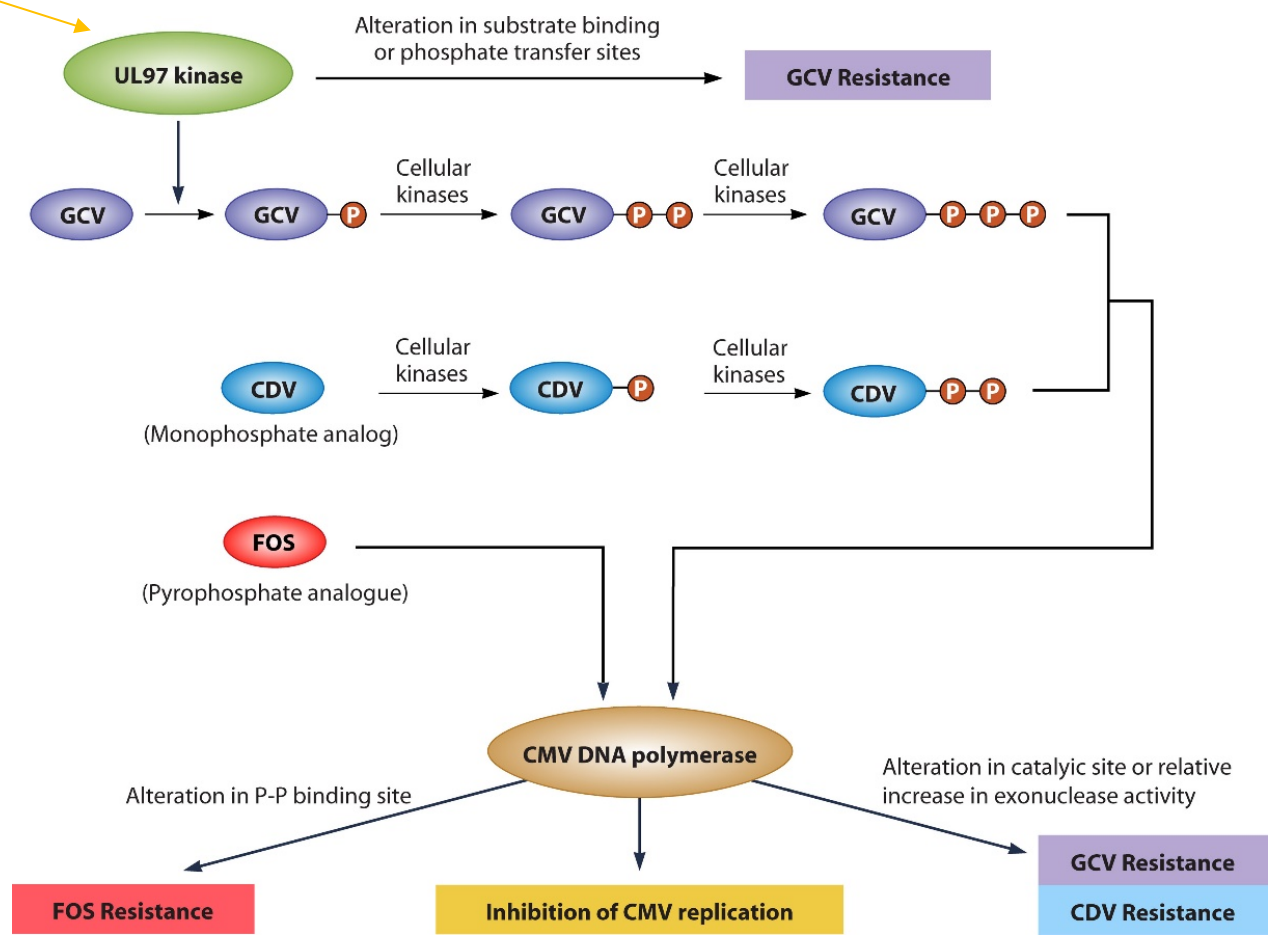


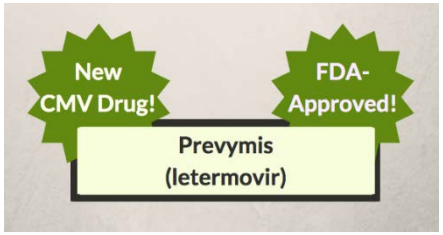
Lurain & Chou, 2010

KU LEUVEN

Mechanism of drug-resistance in CMV

Maribavir





Lurain & Chou, 2010

KU LEUVEN

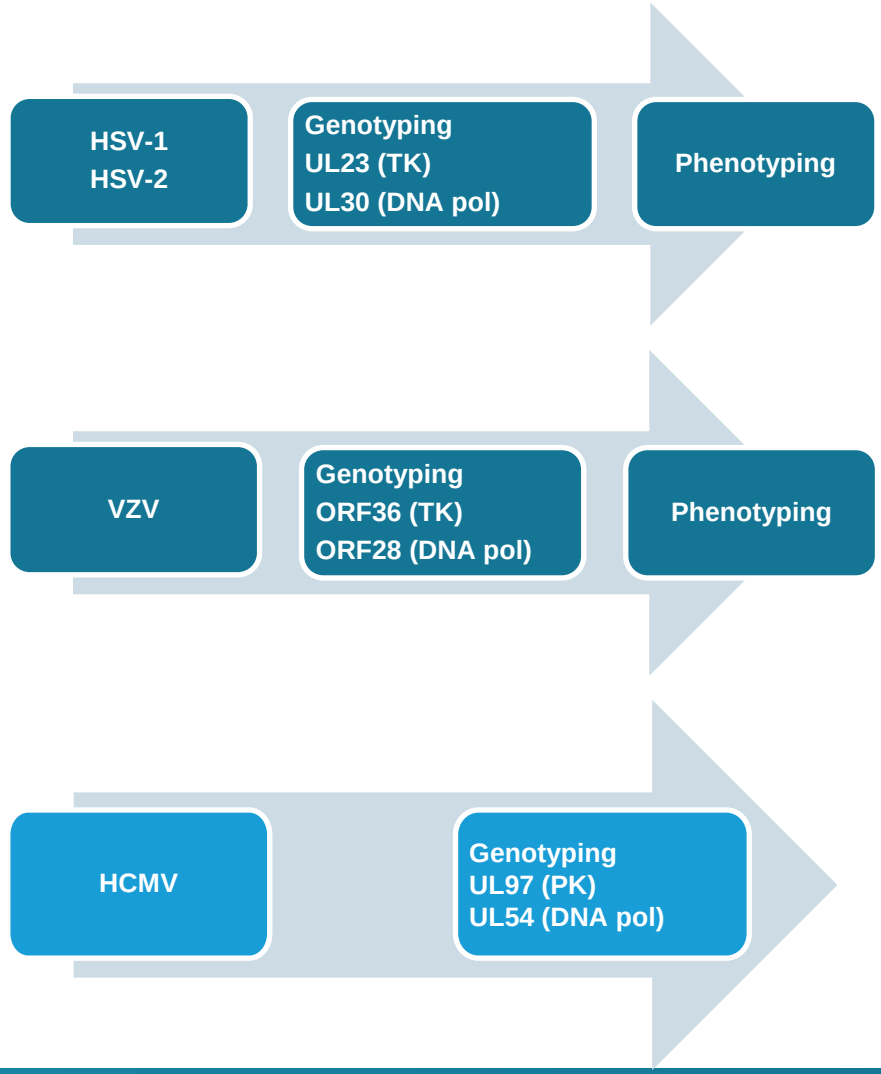
A **Reference and Service Center** created in 2009 by the funding received from the **Belgian National Cancer Plan** (Federal Public Service “PUBLIC HEALTH, FOOD CHAIN SAFETY and ENVIRONMENT”)

Our aim:

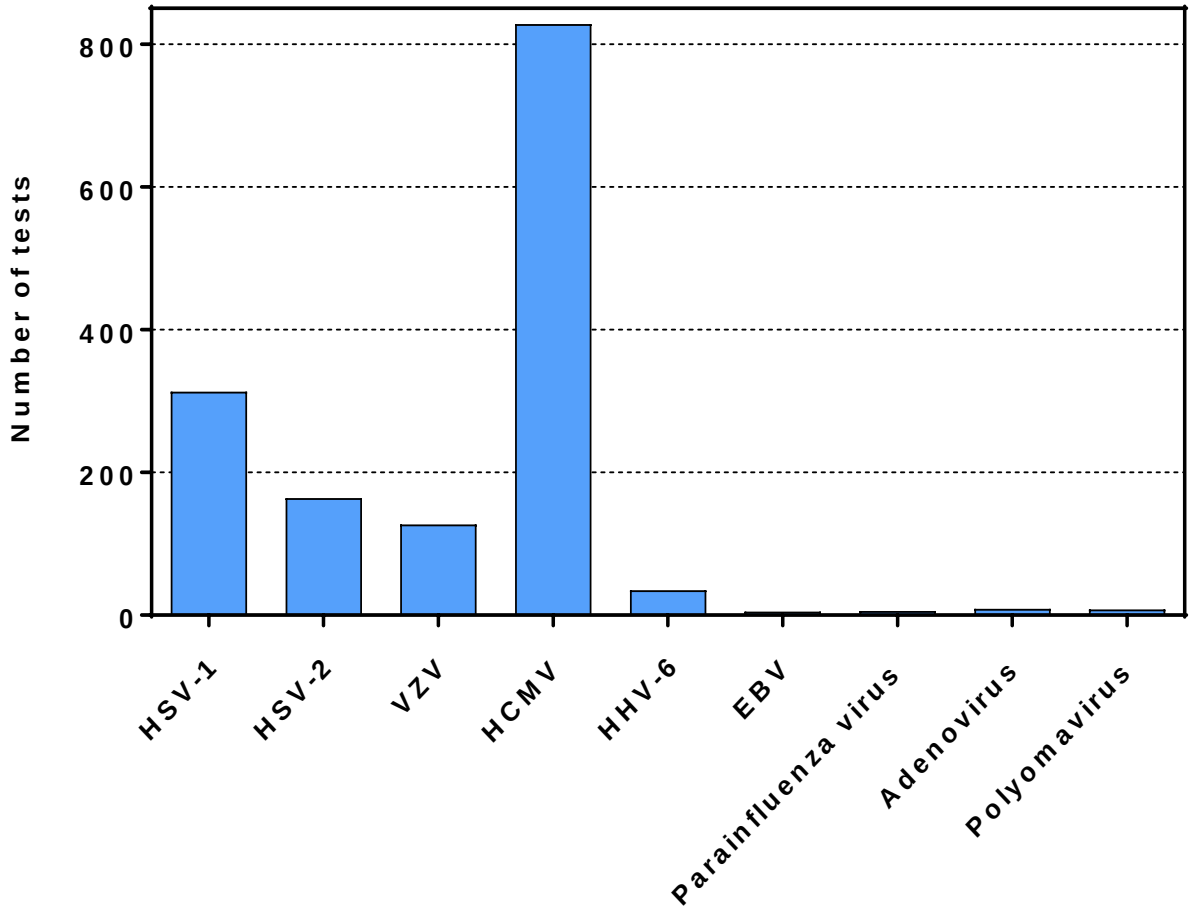
- Typing **herpesvirus drug-resistance** in immunocompromised patients that fail antiviral therapy

- Providing **rapid genotypic** tests to clinicians in order to adapt antiviral treatment.

Antiviral resistance tests available



Number of tests per virus performed by RegaVir (January 2009- April 2018)



KU LEUVEN

Drug-resistance in human cytomegalovirus (HCMV)

- Virtually not observed in immunocompetent individuals
- Well-recognized problem among ≠ populations of immunocompromised patients

Objectives

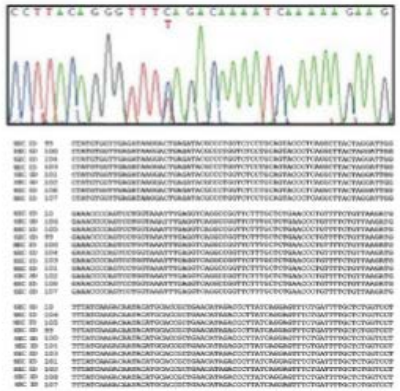
- ✓ Evaluate the emergence of (**multi)-drug resistance** HCMV
- ✓ Search for **herpesvirus co-infections**
- ✓ Investigate **dynamics and compartmentalization** of HCMV drug-resistant mutations arising during antiviral therapy

KU LEUVEN

Evolution & heterogeneity of the viral populations

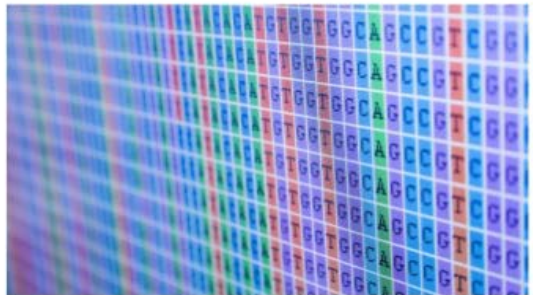
- **Evaluation of multiple samples**
  - **Virus compartmentalization:** ≠ body sites
  - **Evolution of viral populations:** ≠ time points
- Limitations of capillary sequencing (Sanger) to detect viral minor populations → **targeted sequencing of UL97 & UL54 (DNA pol) with Illumina Miseq**

Capillary sequencing



- **Approximately 300 bp / analysis**
- **Detects variants present at ~30%**

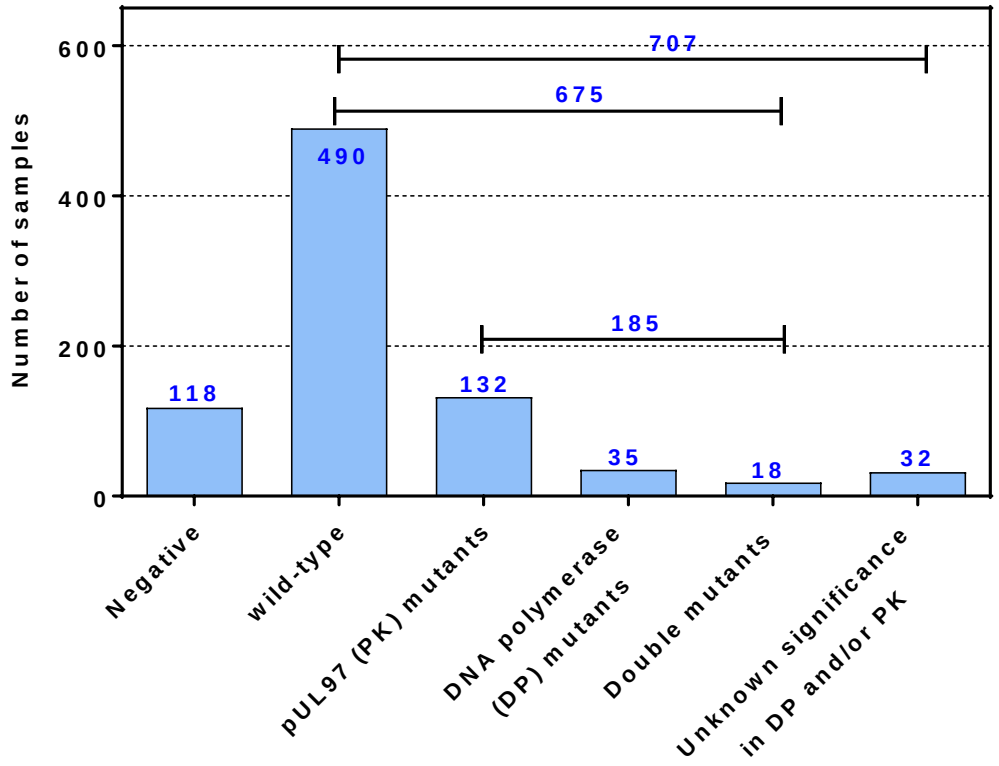
NGS



- **Approximately 75-400 bp per read x millions of reads in one assay**
- **Detects variants present at ~1%**

KU LEUVEN

Genotypic characterization of CMV samples from patients that fail antiviral therapy



Total samples analyzed: 828
Drug-resistant viruses: 185/675 → 27.4%
Drug-resistant viruses: 185/707 → 26.2%

KU LEUVEN

Emergence of multi-drug-resistance

- Clinical challenge to manage multi-drug-resistance
- Alternatives:
  - terminase inhibitor letermovir (FDA approved in November 2017)
  - maribavir (investigational drug)
- Drug repositioning (drug repurposing)
- Need for novel antivirals?

KU LEUVEN

Emergence of HCMV multi-drug-resistance

- **Confered by single mutations associated with resistance to ≠ antivirals**
  - HCMV DNA pol: del 981-982
    - Pediatric HSCT patient
    - Renal transplant recipient (D^+/R^-)
    - Lung transplant recipient with primary HCMV infection
  - HCMV DNA pol: A834P
    - Pediatric HSCT patient
    - Adult HSCT patient

- **Confered by co-infection with ≠ strains bearing specific mutations**
  - Lung transplant recipient
  - Adult bowel and transplant recipient

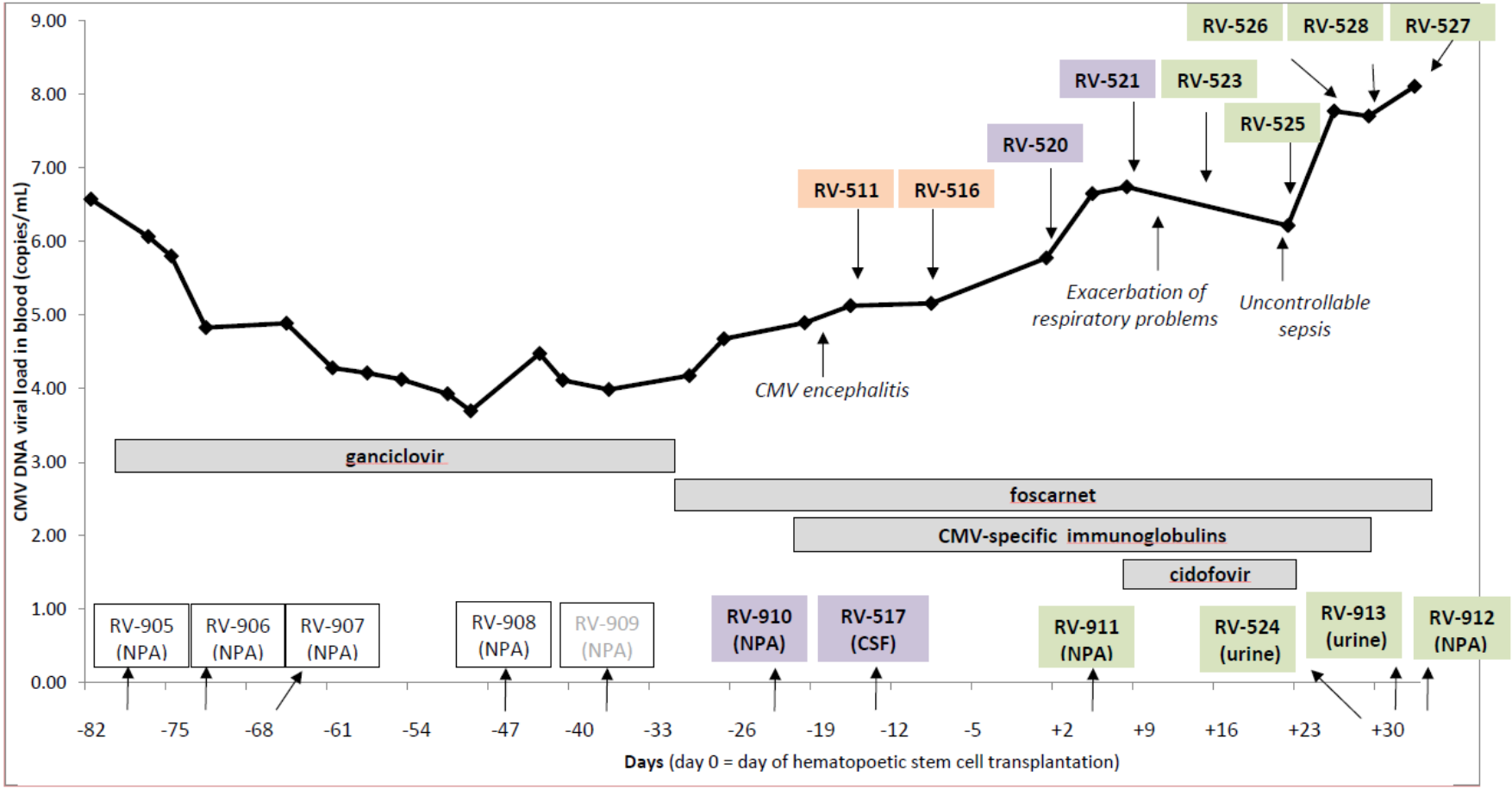
Emergence of HCMV multi-drug-resistance

- **Confered by single mutations associated with resistance to ≠ antivirals**
  - HCMV DNA pol: del 981-982
    - ***Pediatric HSCT patient***
    - ***Renal transplant recipient (D^+/R^-)***
    - ***Lung transplant recipient with primary HCMV infection***
  - HCMV DNA pol: A834P
    - ***Pediatric HSCT patient***
    - Adult HSCT patient

- **Confered by co-infection with ≠ strains bearing specific mutations**
  - ***Lung transplant recipient***
  - Adult bowel and transplant recipient

Pediatric HSCT patient

Multi-drug-resistance CMV infection (DNA pol del. 981-982)



Wild-type | Not amplifiable | GCV-R: UL97 (M460V) + UL54 (wt) | GCV-R / CDV-R / PFA-R: UL97 (M460V) + UL54 (del 981-982) | GCV-R / CDV-R / PFA-R: UL97 (wt) + UL54 (del 981-982)

Abbreviations: NPA (nasopharyngeal aspirate), CSF (cerebrospinal fluid)

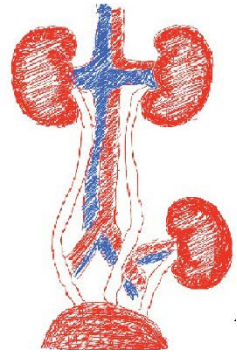
KU LEUVEN

Bauters et al, Antiviral Res. 2016

Renal transplant recipient (D^+/R^-)

Multi-drug-resistance CMV infection (DNA pol del. 981-982)

RegaVir code	Date	Days post-transplantation	Type of sample	HCMV Genotyping	Results genotyping
RV-1050	11/08/2016	66	blood	UL97 + DNA pol	wild-type
RV-1057	01/09/2016	91	blood	UL97 + DNA pol	**UL97: C592G***
RV-1090	04/11/2016	153	blood	UL97 + DNA pol	**UL97: A594V***
RV-1103	17/11/2016	166	blood	UL97 + DNA pol	**UL54: del 981-982*** **UL97: A594V***
RV-1119	14/12/2016	193	blood	UL97 + DNA pol	**UL54: del 981-982*** **UL97: A594V***
RV-1125	20/12/2016	199	blood	UL97 + DNA pol	**UL97: A594V***
RV-1128	26/12/2016	206	blood	UL97 + DNA pol	**UL97: A594V***
RV-1135	02/01/2017	212	blood	UL97 + DNA pol	wild-type
RV-1145	09/01/2017	219	blood	UL97 + DNA pol	**UL54: P522T** **UL97: A594V**
RV-1141	16/01/2017	226	blood	UL97 + DNA pol	**UL54: del 981-982*** **UL97: A594V***



- Graft loss
- ↓immunosuppression
- Stop antiviral therapy

*Heterogeneous population

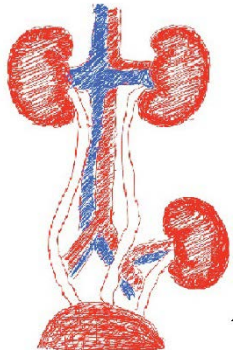
KU LEUVEN

Renal transplant recipient (D^+/R^-)

Multi-drug-resistance CMV infection (DNA pol del. 981-982)

RegaVir code	Capillary (Sanger) sequencing	NGS (MiSeq Illumina)
RV-1050	UL54: wild-type UL97: wild-type	UL54: wild-type UL97: wild-type
RV-1057	UL54: wild-type UL97: C592G*	UL54: wild -type UL97: C592G A594V (10.8%) (11.1%)
RV-1090	UL54: wild-type UL97: A594V*	UL54: T503A (1.5%) P522S (1.7%) del 981-982 (3.0%) UL97: A594V (68.4%) C592G (2.1%)
RV-1103	UL54: del 981-982* UL97: A594V*	UL54: del 981-982 (37.4%) P522S (1.3%) UL97: A594V (22.8%)
RV-1119	UL54: del 981-982* UL97: A594V*	In progress
RV-1125	UL54: wild-type UL97: A594V*	In progress
RV-1128	UL54: wild-type UL97: A594V*	In progress
RV-1135	UL54: wild-type UL97: wild-type	In progress
RV-1145	UL54: P522T UL97: A594V	In progress
RV-1141	UL54: del 981-982 UL97: A594V*	In progress

*Heterogeneous population

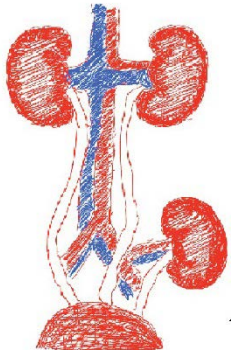


- Graft loss
- ↓immunosuppression
- Stop antiviral therapy

KU LEUVEN

Renal transplant recipient (D^+/R^-)

Multi-drug-resistance CMV infection (DNA pol del. 981-982)



Biopsy type	HCMV genotyping
Transplant kidney (at moment of transplantation)	negative
Transplant kidney after 3 months (14/09/2017)	wild-type
Transplant kidney after 4 months (14/10/2017)	UL97: A594V*
Transplant kidney after 5 months (14/11/2017)	negative
Duodenum	wild-type
Stomach	negative
Transplant kidney (after transplantectomy) (16/11/2017)	UL97: A594V* UL54: T503A*

*Heterogeneous population

Compartmentalization

UL97: C592G*

UL97: A594V*

UL97: A594V*
UL54: del 981-982*

Blood samples

KU LEUVEN

Lung transplant recipient – CMV primary infection

Multi-drug-resistance CMV infection (DNA pol del. 981-982)

RegaVir code	Date	Type of sample	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
RV-1091	28/10/2016	blood	**A594V**	Wild-type
RV-1137	05/01/2017	blood	**A594 V**	**Del 981-982** (heterogeneous population, R ~ WT)
RV-1179	09/03/2017	blood	**A594 V**	**Del 981-982**
RV-1206	18/04/2017	Eye fluid	**A594 V**	Wild-type

Compartmentalization

KU LEUVEN

Pediatric HSCT recipient
Multi-drug-resistance CMV infection

RegaVir code	Date (blood sample)	Virus	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
RV-933	30/12/2015	HHV-6	Wild-type	Wild-type
RV-934	04/01/2016	HHV-6	Wild-type	Wild-type
RV-935	11/01/2016	HHV-6	Wild-type	Wild-type
RV-936	18/01/2016	HHV-6	Wild-type	Wild-type
RV-975	21/03/2016	CMV	Wild-type	Wild-type
RV-1014	08/06/2016	CMV	**A594T*** **L595W***	**A834P**
RV-1028	22/06/2016	CMV	**A594T***	**A834P**
EN_CS_2_1	06/07/2016	CMV	Wild-type	**A834P**
EN_CS_2_2	13/07/2016	CMV	Wild-type	**A834P**
EN_CS_2_3	18/07/2016	CMV	Wild-type	**A834P**

*Heterogeneous population

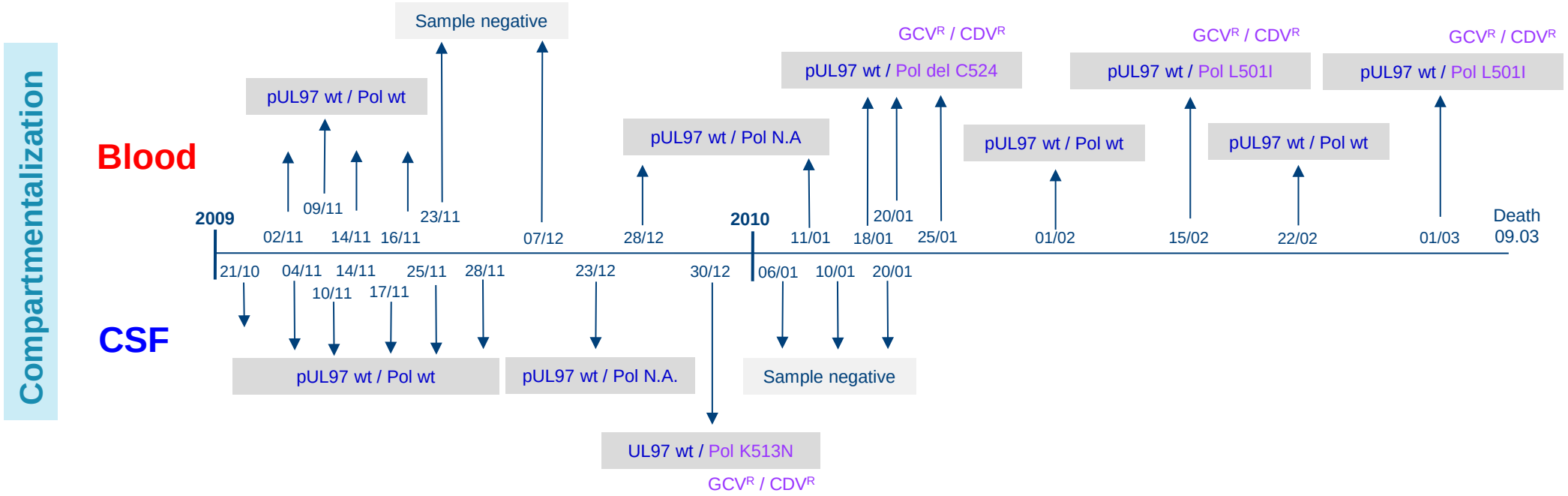
Lung transplant recipient – CMV reactivation
Multi-drug-resistance CMV infection

RegaVir code	Date	Type of sample	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
RV-901	18/11/2015	blood	**L595S**	Wild-type
RV-924	01/01/2016	blood	**L595S** (mixed, WT >> R)	**K513N (GCV^{R} / CDV^{R})** (heterogeneous, R > WT) **V787L (GCV^{R} / PFA^{R})** (heterogeneous, R > WT)

Multi-drug resistance confered by co-infection with ≠ strains bearing specific mutations

HCMV in a severe immune deficiency neonate

HCMV compartmentalization



Herpesvirus co-infections in immunocompromised patients

- HCMV and HHV-6 are known to potentiate their replication
- Other herpesvirus co-infections may occur
- Reactivation from one herpesvirus may favor reactivation of another herpesvirus

KU LEUVEN

HSV & HCMV in the BAL of a non-Hodgkin lymphoma patient

Example of co-infection

RegaVir code	Virus	Date	Type of sample	Genotyping
RV-1106	HSV-1	18/11/2016	BAL	**TK: Q342stop resistance** DNA pol: wild-type
RV-1106	HCMV	18/11/2016	BAL	**UL97: R524stop* (new resistance mutation)** L365F* (unknown significance) **DNA pol: V787A* (new resistance mutation)**

*Heterogeneous population

KU LEUVEN

HSV & HCMV in a mouth swab of an HSCT patient

Example of co-infection

RegaVir code	Virus	Date	Type of sample	Genotyping
RV-295	HSV-1	28/11/2011	Mouth swab	TK: wild-type DNA pol: wild-type
RV-296	HCMV	28/11/2011	Mouth swab	**UL97 : L595S (GCV-R)** **DNA pol : E756D* (PFA-R)**

*Heterogeneous population

Emergence of resistance to maribavir

➢ Adult HSCT recipient

Specimen	Date (blood sample)	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
RV-451	21/01/13	**T409M (MBV-R)**	Wild-type

➢ Adult liver transplant recipient

Specimen	Date (blood sample)	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
RV-500	21/01/13	**T409M (MBV-R)** **C480F (MBV-R / GCV-R)**	Wild-type

Emergence of resistance to maribavir

➢ Adult Hodgkin lymphoma undergoing HSCT

Specimen	Date (blood sample)	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
RV-1294	28/08/17	**C480F* (MBV-R / GCV-R)**	Wild-type
RV-1295	30/08/17	**T409M* (MBV-R)** **C480F* (MBV-R / GCV-R)**	Wild-type
RV-1297	04/09/17	**T409M* (MBV-R)** **C480F (MBV-R / GCV-R)**	Wild-type
RV-1334	16/10/17	**C480F (MBV-R / GCV-R)**	Wild-type
RV-1335	18/10/17	**C480F (MBV-R / GCV-R)**	Wild-type

* Heterogeneous population
The C480F amino acid substitution has not previously been described but the C480R change is known to confer resistance to both GCV and MBV

Emergence of similar pattern of HCMV drug-resistance in 3 SOT patients with a shared donor

Patient	Specimen	Date (blood sample)	UL97 genotyping (protein kinase)	UL54 genotyping (DNA pol)
#1: 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)***
#2: 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)
#3: lung transplantation on 19/03/2017	**RV-1389**	17.01.2018	**L595S***	A505G (GCV-R / CDV-R)*
	RV-1445	17.04.2018	**T409M (MBV-R)**	A505G (GCV-R / CDV-R)

Multi-drug resistance

Letermovir

Maribavir resistance

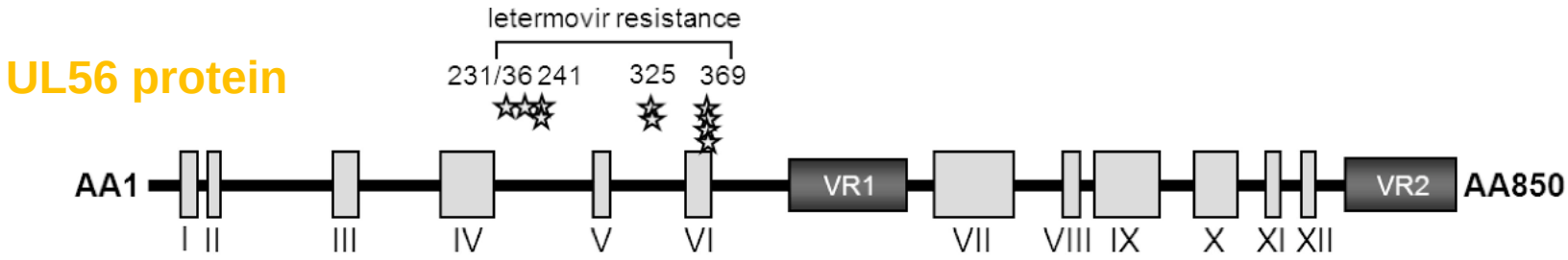
* Heterogeneous population
The A505V amino acid substitution has not previously been described but the A505R change is known to confer resistance to both GCV and CDV

KU LEUVEN

Preparedness for testing letermovir resistance

- Letermovir targets the terminase complex: UL51, UL56, UL89

- Most of letermovir-resistance mutations map to the UL56 protein but also described in UL89 and UL51 proteins



Conclusions & Perspectives

- Usefulness of **rapid HCMV genotyping** → adjustment of antiviral therapy
- Emergence of **multiple drug-resistance**
- Viral *compartmentalization*
- Advantage of **NGS** for detecting minor HCMV drug-resistant populations
- Appearance of **novel mutations** in the UL97 and UL54 genes
- Herpesvirus **co-infections**
- Emergence of **resistance to new anti-HCMV drugs**
- Urgent need for **novel anti-HCMV agents** → reduce morbidity and mortality caused by drug-resistant HCMV

KU LEUVEN

RegaVir

- **Acknowledgements**

Rega Institute

- Laboratory Virology and Chemotherapy

Sarah Gillemot

Seppe Kelchtermans

Ellen De Waegenaere

Graciela Andrei

- Laboratory Immunobiology

Pierre Fiten

Ghislain Opdenakker

Contact: RegaVir

Rega Institute for Medical Research

O&N Rega, Herestraat 49, postbus 1043

B-3000, Leuven

Belgium

Website: www.regavir.org

Fundings: Action 29 of the National Cancer Plan (Federal Public Service “Public health, food chain safety, and environment”) for translational research