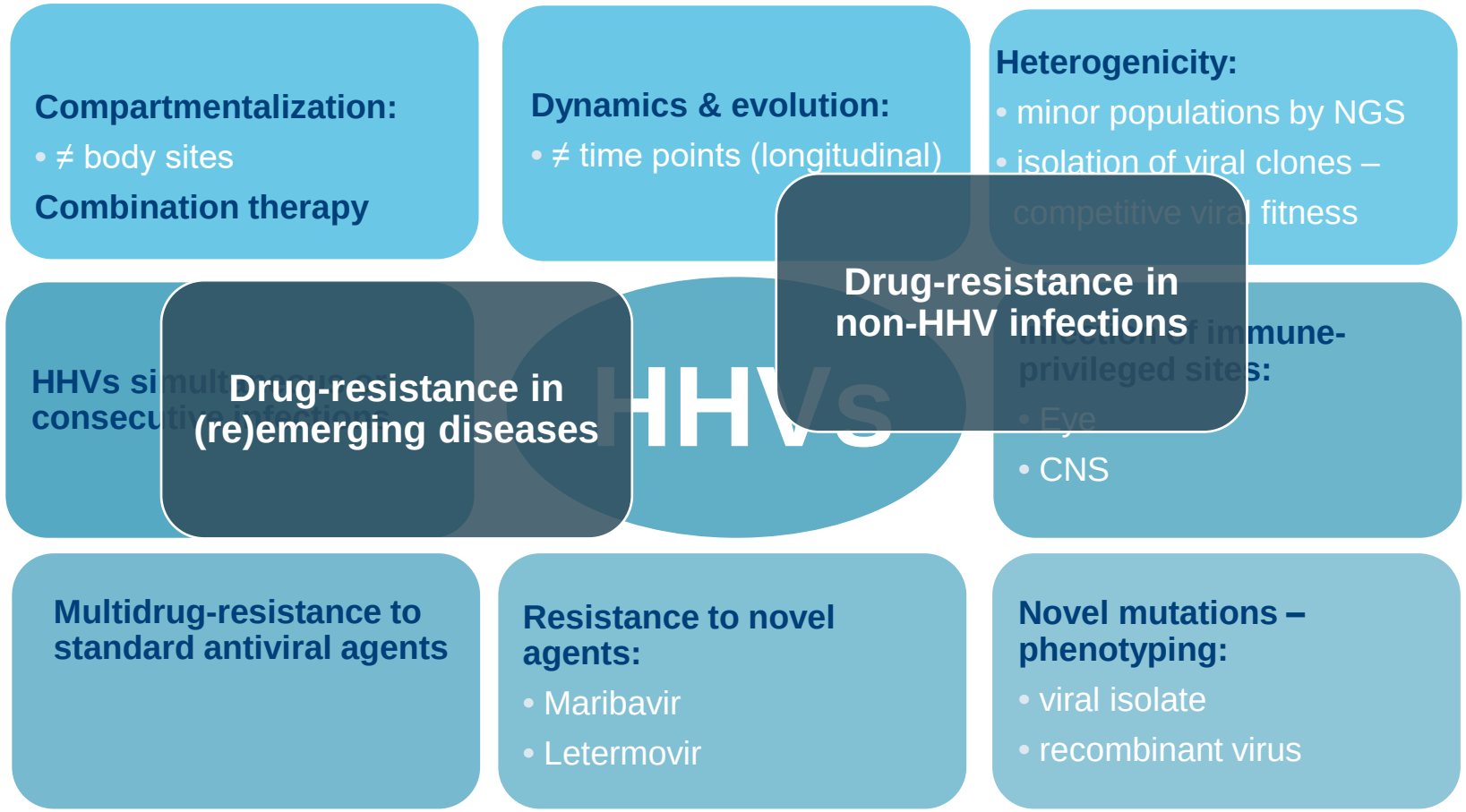
KU LEUVEN

The translational Research platform RegaVir

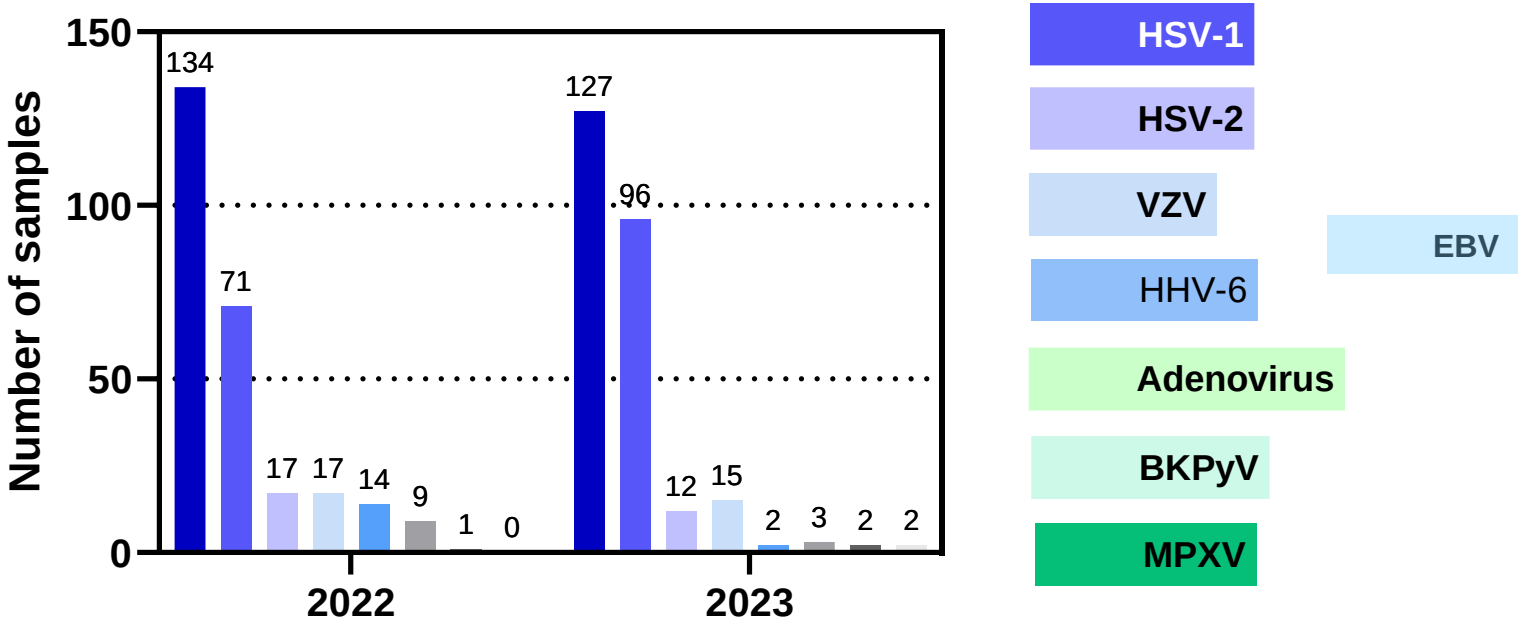
Robert Snoeck & Graciela Andrei

Leuven, April 30, 2024

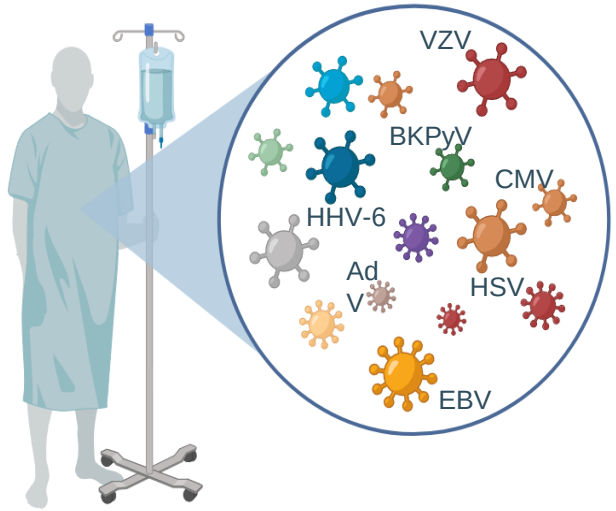
RegaVir platform for translational research



Activity report 2022-2023



CMV
HSV-1
HSV-2
VZV
EBV
HHV-6
Adenovirus
BKPyV
MPXV

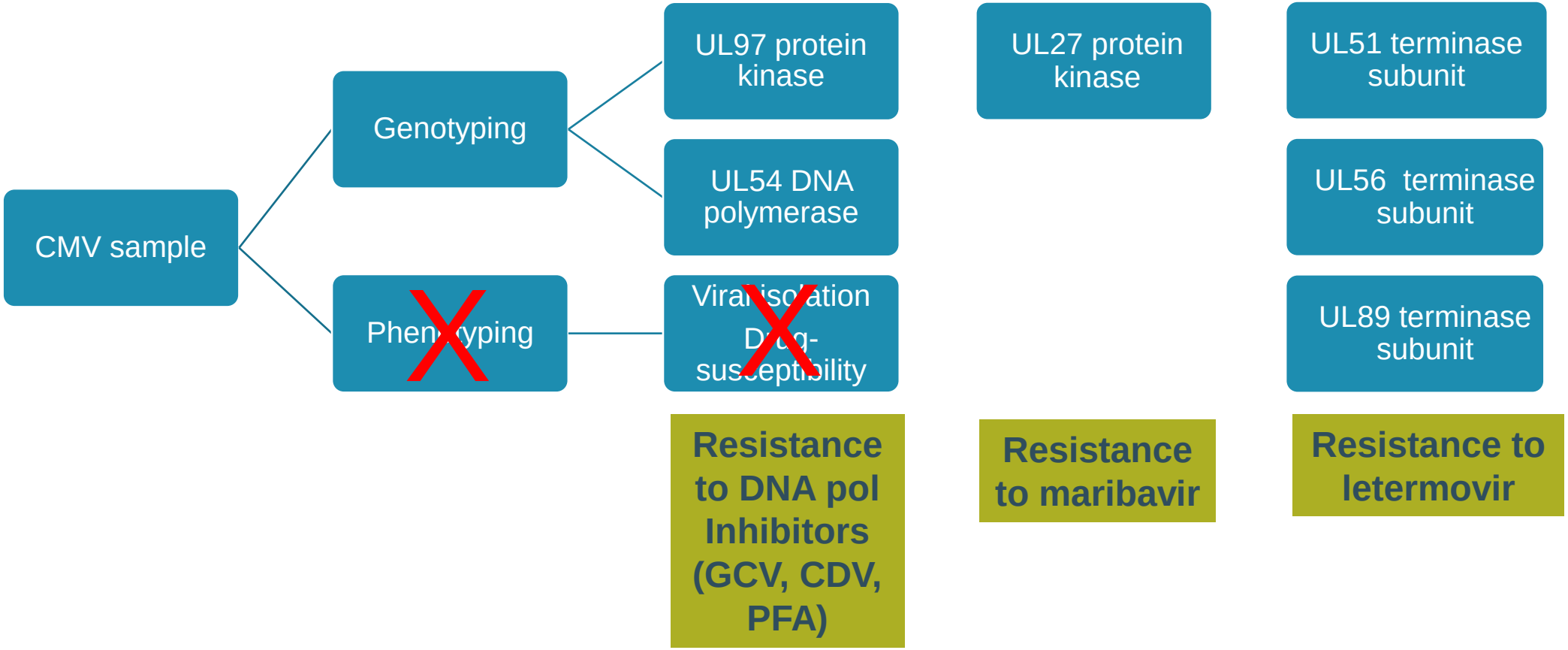


KU LEUVEN

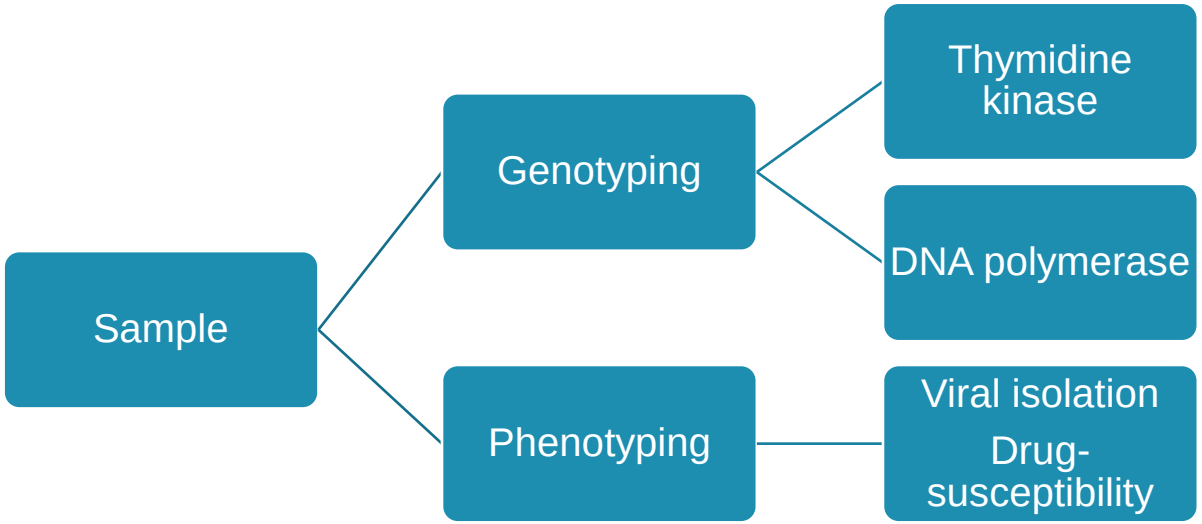
Drugs to manage DNA virus infections

	DNA polymerase inhibitors					Terminase inhibitor	UL97 PK inhibitor	P37
	Acyclovir Valacyclovir	Penciclovir Famciclovir	Ganciclovir Valganciclovir	Cidofovir	Foscarnet	Letermovir	Maribavir	Tecovirimat
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 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			
Adenovirus				off-label				
BKPyV				off-label				
MPXV				approved				approved

RegaVir CMV workflow



RegaVir HSV / VZV workflow



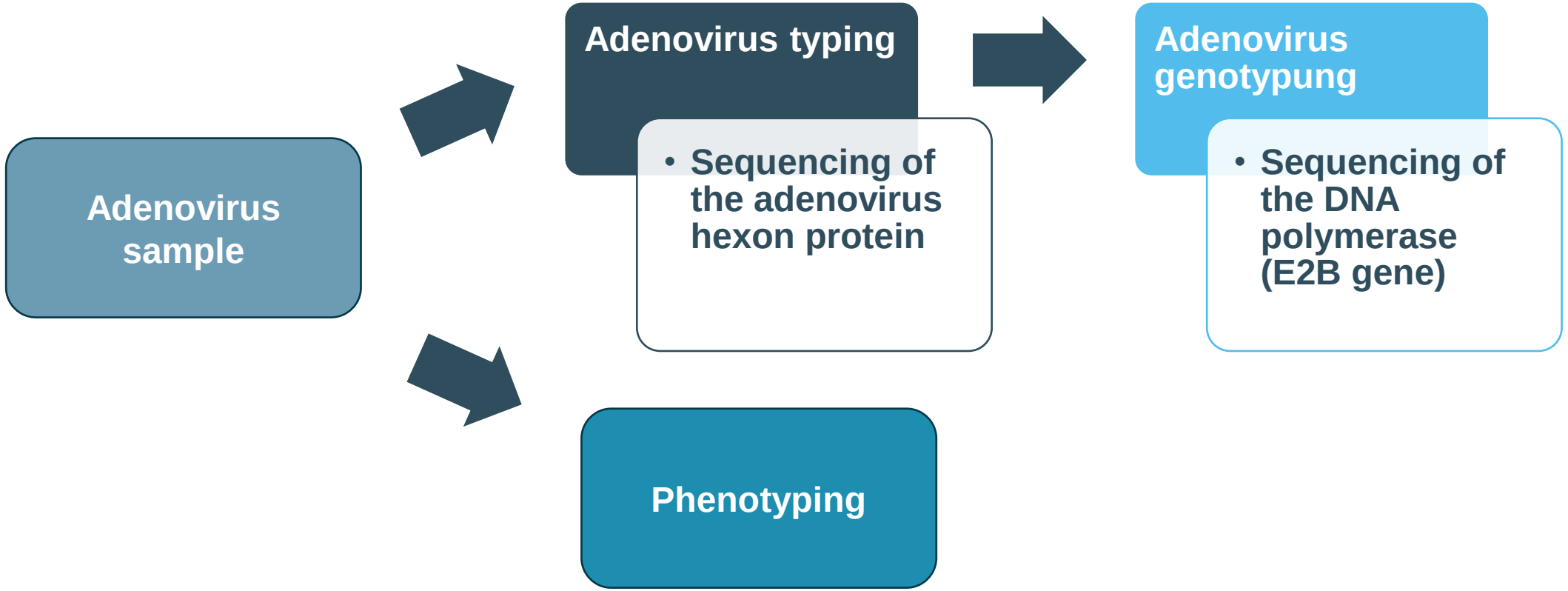
UL23 (HSV)
ORF36 (VZV)

UL30 (HSV)
ORF28 (VZV)

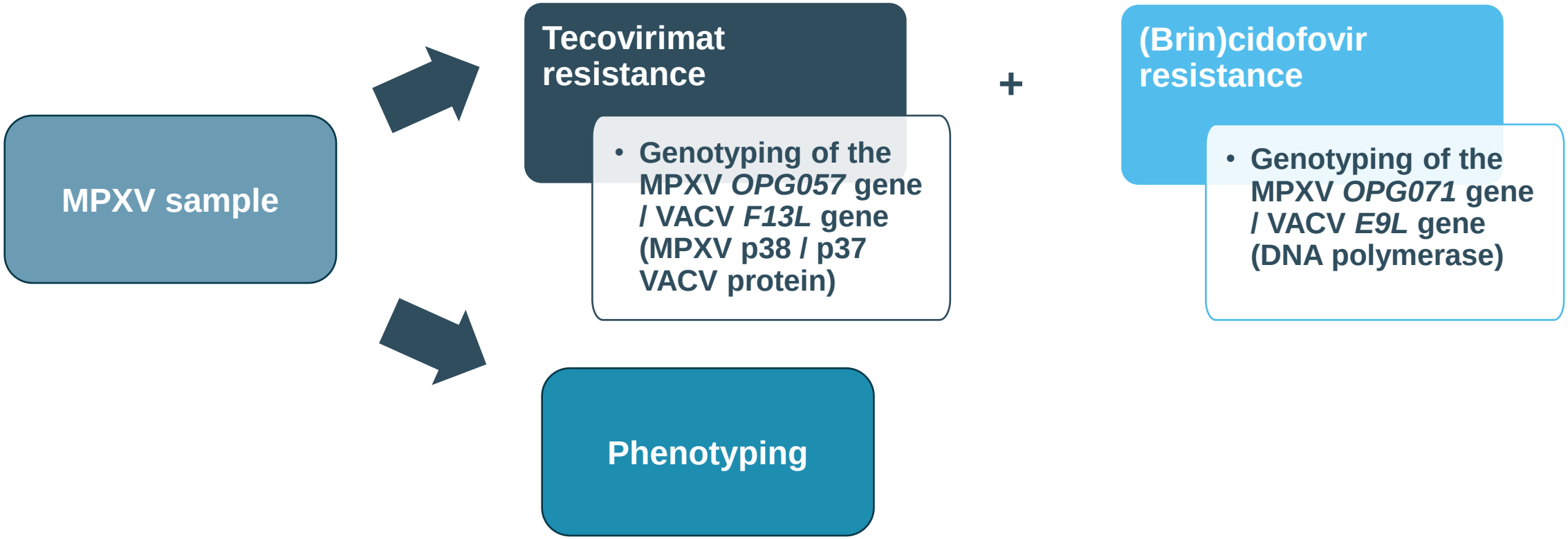
Intermediate report

Final report

RegaVir adenovirus workflow

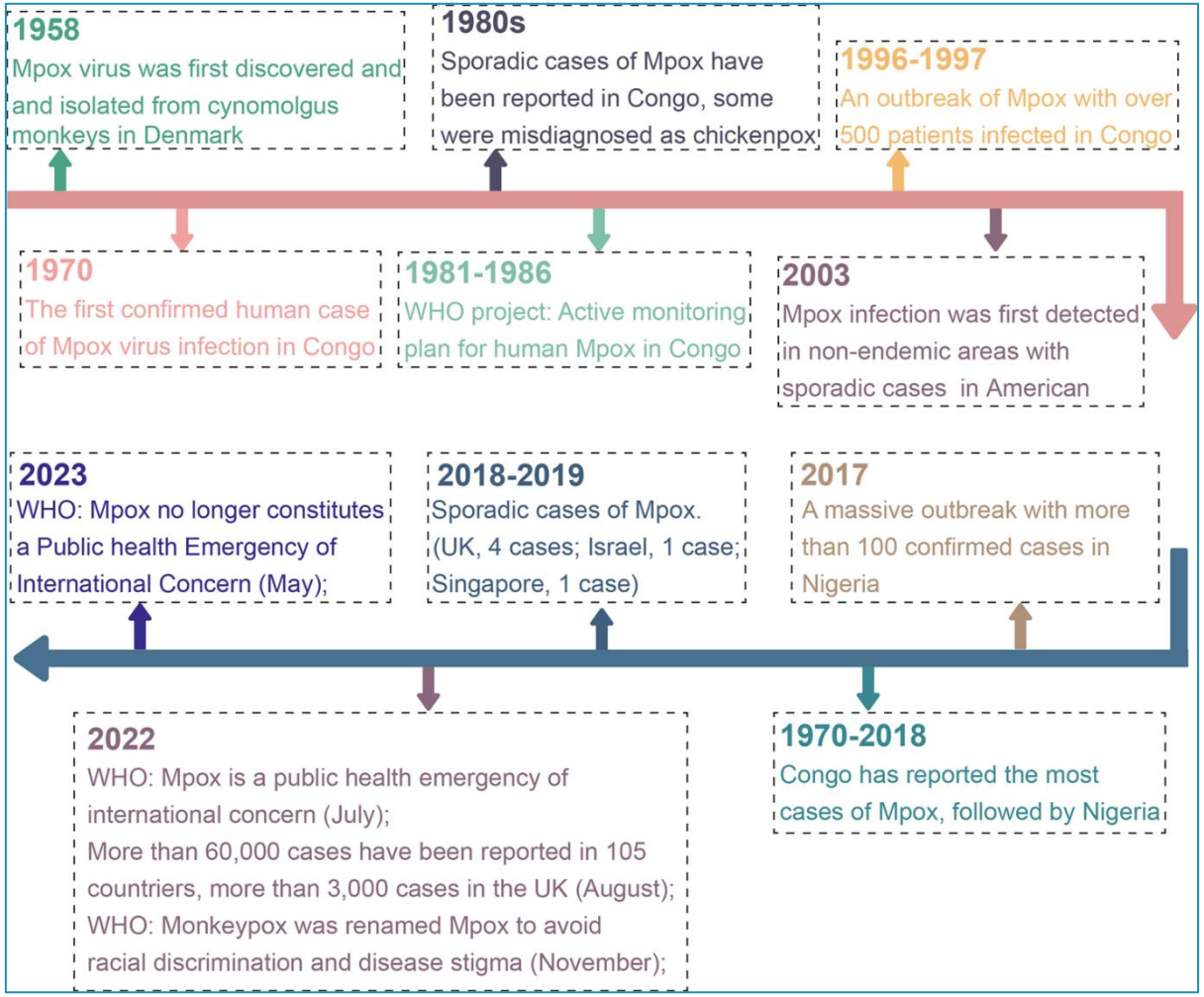


RegaVir MPXV workflow



KU LEUVEN

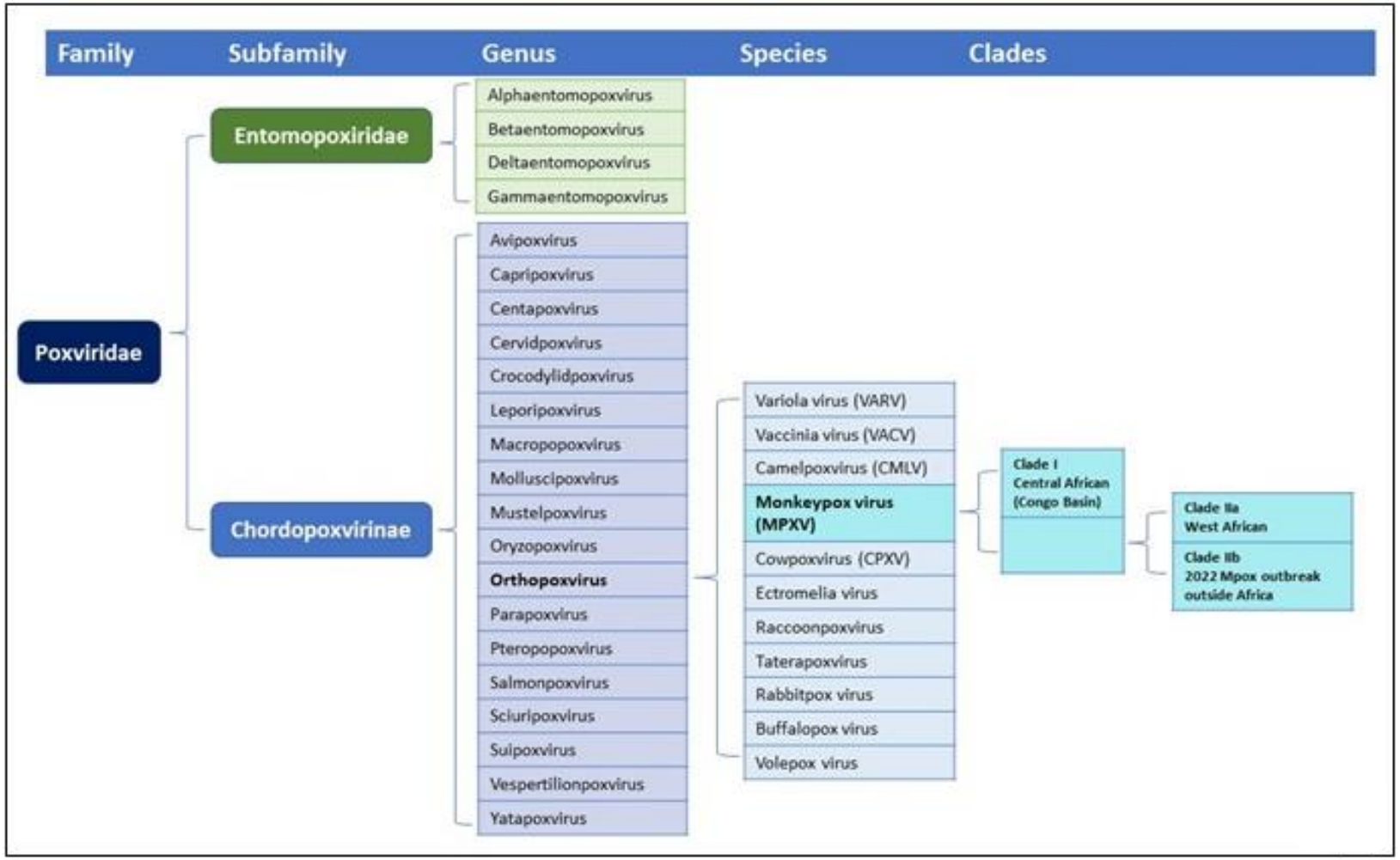
Timeline of the major milestones in Mpox (formerly monkeypox)



Junjie Lu et al , Sig Transduct Target Ther 2023

KU LEUVEN

MPXV classification



Andrei & Snoeck, Trends in Pharmacol. Sci. 2023

KU LEUVEN

Comparative features of classical mpox and the 2022 mpox outbreak

	Classical mpox		2022 Global mpox outbreak
WHO recommended nomenclature	**Clade I**	**Clade IIa**	**Clade IIb**
Original clade nomenclature	Central African, Congo Basin (clade 1)	West African (clade 2)	West African variant (clade 3)
Geographic distribution	Democratic Republic of Congo (DRC), Central African Republic (CAR), Cameroon, South Sudan, Gabon	Nigeria, Liberia, Sierra Leone, Ivory Coast, Cameroon	In non-endemic regions (Europe, North America, South America, Middle East, Pacific regions)
Epidemiological characteristics	Endemic Sporadic cases Outbreaks	Endemic Sporadic cases Outbreaks	**Pandemic**
Transmission	Zoonotic through contact with: - infected reservoir hosts (mostly rodents, e.g., squirrels, rats) - intermediate or incidental hosts (monkeys or African apes) - exotic pets (infected prairie dogs) Limited human-to-human transmission	Zoonotic through contact with: - infected reservoir hosts (mostly rodents, e.g., squirrels, rats) - intermediate or incidental hosts (monkeys or African apes) - exotic pets (infected prairie dogs)	**Exclusively human-to-human transmission (close contact)**
Dissemination	Limited nosocomial dissemination, mostly intra-families	Limited nosocomial dissemination, mostly intra-families	**Mostly sexually transmitted**, mainly involving men who have sex with men (MSM) with multiple partners
Case fatality rate	Up to 10%	~1%	**0.025%**

A. Typical symptomatic presentation of mpox seen in endemic countries in Africa

Infectious period

Exposure				
Average 6-13 days Range 5-21 days	Average 1-4 days Range 0-5 days	Average 2-4 weeks – Begins within 1-3 days of onset of fever – Potential overlap period with invasion phase	Days to weeks
INCUBATION PHASE	**PRODROMAL PHASE (Invasion phase)**	**RASH PHASE (Skin eruption phase)**	**RECOVERY**
Initial viremia: ⇒ MPXV enters human body ⇒ Replication at inoculation site ⇒ Spread to local lymph nodes No symptoms	Secondary viremia Fever Lymphadenopathy Headache Chills Sore throat Malaise Fatigue	Lesions usually appear first on oropharynx, centrifugal progression of rash (mouth ⇒ face ⇒ extremities (arms/legs) ⇒ trunk, hands/feet (including palms/soles) ⇒ MACULES – flat based lesions, red spots (last for 1-2 days) ⇒ PAPULES – spots become harder, slightly raised lesions (last for 1-2 days) ⇒ VESICLES – bumps get larger, blisters filled with clear fluid (last for 1-2 days) ⇒ PUSTULES – blisters filled with pus (last 5-7 days) ⇒ CRUSTS – spots become scabs that eventually fall off (last 7-14 days) Lesions change synchronously, develop uniformly throughout the disease (monomorphic pustular rash) Serum antibodies can be detected by the time the lesions appear	No symptoms

B. Atypical symptomatic presentation of mpox in the 2022 outbreak

Infectious period

Exposure			
Average 6-13 days Range 5-21 days			
INCUBATION PHASE	**NO PRODROMAL PHASE**	Average 2-4 weeks	Days to weeks
Initial viremia: ⇒ MPXV enters human body ⇒ Replication at inoculation site ⇒ Spread to local lymph nodes No symptoms	Appearance of lesions before the onset of fever, malaise and other constitutional symptoms Lymphadenopathy and fever not common	**RASH PHASE (Skin eruption phase)**	**RECOVERY**
		• Presentation of only a few or even just a single lesion • Rash either scattered or diffuse; sometimes limited to one body site or mucosal areas (anogenital or lips/face) • In some cases, absence of skin lesions • Sometimes complaints of anorectal pain or tenesmus • Lesions localized in genital or perineal/perianal area not further spreading • Lesions appearing at different (asynchronous) stages of development – regional polymorphism) • Some co-infections with sexually transmitted diseases	No symptoms

Andrei & Snoeck, Trends in Pharmacol. Sci. 2023

Vaccines for mpox

ACAM2000	Jynneos (Imvanex in Europe)
Second generation vaccine	Third generation vaccine
Live replicating VACV	Live nonreplicating VACV, modified vaccinia Ankara (MVA) vaccine
Percutaneous administration with punctuated needle (one dose)	Intradermal administration (2 doses)
FDA-approved for immunization against smallpox and available for mpox under Expanded Access Investigational New Drug (EA-IND) protocol.	FDA- and EMA-approved for smallpox and mpox disease prevention in adults ≥18 years old at high risk for VARV or MPXV infection
Not approved in the European Union	An individual is considered fully vaccinated ~2 weeks after second dose of Jynneos (two doses administered subcutaneously 4 weeks apart)

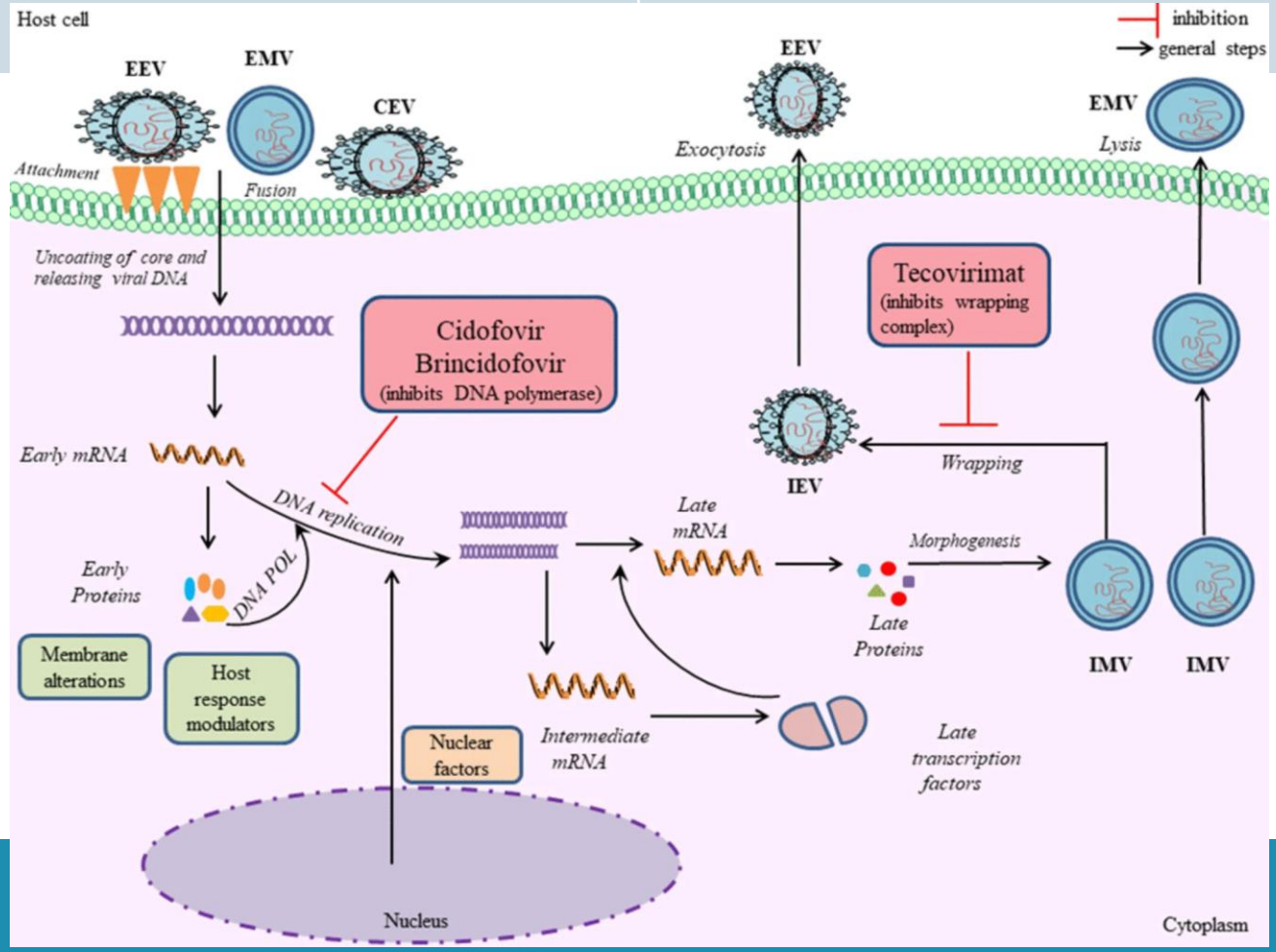
Antivirals for mpox

Tecovirimat (TPOXX or ST-246)	Cidofovir (CDV, Vistide)	Brincidofovir (Tembexa or CMX001)
FDA-approved (2018) for treatment of **smallpox; stockpile**	Available through **FDA IND** protocol for smallpox treatment; **stockpile**	**FDA-approved** (2021) for smallpox treatment (not approved in Europe)
Available under **CDC's EA-IND** protocol for **mpox disease** treatment	Available through **FDA EA-IND** protocol for **mpox disease** treatment	Available through single-patient FDA emergency use Investigational New Drug (e-IND) for mpox disease
EMA approved in 2022 in Europe for *Orthopoxvirus* (smallpox, cowpox, vaccinia, mpox) outbreaks	**Not EMA approved**	**Not EMA approved** – orphan drug designation
Administered orally or intravenously Course duration: 14 days	Administered intravenously 5mg/kg 1x/week Poor oral bioavailability Course duration: 2 consecutive weeks	Oral prodrug of cidofovir (no IV form available) >48kg: 200 mg 1x/week Course duration: 2 consecutive weeks

IND (investigational new drug)
EA-IND (expanded access investigational new drug

Antivirals for mpox

Tecovirimat (TPOXX or ST-246)	Cidofovir (CDV, Vistide)	Brincidofovir (Tembexa or CMX001)
Targets the specific *Orthopoxvirus* p37 phospholipase required for extracellular virus formation, prevents viral release and spread, but does not inhibit intracellular virus production.	Broad-spectrum anti-DNA virus activity; inhibitor of viral DNA synthesis, targets viral DNA polymerases	Same antiviral activity spectrum and mechanism of action as cidofovir



Isha Rani et al, Archives of Pharmacology, 2023

Antivirals for mpox

Tecovirimat (TPOXX or ST-246)	Cidofovir (CDV, Vistide)	Brincidofovir (Tembexa or CMX001)
Highly effective against currently circulating mpox strains and good safety profile	Safety concerns: potential nephrotoxicity	Shows no nephrotoxicity but is associated with gastrointestinal and hepatocellular toxicities
Currently undergoing several clinical trials for mpox	/	/
Tecovirimat-resistant mutations selected in cell culture map to the VACV p37 protein	Cidofovir-resistant mutations selected in vitro map to the DNA polymerase	Same pattern of resistance as cidofovir

MPXV case – CHU Saint Pierre, Brussels

- 32 years-old man
- HIV positive (CD4 count: 64 cells/mm^3)
- Severe mpox (07/2022)
- Cidofovir 1x
- Tecovirimat 600mg 2x/day since 22/12/2022

MPXV case – CHU Saint Pierre, Brussels

- 2 samples received by RegaVir on 06/01/2023

RegaVir Identification	Original Identification	Date	Type
RV-2660	12108754-9100	05/03/2023	Blood EDTA
RV-2661	12108754-9200	05/03/2023	Swab skin lesion

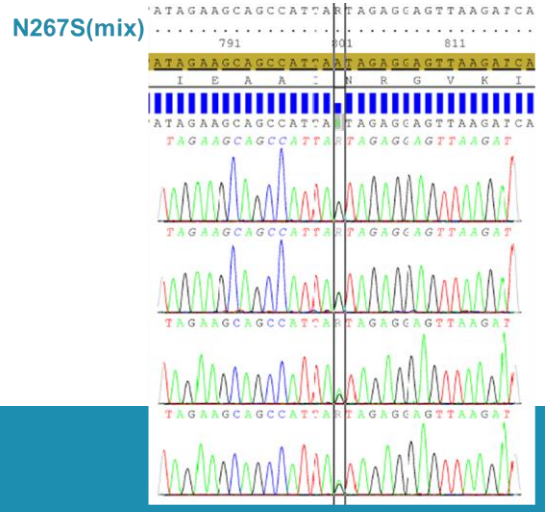
MPXV case – CHU Saint Pierre, Brussels

	Amino acid changes in OPG071 (DNA polymerase) 1006 amino acids – complete sequence	
	Known to be related to genetic polymorphism (inter-strain variability)	**Known to be associated with drug-resistance**
RV-2660 (blood)	L108F	None
RV-2661 (skin swab)	L108F	None

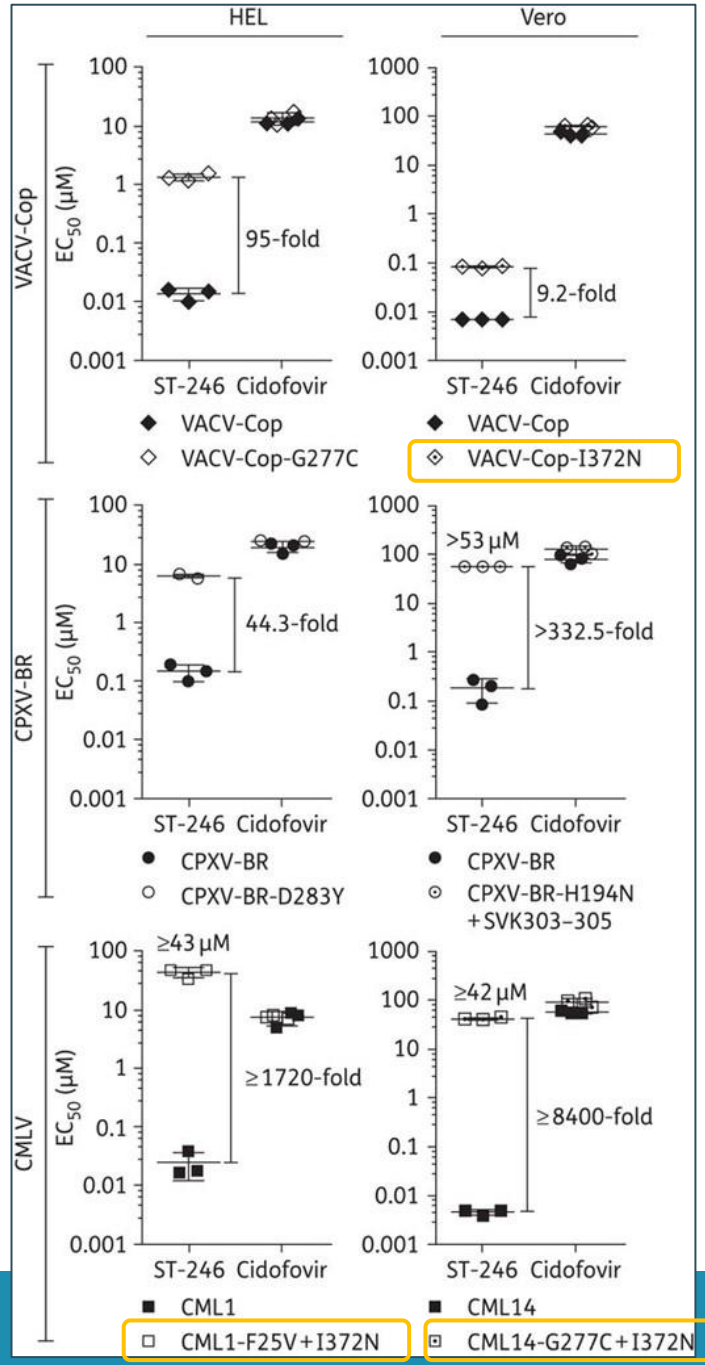
MPXV case – CHU Saint Pierre, Brussels

	Amino acid changes in OPG057 (p38 protein) 1006 amino acids – complete sequence		
	Known to be related to genetic polymorphism (inter-strain variability)	**Known to be associated with drug-resistance**	**Novel**
RV-2660 (blood)	E353K	**I372N_mix**	None
RV-2661 (skin swab)	E353K	None	**N267S_mix** **A290V_mix**

Compartmentalization



Tecovirimat resistance mutations



Duraffour et al, J Antimicrob Chemother 2015

Phenotyping RV-2661 (p38 N267S_mix A290V_mix)

Compound	Antiviral activity EC_{50} in HEL cells		
	RV-2661	MPXV # 2 Reference (2022 outbreak)	Ratio sample/wild type
ST-246 (Tecovirimat)	**0,65 µg/ml**	0,017 µg/ml	**38,24**
	0,65 µg/ml	0,0060 µg/ml	**108,33**
	0,59 µg/ml	0,0060 µg/ml	**98,33**
CDV (Cidofovir)	7,31 µg/ml	1,64 µg/ml	4,46
	3,46 µg/ml	1,79 µg/ml	1,93
	2,53 µg/ml	1,71 µg/ml	1,48
BCDV (Brincidofovir)	0,22 µM	0,16 µM	1,38
	0,22 µM	0,093 µM	2,37
	0,40 µM	0,15 µM	2,67

Amino acid substitution	Isolates	Patients	EC_{50}, µmol/L	Fold change†
A288P (25,26)	6	4	0.5 to >500	29 to >29,000
A288P, A290V, D294V (26)	3	1	0.66 to >500	38 to >29,000
A288P, A290V, L297ins (26)	1	1	>500	>29,000
A288P, A290V, I372N	1	1	15	880
A288P, D294V, A295E	1	1	1.4	83
A288P, D294V, D301del (26)	1	1	>500	>29,000
A288P, I372N	1	1	>150	>8,600
A290V (25,26)	9	9	0.17–43	10–2,500
A290V, I372N (25)	5	5	30–32	1,700–1,800
A295E	3	3	2.0–3.3	110–190
D100N	2	2	0.008	−2
D217N	11	11	0.007–0.012	−2.4 to −1.3
D248N	1	1	0.007	−2.4
D256N	3	3	0.009	−1.8
D283G	2	1	7.1–7.3	404–420
D294V (25)	8	7	0.23–1.4	13–78
D294V, A295E	1	1	1	59
H238Q (25)	4	4	0.54–0.6	28–34
H238Q, A288P, D294V, I372N (25)	1	1	≈5.2	≈290
H238Q, N267D, A295E	1	1	24	1,400
I372N (25)	12	9	0.04–>150	2.3 to >8600
K174N, N267D	1	1	12	720
N267D (25)	3	3	10–11	570–630
N267D, A288P (25,26)	4	3	1.2–16	71–900
N267D, A290V	1	1	2.0	110

Amino acid substitution	Isolates	Patients	EC_{50}, µmol/L	Fold change†
N267D, D294V	1	1	12	680
N267D, A288P, A290V, D294V (26)	1	1	>500	>29,000
N267D, A288P, A290V, A295E, L297ins (26)	1	1	>500	>29,000
N267D, A288P, A290V, A295E, I372N	1	1	>500	>29,000
N267del (27)	8	7	1.5–4.0	85–230
N267del, N267D	1	1	Not tested	
N267del, N267D, A295E	2	2	2.9–18	160–1,000
N267del, N267D, A288P, A295E	1	1	Not tested	
N267del, N267D, D294V, A295E	1	1	2.5	140
N267del, A288P, A295E	1	1	>500	>29,000
N267del, T289A, A295E	1	1	0.26	15
N267del, A290V	1	1	0.13	7.5
N267del, A290V, I372N	1	1	3.1	180
P243S, A288P, A290V (26)	1	1	0.56	32
S215F, T289A, A290V, I372N	1	1	Not tested	
S369L	3	3	0.006	−2.9
T245I, A290V	1	1	0.17	10
T289A	3	3	0.078–0.14	3.7–7.8
T289A, I372N	1	1	Not tested	
T289A, R291K	1	1	1.7	98
Y258C	1	1	18	1,000
Y285H, I372N	1	1	0.045	2.6

MPXV F13 mutations identified from 76 patients with mpox, United States, 2023 ***(Smith et al, Emerg Infect Dis. 2023)***

All specimens belong to MPXV clade IIb lineage B.1 and contain E353K substitution in addition to the listed substitutions. All substitutions detected from a specimen are listed regardless of their proportion in the viral population. Insertions (ins) and deletions (del) were detected in addition to substitutions. EC_{50}, 50% effective concentration; MPXV, monkeypox virus. †Fold change was calculated based on the EC_{50} of the reference strain MPXV clade IIa (U.S., 2003)

Tecoviriamt resistance-associated mutations

- **A low barrier to tecovirimat resistance** by mutations in the VP37 protein encoded by the F13L gene has been demonstrated through in vitro and animal studies.

- **Multiple VP37 mutations associated with tecovirimat resistance** have been reported within the current global mpox outbreak in immunocompromised individuals with advanced HIV infection.

- In many of these cases, **resistance mutation heterogeneity** was observed following tecovirimat exposure, suggesting resistance emerged under selective pressure during treatment.

Community spread of a human monkeypox virus variant with a tecovirimat resistance-associated mutation

- Genomic surveillance network established to monitor circulating MPXV within California

  → clinical and commercial laboratories provided positive specimens for whole-genome sequencing

  → identification of 11 mpox cases in southern California with the same tecovirimat resistance-associated mutation VP37 protein (OPG057 gene) - VP37:N267del.

- VP37:N267del was the only tecovirimat resistance-associated mutation detected in identified specimens and had allele frequencies greater than 89% in all instances → infections may have occurred with predominantly mutant virus.

Garrigues et al, Virology 2023

Patient and laboratory information for southern California MPXV specimens with the VP37:N267del mutation

Case	Symptom onset date	No. JYNNEOS doses[a]	Tecovirimat received[b]?	Specimen[c]	Specimen collection date	Specimen source
A	2022–11-28	0/2	No	A-1	2022–12-14	Foot
B	2022–11-29	0/2	No	B-1	2022–12-02	Unknown
C	2022–12-01	0/2	No	C-1	2022–12-14	Left upper arm
D	2022–12-13	0/2	No	D-1	2022–12-19	Right ankle
				D-2	2022–12-19	Left wrist
				D-3	2022–12-19	Right breast
E	2022–12-19	0/2	No	E-1	2023–01-02	Unknown
F	2023–01-02	2/2	No	F-1	2023–01-08	Unknown
G	2023–01-04	0/2	No	G-1	2023–01-06	Unknown
				G-2	2023–01-06	Left buttock
H	2023–01-04	1/2	No	H-1	2023–01-10	Unknown
I	2023–01-06	0/2	No	I-1	2023–01-10	Left buttock
J	2023–01-08	2/2	No	J-1	2023–01-13	Mouth
K	2023–01-09	0/2	No	K-1	2023–01-10	Right side face

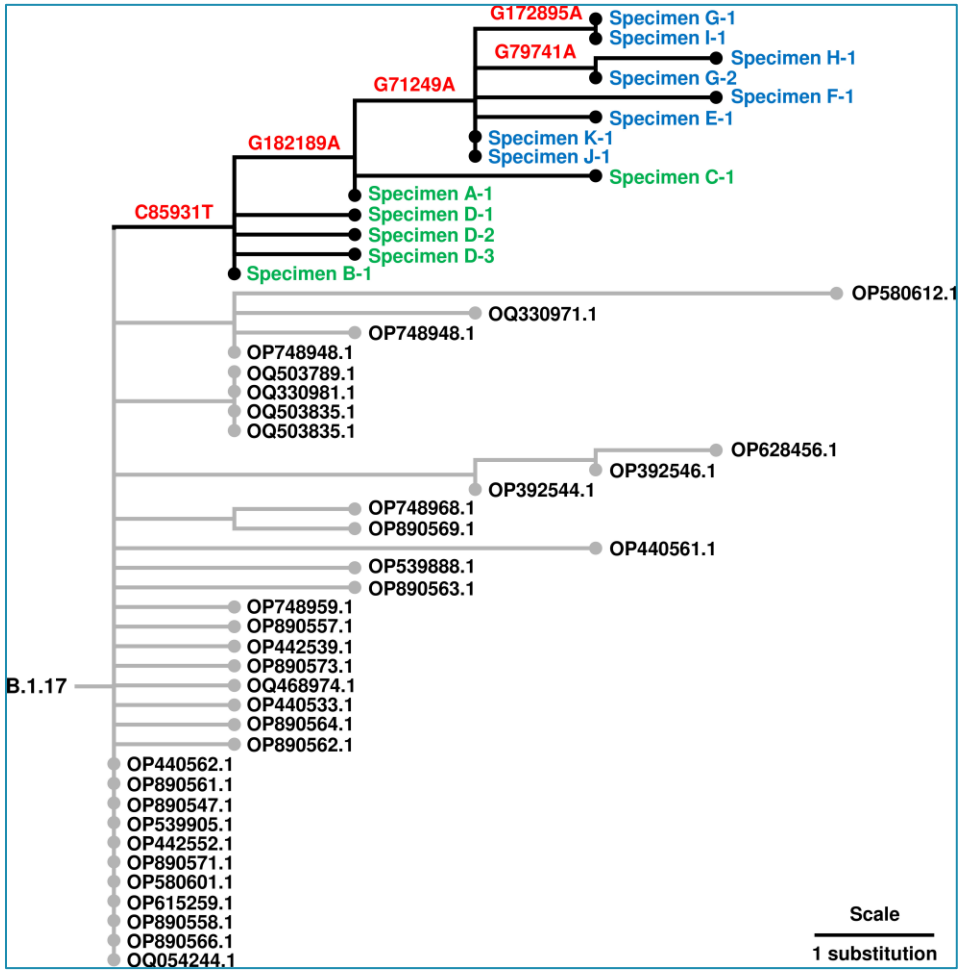
Garrigues et al, Virology 2023

- Phenotypic testing in vitro confirmed tecovirimat resistance in ten identified specimens with EC_{50} values ranging from 1.488 to 3.977 µM, corresponding to an 85- to 230-fold change compared to wild-type isolates.

[a]JYNNEOS doses received 14+ days before symptom onset.
[b]Tecovirimat received before specimen collection.
[c] All specimens were lesion swabs.

Community spread of a human monkeypox virus variant with a tecovirimat resistance-associated mutation

- Four patients had conditions associated with reduced immune function, including HIV infection.
- No patients in this study required hospitalization, suggesting resistance alone does not result in increased virulence.
- Seven cases were epidemiologically linked to a group sex event, indicating person-to-person transmission.
- Supporting their relatedness, phylogenetic analysis showed that all identified VP37:N267del specimens cluster within the B.1.17 lineage of Clade IIb, and the seven cases with confirmed epidemiologic links cluster further.



Garrigues et al, Virology 2023

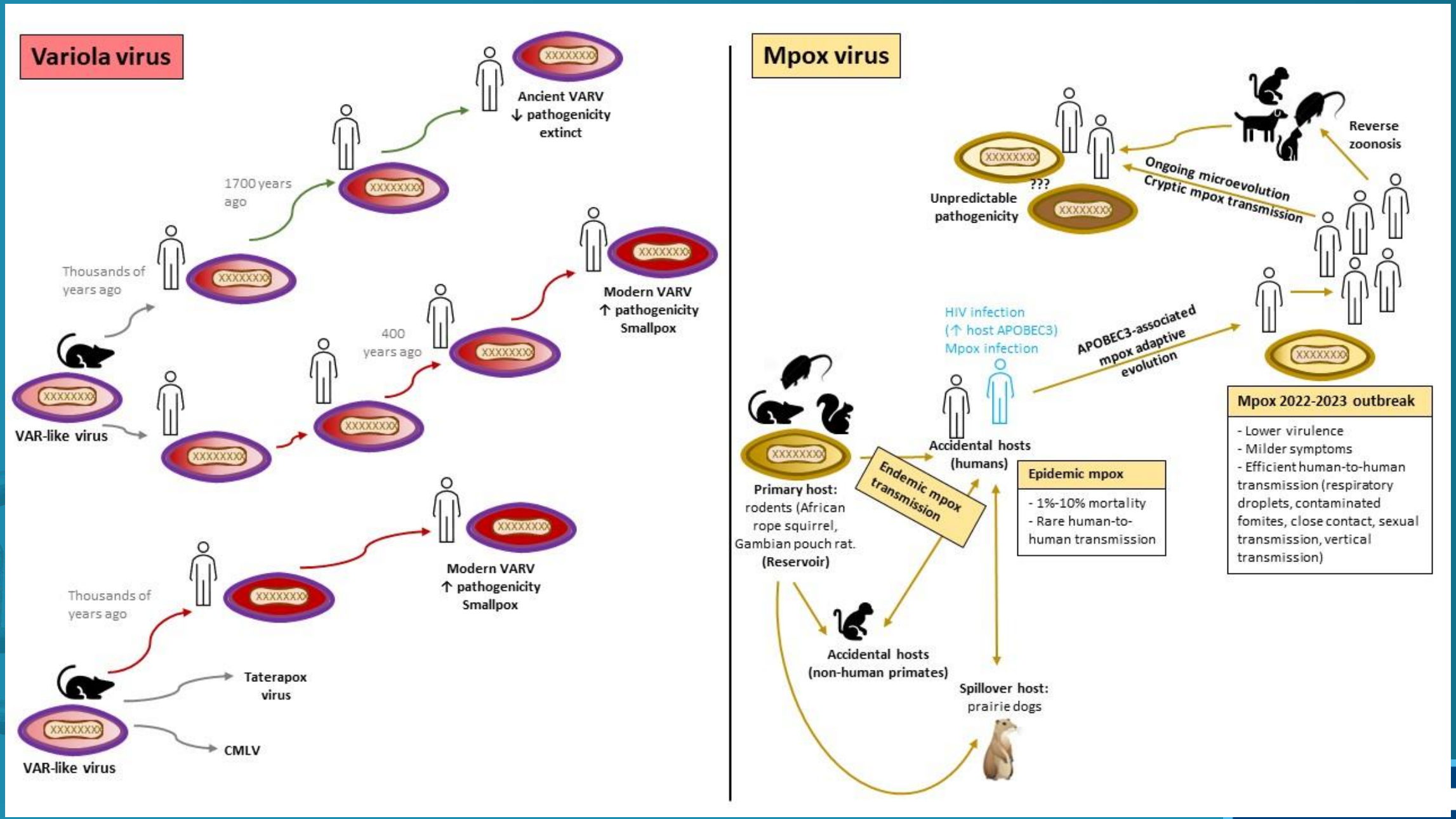
KU LEUVEN

Mpox infections in heavily immunosuppressed patients

- **Immunocompromised hosts:** mpox is associated with hospitalization, **severe disease,** including progressive or disseminated rash, protracted course, and c**omplications including sepsis, ocular disease, encephalitis, and death.**

- Of the people with severe mpox manifestations for whom CDC has been consulted, the majority have had **HIV with CD4 counts 200 cells/ml**, indicating substantial immunosuppression.

- Drug-resistance can emerge following tecovirimat treatment for mpox in severely immunocompromised patients.



Andrei & Snoeck, Trends in Pharmacol. Sci. 2023

Outbreak of mpox caused by Monkeypox virus clade I in the Democratic Republic of the Congo

- **Since 2023 and as of 29 March 2024, DRC reported a total of 18 922 suspected mpox cases including 1 007 deaths.**
- **In 2024, and as of 29 March, 4 488 cases have been reported, of which 319 have been confirmed. A total of 279 deaths have been reported in the country in 2024 (CFR: 6.7%). Mpox cases have been reported in 23 of 26 provinces of the DRC.**
- **Despite this increase in cases, the overall risk from this outbreak in the DRC for the general population in the EU and for MSM with multiple sexual partners in the EU remains low.**
- **Sporadic introduction of mpox in the EU connected to the ongoing DRC outbreak cannot be excluded.**
- **Public health authorities in the EU should continue preparedness and awareness activities to be able to rapidly respond in case of an introduction of MPXV clade I infection.**

Preparedness and awareness activities to be able to rapidly respond in case of an introduction of MPXV clade I infection

- **Raise clinician awareness about the ongoing outbreak and the possibility of travel-associated mpox cases.**
  → possibility of different clinical presentations & more severe disease due to MPXV clade I.

- **Ensure effective surveillance, testing, and contact tracing capacities.**
  → Sequencing of samples from detected mpox cases should be performed with prompt sharing of the detected sequences

- **Continue risk communication activities and working with civil society organizations to engage with at-risk groups.**

- **In the event of detection of mpox case(s) with increased severity and/or the detection of a MPXV clade I infection, this should be promptly communicated at the EU level via the alert systems.**

Thank you for your attention

Any questions?

KU LEUVEN