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RegaVir platform: Case discussions antiviral resistance testing

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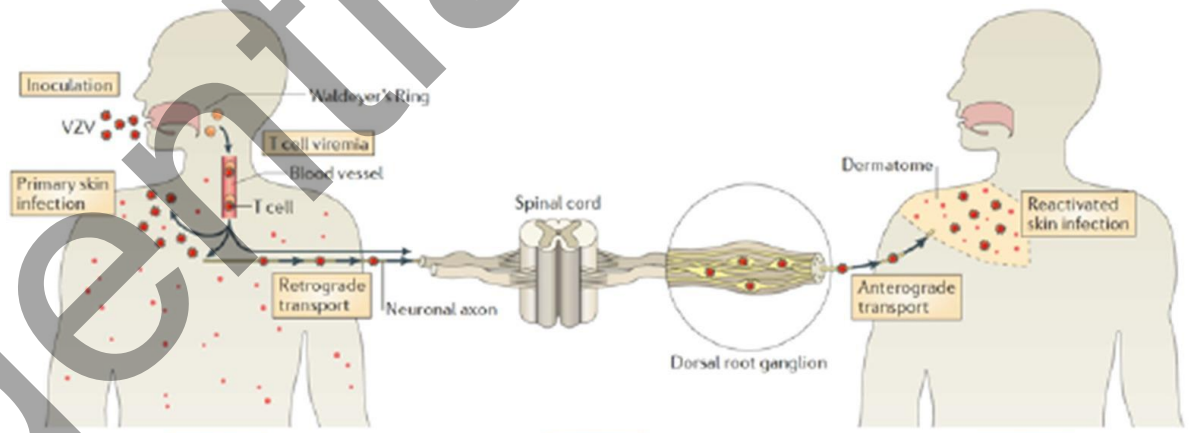
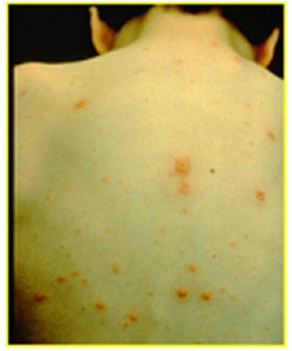
Leuven, March 28, 2023

Pathogenesis of **varicella-zoster virus (VZV)**

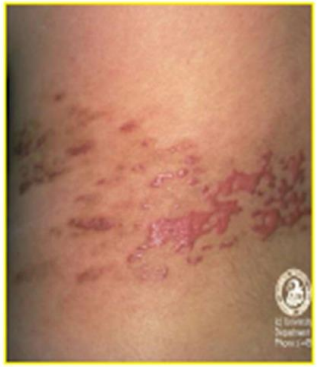
- Initial replication is in the respiratory tract.
- VZV infects epithelial cells, fibroblasts, T cells, and neurons.
- VZV can form syncytia and spread directly from cell to cell.
- VZV is spread by viremia to skin, causes lesions of chickenpox.
- VZV can escape antibody clearance, and cell-mediated immune response is essential to crontrol infection.





Varicella "Chicken pox"

- T cell tropism
- Skin tropism
- Neurotropism



Zoster "Shingles"

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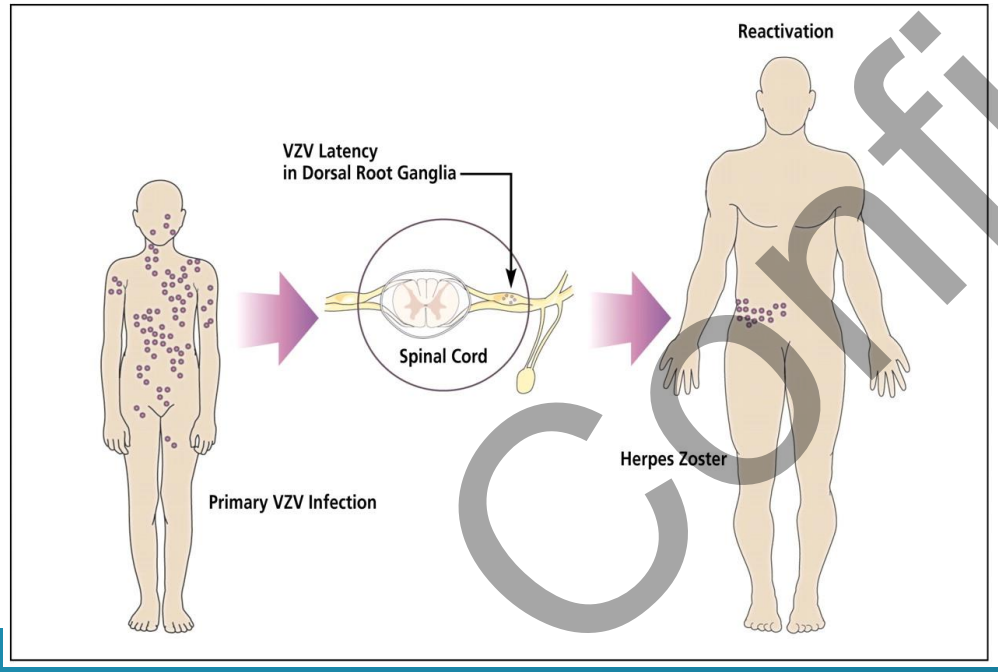
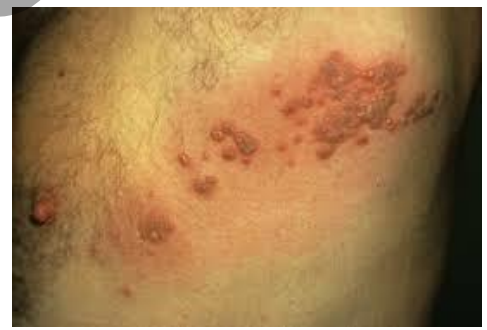
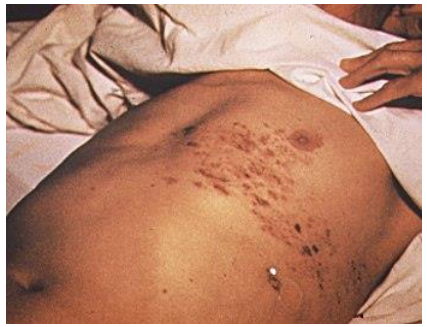
Pathogenesis of VZV

- Latency in ganglia: trigeminal cranial ganglia & multiple dorsal root ganglia adjacent to the spinal cord.
- Since varicella lesions can be present on all areas of the body, VZV has potential access to, and the ability to reactivate from, ganglia over the entire neuraxis.
- Herpes zoster is a recurrent disease; it results from virus replication along the entire dermatome.
- Typically, the virus remains latent in a dorsal root ganglion, and reactivation manifests as painful skin lesions following a dermatomal distribution.







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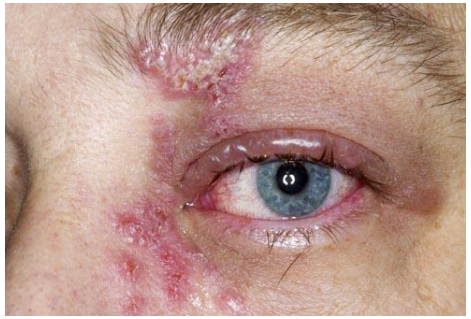
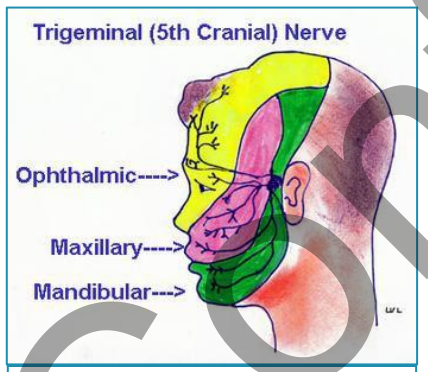
Pathogenesis of VZV

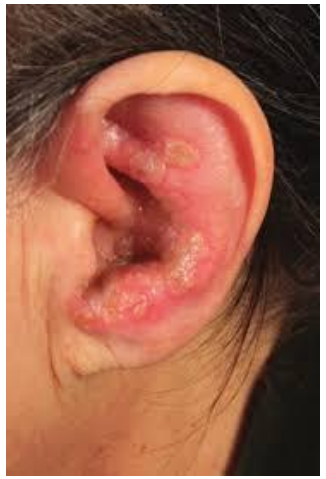


Herpes zoster ophthalmicus:
- accounts for 10-20% of cases of HZ infection.
- patients usually present with painful, vesicular, dermatomal rashes affecting the **ophthalmic division of the trigeminal nerve (V1).**

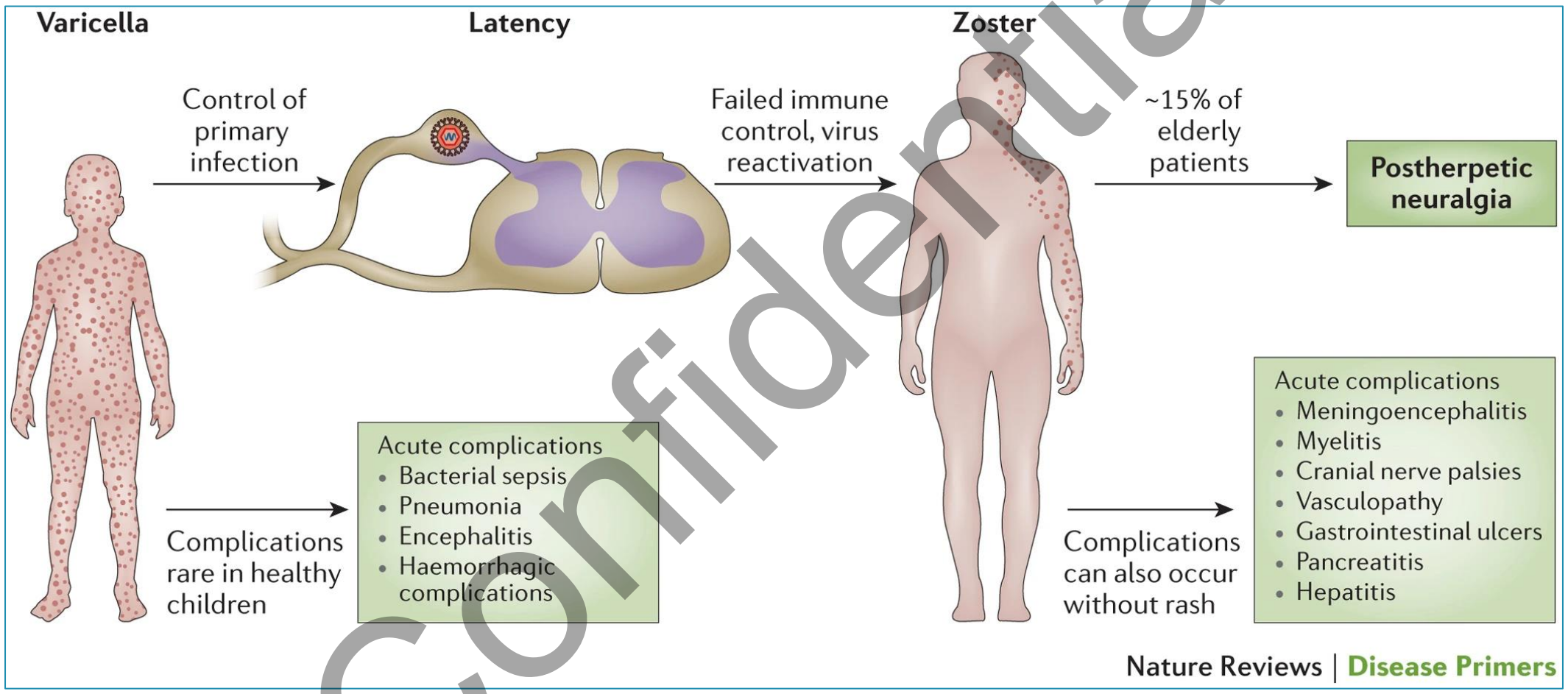


The three major sensory divisions of the trigeminal nerve

Herpes zoster octicus:
- uncommon manifestation of HZ
- affects the **8th cranial nerve ganglia and the geniculate ganglion of the 7th (facial) cranial nerve.**

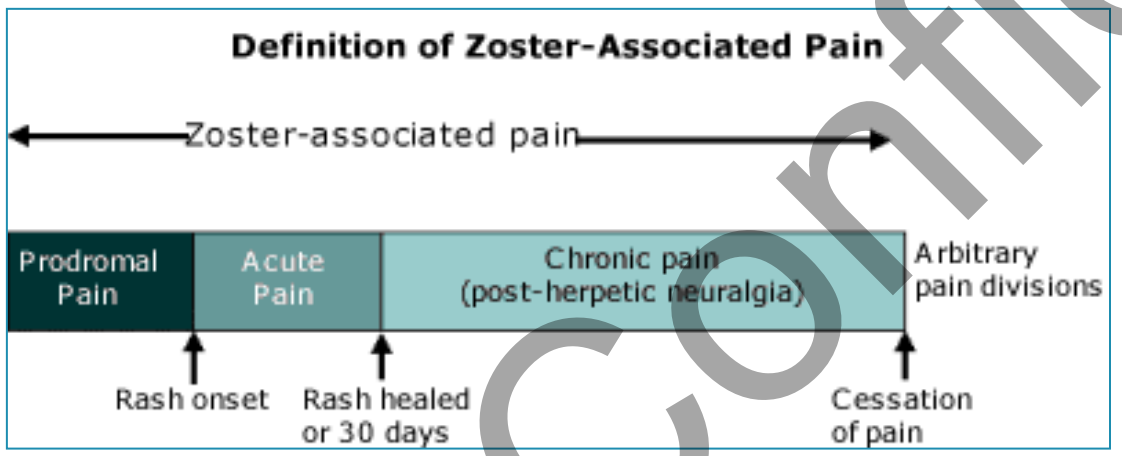


Different phases of VZV infection



VZV: who is at risk?

- Children (ages 5 to 9): mild classical disease.
- Teenagers and adults: more severe disease, potential pneumonia.
- Immunocompromised persons and newborns: life-threatening pneumonia, encephalitis, and **progressive disseminated varicella.**
- Elderly and immunocompromised people: recurrent disease (herpes zoster or shingles).
- Patients developing herpes zoster: post-herpetic neuralgia (PHN)



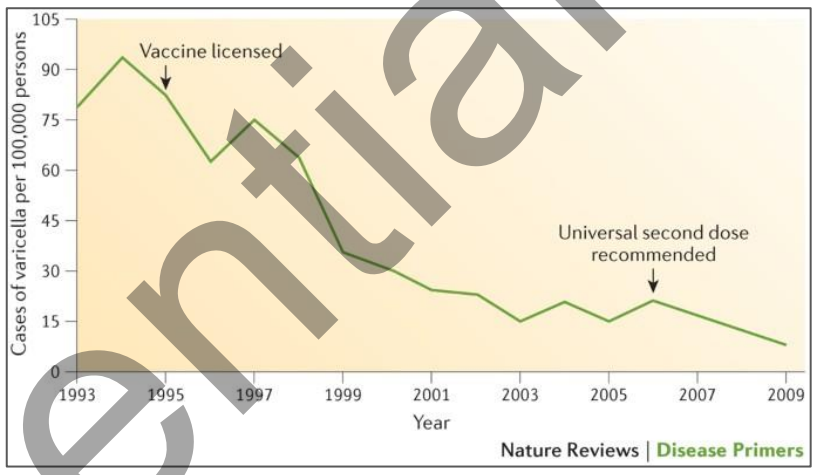
- Burning pain in nerves and skin.
- **Pain that lasts three months or longer after the shingles rash has healed.**
- Pain lasts long after the rash and blisters of shingles go away.

Risk factors for herpes zoster

Older age
CMI dysfunction (immunosuppressed individuals)
Diabetes
Female gender
Mechanical trauma
Psycological stress
White race

Control of VZV - Vaccines

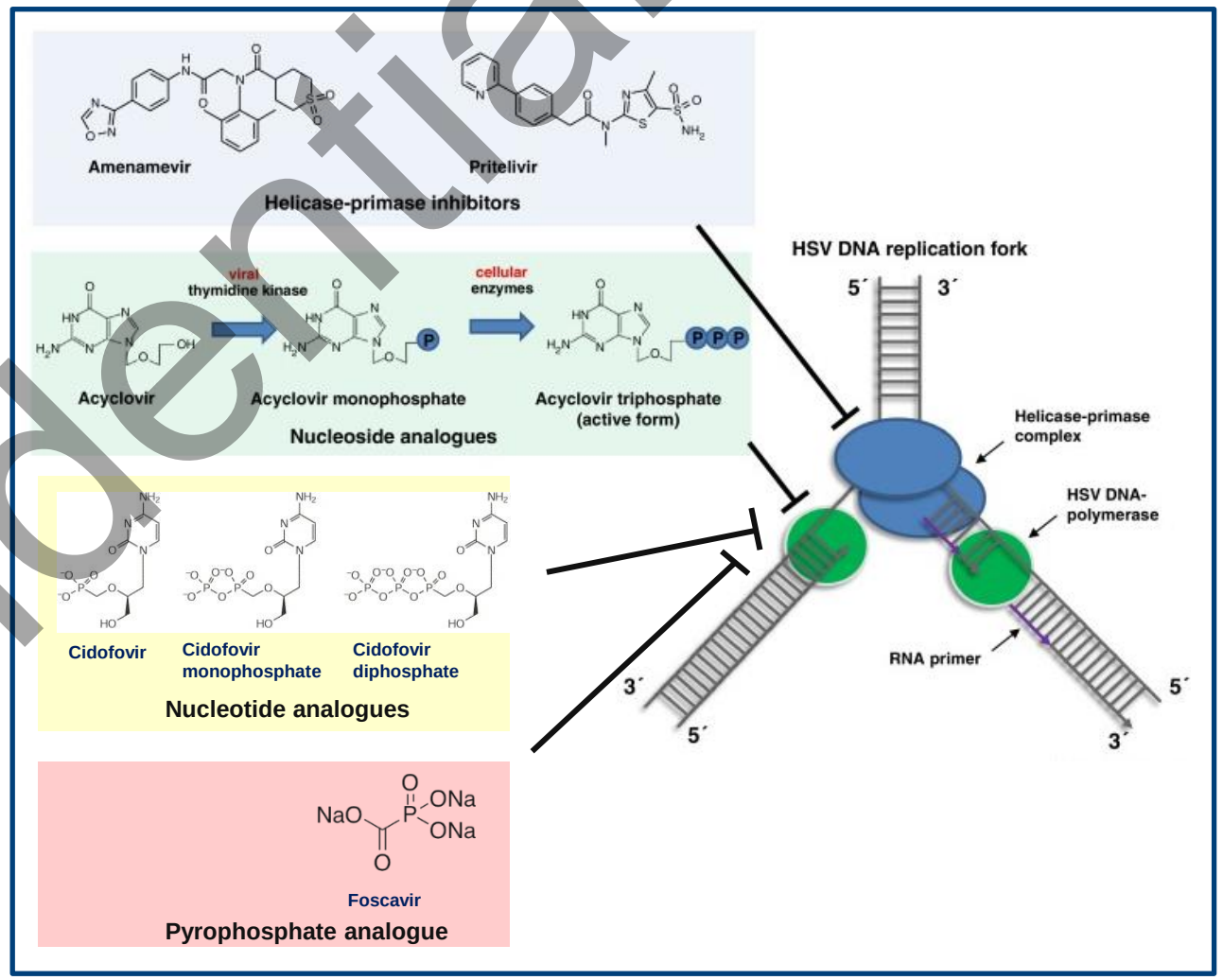
- **Prevention of varicella in children** – Live attenuated vaccine (Oka strain)
  - Merck (Varivax)
  - GSK (Varilrix)
- **Prevention of herpes zoster in the elderly:**
  - Life vaccine (Oka strain)
  - Subunit vaccine for the elderly



Zostavax	Shingrix
Merck	GSK
Live-attenuated vaccine	Non-live, recombinant subunit vaccine
Single subcutaneous infection	2 IM injections at 0 and 2-6 months
FDA approved: ≥50 years old CDC recommendation: ≥60 years old	FDA approved: ≥50 years old CDC recommendation: ≥50 years old
Recommended for the prevention of herpes zoster and related complications in adults aged ≥19 years† who are or will be **immunodeficient or immunosuppressed** because of disease or therapy	Contraindicated for immunocompromised patients
Efficacy against shingles prevention & PHN (age-dependent)	Efficacy against shingles prevention & PHN (age-independent)

Control of VZV

- Antiviral drugs are available:
  - ✓ Acyclovir / Valacyclovir
  - ✓ Penciclovir / Famciclovir
  - ✓ Brivudin (in some European countries)
  - ✓ Foscavir (only for drug-resistant virus)
  - ✓ Cidofovir (only for drug-resistant virus)
  - ✓ Amenavivir (only in Japan)



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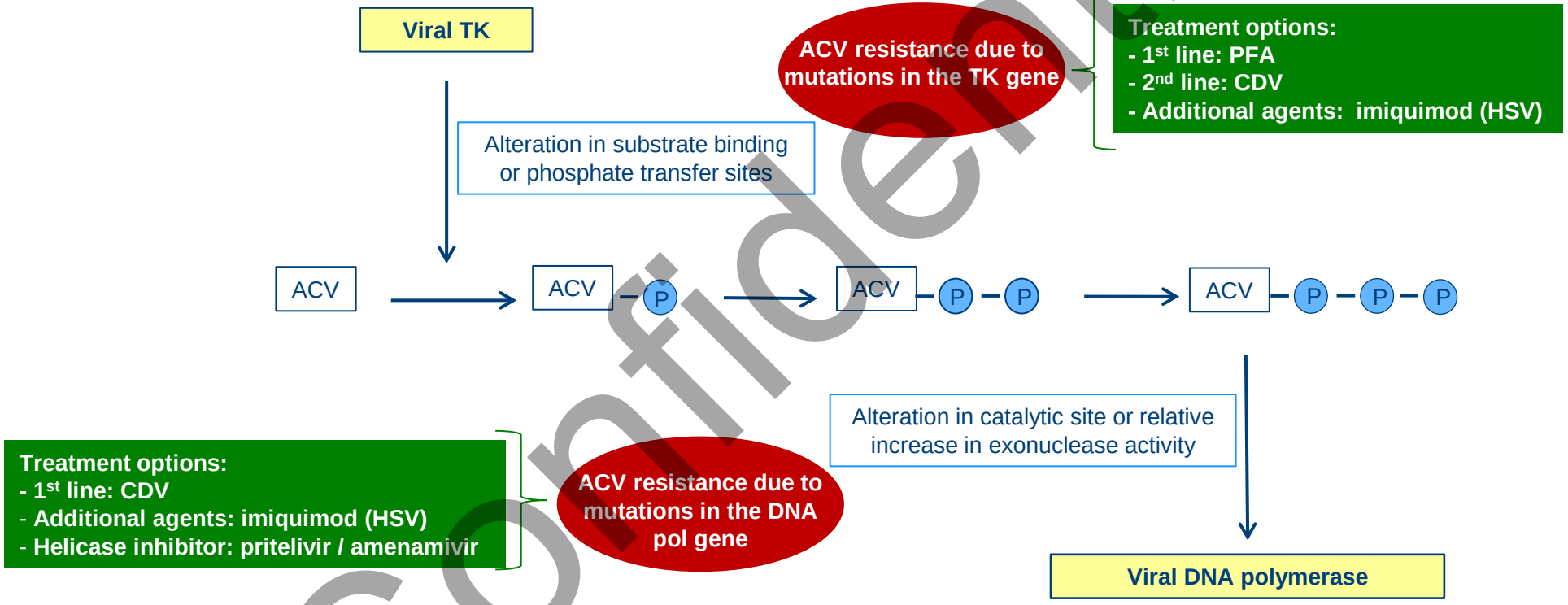
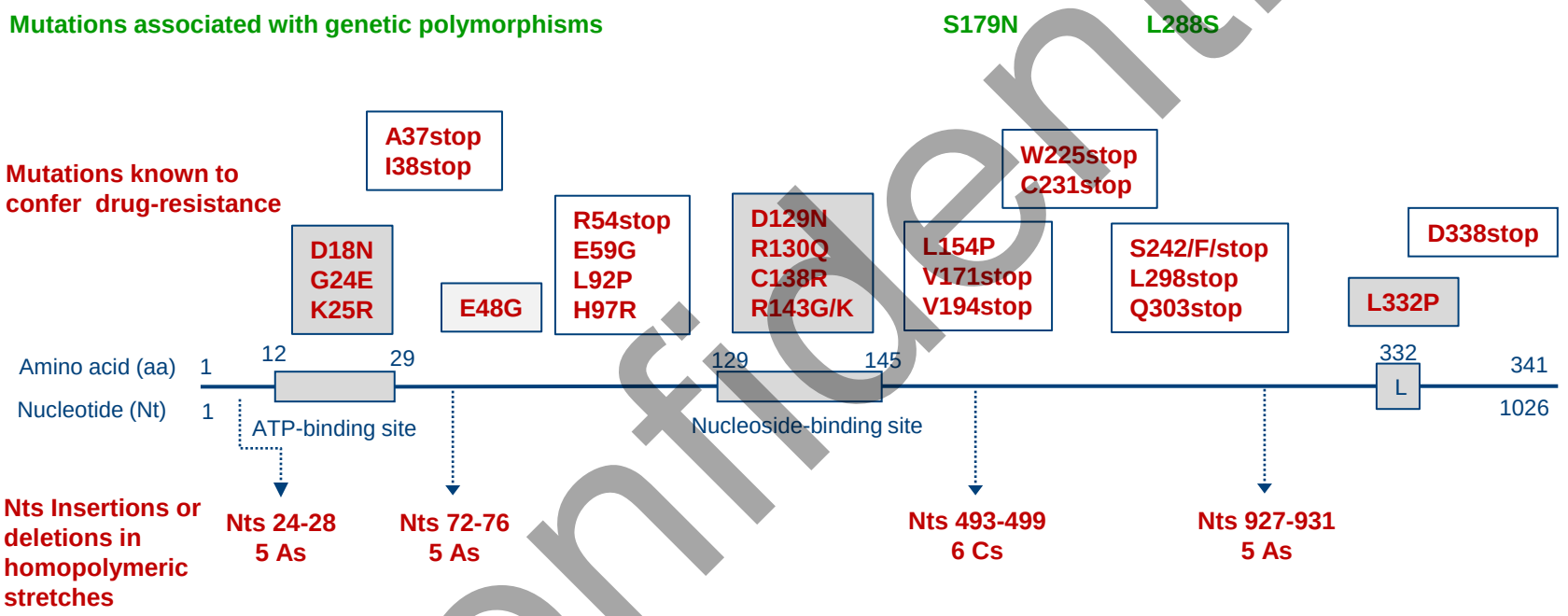
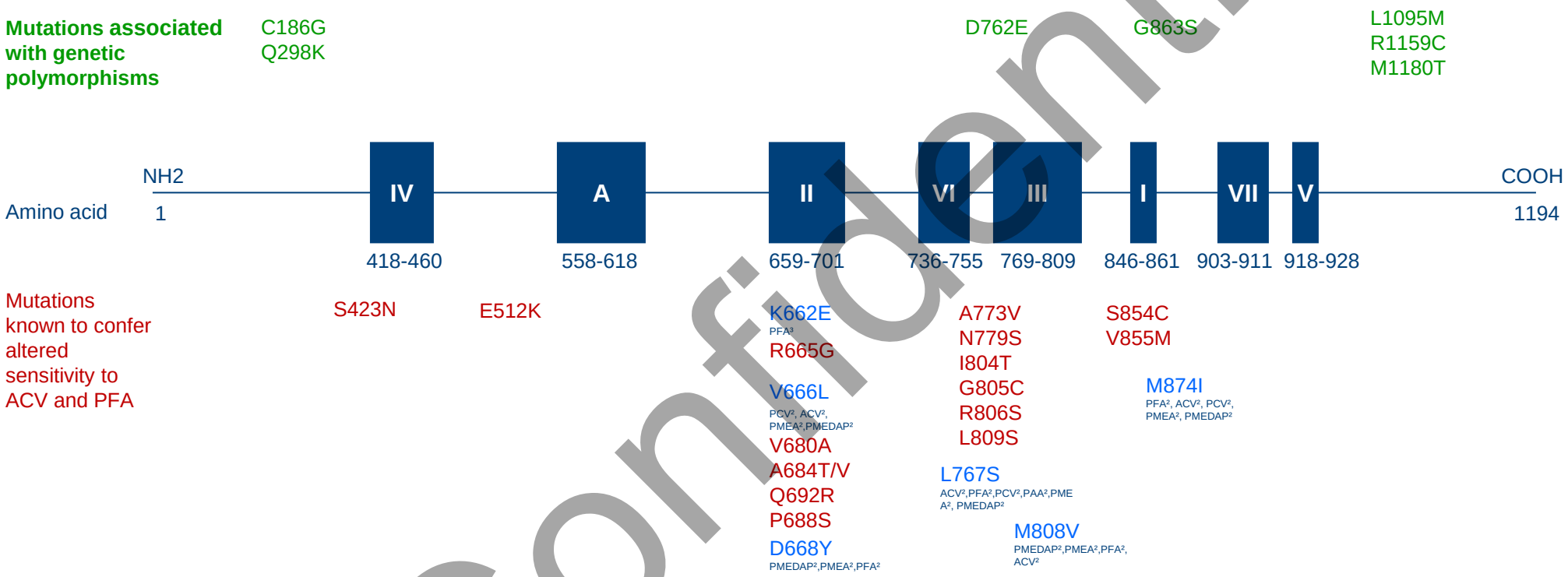
Mechanisms of drug-resistance in HSV and VZV



VZV thymidine kinase



VZV DNA polymerase



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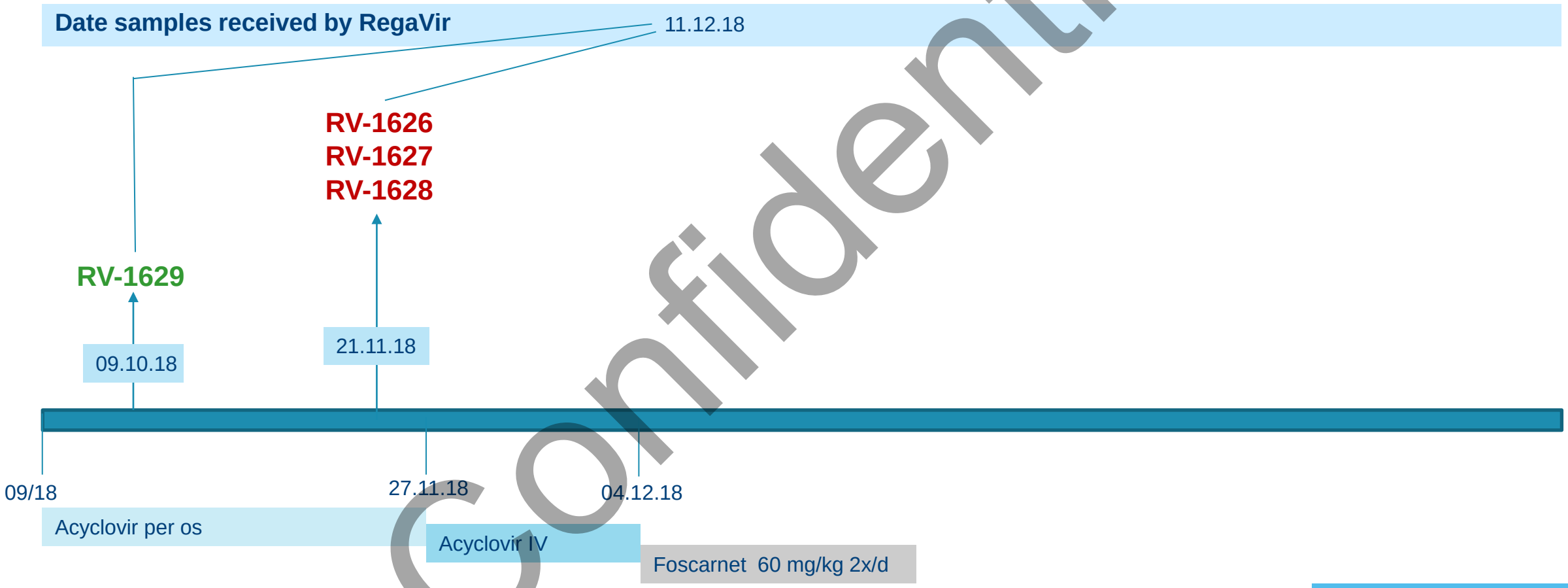
72-years old male patient with herpes zoster

- Patient from Uppsala University Hospital (Department of Infectious Diseases).
- DLBCL (Diffuse large B-cell lymphoma).
- CHOP treatment - cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin), and prednisone.
- Severely immunocompromised, received regularly Privigen (IVIg infusion therapy).
- Developed a generalized herpes zoster in September 2018.

72-years old male patient with herpes zoster

Generalized herpes zoster



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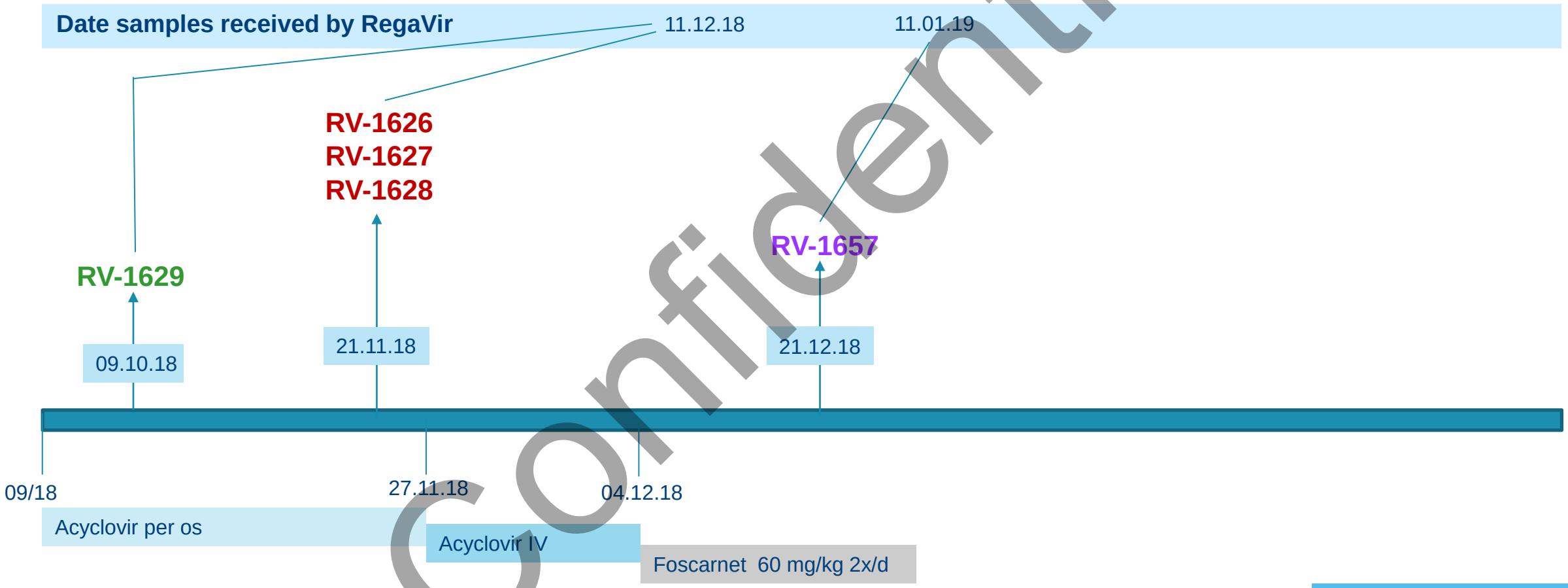
Analysis of samples received on 11.12.18

- PCR Ct VZV: 20-25

			Amino acid changes in ORF36 (thymidine kinase): 341 amino acids (complete sequence)		Amino acid changes in ORF28 (DNA polymerase): 1194 amino acids (partial sequence - all positions verified)	
	Date	Type	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs
RV-1629	09.10.28	Vesicle fluid	none	none	none	none
RV-1626	21.11.28	Vesicle fluid	none	**C insertion at Nts 493-498 (heterogeneous population)**	none	none (all positions verified)
RV-1627	21.11.28	Vesicle fluid	none	**C insertion at Nts 493-498 (homogeneous population)**	none	none (all positions verified)
RV-1628	21.11.28	Vesicle fluid	None	**R289 stop (homogeneous population)**	none	none (all positions verified)

72-years old male patient with herpes zoster

Generalized herpes zoster



Analysis of samples received on 11.01.19

- PCR Ct VZV: not tested

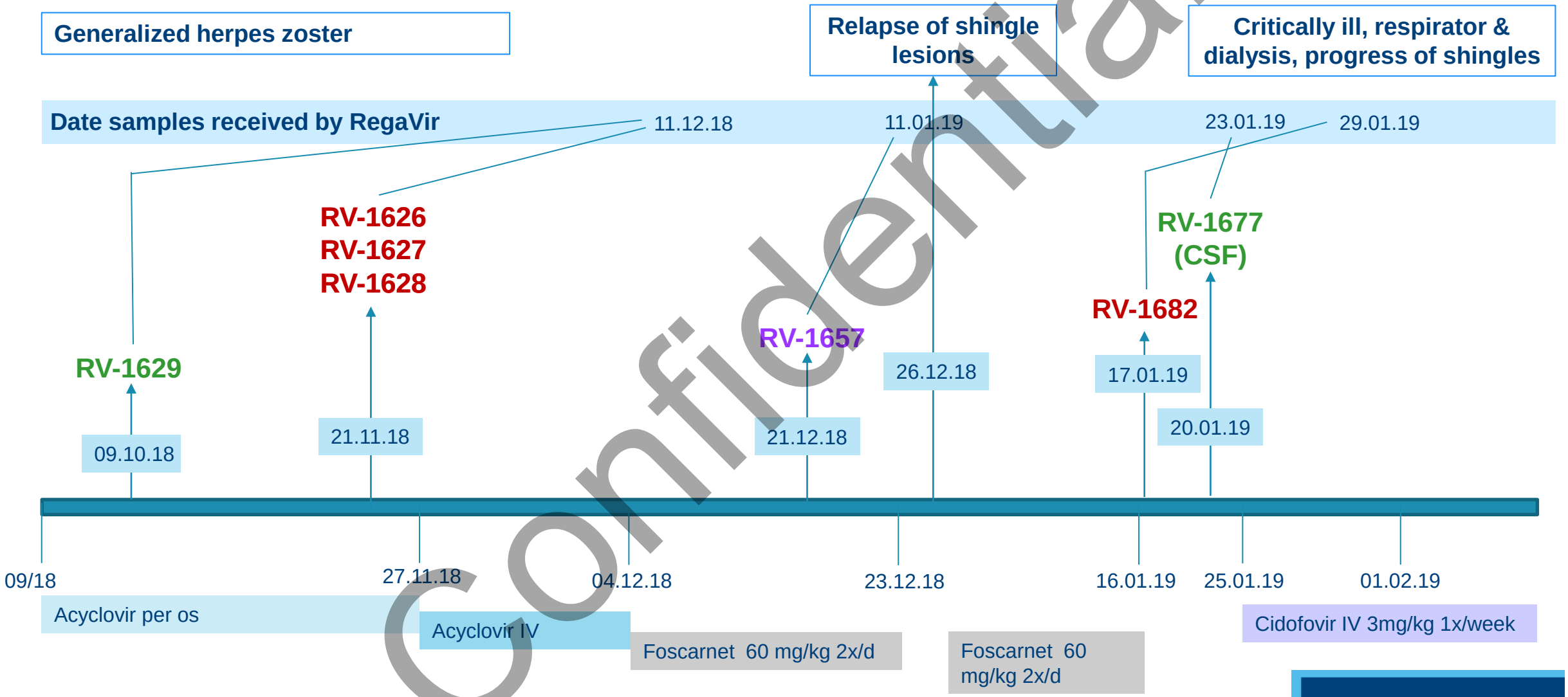
			Amino acid changes in ORF36 (thymidine kinase) 341 amino acids (complete sequence)		Amino acid changes in ORF28 (DNA polymerase) 1194 amino acids (partial sequence - all positions verified)		
	Date	Type	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Novel
RV-1657	21.12.18	Vesicle fluid right arm	none	none	none	none	R813G E872K (both detected as heterogeneous populations)

The **R-813** is conserved only among α-herpesviruses and no drug-resistance mutations in other herpesviruses have been described at homologous positions.

The **E-872** is not conserved among herpesviruses. However, the homologous positions in HSV have been found to be linked to ACV-R / PFA-R

- D907V (HSV-1)
- D912V (HSV-2).

72-years old male patient with herpes zoster

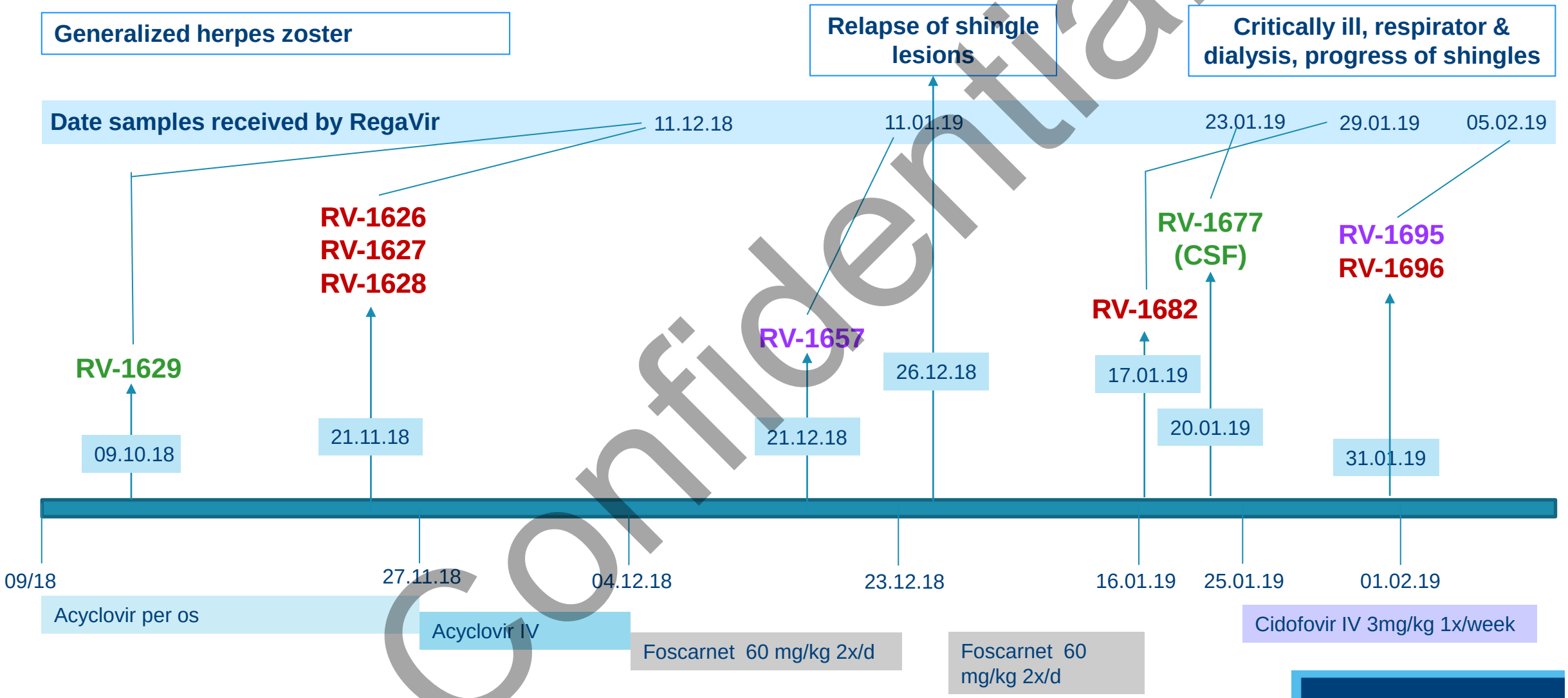


Analysis of samples received on 23.01.19 (RV-1677) & 29.01.19 (RV-1682)

- PCR Ct VZV: 22 (RV-1677) & unknown (RV-1682)

			Amino acid changes in ORF36 (thymidine kinase) 341 amino acids (complete sequence)		Amino acid changes in ORF28 (DNA polymerase) 1194 amino acids (partial sequence - all positions verified)		
	Date	Type	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Novel
RV-1677	20.01.19	**CSF**	none	none	none	none (all positions verified)	none (all positions verified)
RV-1682	17.01.19	Lesion swab	none	none	none	**R665G (ACV-R / PFA-R)**	none

72-years old male patient with herpes zoster



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Analysis of samples received on 05.02.19

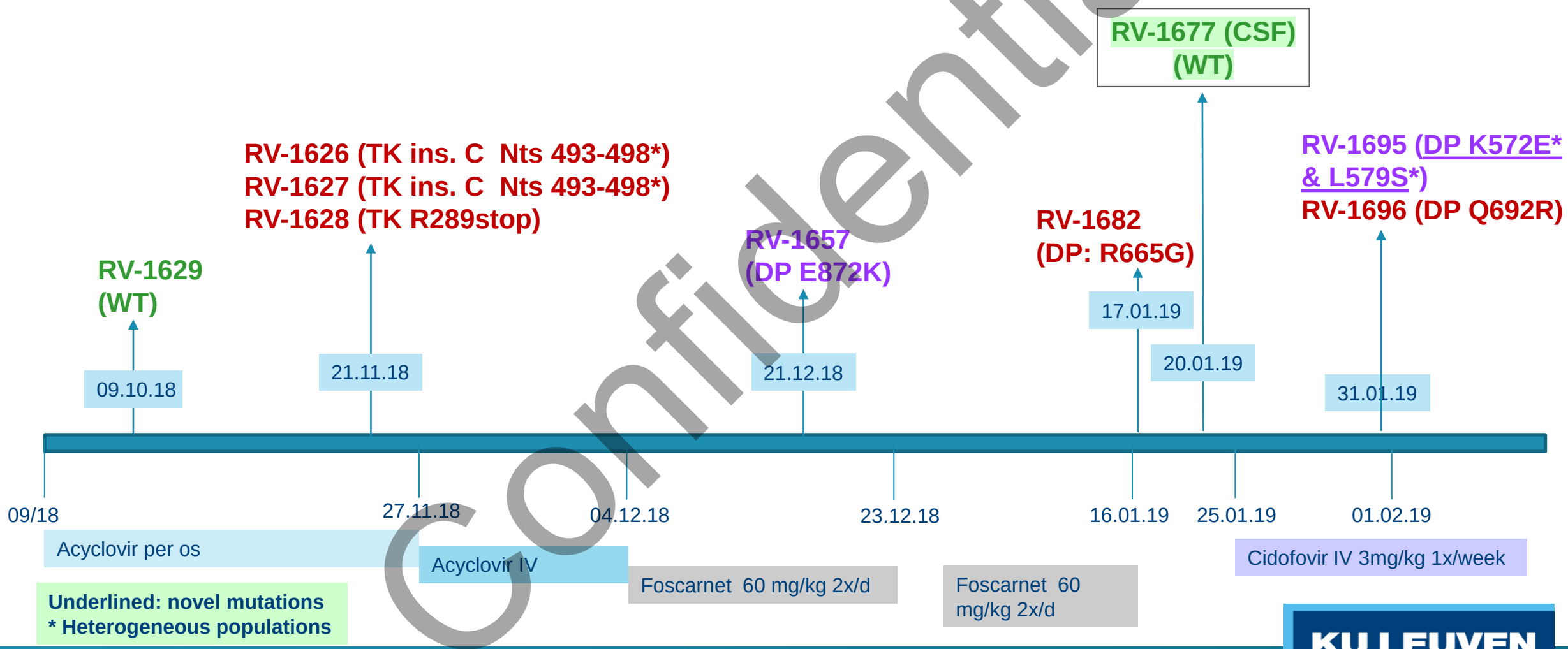
- PCR Ct VZV: unknown

			Amino acid changes in ORF36 (thymidine kinase) 341 amino acids (complete sequence)		Amino acid changes in ORF28 (DNA polymerase) 1194 amino acids (partial sequence - all positions verified)		
	Date	Type	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Novel
RV-1695	31.01.19	Lesion swab	none	none	none	none (all positions verified)	**K572E L579S (both as heterogeneous populations)**
RV-1696	31.01.19	Lesion swab	none	none	none	**Q692R (ACV-R / PFA-R)**	none

The K-572 and L-579 positions map to the conserved region A in the 3'-5'exonuclease domain of the viral enzyme.

Lys-572 and Leu-579 are conserved among α-herpesviruses. Therefore, these changes are expected to confer resistance to acyclovir and foscavir.

72-years old male patient with herpes zoster



Underlined: novel mutations
*** Heterogeneous populations**

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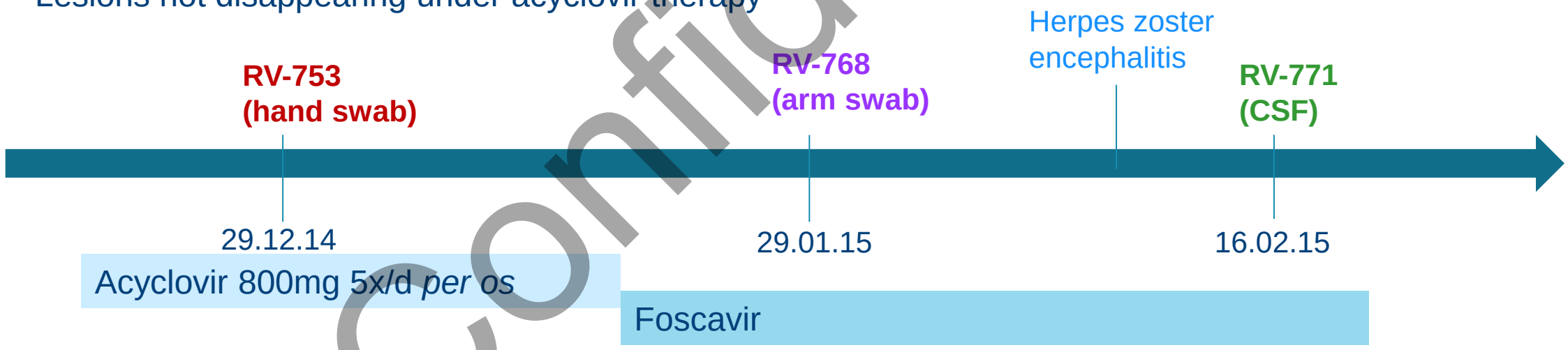
25-years old male patient with herpes zoster (UZ Brussel)

- Non-Hodgkin lymphoma (DLBCL)
- Herpes zoster (hand and arm)
- Antiviral treatment: acyclovir 800mg 5x/d *per os*
- Lesions not disappearing under acyclovir therapy



25-years old male patient with herpes zoster (UZ Brussel)

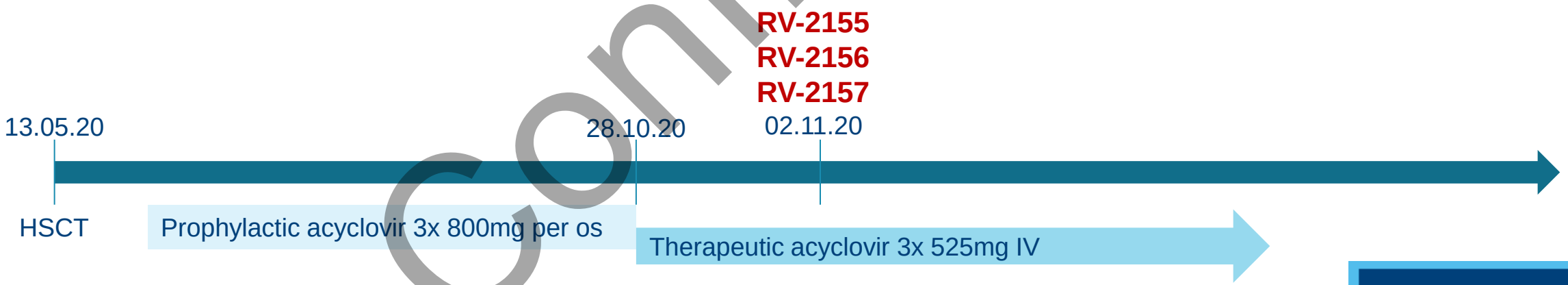
	Sample		Amino acid changes in ORF36 (thymidine kinase) 341 amino acids (complete sequence)		Amino acid changes in ORF28 (DNA polymerase) 1194 amino acids (partial sequence - all positions verified)		
	Date	Type	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Novel
RV-753 (06.01.15)	29.12.14	Hand swab	none	**G191stop (heterogeneous population)**	none	none	none
RV-768 (13.02.15)	29.01.15	Arm swab	none	none	none	none	**L579S**
RV-771 (23.02.15)	16.02.15	CSF	none	none	none	none	none

Phenotyping results

Sample		Acyclovir	Penciclovir	Brivudin	Ganciclovir	Foscavir	Cidofovir
RV-753 (hand swab, 29.12.14) **TK G191 stop**	EC_{50} (µg/ml)	**4.83 ± 1.48**	**2.30 ± 0.24**	**0.95 ± 0.16**	Not done	13.27 ± 0.88	0.18 ±0.02
Oka reference strain	EC_{50} (µg/ml)	0.4	0.21	0.036	Not done	13.8	0.2
	Fold-resistance	**12.1**	**11.0**	**26.4**	Not done	1.0	0.9
RV-768 (arm swab, 29.01.15) **DNA pol L579S**	EC_{50} (µg/ml)	**3.39 ± 1.76**	**3.13 ± 1.99**	0.0054 ± 0.0014	**1.13 ± 0.65**	**≥46.5 ± 8.5**	**0.58 ± 0.23**
Oka reference strain	EC_{50} (µg/ml)	0.61 ± 0.40	1.04 ± 0.56	0.0058 ± 0.0018	0.15 ± 0.11	14 ± 8.7	0.11 ± 0
	Fold-resistance	**5.6**	**3.0**	**0.9**	**7.5**	**≥3.3**	**5.3**

50-years old female patient with herpes zoster (AZ Delta Roeselare)

- HSCT 13.05.20
- Herpes zoster
- Antiviral treatment:
  - Prophylactic acyclovir 3x 800mg *per os* (24.09.20 → 28.10.20)
  - Therapeutic acyclovir 3x 525mg IV (29.10.20 →)
- Immunosuppressive treatment: Mycophenolate mofetil
- Multiple lesions under acyclovir therapy: 3 swab samples sent to RegaVir on 04.11.20



50-years old female patient with herpes zoster (AZ Delta Roeselare)

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	Date	Type	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Novel	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs
RV-2155	02.11.20	Mouth swab	none	none	**R17W* E259K* S285P***	none	none
RV-2156	02.11.20	Belly lesion swab	none	none	**S51L R54C L56F**	none	none (all positions verified)
RV-2157	02.11.20	Vulvar lesion swab	none	none	**T deletion Nts 414-417* 2C's deletion Nts 904-907***	none	none (all positions verified)

* Heterogeneous populations

68 years old male patient with ALL

- Hôpital de la Citadelle - Site Citadelle (CHR)
- **ALL**
- **Patient under Campath® (Alemtuzumab)**, a recombinant DNA-derived humanized monoclonal antibody (Campath-1H) that is directed against the 21-28 kD cell surface glycoprotein, CD52.
- **Herpes zoster** (skin lesions)
- **Antiviral treatment:** acyclvoir 500 mg 4x/day iv → foscavir 5April 2012)

68 years old male patient with ALL

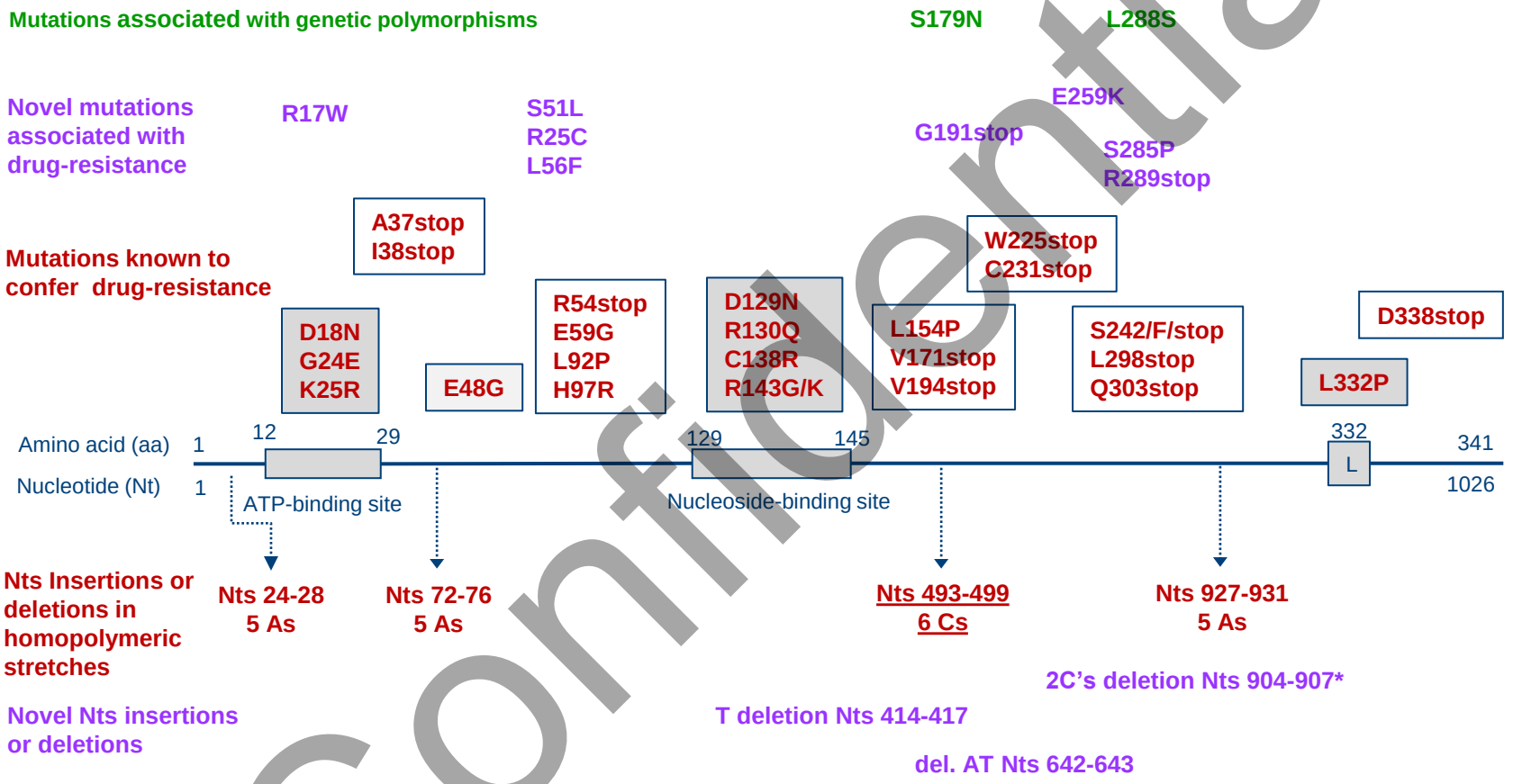
	Sample		Amino acid changes in ORF36 (thymidine kinase) 341 amino acids (complete sequence)		Amino acid changes in ORF28 (DNA polymerase) 1194 amino acids (partial sequence - all positions verified)	
	Date	Type	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs	Known to be related to genetic polymorphism (inter-strain variability)	Known to be associated with resistance to antiviral drugs
RV-337	23.03.12	Skin lesion swab	None	**AT deletion at Nts 642-643**	none	none
RV-356	16.04.12	Skin lesion biopsy	none	none	none	**A684T**
RV-365	03.05.12	CSF	none	none	none	none
RV-370	09.05.12	CSF	Not amplifiable		none	none
RV-371	09.05.12	Skin lesion biopsy	none	none	none	none
RV-374	22.05.12	CSF	Not amplifiable			

Phenotypic characterization RV-356

Sample		Acyclovir	Brivudin	Cidofovir	Foscavir	Adefovir
RV-356 (DNA polymerase: A684T	EC_{50} (µg/ml)	**4.29**	0.0069	0.038	**75.04**	**3.3**
		3.06	0.014	0.10	**89.4**	**3.99**
		6.34	0.018	0.072	**40**	**4.89**
		4.0	0.018	0.12	**89.4**	**5.47**
Oka (VZV reference strain)	EC_{50} (µg/ml)	0.305	0.088	0.185	11.31	0.45
	Fold resistance	**14.5**	**0.2**	**0.4**	**6.5**	**9.8**

EC_{50}: 50% effective concentration or concentration of compound required to reduce viral plaque formation by 50% in human embryonic lung fibroblasts.

VZV thymidine kinase



Known drug-resistance mutations found by RegaVir are underlined

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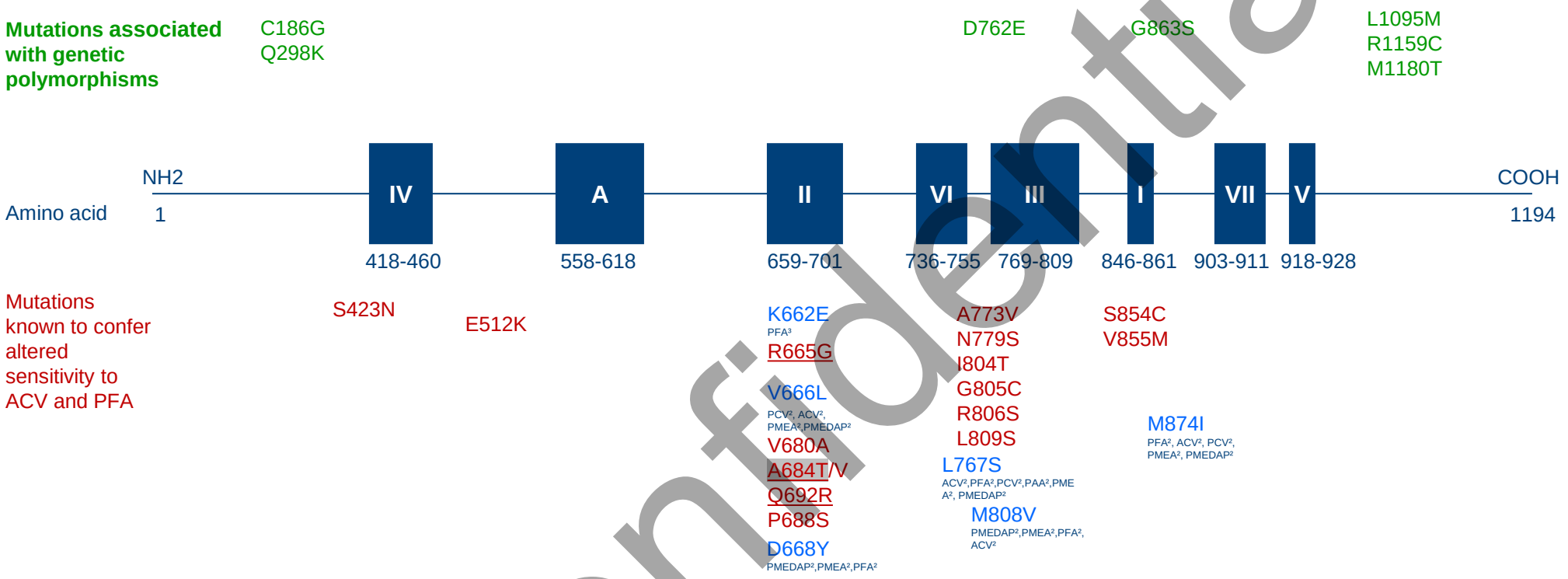
VZV DNA polymerase



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