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First description of letermovir resistance mutation in *UL51* gene from a HSCT-patient and study of its impact on the terminase complex structure

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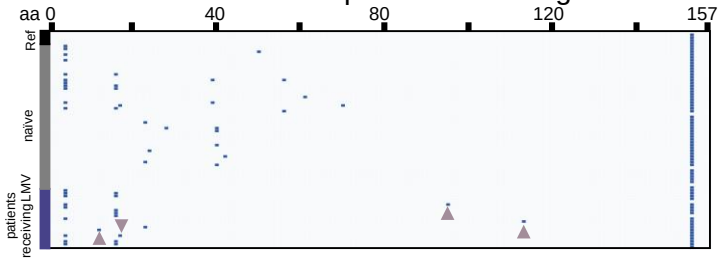
Purpose

- **Letermovir** (LMV) is a human cytomegalovirus (HCMV) terminase inhibitor indicated as prophylaxis for **HCMV-positive stem-cell recipients**.
- Its mechanism of actions involves at least the **viral terminase proteins** pUL56, pUL89 and pUL51. Despite its efficiency, **resistance mutations** were characterized *in vitro* and *in vivo*, largely focused on pUL56. To date, **the involvement of pUL51 in clinical resistance remains to be demonstrated**.

1-Identification of pUL51 polymorphism

- Polymorphism study by sequencing ***UL51*** from 77 strains (Next generation sequencing method):
  - **5** reference (ref) strains / **56** naïve strains / **16** strains from patients receiving LMV.



- Identification of 4 undescribed mutations: **D12E, 17del, A95V, V113L.** 17del is on a known polymorphism position.
- The clinical strain with A95V in UL51 has also a resistance mutation in UL56 (**L257I**)

2-Impact of theses mutations on LMV activity

- **Recombinant viruses** (BAC technology) building to measure the impact of the mutations on LMV activity.

Strains	Genotype		EC50 nM LMV			
	UL51	UL56	Mean	SD[1]	N[2]	Ratio[3]
AD169	WT	WT	**2.123**	**0.633**	**7**	**1.0**
AD169	D12E	WT	3.018	0.373	3	1.4
AD169	A95V	WT	**29.246**	**0.788**	**4**	**13.8**
AD169	V113L	WT	3.206	0.453	4	1.5
AD169	A95V	L257I	**271.39**	**41.05**	**5**	**127.8**

[1] Standard deviation of EC50 values
[2] Number of replicates of testing in triplicates (over at least 3 separate dates)
[3] Ratio of EC50 value to that of wild type control strain

- D12E and V113L do not confer LMV resistance
- A95V confers **13.8-fold** increase LMV resistance by it self
- The mutant combining UL51-A95V and UL56-L257I has an EC50 of **271.39±41.05 nM**.

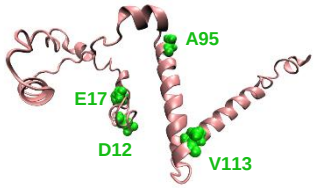
Conclusion

- A single **mutation** in pUL51 can lead to **LMV resistance**
- It is essential to **systematically sequence** the 3 genes encoding the complex terminase.
- With terminase modelling, we make the hypothesis that **LMV could bind to domains were both mutations were localized.**

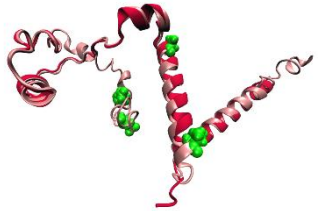
3-Protein modelling

- **Homology protein modelling** of **pUL51** and structural alignment of pUL51 and HSV-1 pUL33 from PDB:6M5R.

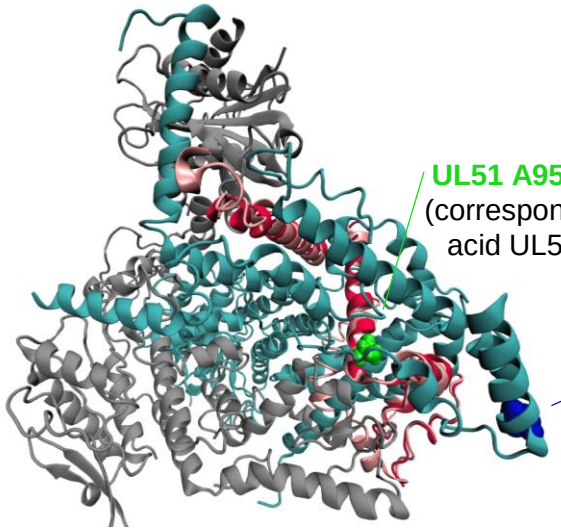
pUL51 model



Alignment pUL33 and pUL51



- To **localize** both mutations in a tridimensional space, we use the **coordinates of HSV-1 terminase model** and the mutations in the homologous proteins were reported



UL51 A95 and **UL28 D253**, (corresponding to the amino acid UL56 L257) are close together

HSV-1 pUL28
HSV-1 pUL15
HSV-1 pUL33
HCMV pUL51