# Surveillance of CMV resistance to antivirals in France 14 years survey and focus on new antivirals

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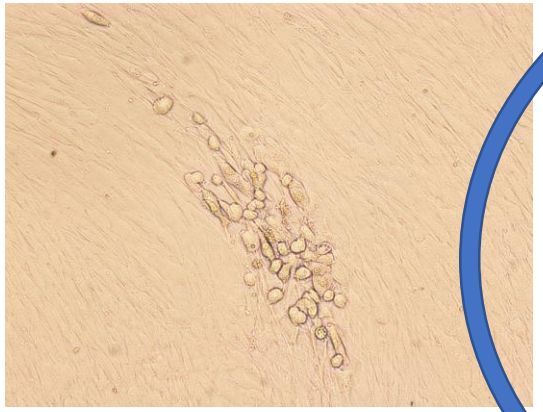
# CMV resistance to current antivirals remains a concern in immunocompromised patients

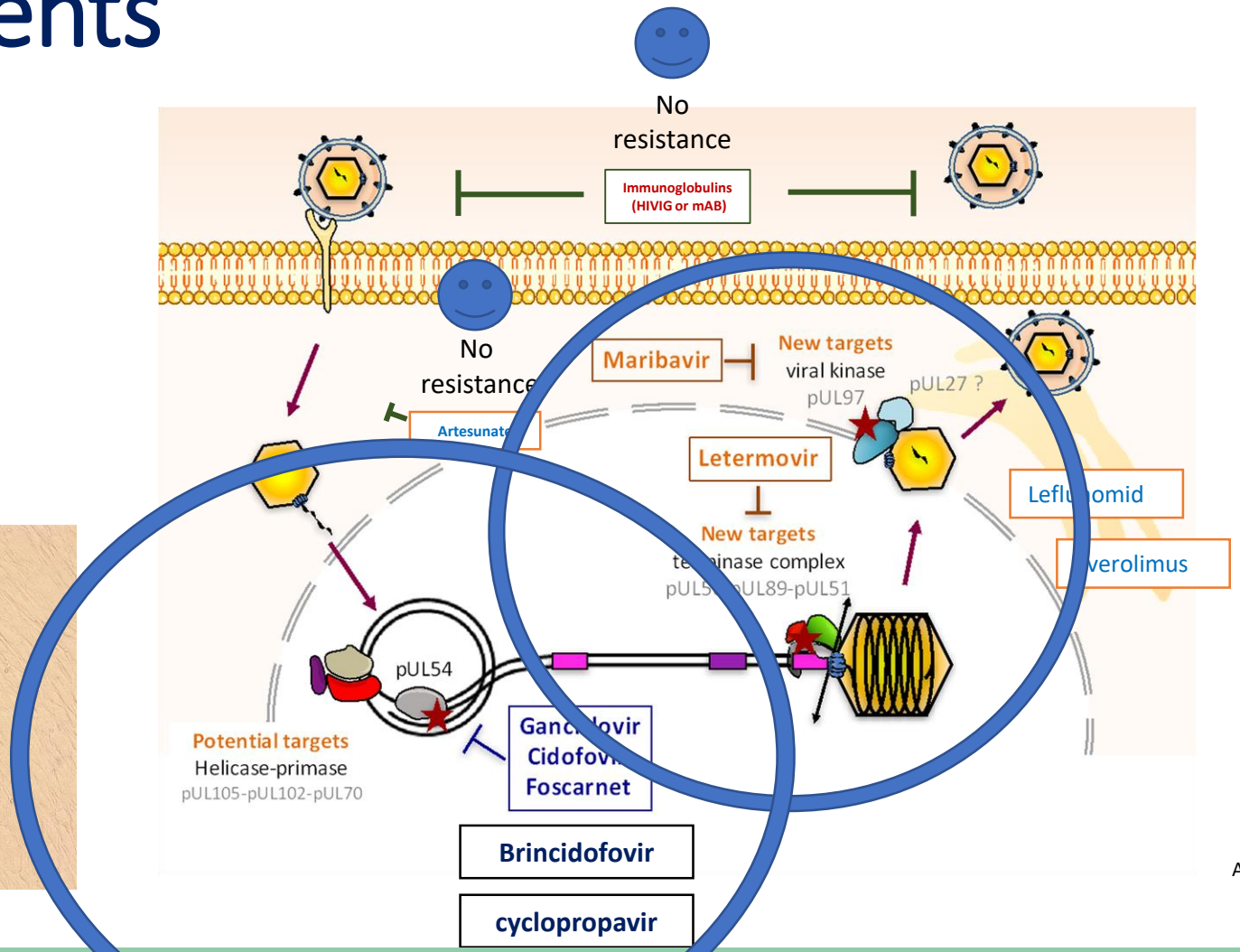
- Prevalence in treated patients :
- Solid organ recipients : (updated from the 2017 consensus)
  - 5% to 12% overall
  - Up to 18% in lung transplant recipients
  - 31% in intestinal and multivisceral organ transplant recipients
  - 0% to 3% range, for 100 to 200 days of ganciclovir or valganciclovir prophylaxis in D+/R- kidney recipient
  - 4% after valaciclovir prophylaxis

- Stem cell recipients :
  - 1- 3% (Orphavic French cohort and Campos 2017- 7/22 NR- in Portugal).

(Lurain, 2001 ; Alain 2004, Limaye, 2000 ; Boivin, 2004, 2009 ; Humar, 2005 ; Gruber, 2005, Hantz, 2009, Hantz 2010, Boivin 2010, Boivin 2012, Schubert BMJ 2013, kotton 2013, Campos 2016, Fisher 2017, Kotton 2017 and French CNR Data

# Mechanisms of resistance to current treatments






No résistance

Adapted from Ligat et aL, FEMS Microbiology reviews, 2018

# Resistance survey network in France

- **Network:**
  - National Reference Center laboratoriesLimoges and Paris (90%) and two hospital laboratories (Nantes and Paris)

- **Genotyping indication: Refractory infections**
  - **Before 2018:** viral replication persisting for more than 3 weeks on treatment

  (CNR recommendations, Hantz et al. 2010 and ORPhaViC cohort)
  - **Since 2018:** «viral replication persisting for more than 15 days in a previously treated patient "

  (International consensus 2017 and IDSA recommendations)

- **Sanger sequencing of full-length genes**
  - UL97, UL54
  - UL97/UL27
  - UL56/UL89 since 2018

- **Expert/participants for the annual QCMD Quality control**

*Clinical Infectious Diseases*
SPECIAL SECTION/INVITED ARTICLE

## Definitions of Resistant and Refractory Cytomegalovirus Infection and Disease in Transplant Recipients for Use in Clinical Trials

Roy F. Chemaly,[1] Sunwen Chou,[2] Hermann Einsele,[3] Paul Griffiths,[4] Robin Avery,[5] Raymund R. Razonable,[6] Kathleen M. Mullane,[7] Camille Kotton,[8] Jens Lundgren,[9] Takashi E. Komatsu,[10] Peter Lischka,[11] Filip Josephson,[12] Cameron M. Douglas,[13] Obi Umeh,[14] Veronica Miller,[15] and Per Ljungman[16,17]; for the Resistant Definitions Working Group of the Cytomegalovirus Drug Development Forum

**Table 2. Summary of the Definitions of Refractory Cytomegalovirus Infection and Disease and Antiviral Drug Resistance for Use in Clinical Trials**

| Term | Definition |
|---|---|
| Refractory CMV infection | CMV viremia that increases[a] after at least 2 wk of appropriately dosed antiviral therapy |
| Probable refractory CMV infection | Persistent viral load[b] after at least 2 wk of appropriately dosed antiviral therapy |
| Refractory CMV end-organ disease | Worsening in signs and symptoms or progression into end-organ disease after at least 2 wk of appropriately dosed antiviral therapy |
| Probable refractory CMV end-organ disease | Lack of improvement in signs and symptoms after at least 2 wk of appropriately dosed antiviral drugs |
| Antiviral drug resistance | Viral genetic alteration that decreases susceptibility to one or more antiviral drugs[c] |

Abbreviation: CMV, cytomegalovirus.

[a]More than 1 $\log_{10}$ increase in CMV DNA levels in blood or serum and determined by $\log_{10}$ change from the peak viral load within the first week to the peak viral load at ≥2 weeks as measured in the same laboratory with the same assay.

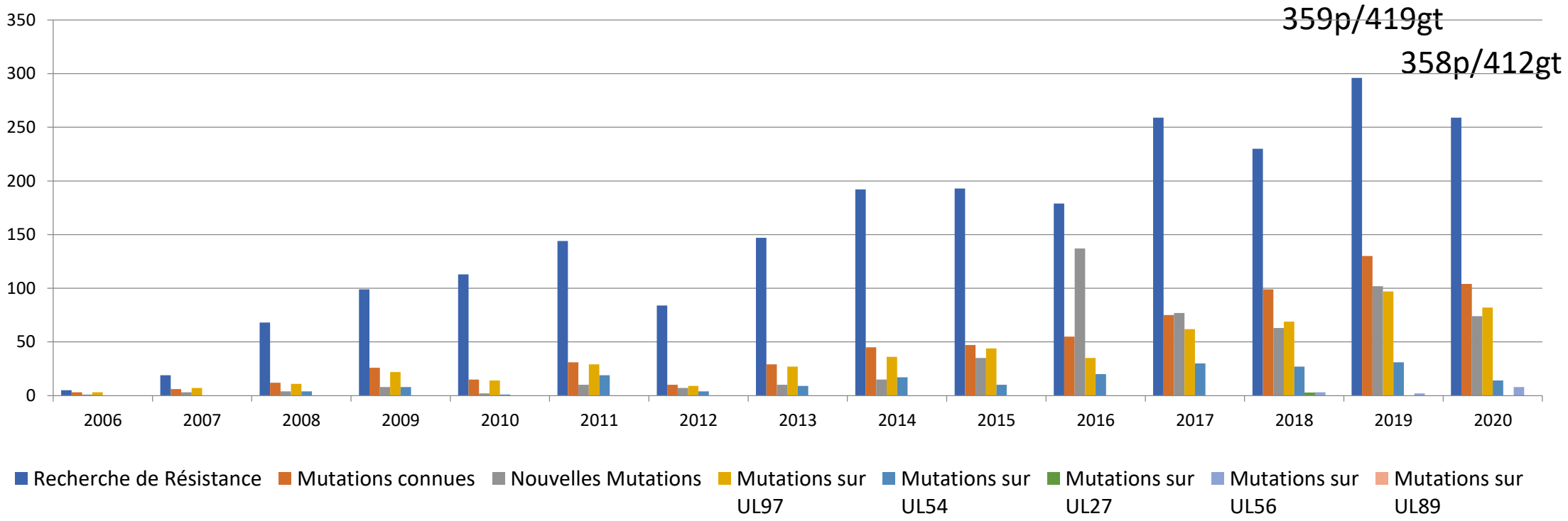
[b]CMV viral load at the same level or higher than the peak viral load within 1 week but <1 $\log_{10}$ increase in CMV DNA titers done in the same laboratory and with the same assay.

[c]Known examples involve genes involved in antiviral drug anabolism (eg, UL97-mediated phosphorylation of ganciclovir), the antiviral drug target (eg, UL54, UL97, UL56/89/51), or compensation for antiviral inhibition of biological function (eg, UL27).

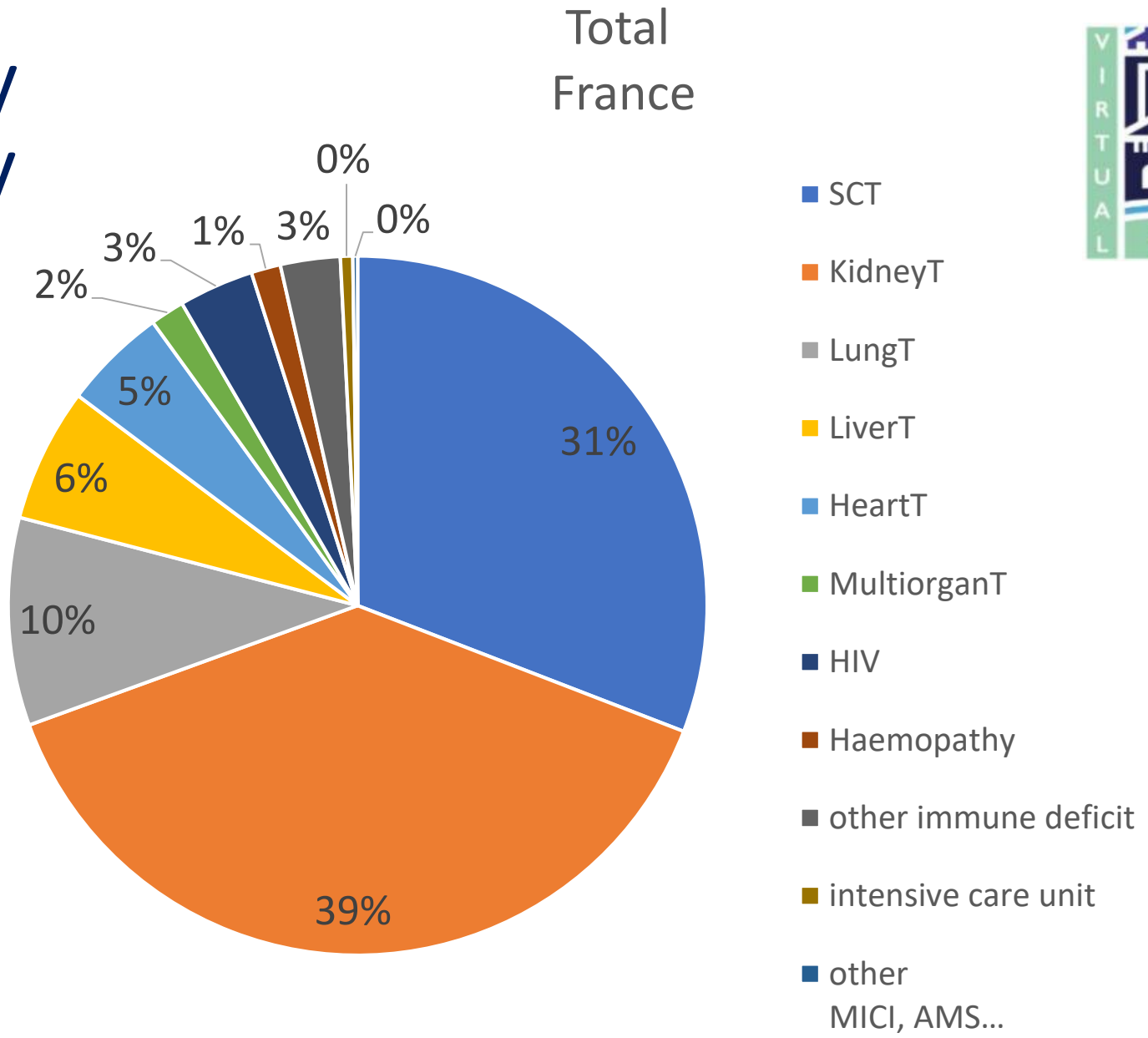
# Global results 2006-2020

No major impact of Covid19 on the burden of resistance

Out of 2287 genotypes performed at the University Hospital of Limoges,
687 (30%) showed the presence of resistance mutations,
547 in UL97 (79.6%), 194 in UL54 (28.2%), 13/181 (7,2%) since 2018 in UL56, 0 in UL89.



# Proportion of refractory infections by pathology

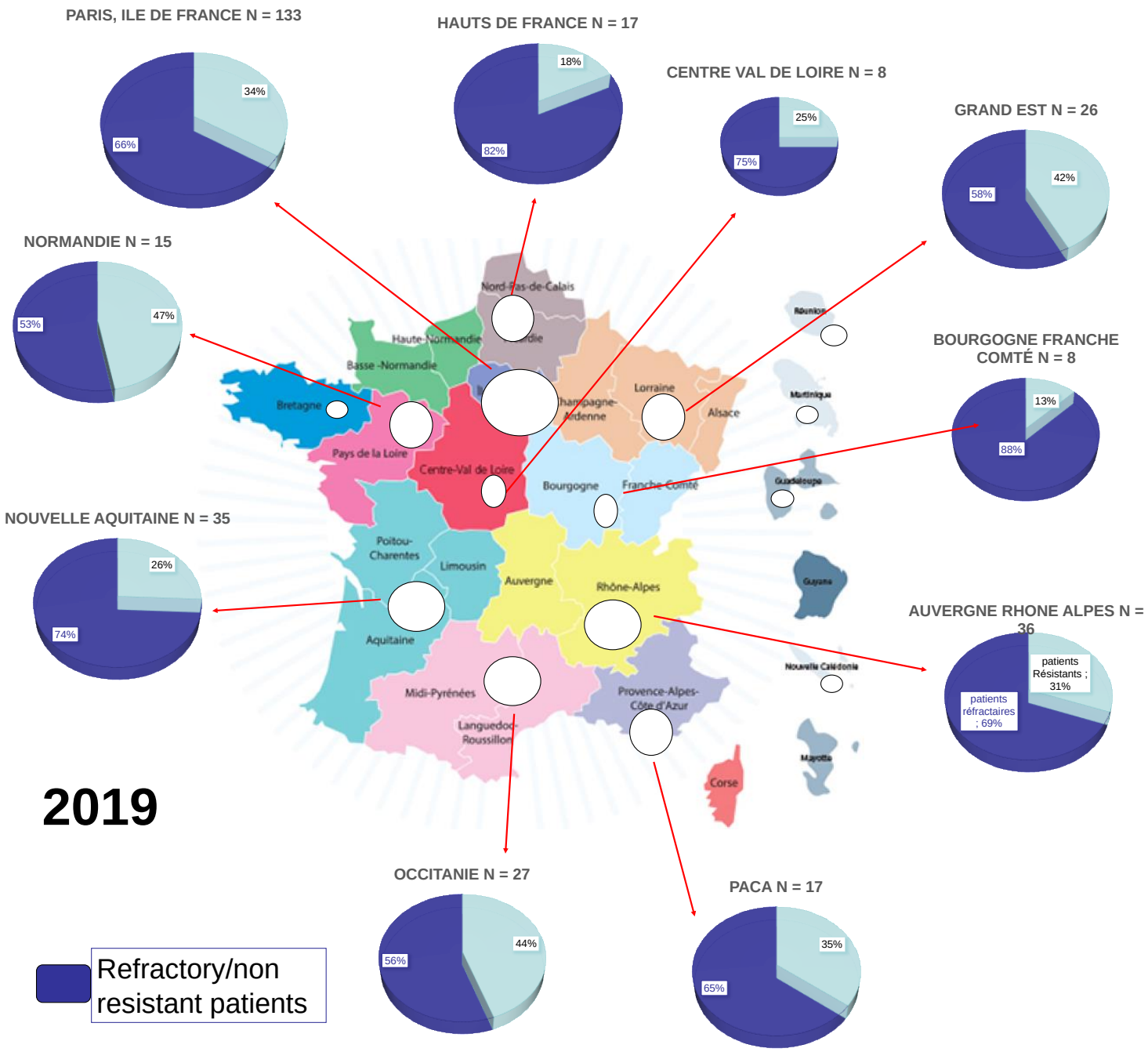



2278 resistance genotyping

2010-2020

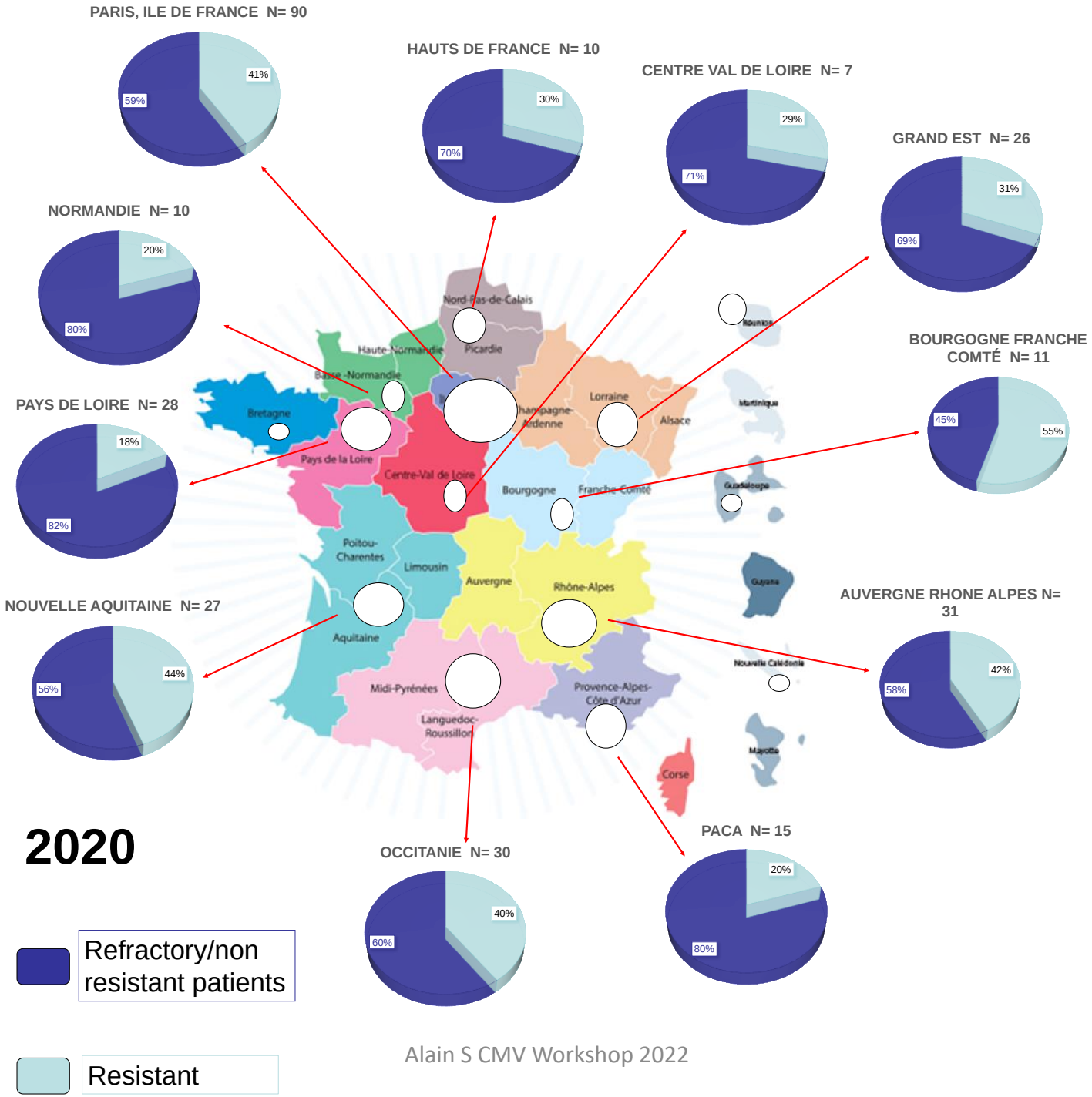
2019-2020
831 Genotypes
274 resistance cases

# Proportion of antiviral resistance amongst refractory patients 2019 : 32 %

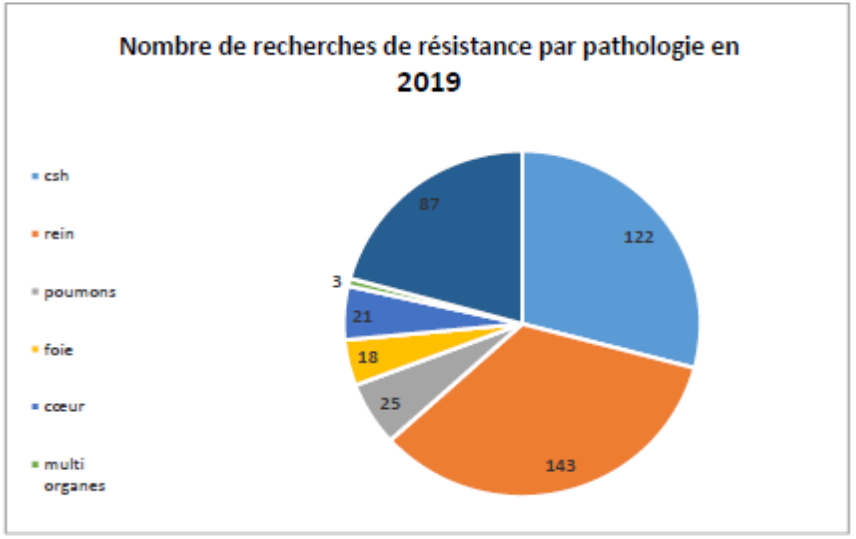


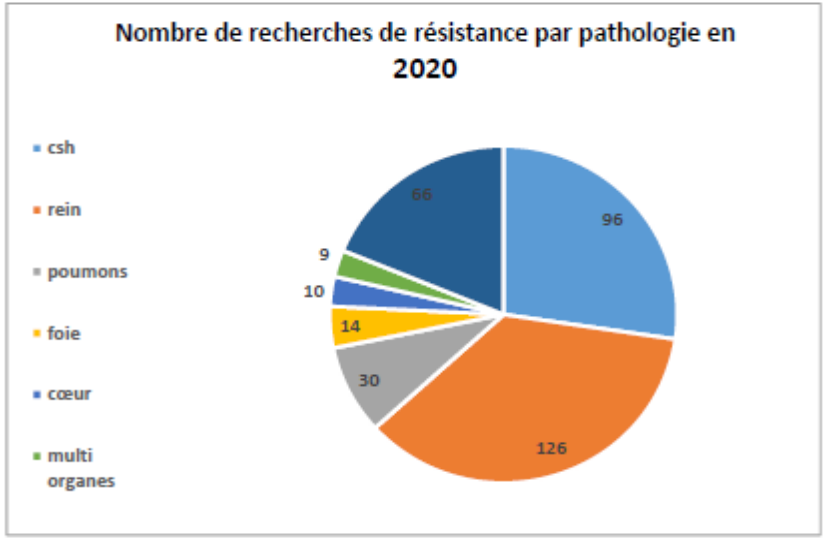
# Proportion of virological resistance amongst refractory patients

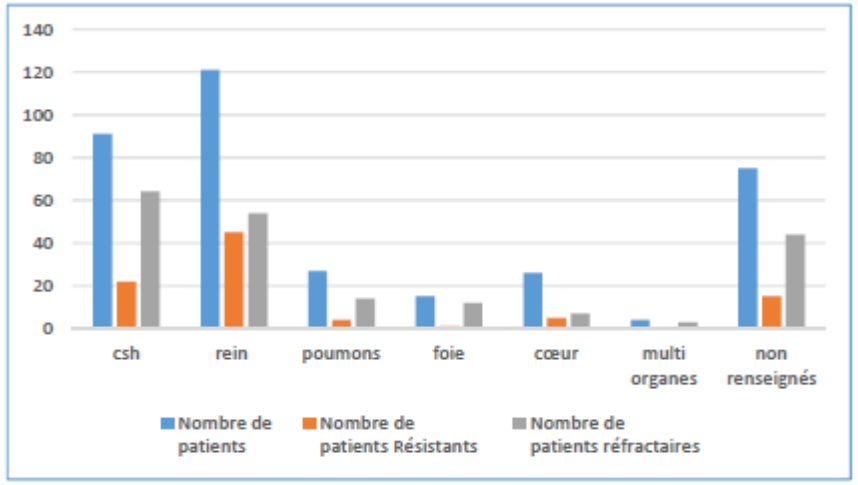
2020: 34%

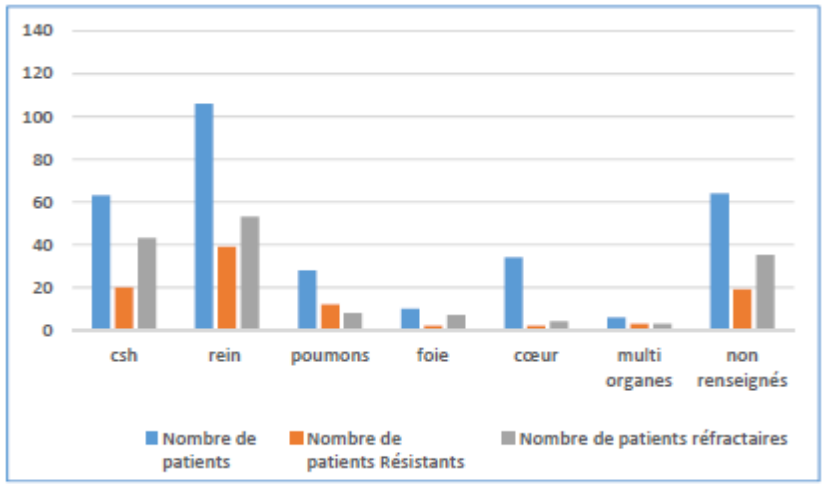


# By their number, kidney and HCT represent the major population for resistance



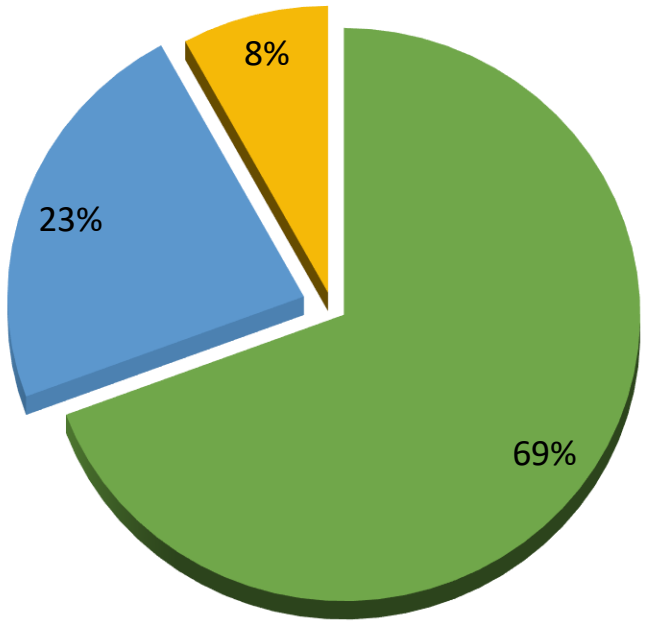



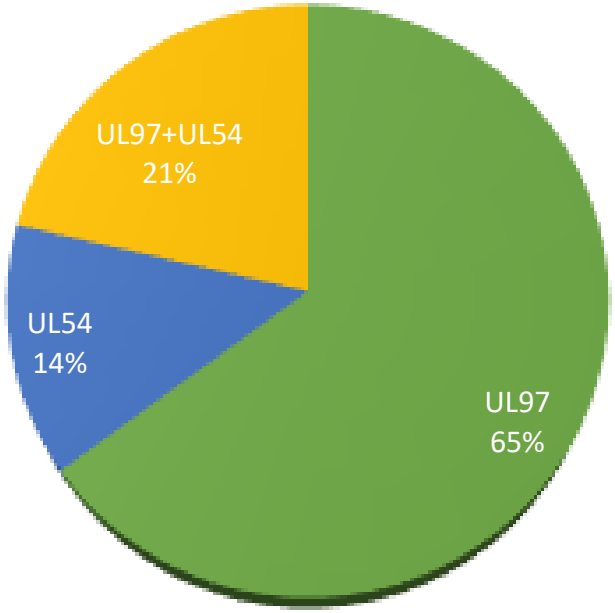




2019
2020

# Distribution of resistance mutations: multidrug resistance

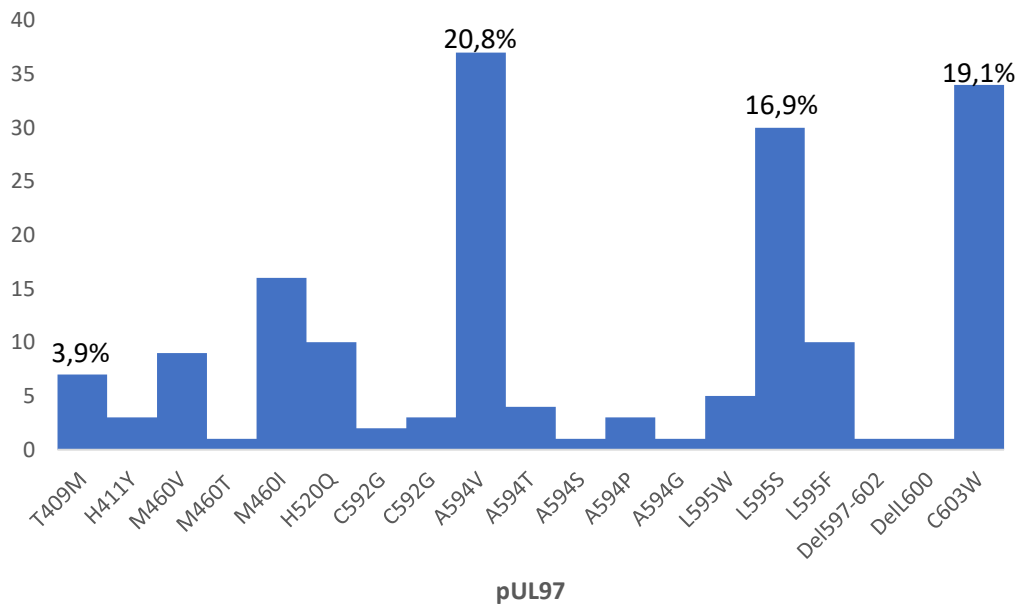


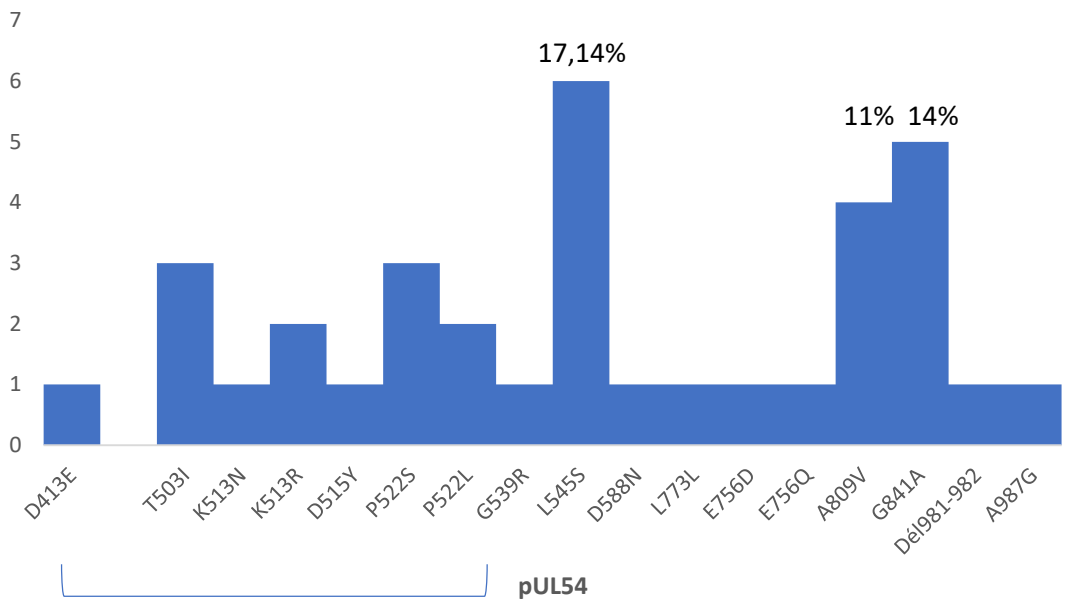

2017-2018




2019 2021

# Aminoacids substitutions in UL97 and UL54 2019-2020




Canonical UL97 GCV R mutations:

594+595+460+520 = 73,6%

594+595+460 = 67,8%

Exonucleasic domains

Associations:

T409M/H411Y/GCVR
T409M/GCV R
H520Q/C603W
594+595

603 W and 411Y always associated

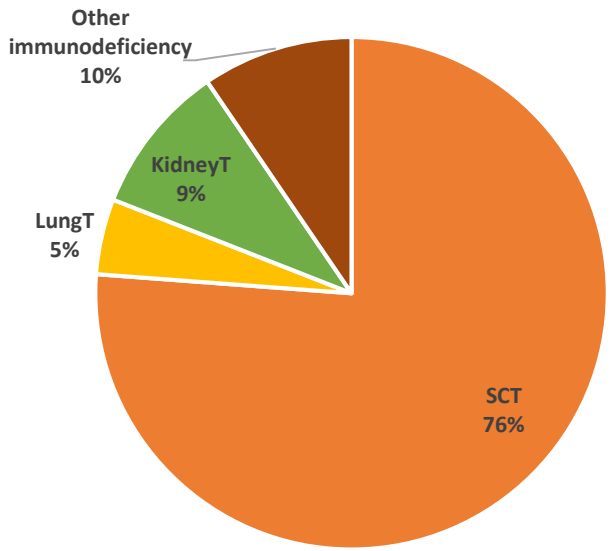
MBV R: 3,9%

22% FOS +/- GCV+/-CDV

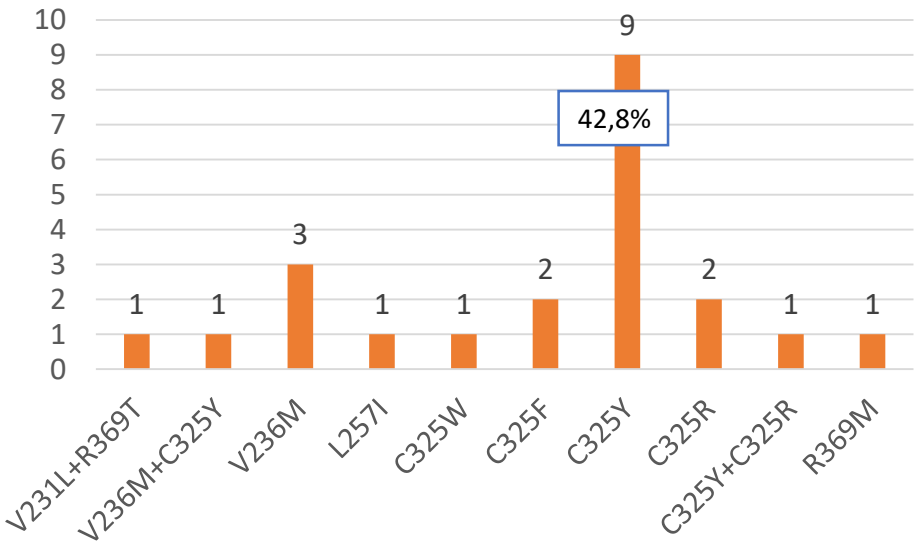
78% GCV/CDV R

# Letermovir resistance 2018-2021

- 21 patients with resistance from 12 centers




- Mutations:
- UL56




- No mutation in UL89, two in UL51 (P91S and a new mutation)

GCV >5
GCV 2-5
MBV >5
MBV2-5
GCV/MBV>5
GCV2-5/MBV>5
Polymorphism/ratio<2
* hypersensitivity to MBV
# Increased replicative capacity
**only in cell culture

# New mutations were studied by marker transfer to CMV bacmids pUL97

F342Y#
H393Y#
C480F

N510S
P536T#

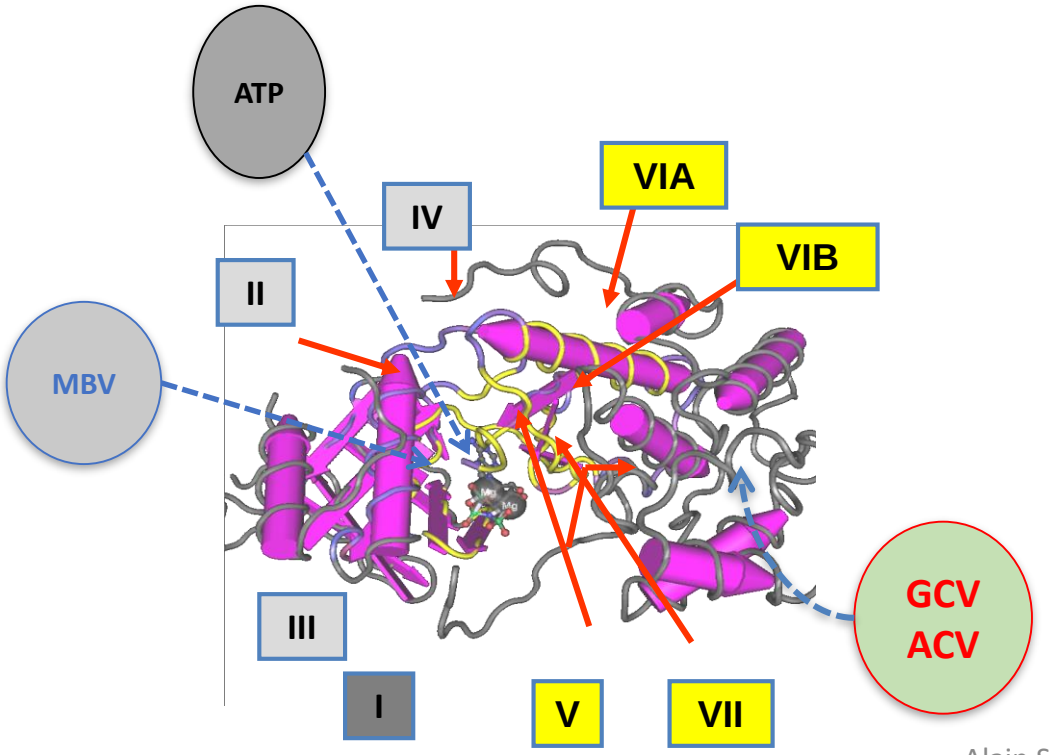
NH2 codon1 — NLS — I II III IV V VI VII VIII IX X XI — COOH 707

L337M**
F342Y
V353A**
V356G**
L397R**
K359N
K359E/Q
E362D*
Y335H
**V345i***
L348V
A378T
V388M
T409M
H411Y
H411N
H411L**
H415Y
A435V
C437Y
L405P

M460V/I,
V466G,
C480R**
C518Y
H520Q,
P521L,

Del(s)590-613
A591del
A591V
C592G,
A594E/S/T
A594V/G,
L595S/F/W/del
E596Y
E596G/del
N597del
K599T

L600del
T601del
C603W/R/S
D605E
C607Y/F
I 610T
A613V
Y617Del**




ATP binding
Autophosphorylation

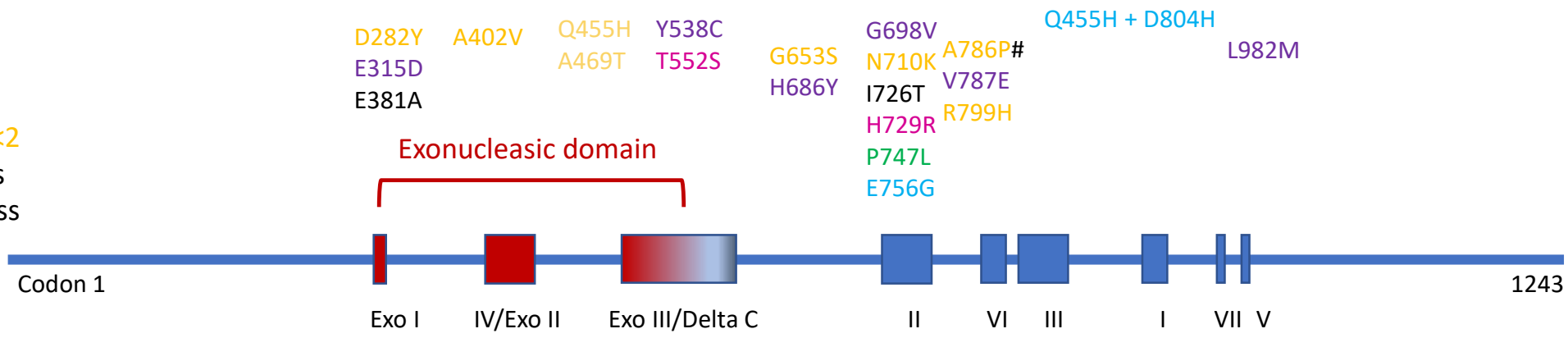
Catalytic domain

Substrate binding site

(Chou.S et al., Antiviral res. 2020, 2021, Champier G. pHD manuscript,)
Santos-Bravo et al. JID 2020

# pUL54:

New mutations in clinical samples:
GCV + CDV
FOS
CDV
FOS + GCV
FOS + GCV + CDV
No resistance or <2
*increased fitness
# decreased fitness




Known resistance mutations:
GCV
GCV + CDV
FOS
CDV
FOS + GCV
FOS + GCV + CDV
**only in lab strains

S290R**
D301N
E303D/G

N408D/K/S
N410K
F412C/L/S
F412V**
D413Y
D413E/A

K488R**
N495K
K500N
L501I
T503I
A505V

K513E/N/R
D515Y/E
L516R**
L516P/W
I521T
P522A/S
C524del
V526L
C539G
C539R**
D542E**
A543P**
L545S/W
T552N**
Q578H
Q578L
S585A
D588N
F595I

T700A
V715A/M
I726T/V
E756K/D/Q

L773V
L776M
V781I
V787L/A
V787E

L802M
K805Q
A809V
V812L
T813S
T821I
P829S**
A834P
T838A
G841A/S
M844T**
M844V**

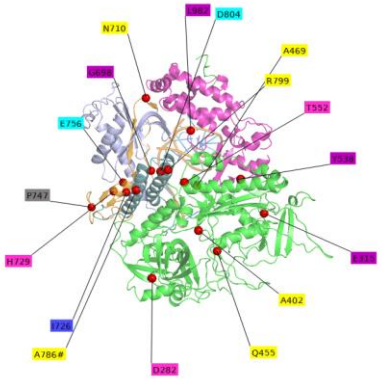
V946L**
E951D**
L957F
del 981-2
A987G

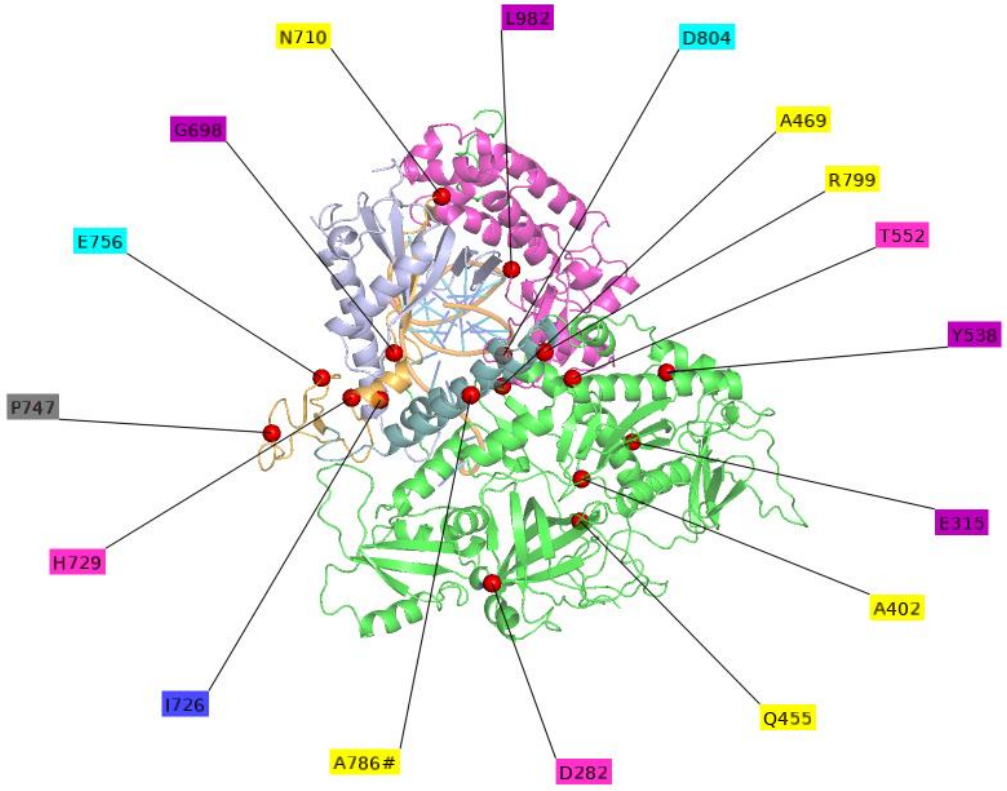
**Exonucleasic domains (codons 296-554)**
Exo I (296-304)
Exo II/domaine IV (379-421)
Exo III/deltaC (492-588)

**Other functional domains (codons)**
II (696 -642)
VI (771-790)
III (805-845)
I (905-919)
VII (962-970)
V (978-988)

Adapted from Chou S. et al. AVR 2020, Piret et al., JID 2019, Paccoud et al., Curr Res in Transl. Med 2022, CNR data

# Location of new aa substitutions in pUL54



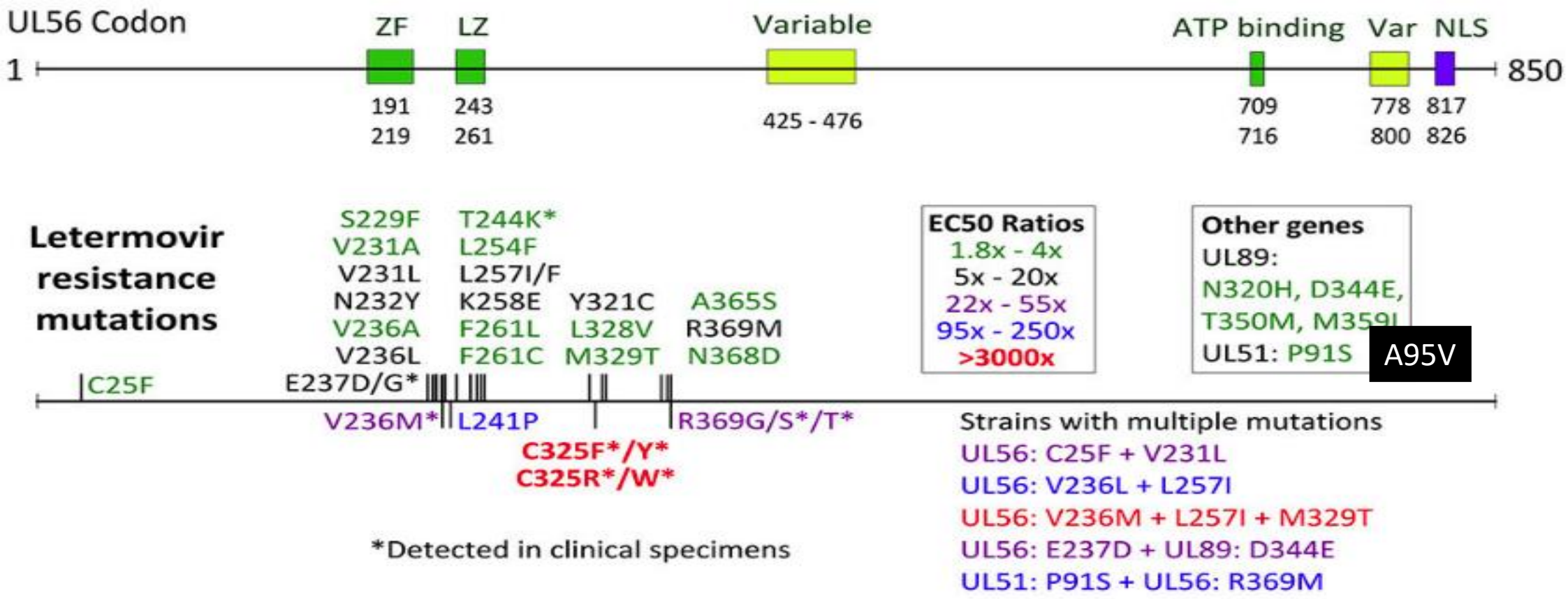



# Terminase complex

No resistance or <2

*** highly increased replication

R246C***






Adapted from Chou S. et al. AVR 2020, Santos Bravo, Spectrum J In press

# Take home messages

- Increasing exhaustivity of data collected, since 2016
- The stable number of resistance genotyping asked by centers illustrates the constant risk of treatment failure and emergence of resistance, even during Covid 19 pandemics, with the risk of lower access to medical structures.
- Resistant cases represent one third of refractory cases
- UL97 GCV resistance remains majority but one third of cases were multidrug resistant
- MBV and LMV resistance does exist and for LMV it represents 7,2% of LMV refractory cases.
- No UL89 mutation in the clinics but UL51 mutations requiring UL56/89/51 genotyping

# 8th International Congenital CMV Conference & 18th International CMV Workshop

28 March - 1 April 2022

VIRTUAL CMV2022

Resistance testing
Melissa Gomez-Mayeras
Matthieu Lafarge

Bioinformatics

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D Diaz

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N Plault

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Françoise Garnier



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CNR HERPESVIRUS

CHU Centre hospitalier universitaire Limoges

Université de Limoges

Inserm