UNIVERSITÀ DI SIENA
1240

New drugs, new mechanisms and new resistance

XVI Workshop Nazionale "TERAPIA E PREVENZIONE DELL' INFEZIONE DA HIV E MICRORGANISMI EMERGENTI"
Firenze, 13 Febbraio 2023

- **FRANCESCO SALADINI, PhD**
  DIPARTIMENTO BIOTECNOLOGIE MEDICHE
  UNIVERSITA' DI SIENA

UNIVERSITÀ DI SIENA 1240

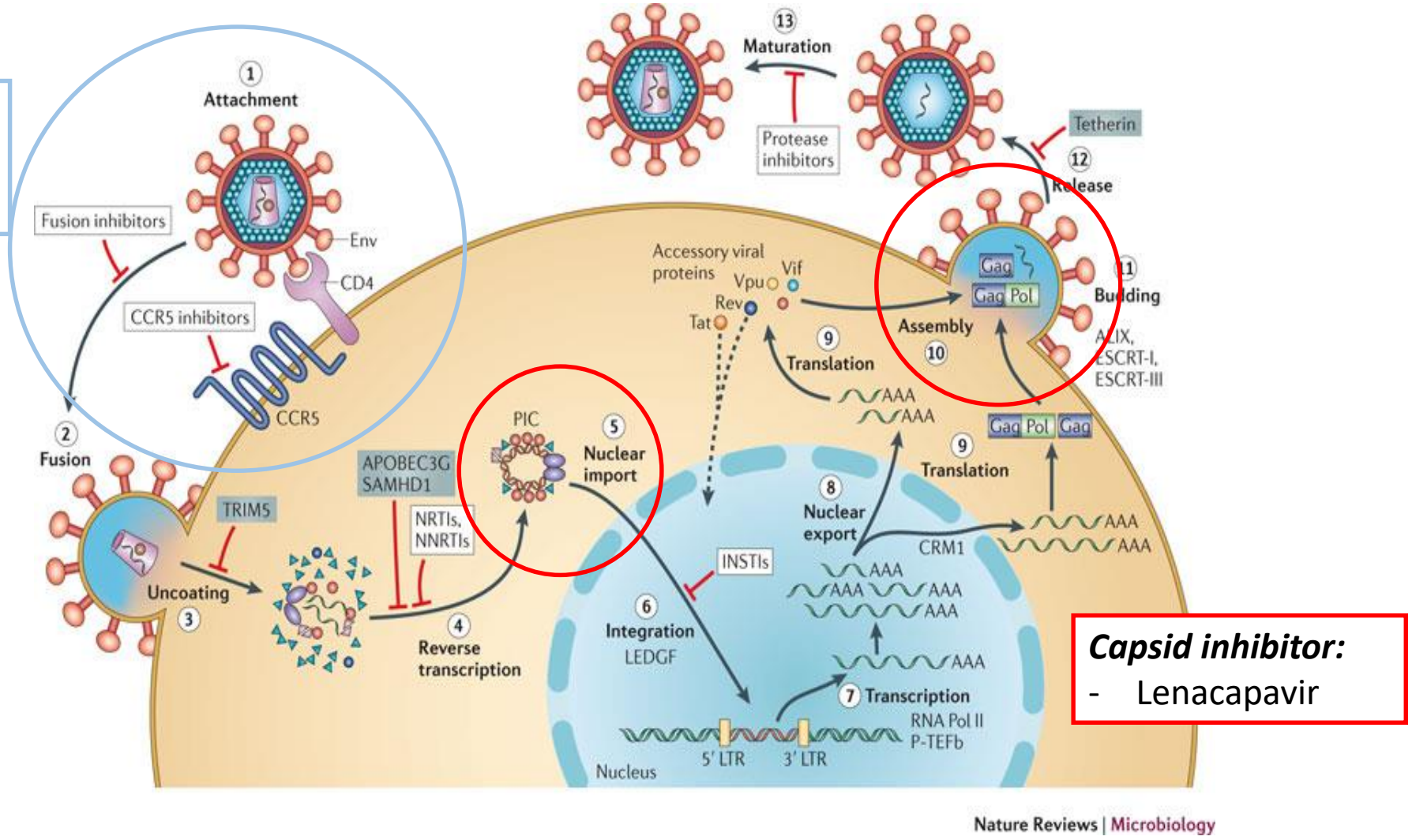
Conflicts of interest

- Dr. Francesco Saladini has no financial relationships with commercial entities to disclose.

UNIVERSITÀ DI SIENA
1240

Recently approved antivirals with new mechanisms of action

Entry inhibitors:
- Ibalizumab
- Fostemsavir

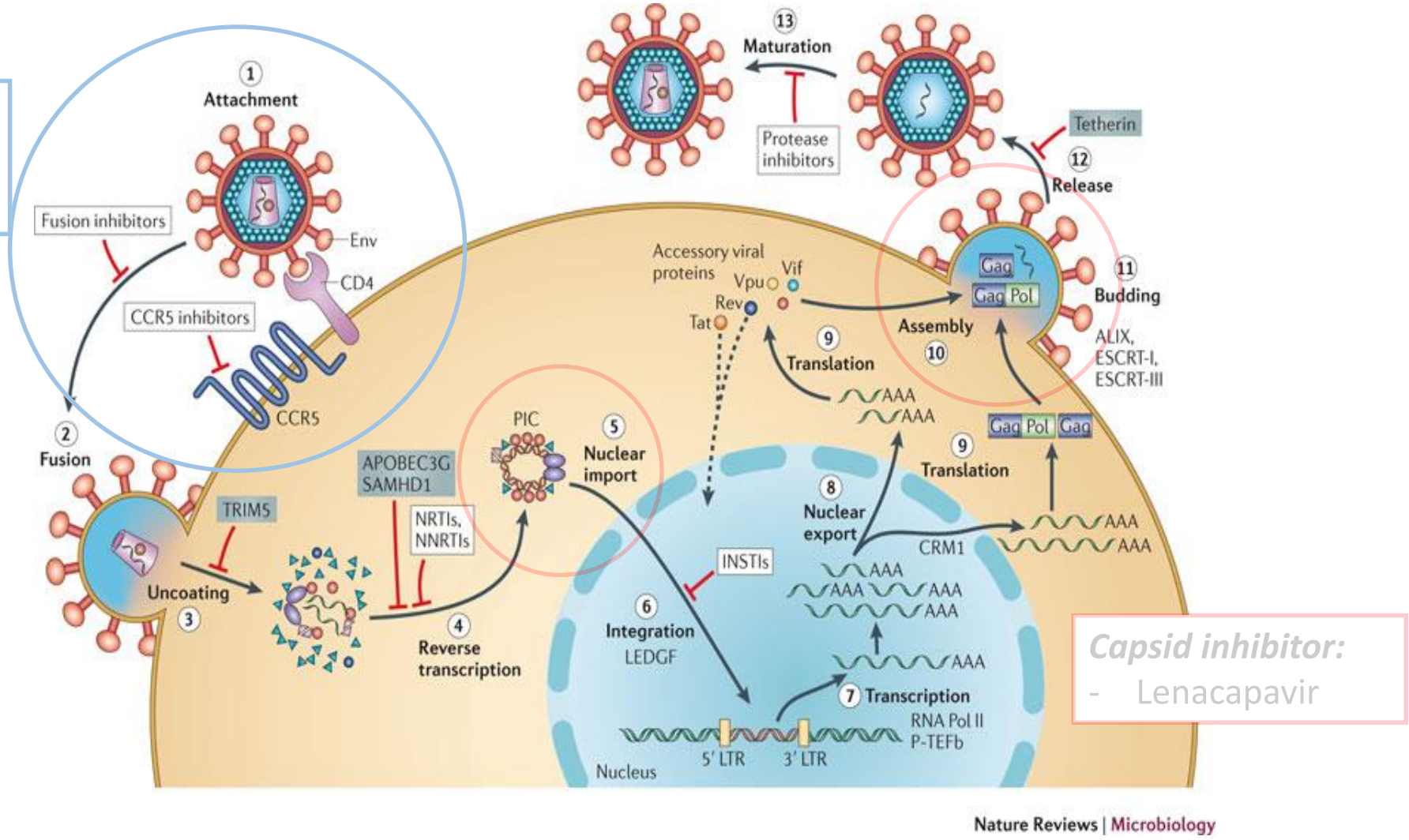


Capsid inhibitor:
- Lenacapavir

UNIVERSITÀ DI SIENA
1240

Recently approved antivirals

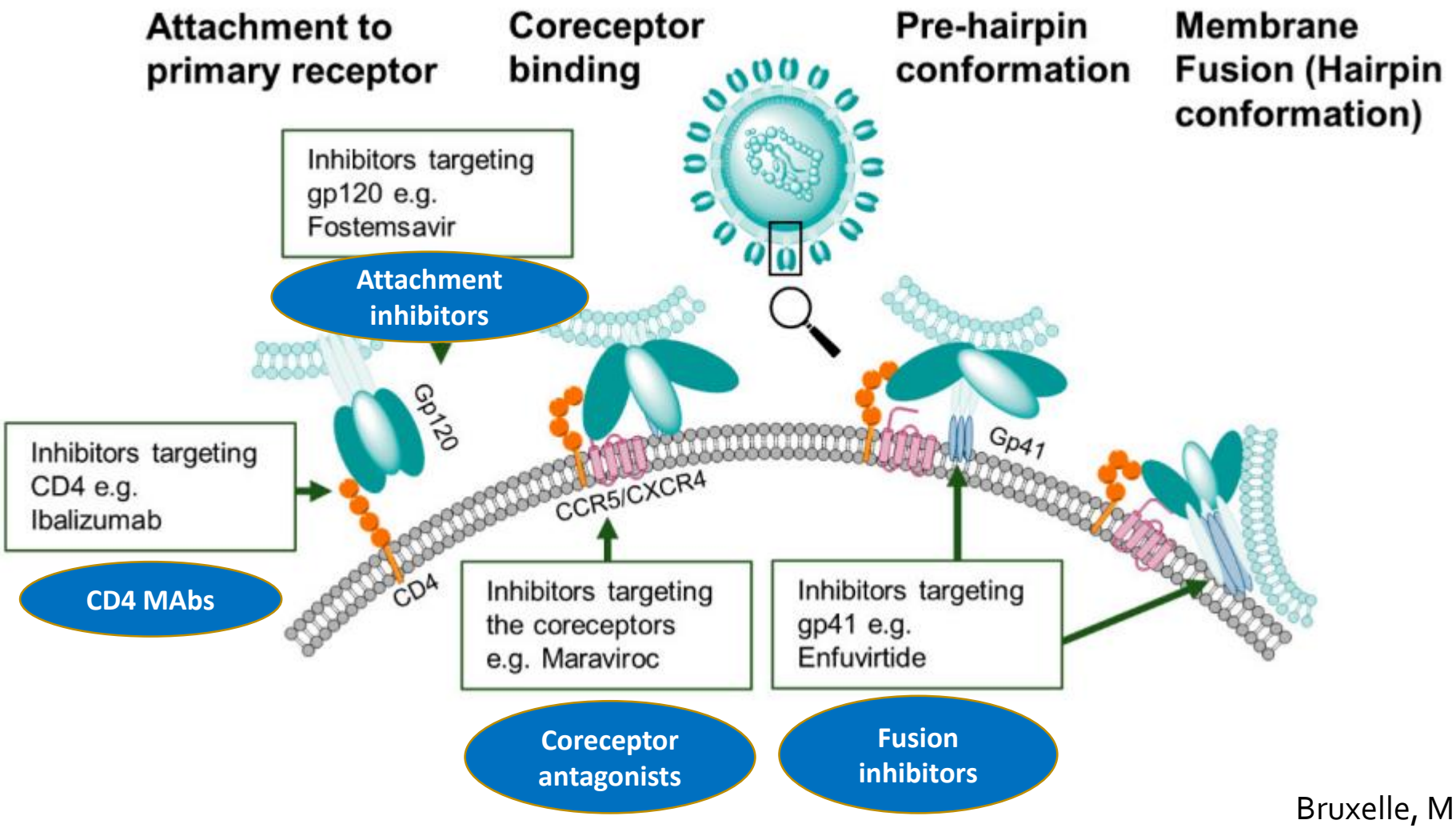
Entry inhibitors:
- Ibalizumab
- Fostemsavir



Capsid inhibitor:
- Lenacapavir

UNIVERSITÀ DI SIENA 1240

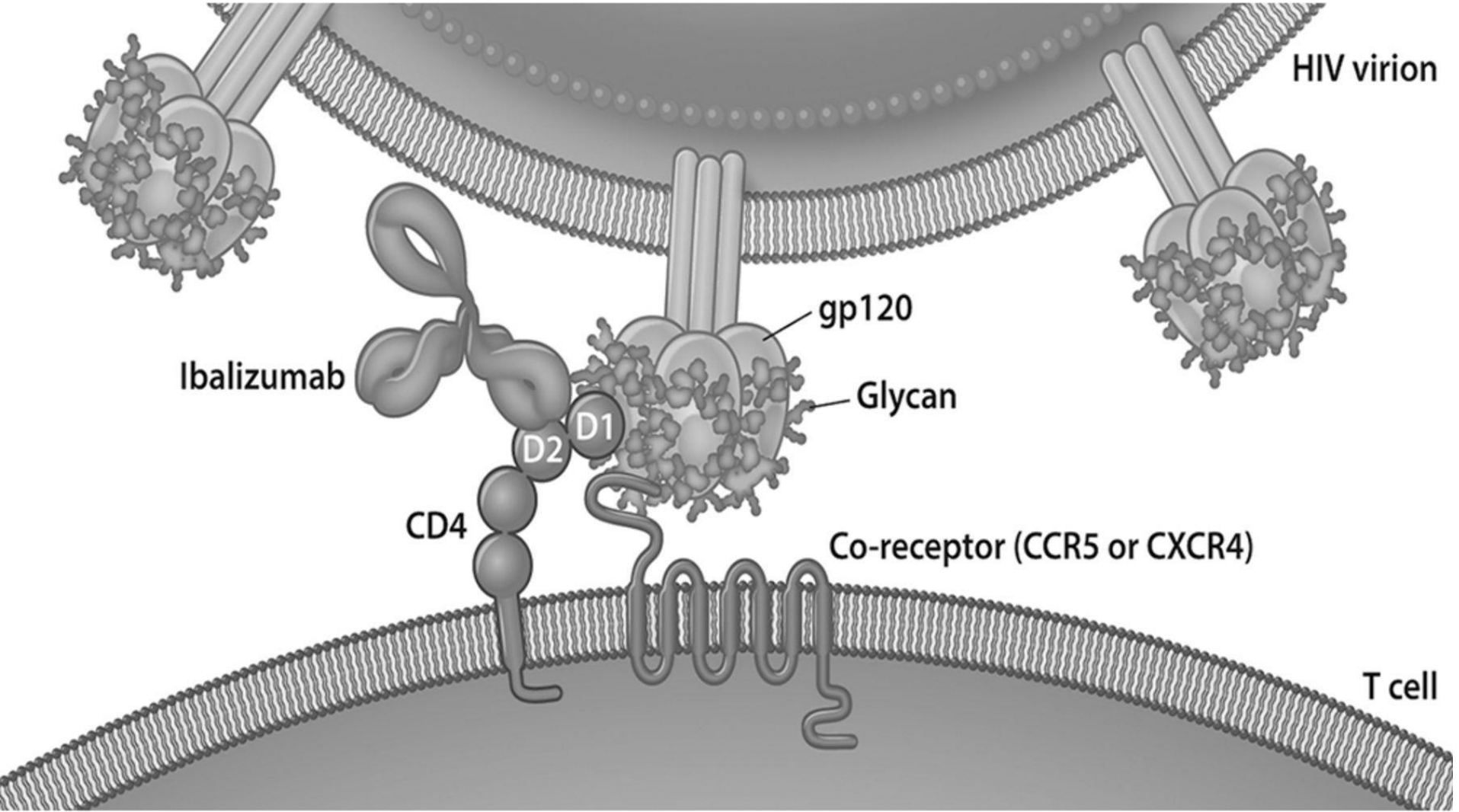
HIV entry steps and entry inhibitors



Bruxelle, Microorganisms 2021

UNIVERSITÀ DI SIENA 1240

Mechanism of action of Ibalizumab

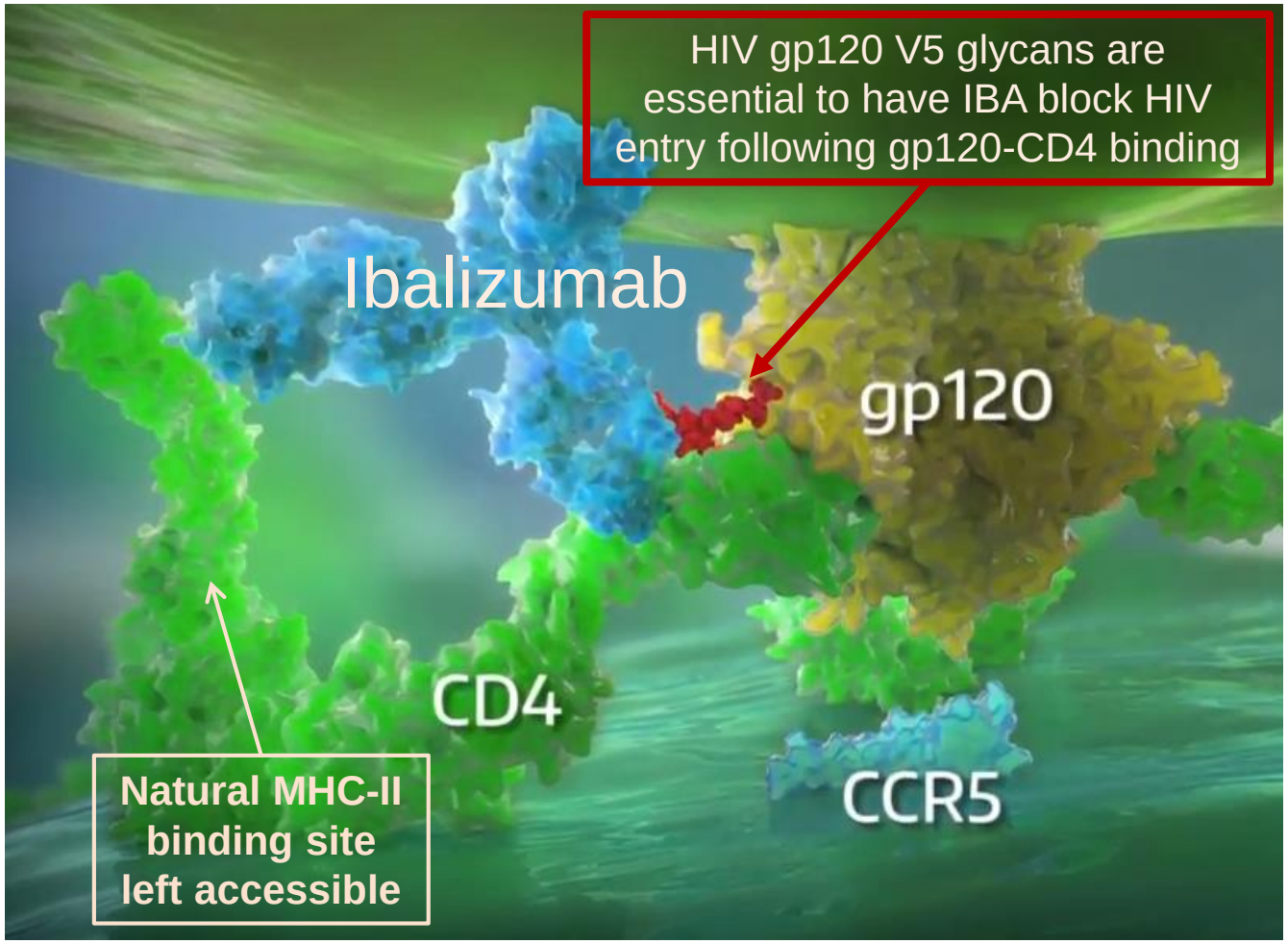


- Ibalizumab is a recombinant humanized immunoglobulin (Ig) G4 Mab
- Binds to the CD4 T cell extracellular domain 2 at four sites and domain 1 at two sites
- Does not interphere with the binding of MHC-II molecule to domain 1
- Prevents conformational changes leading to the exposure of V3 domain required for co-receptor binding

Beccari AAC 2019

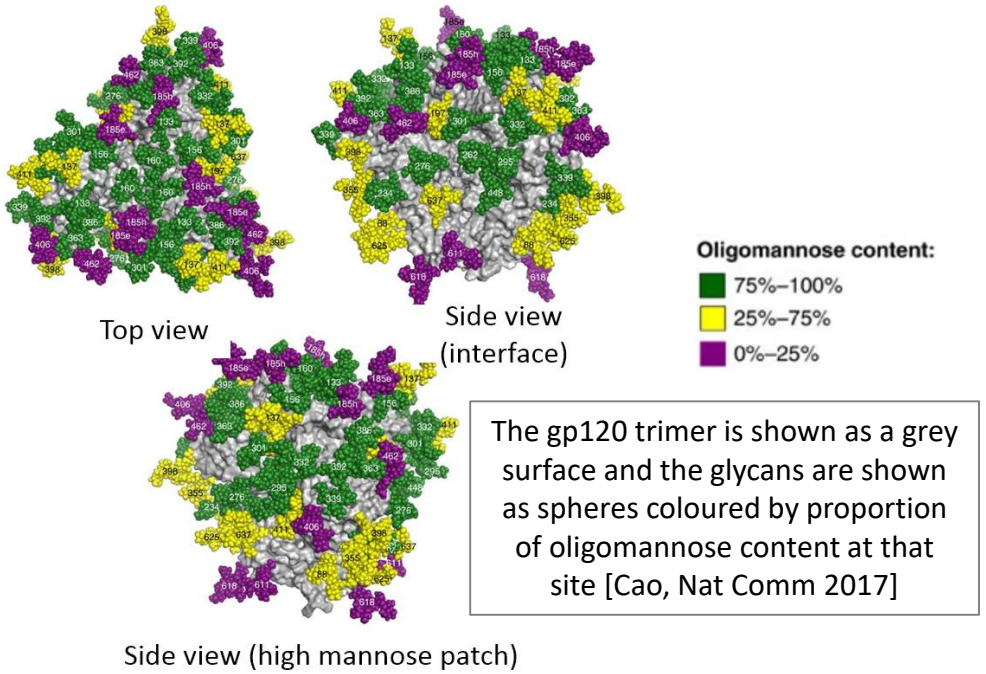
UNIVERSITÀ DI SIENA 1240

Mechanism of action of Ibalizumab



UNIVERSITÀ DI SIENA
1240

Gp120 Env glycosylation



The gp120 trimer is shown as a grey surface and the glycans are shown as spheres coloured by proportion of oligomannose content at that site [Cao, Nat Comm 2017]

Potential N-linked glycosylation site (PNGS)

Asn-any-Ser OR **Asn-any-Thr**

N – X – S OR **N – X – T**

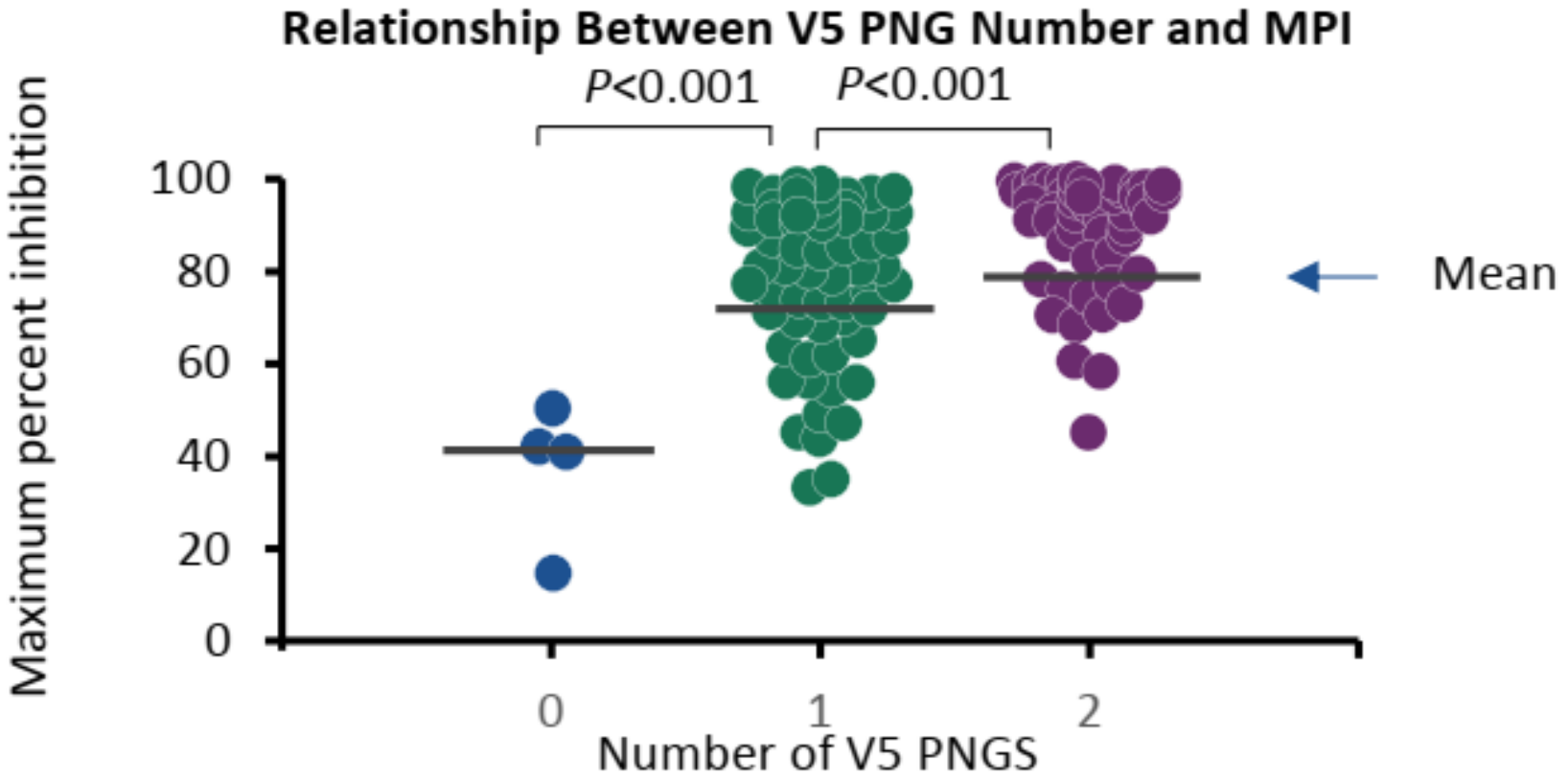
The process of glycosylation is host-derived, which may result in the immune system recognizing viral glycans as **self**

Enveloped viruses successfully infecting humans have evolved the ability to use a **glycan shield** to prevent immune recognition

Glycans can play a role in **virus-cell attachment**

UNIVERSITÀ DI SIENA 1240

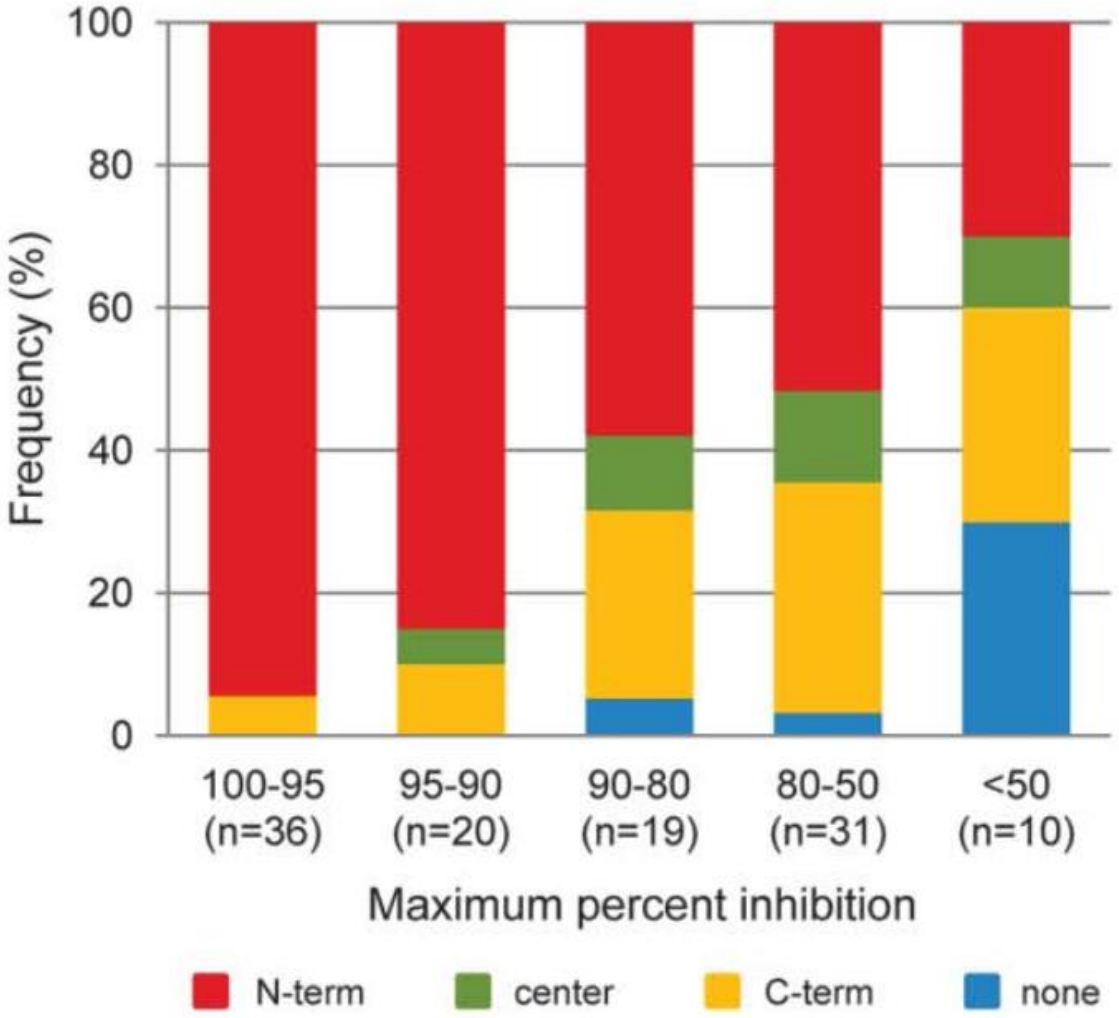
Ibalizumab maximum percentage inhibition and gp120 V5 glycosylation



Ibalizumab activity, as maximum percentage inhibition (MPI) for a panel of HIV-1 pseudoviruses (n = 116) grouped by number of gp120 V5 PNGS

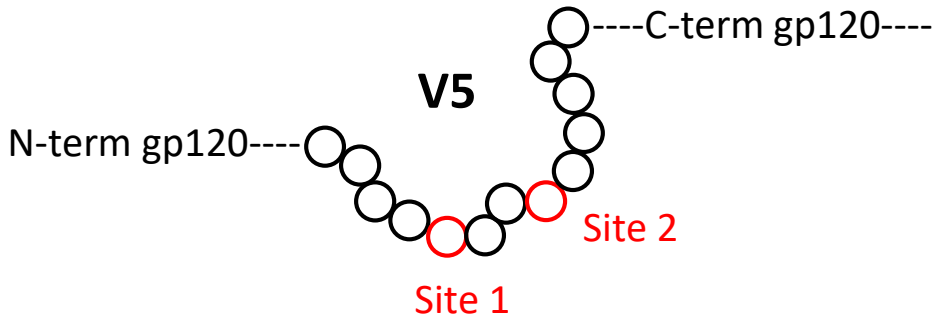
Pace, J AIDS 2013

Ibalizumab maximum percentage inhibition and gp120 V5 glycosylation



V5 PNGS affect susceptibility to Ibalizumab

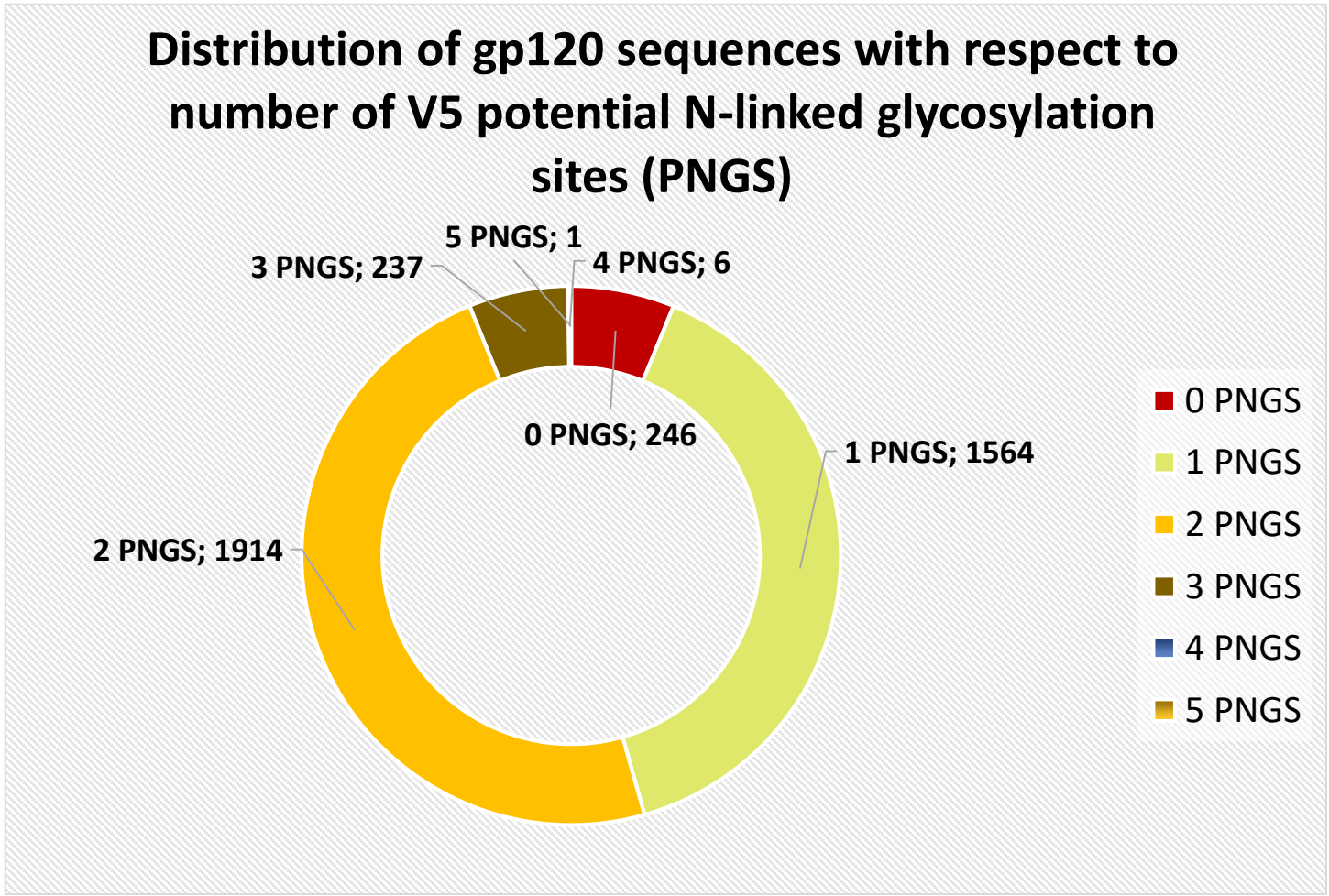
- Larger numbers of PNGS
- Closer proximity of PNGS to V5 N-terminus (“site 1”)



Pace, JAIDS 2013

UNIVERSITÀ DI SIENA 1240

Number of PNGS in HIV-1 gp120 V5

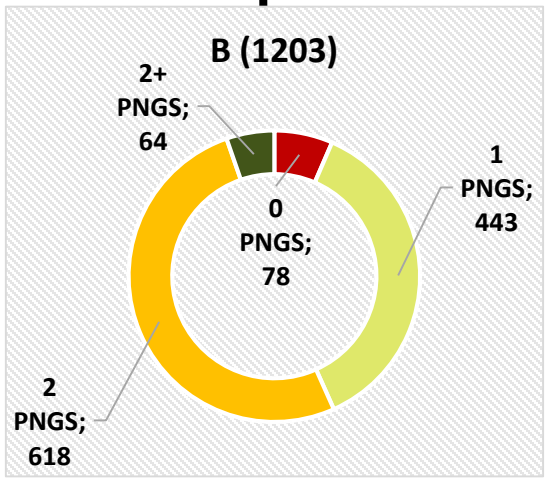


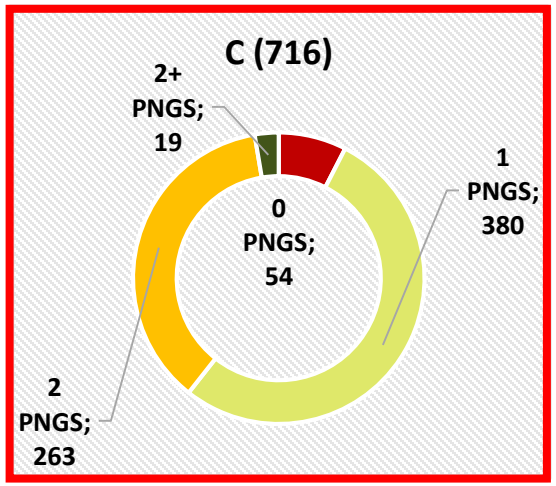
- HIV-1 gp120 dataset: n = 3967
- Representative of most common subtypes and CRFs
- 6.2% with no PNGS
- 39.4% with one PNGS
- 54.4% with two or more PNGS

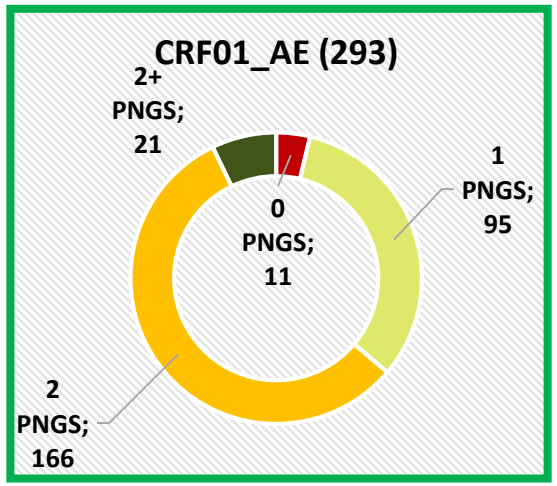
LANL HIV database, 2018 alignments. Accessed Oct 6, 2020

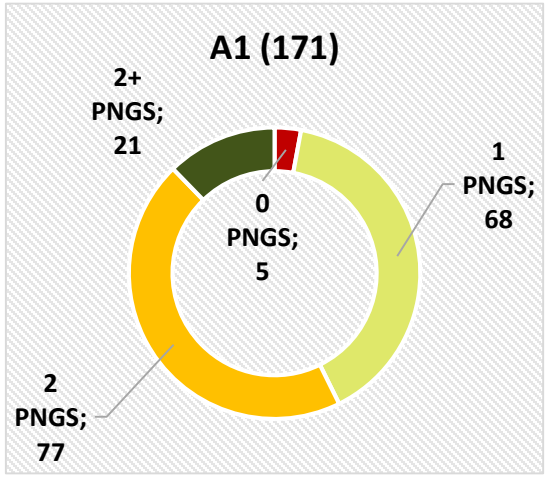
UNIVERSITÀ DI SIENA 1240

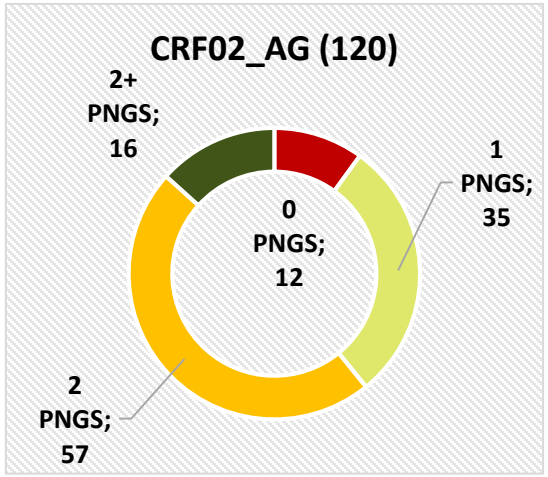
Distribution of V5 PNGS in different subtypes
Top 8 most common in the LANL 2018 alignment

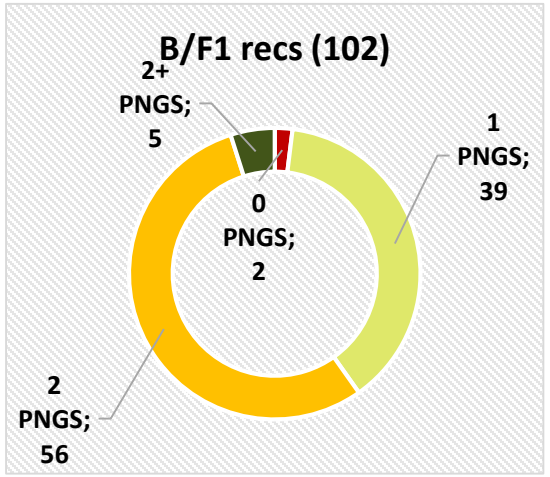


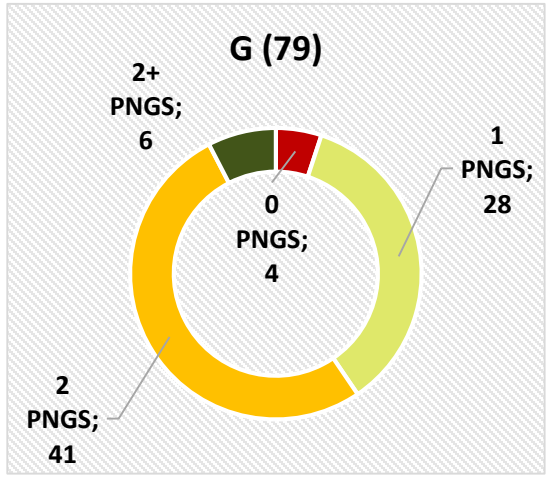


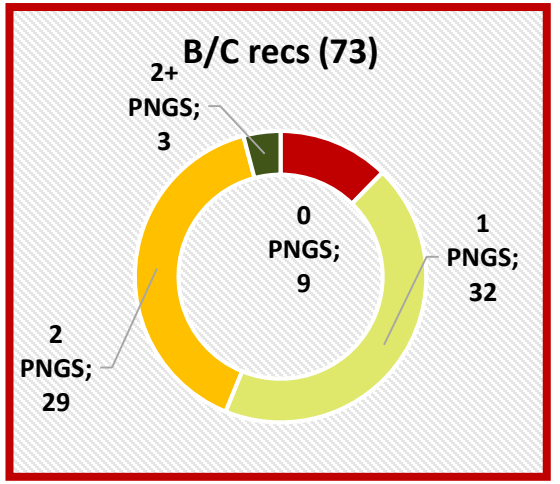








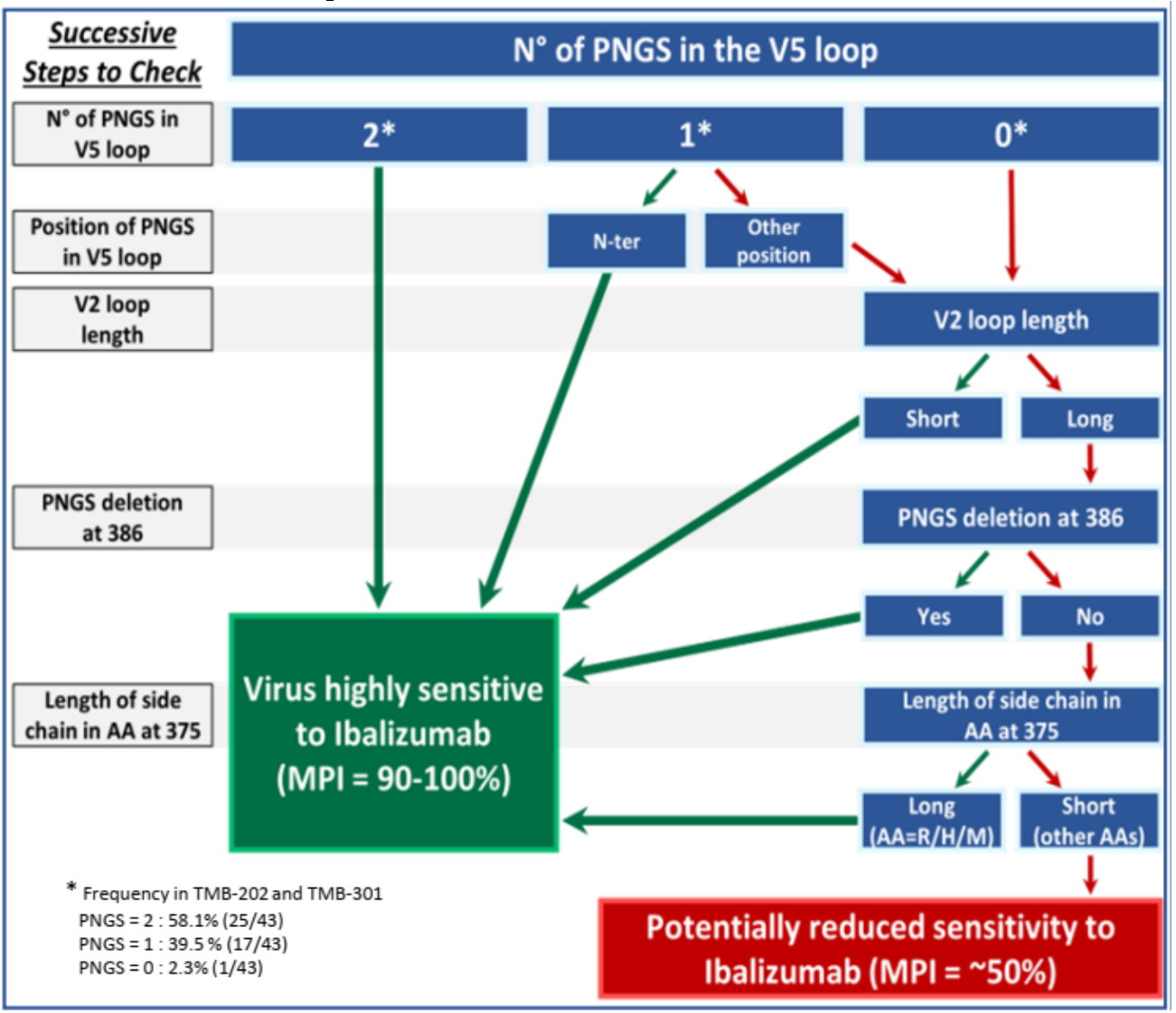




LANL HIV database, 2018 alignments. Accessed Oct 6, 2020

UNIVERSITÀ DI SIENA

Assessment of genotypic patterns associated with HIV-1 sensitivity to ibalizumab



Higher susceptibility to IBA associated with **PNGS located closer to the N-terminus of V5**

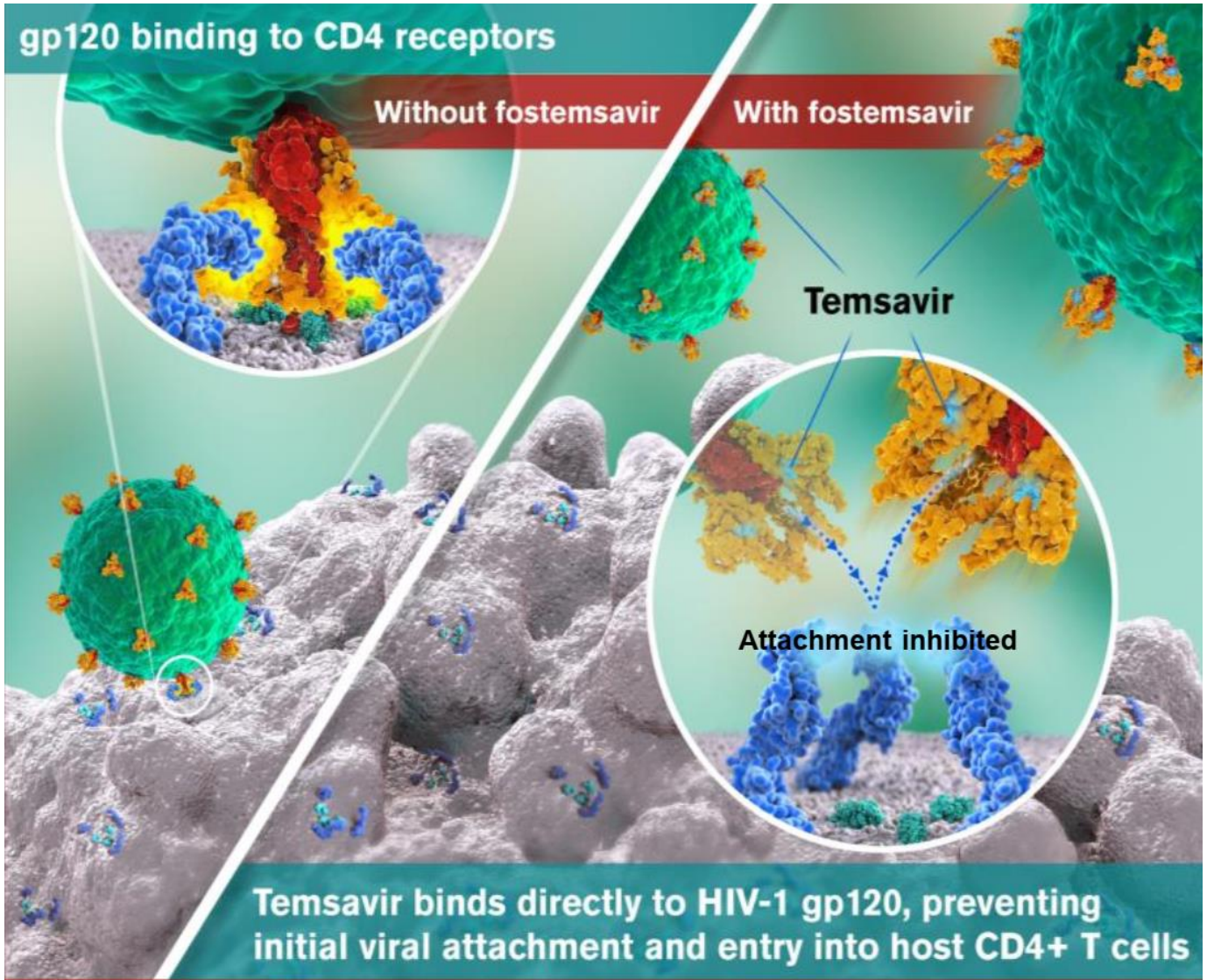
HIV with only 1 V5 PNGS can exhibit complete or partial susceptibility to IBA (Pace et al, 2013; Toma et al, 2011; and TMB-202 and TMB-301 studies)

Higher susceptibility to IBA associated with **shorter V2 regions**, **PNGS deletion at position 386**, or **long side chain AA (H/R/M) at position 375, but only when V5 N-terminal PNGS were absent or deleted**

Jullien, European Meeting on HIV & Hepatitis 2020

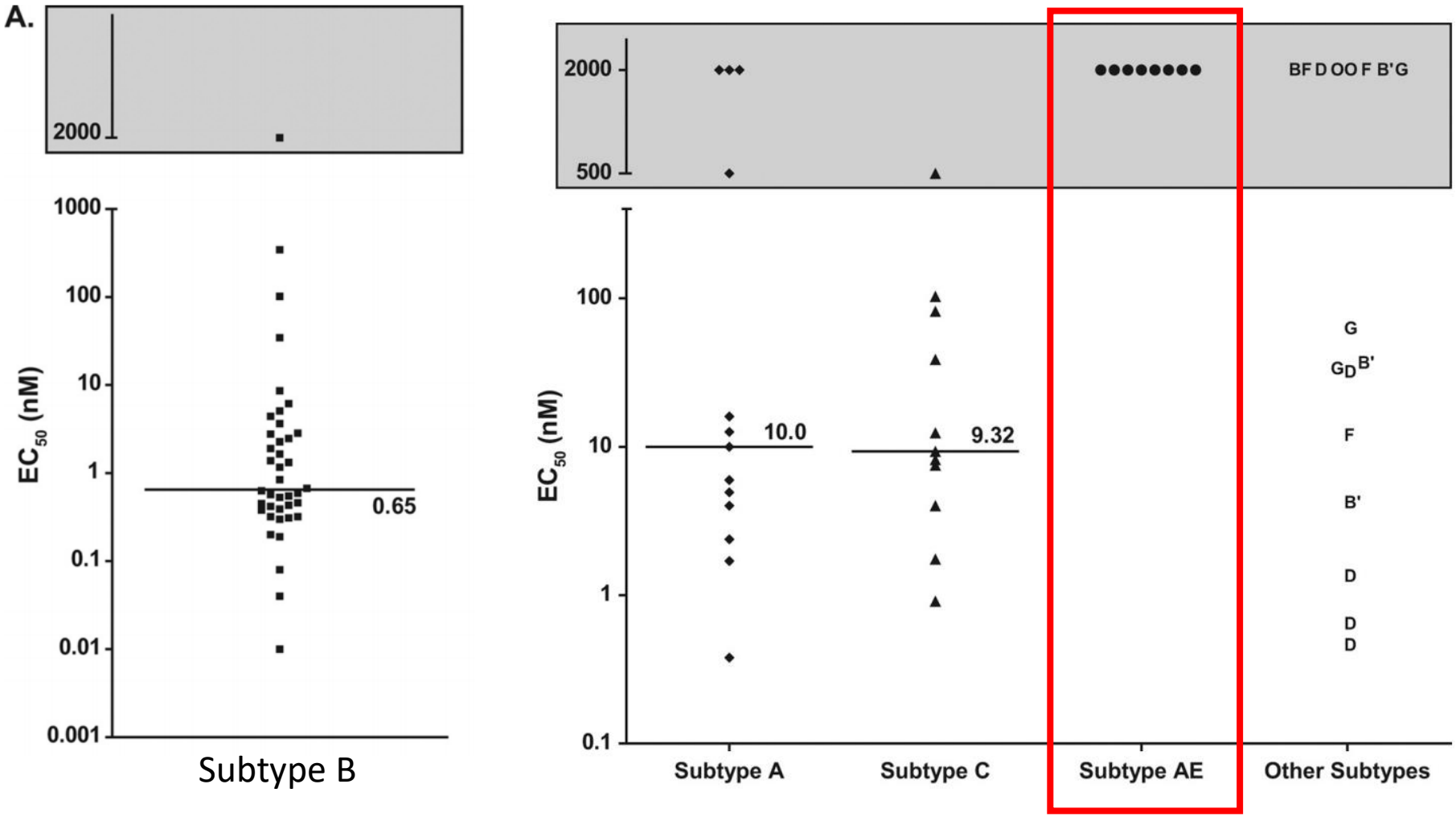
UNIVERSITÀ DI SIENA
1240

Mechanism of action of Fostemsavir



UNIVERSITÀ DI SIENA 1240

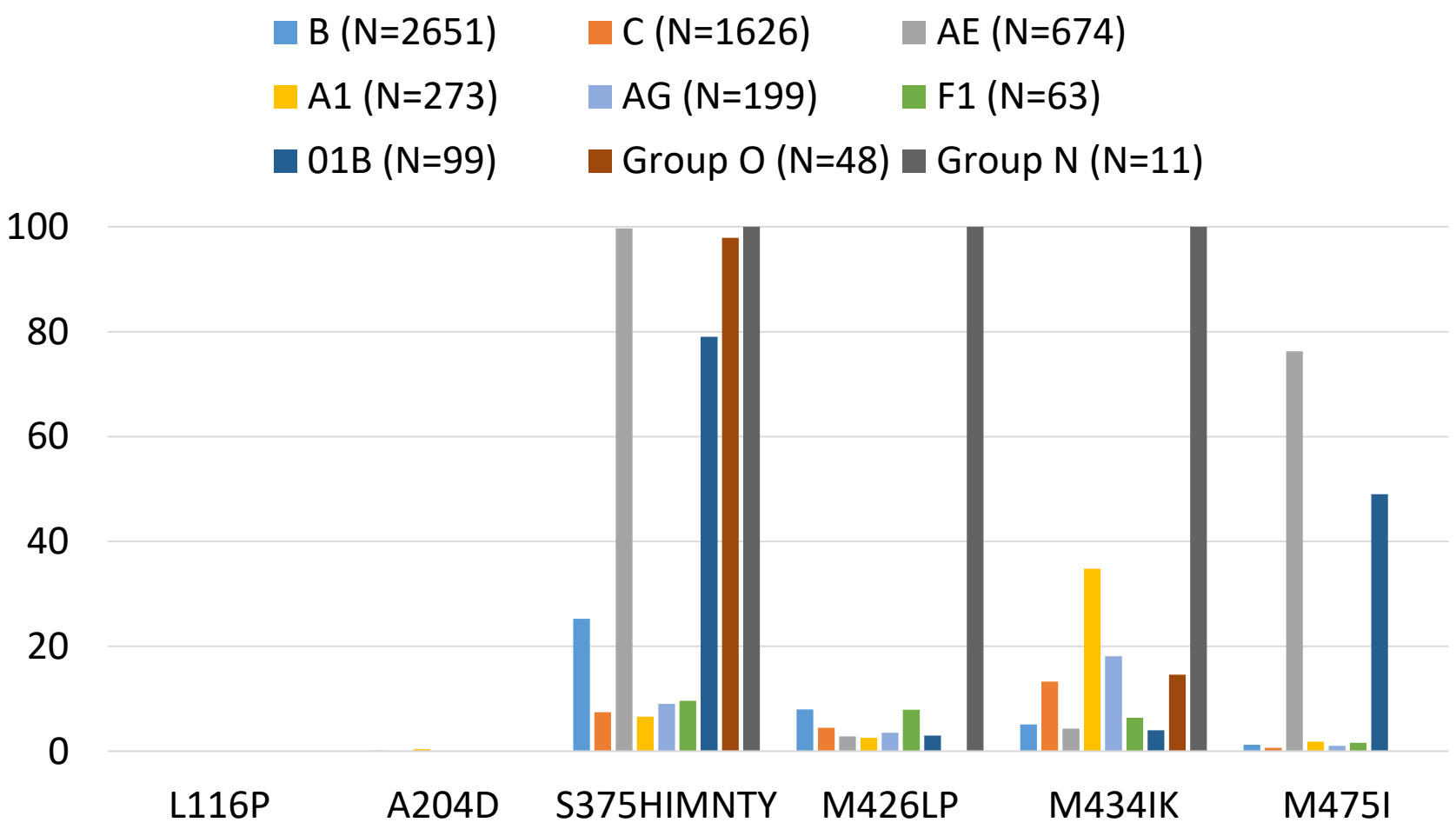
Natural susceptibility to Fostemsavir in different HIV1 subtypes



Nowicka-Sanz, Antimicrobial Agents Chemother 2017

UNIVERSITÀ DI SIENA 1240

Prevalence of gp160 polymorphisms known to be related to decreased susceptibility to temsavir in different subtypes of HIV-1 in the Los Alamos National Laboratory HIV Sequence Database



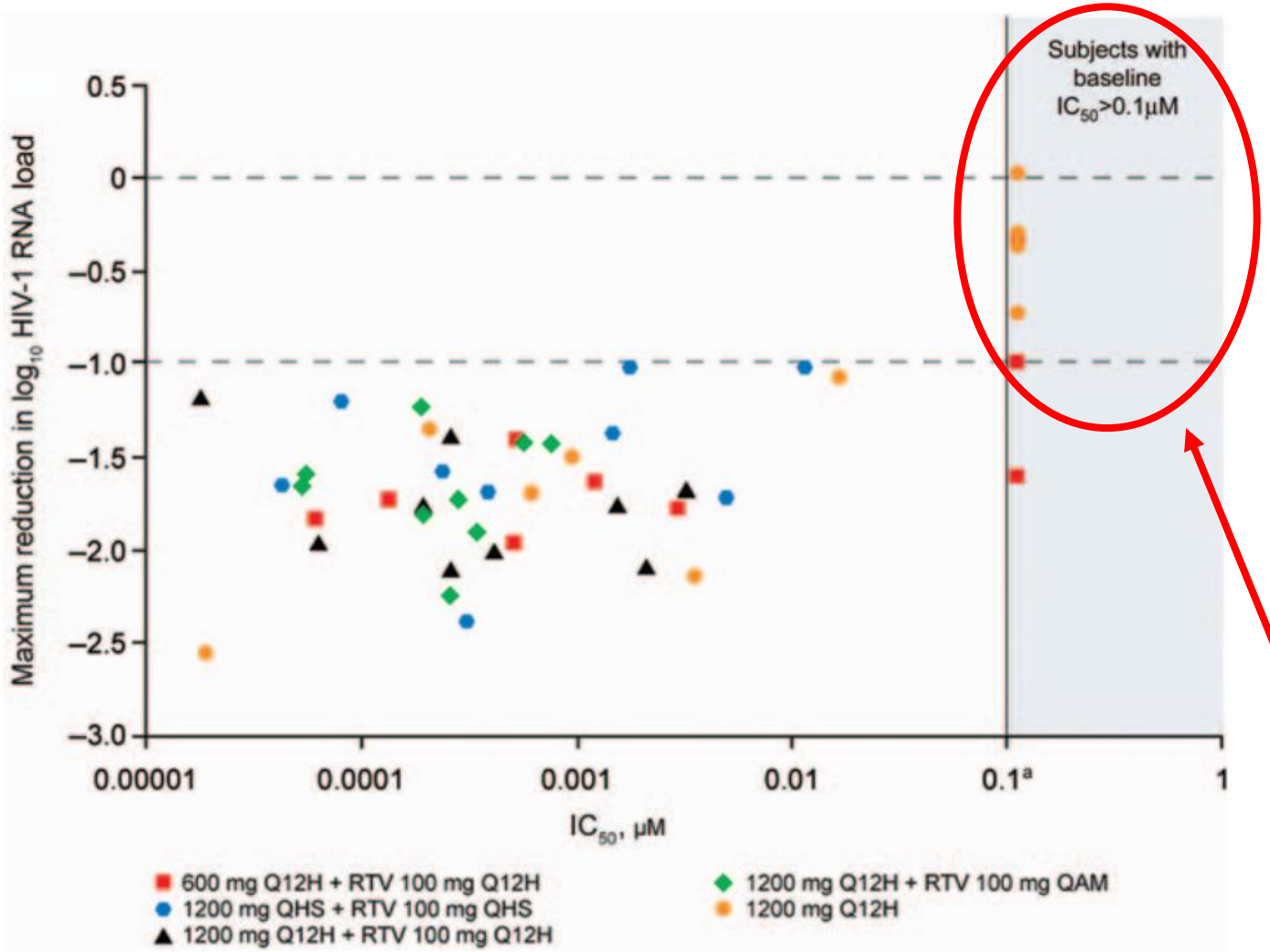
Provisional FTR resistance signatures include: L116P, A204D, **S375H/I/M/N/T/Y, M426L/P, M434I/K, M475I**

S375H and **M475I** predominant among **CRF01_AE** (S375H, 99.3%; M475I, 76.3%) and **01B** (S375H, 71.7%; M475I, 49.5%)

Group O and **Group N** predicted to be not susceptible

Adapted from Gartland, J Antimicrob Chemother 2021

Pharmacodynamics, Safety, and Pharmacokinetics of BMS-663068, an Oral HIV-1 Attachment Inhibitor in HIV-1–Infected Subjects



Open-label, multiple-dose, parallel **8-day monotherapy** study

50 HIV-1–infected subjects randomized to 1 of 5 regimen groups (FTR/rtv 600/100 Q12H, FTR/rtv 1200/100 Q24H, FTR/rtv 1200/100 Q12H, FTR/rtv 1200/100 Q12H/Q24H, FTR 1200 Q12H)

Median maximal decrease in plasma HIV-1 RNA load from baseline ranged from **1.21 to 1.73 log**

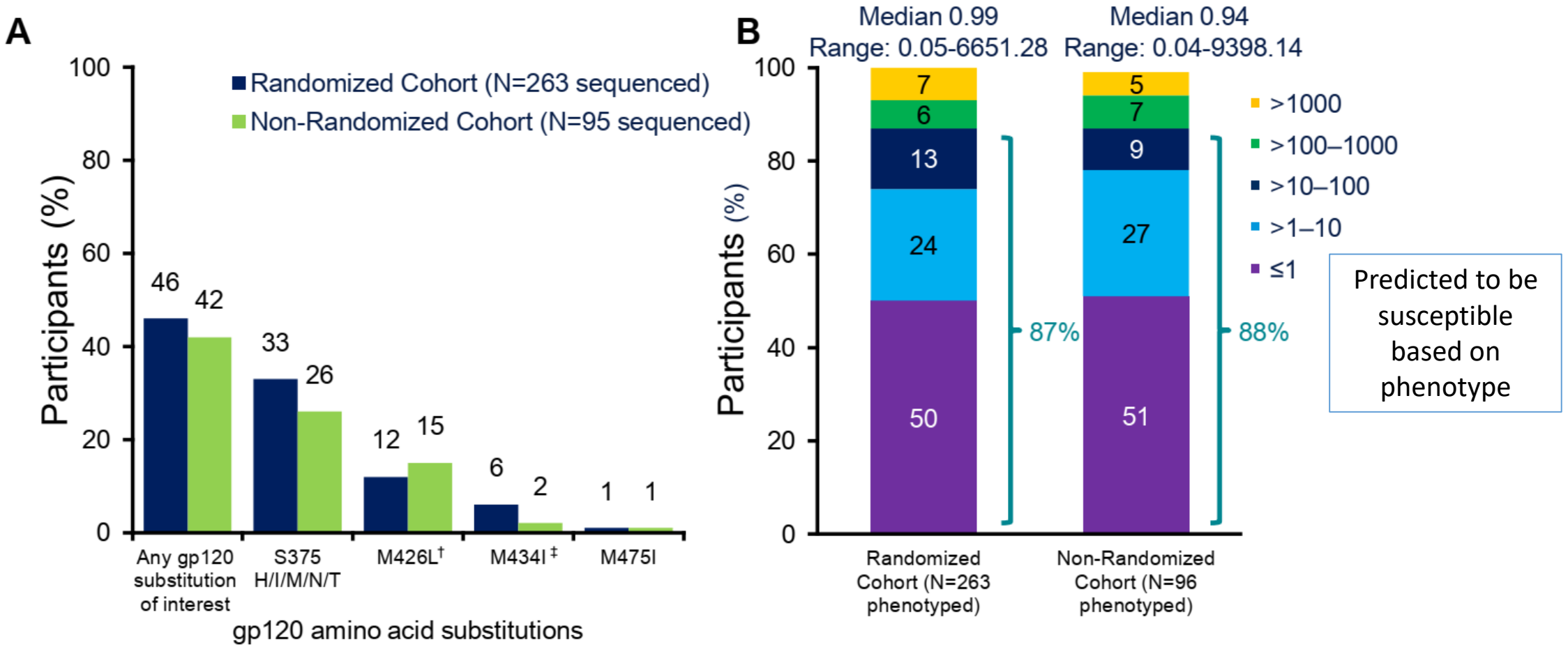
Higher **intrasubject variability** in susceptibility to BMS-626529 compared with other classes of entry inhibitors

Lower response with baseline IC_{50} >100 nM

Nettles, J Infect Dis 2012

Fostemsavir in HTE individuals: the BRIGHTE study

(A) Baseline gp120 Substitutions of Interest* (B) Baseline TMR IC_{50} FC

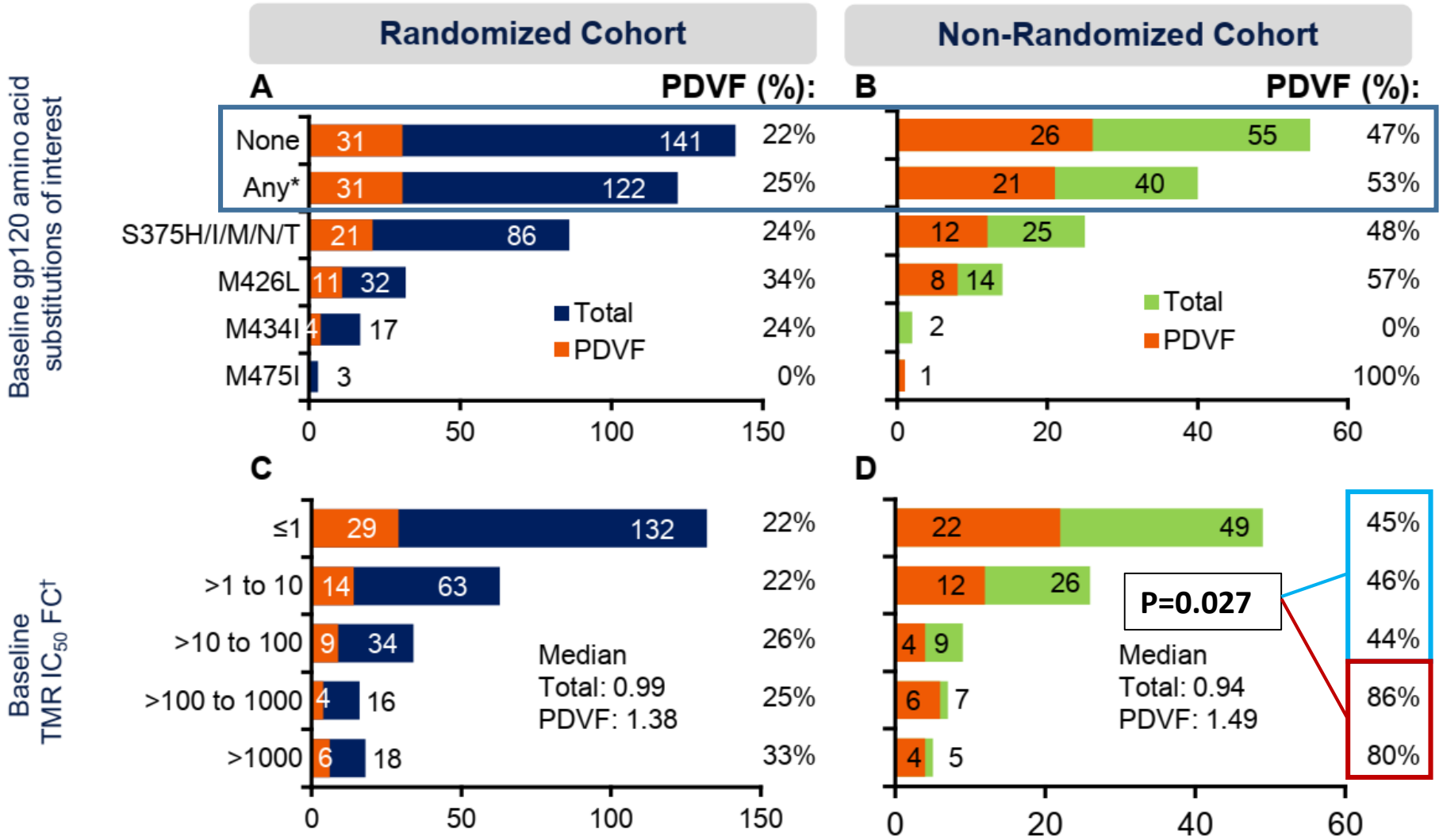


*S375H/I/M/N/T, M426L/P, M434I/K, and M475I. Numbers include mixtures. †No M426P was detected. ‡No M434K was detected.

Gartland, AAC 2022

UNIVERSITÀ DI SIENA 1240

Fostemsavir in HTE individuals: the BRIGHTE study



PDVF Through Week 96 for Randomized and Non-Randomized Cohorts by **Baseline gp120 Substitutions of Interest** (A and B) and **TMR IC_{50} FC** (C and D)

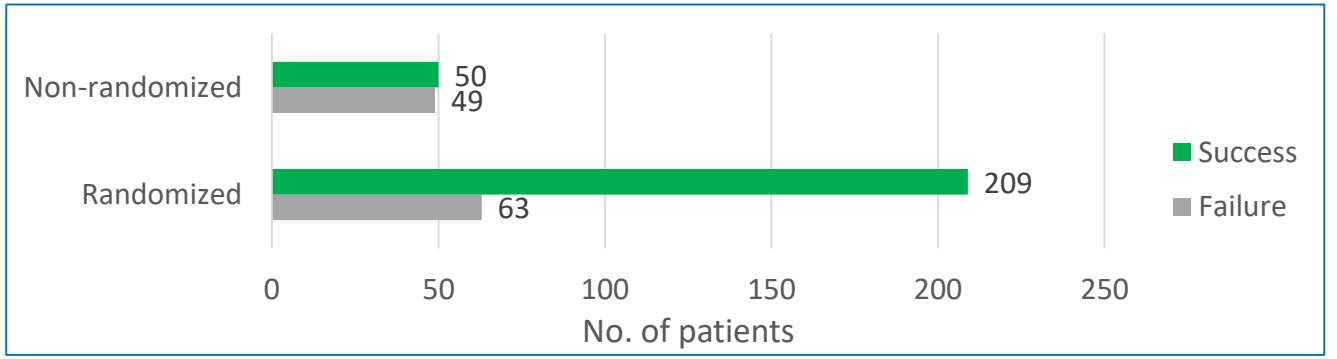
No correlation between baseline **genotypic** resistance and response

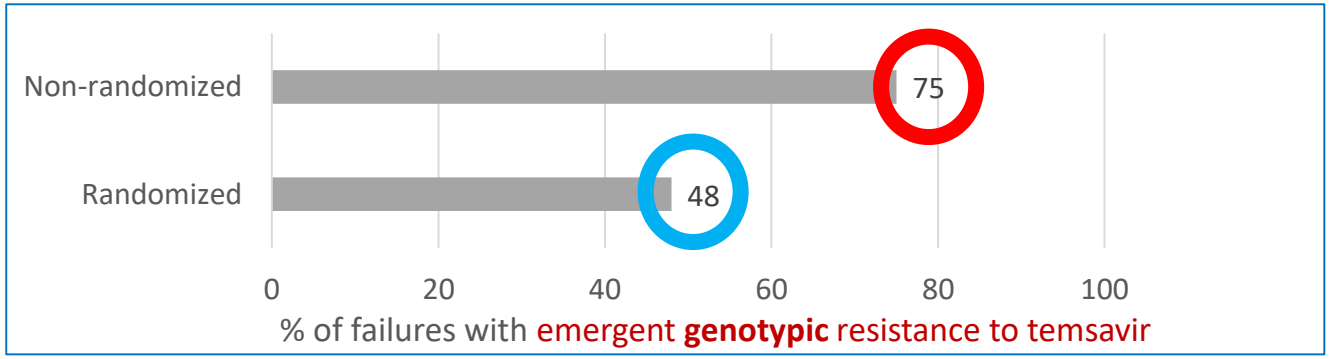
Correlation between baseline **phenotypic** resistance (>100 FC IC_{50}) and response limited to the non-randomized cohort

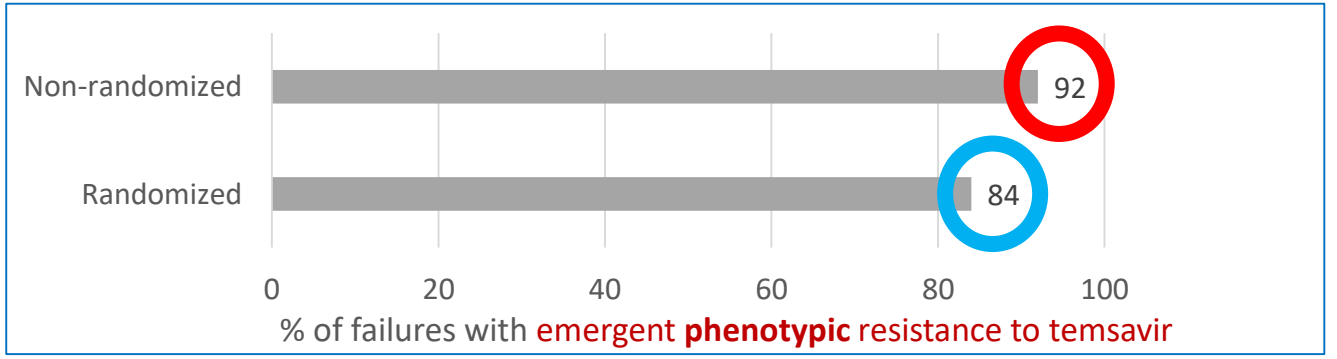
Gartland, AAC 2022

Emergent resistance to temsavir in the BRIGHTE study (wk 96)

Emergent substitutions of interest in gp120	Randomised cohort (n=63)	Non-randomised cohort (n=49)	Total (n=112)
Sequenced*	50 (79%)	44 (90%)	94 (84%)
Substitutions of interest in gp120†	24 (48%)	33 (75%)	57 (61%)
Ser375	15 (30%)	22 (50%)	37 (39%)
Ser375His	0	1 (2%)	1 (1%)
Ser375His/Asn	1 (2%)	1 (2%)	2 (2%)
Ser375Met	0	3 (7%)	3 (3%)
Ser375Asn	7 (14%)	8 (18%)	15 (16%)
Ser375Asn/Thr	1 (2%)	2 (5%)	3 (3%)
Ser375Ser/Ile	0	1 (2%)	1 (1%)
Ser375Ser/Met/Thr	1 (2%)	0	1 (1%)
Ser375Ser/Asn	4 (8%)	6 (14%)	10 (11%)
Ser375Ser/Thr	1 (2%)	0	1 (1%)
Met426	16 (32%)	21 (48%)	37 (39%)
Met426Leu	10 (20%)	13 (30%)	23 (24%)
Met426Met/Leu	7 (14%)	8 (18%)	15 (16%)
Met434	5 (10%)	4 (9%)	9 (10%)
Met434Ile	1 (2%)	1 (2%)	2 (2%)
Met434Met/Ile	4 (8%)	3 (7%)	7 (7%)
Met434Met/Ile/Thr	1 (2%)	0	1 (1%)
Met475	6 (12%)	5 (11%)	11 (12%)
Met475Ile	4 (8%)	1 (2%)	5 (5%)
Met475Met/Ile	2 (4%)	4 (9%)	6 (6%)



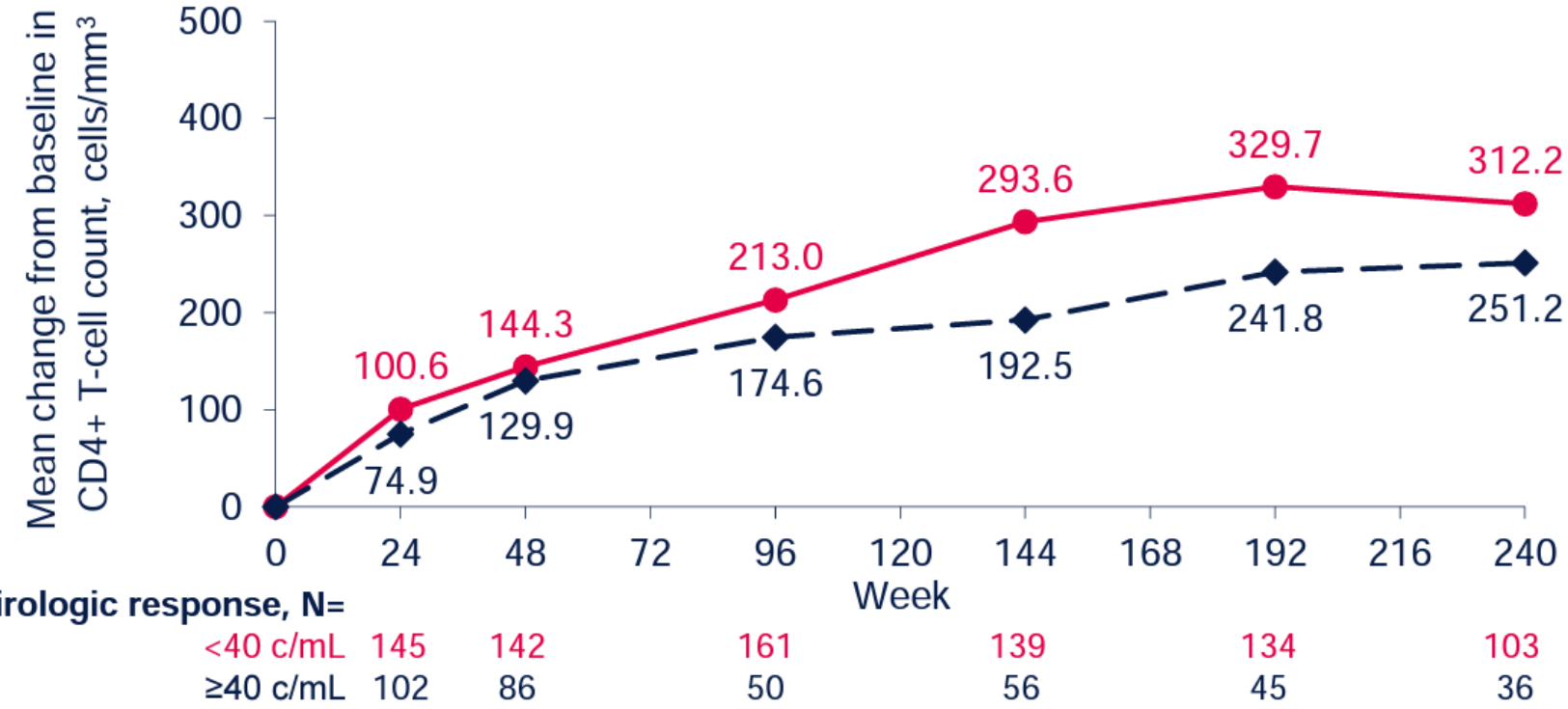




Gartland, AAC 2022

Fostemsavir in HTE individuals: the BRIGHTE study

Figure 6. Change in CD4+ T-Cell Count From Baseline to Week 240 by Virologic Response at the Same Time Point (Randomized Cohort, Observed Analysis)



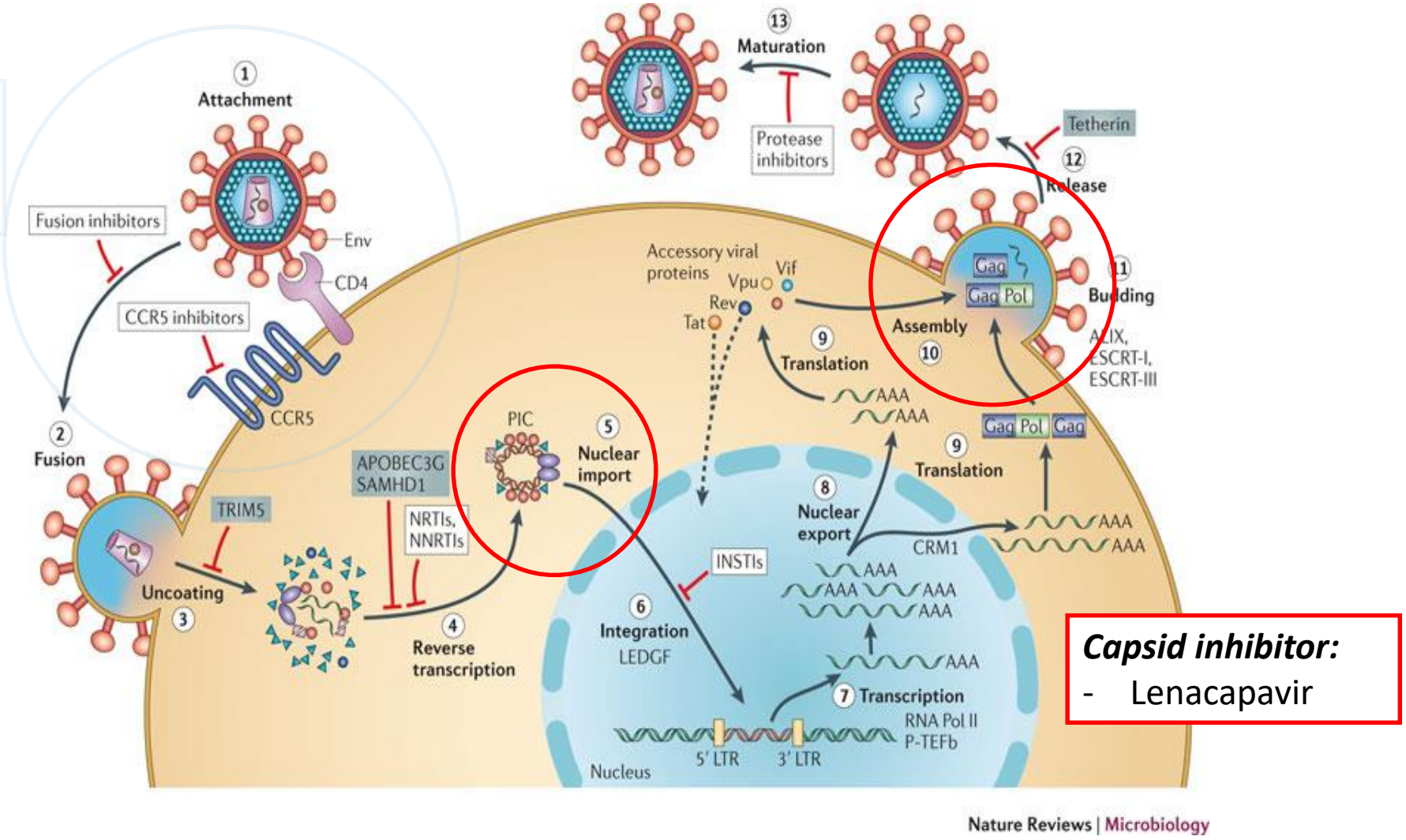
At week 240, participants with HIV-1 RNA ≥40 cp/mL experienced CD4+ T-cell count recovery similar to those with HIV-1 RNA <40 cp/mL

Abert, HIV Glasgow 2022, poster #P061

UNIVERSITÀ DI SIENA
1240

Recently approved antivirals

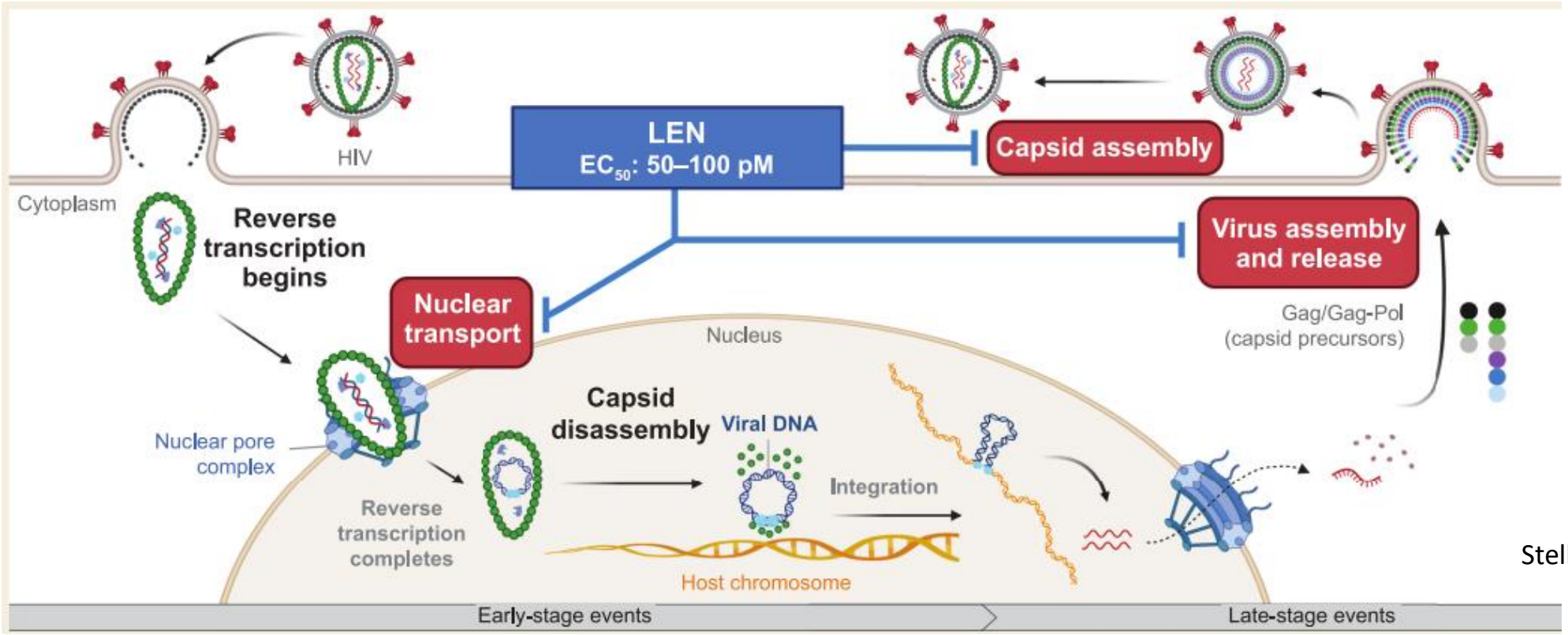
Entry inhibitors:
- Ibalizumab
- Fostemsavir



Capsid inhibitor:
- Lenacapavir

UNIVERSITÀ DI SIENA
1240

Lenacapavir mechanism of action



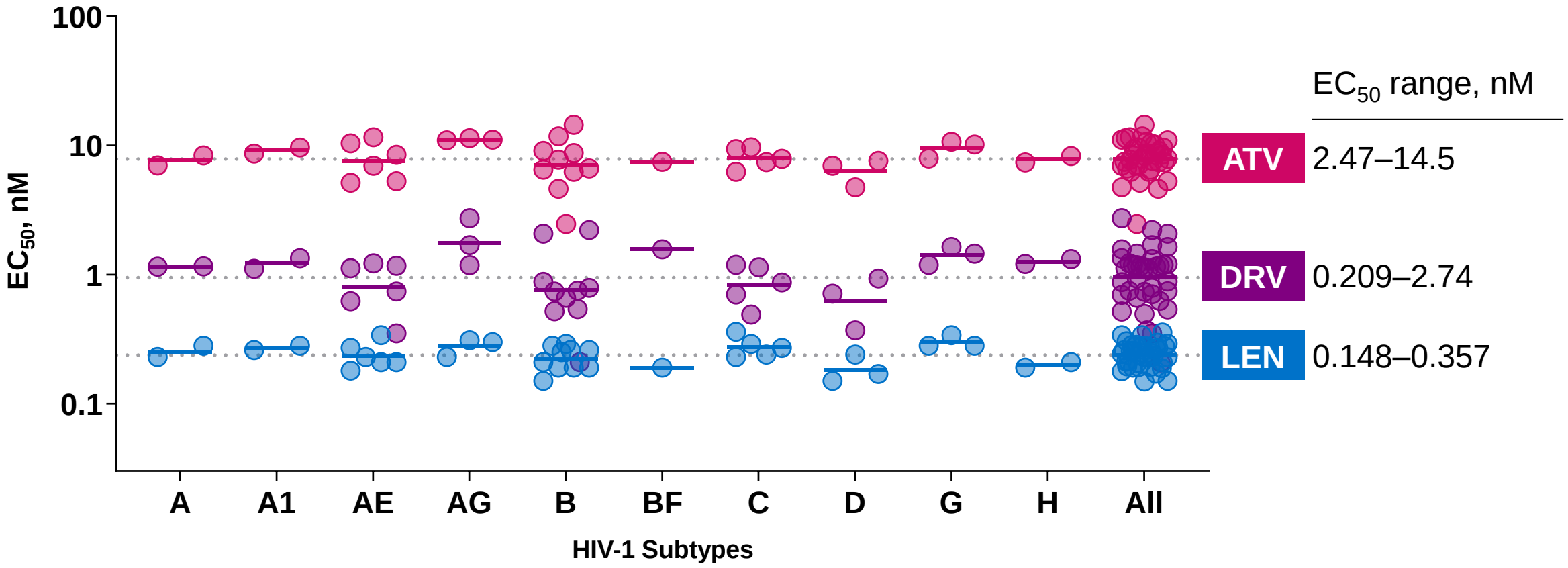
Stellbrink, EACS 2021

First-in-class capsid (CA) inhibitor approved for the treatment of multidrug resistant HIV-1

Picomolar potency (EC_{50} = 50-100 pM)

LEN inhibits CA-mediated nuclear entry of HIV DNA (Selyutina, iScience 2022), HIV assembly and proper capsid formation

Lenacapavir is active against all HIV-1 subtypes



EC_{50} values determined with PhenoSense Gag-Pro assay. n=37 clinical isolates.
Each dot represents an individual isolate; solid lines represent geometric mean EC_{50} for each subtype; dotted lines indicate geometric mean EC_{50} for all subtypes tested.

VanderVeen L, et al. vCROI 2021. Oral #128

UNIVERSITÀ DI SIENA 1240

Lenacapavir resistance

- *In vitro* resistance selections identified 7 mutations arising at 6 amino acids in capsid
  - **L56I, M66I, Q67H, K70N, K74S/D, T107N**
- Three main patterns of emergent mutations during CAPELLA study (72 participants, 20 with resistance test, 8 with emergent RAMs at week 26):
  - **M66I** ± Q67H/K/N, K70N/R/S, N74D, A105T, T107A/C (n.6)
  - **Q67H+K70R** (n.1) (*+2 cases in the CALIBRATE study*)
  - **K70H+A105S/T+T107N** (n.1)
- All mutations map to LEN binding site
- Resistance correlated with low replication capacity for most mutants

Resistance Selected by LEN

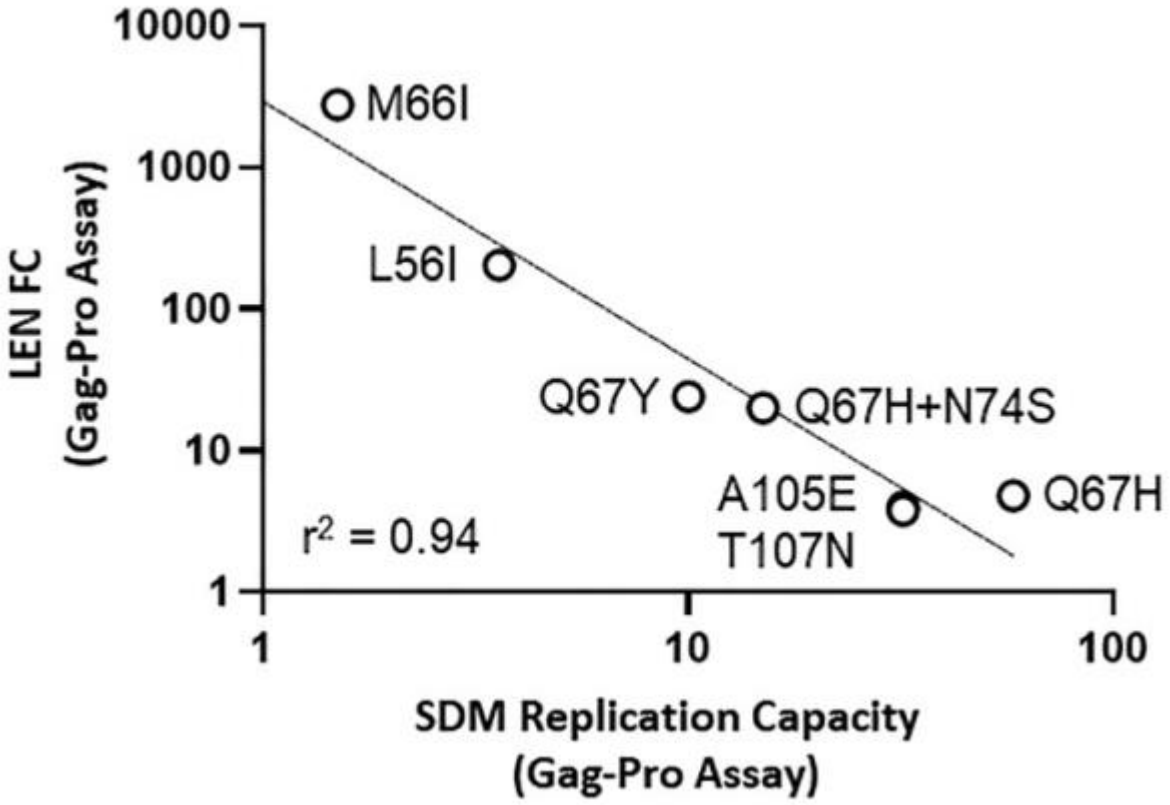
	PhenoSense Gag-Pro (single cycle)*	
HIV-1 Capsid Sequence	LEN Fold-Resistance†	Replication capacity, % WT‡
T107N	3.8	32
Q67H	4.8	58
N74D	16	ND
Q67H+N74S	20	15
Q67H+T107N	87	ND
L56I	204	3.6
Q67H+M66I	1,594	ND
Q67H+N74D	>2,700	ND
M66I	>2,700	1.5

WT, wild-type; ND, not determined; SC, single cycle
* Results were consistent across single- and multi-cycle assay formats, and across primary cells and cell lines
† Ratio of Mutant/WT EC_{50}, determined with SC reporter HIV-1 in PhenoSense Gag-Pro assay
‡ Percentage of reference strain, determined with SC reporter HIV-1 in PhenoSense Gag-Pro assay

Link Nature 2020; Margot JAC 2022; Margot JID 2022

UNIVERSITÀ DI SIENA 1240

Lenacapavir: the higher the resistance, the lower the fitness resistance



Correlation between replication capacity and fold change in lenacapavir susceptibility in the Gag-Pro assay. Replication capacity (RC) and lenacapavir change in susceptibility for the seven mutants with dual data in the single-cycle Gag-Pro assay are plotted and analysed by linear regression. An inverse relationship was observed between RC and susceptibility to lenacapavir (r2=0.94). FC, fold change; LEN, lenacapavir; SDM, site-directed mutant.

Margot, JAC 2022

UNIVERSITÀ DI SIENA 1240

LEN RAMs are not natural polymorphisms

HIV-1 subtype distribution	ART naive (*N* = 500), % (*n*)	ART experienced without PI use (*N* = 500), % (*n*)	ART experienced with history of PI failure (*N* = 500), % (*n*)
B	37 (185)	42 (210)	56 (280)
CRF02_AG	46 (230)	48 (240)	37 (185)
F1	4.6 (23)	2.4 (12)	—
CRF06	4.4 (22)	3.8 (19)	3.4 (17)
A1	2.8 (14)	—	—
D	2.2 (11)	2.2 (11)	1.6 (8)
Other non-B	3.0 (15)	1.6 (8)	1.0 (5)

- CA mutations conferring resistance to LEN *in vitro* (**L56I, M66I, Q67H, K70N, N74D, N74S, T107N**) evaluated in 1500 patients
  - 500 ART naïve
  - 500 PI naïve ART experienced
  - 500 with history of PI failure
- **None detected**

Marcelin, J Antimicrob Chemother 2020

UNIVERSITÀ DI SIENA 1240

LEN RAMs are not natural polymorphisms

WT aas and their positions	Resistant variants[a]	HIV-1 group M subtypes							
		A ($n=157$)	B ($n=205$)	C ($n=834$)	D ($n=71$)	F1 ($n=14$)	CRF07_BC ($n=267$)	CRF01_AE ($n=446$)	ORFs ($n=37$)
L56	I	—	—	F (3), V (2), M (1)	—	—	—	K (1)	—
M66	I	C (1)	C (5)	C (7), H (2), **I (1)**	C (71)	—	V (1)	A (1), C (1)	—
Q67	H	K (1)	K (5)	K (4), R (3), A (2)	K (67)	—	—	N (1), K (1), **H (1)**	—
K70	N	—	—	R (1)	R (5)	—	—	R (1), I (1)	—
N74	D, S	M (1)	M (5)	M (7), Q (2)	R (57)	—	—	M (1)	—
T107	N	L (1)	A (1), V (2), L (4), P (1), S (1)	A (1), S (2), L (7), **N (1)**	L (69), P (3)	—	—	S (9), L (2), Q (1)	—

Other recombinant forms (ORFs) refers to CRF89_BF1 ($n=18$), CRF02_AG ($n=11$), CRF_49.cpx ($n=3$), CRF76_01B ($n=3$) and CRF65_cpx ($n=2$). Numbers in brackets are the number of sequences harbouring the variant. Reference to HXB2. In bold they are amino acid substitution associated to Lenacapavir drug resistance.
[a]Resistant variants were defined according to previous studies.[9,13,17]

- Analysis of 2031 p24 sequences belonging to different HIV-1 subtypes and CRFs were retrieved from LANL database
- Only 3/2031 (0.14%) sequences harboured known LEN RAMs emerged ***in vitro*** (boxes indicate mutations emerged ***in vivo****)
- Several polymorphisms at LEN RAMs positions have been identified → unknown impact on LEN susceptibility

Nka, J Antimicrob Chemother 2023. *Margot, JID 2022

UNIVERSITÀ DI SIENA 1240

Take home messages

- **Natural susceptibility to new drugs is good/excellent**
  - Reduced susceptibility to IBA expected in 5-10% of isolates and to FTR in CRF01_AE, no issues for LEN
- **Emergent resistance to new drugs at treatment failure is less reassuring**
  - None may be labelled as high genetic barrier drug
- **Genotype may predict drug susceptibility with good/high accuracy**
  - …except for FTR
- **Phenotyping may help to evaluate drug activity**
  - To be considered in deep salvage with complex MDR