UNIVERSITÀ
DI SIENA
1240

Aspetti virologici e diagnostici nei pazienti HTE

1° Congresso Nazionale
AGGIORNAMENTI INFETTIVOLOGICI: HIV, EPATITI & …..
Firenze, 16 Gennaio 2024

▶ **FRANCESCO SALADINI**
DIPARTIMENTO BIOTECNOLOGIE MEDICHE
UNIVERSITA' DI SIENA

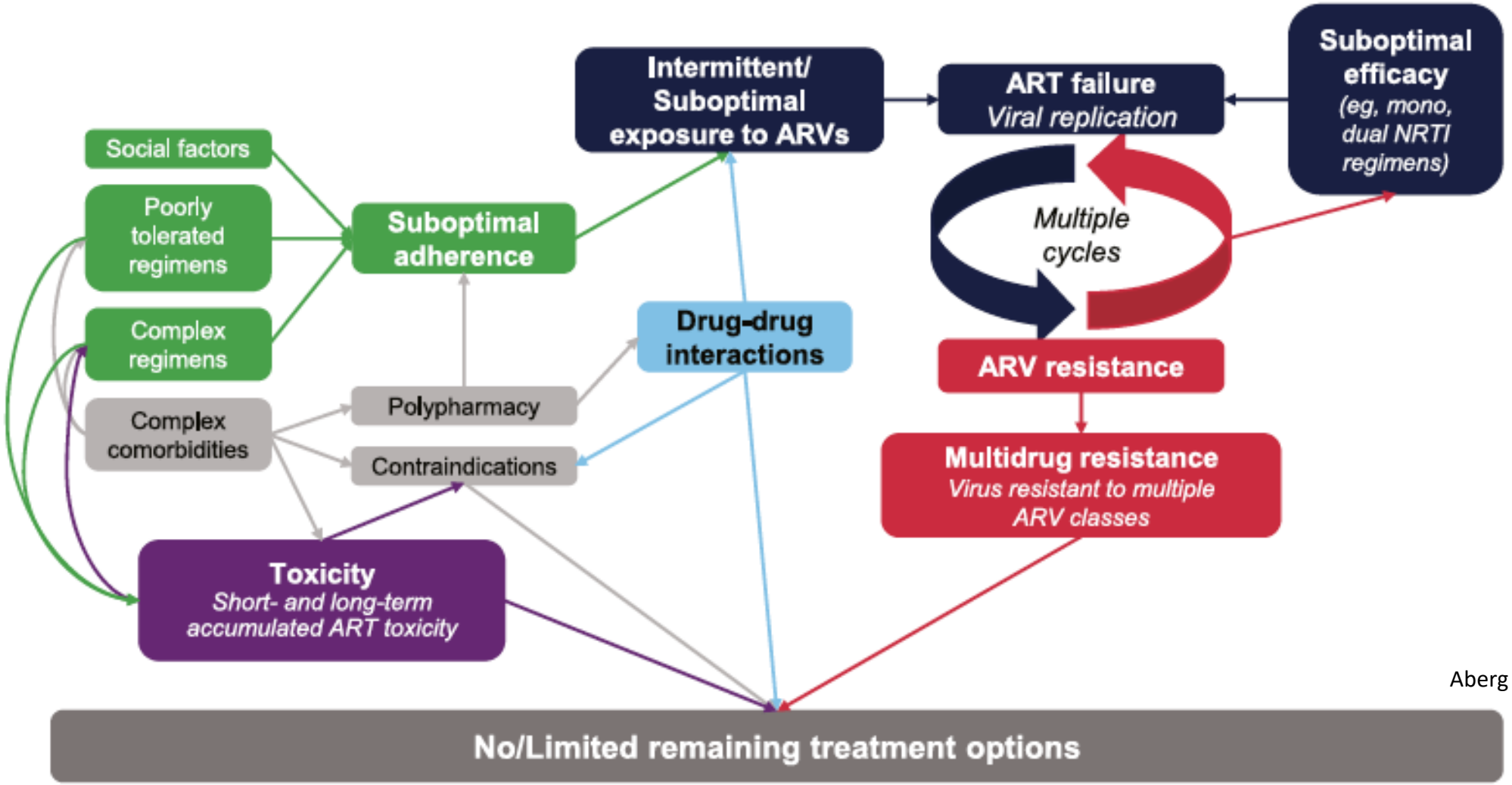
UNIVERSITÀ DI SIENA 1240

Conflicts of interest

- Dr. Francesco Saladini has received personal fees for speaker’s bureau from ViiV Healthcare

UNIVERSITÀ DI SIENA 1240

Clinical challenges to optimal ARV treatment in heavily treatment-experienced PLWH



Aberg *Antivir Ther* 2023

Towards undetectability in viremic MDR PLWH

Current salvage therapies include:

- new ARVs with ***innovative mechanism of action***
- drugs with full/partial activity as determined by ***genotypic/phenotypic assays***
- DTG and/or DRV bid even in presence of RAMs

Viral suppression

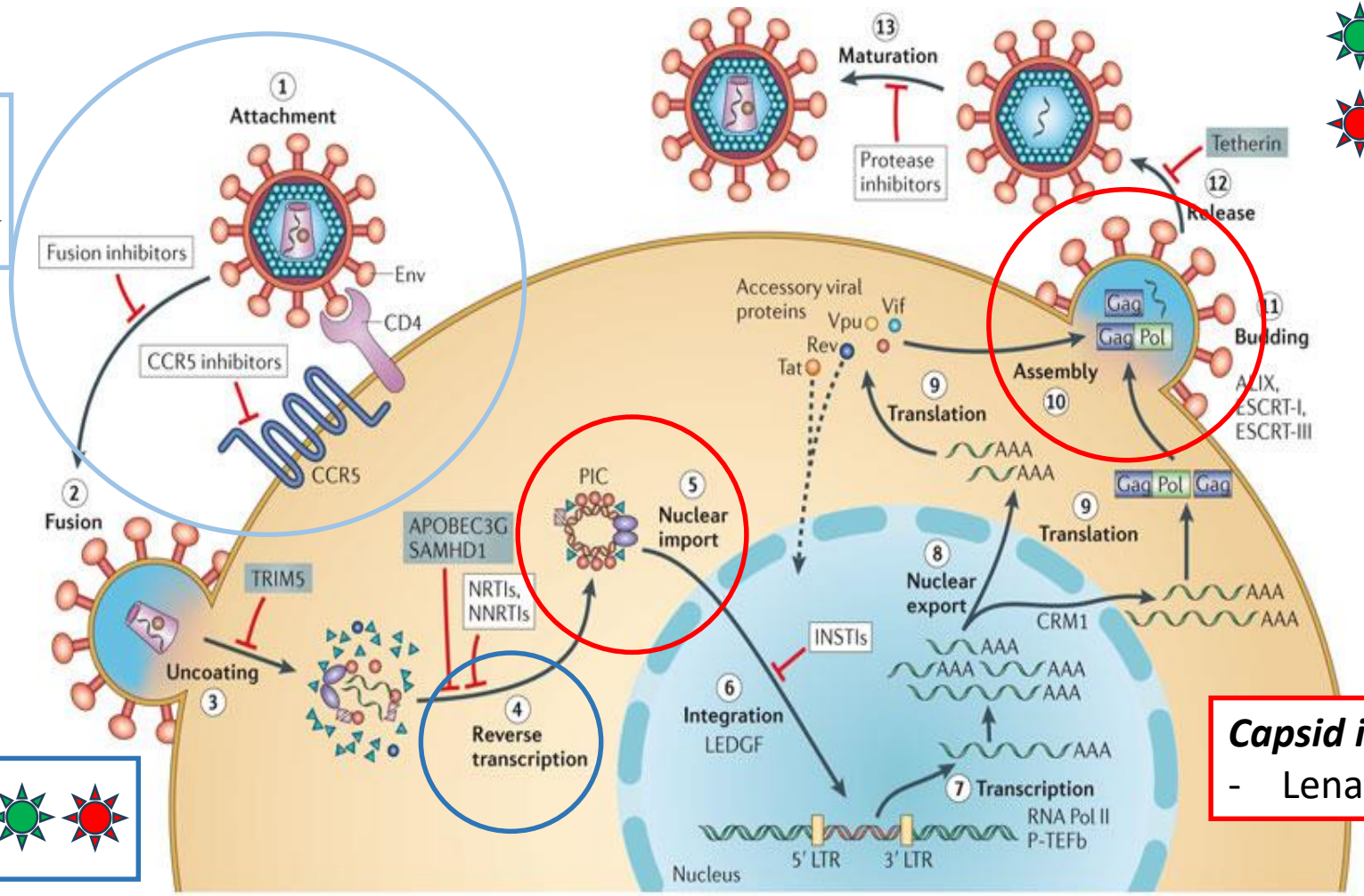
UNIVERSITÀ DI SIENA 1240

Towards undetectability in viremic MDR PLWH: new ARVs

- First-line therapy
- Maintenance therapy
- Salvage therapy

Entry inhibitors:
- (Ibalizumab)*
- Fostemsavir

* EU marketing withdrawal from January 1, 2023



Nature Reviews | Microbiology

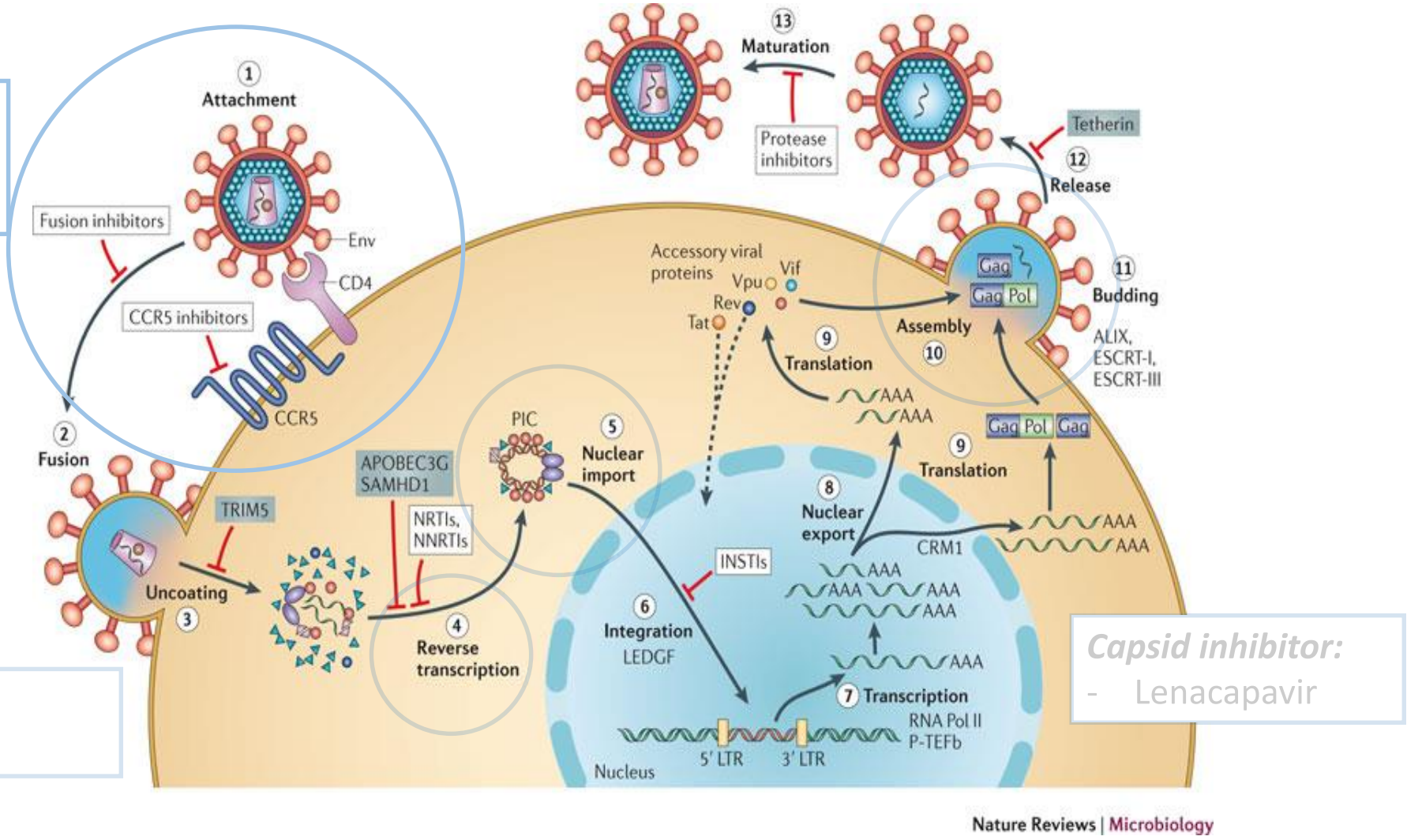
NNRTIs:
- Doravirine

Capsid inhibitor:
- Lenacapavir

UNIVERSITÀ DI SIENA
1240

Towards undetectability in viremic MDR PLWH: new ARVs

Entry inhibitors:
- Ibalizumab
- Fostemsavir

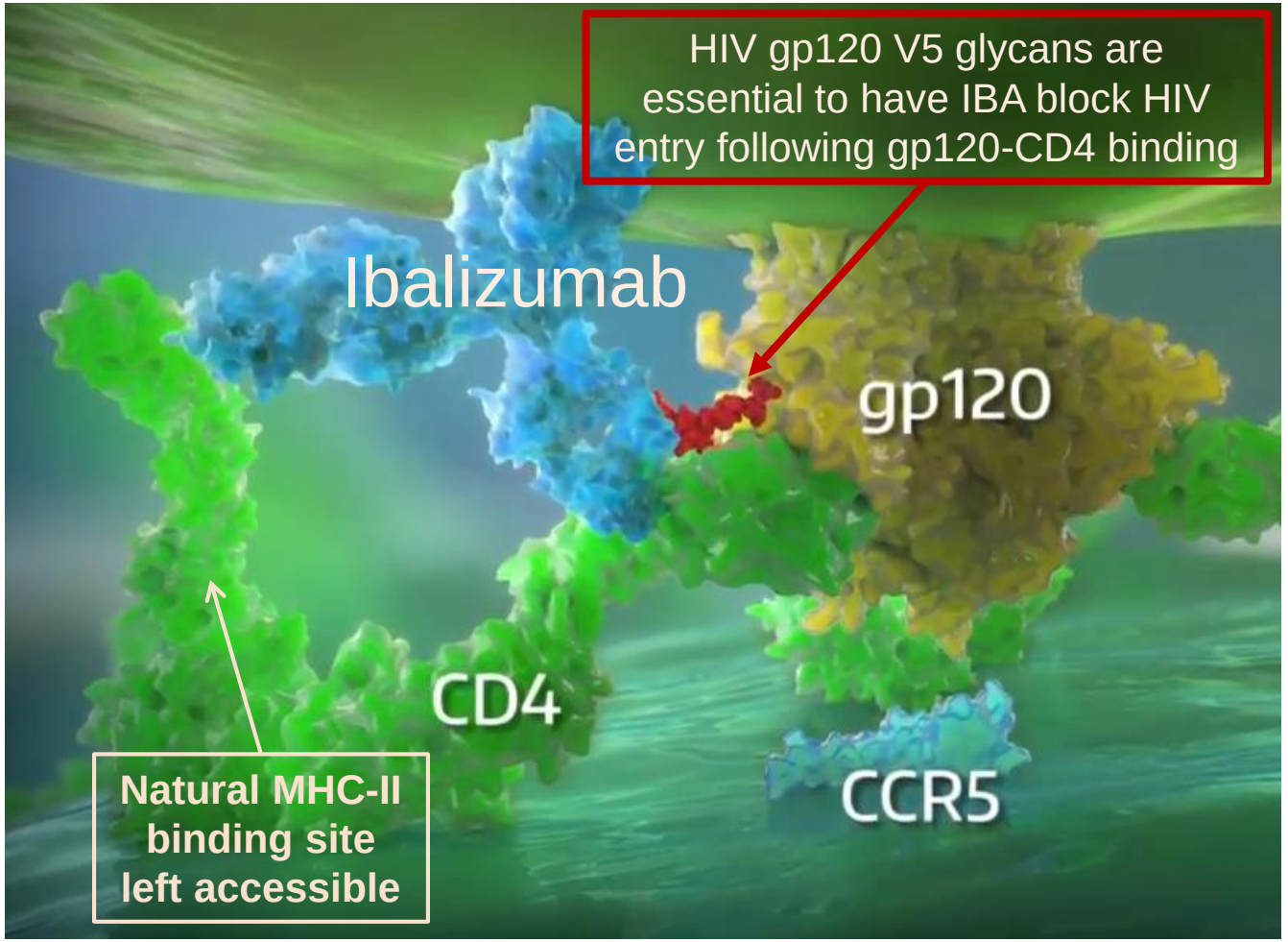


NNRTIs:
- Doravirine

Capsid inhibitor:
- Lenacapavir

UNIVERSITÀ DI SIENA 1240

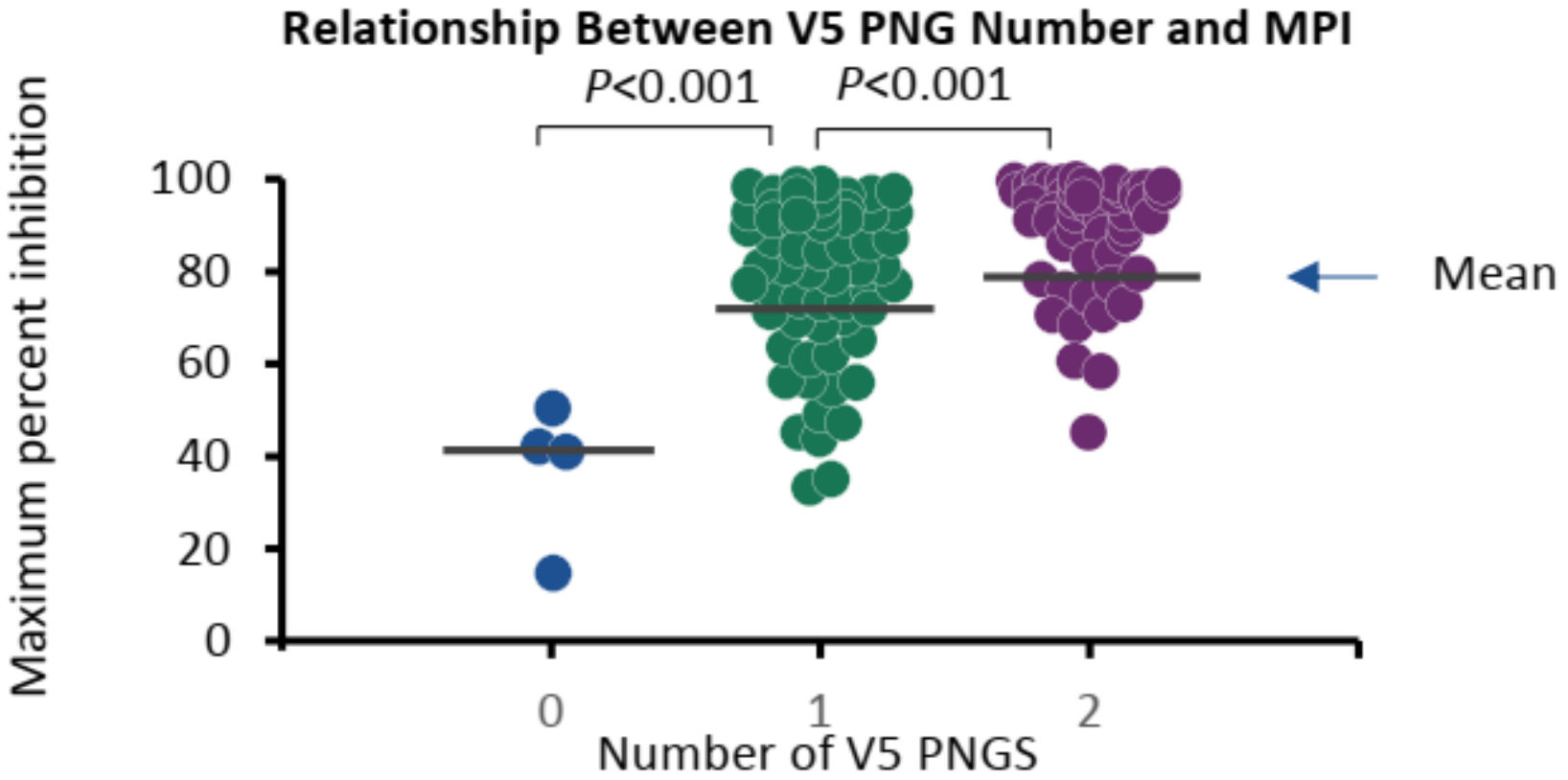
Mechanism of action of Ibalizumab



- 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

UNIVERSITÀ DI SIENA 1240

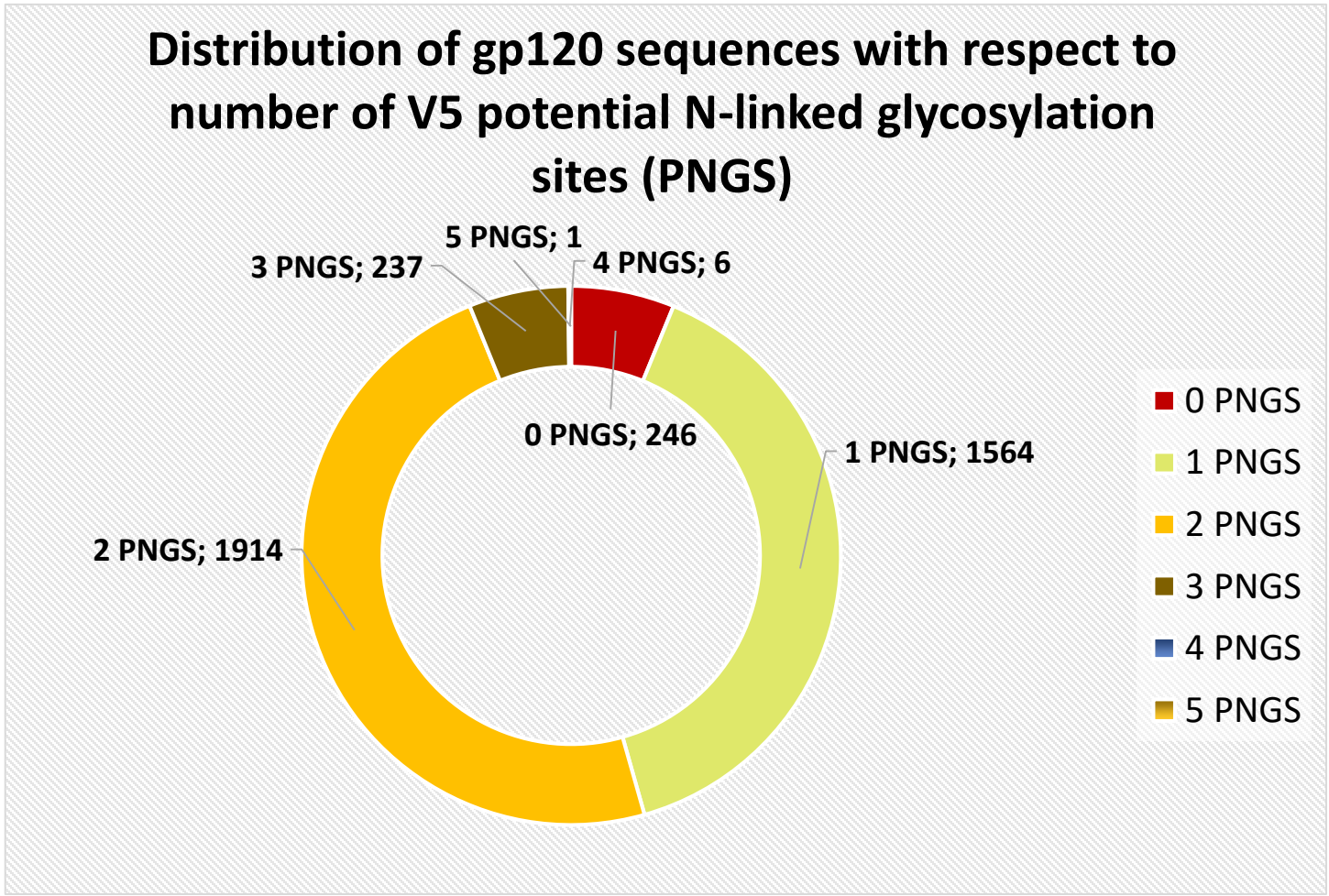
Ibalizumab maximum percentage of inhibition and gp120 V5 glycosylation



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

Pace *JAIDS* 2013

Number of PNGS in HIV-1 gp120 V5

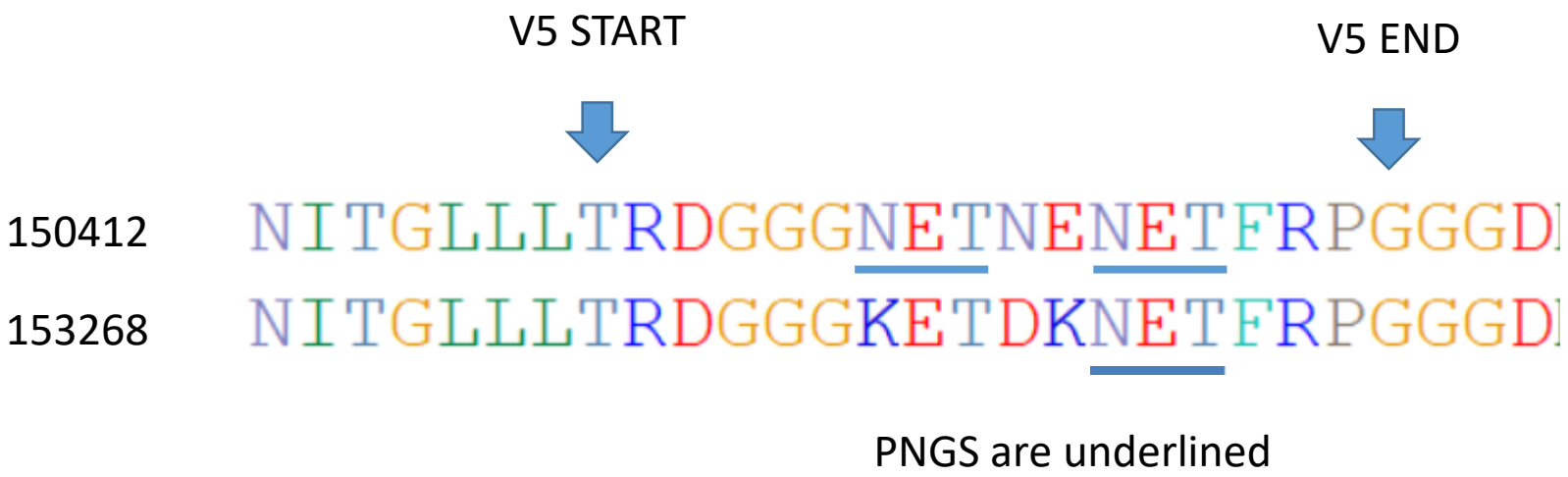


- HIV-1 gp120 dataset: n = 3967
- Representative of most common subtypes and CRFs
- 6.2% with no PNGS
- 39.4% with one PNGS
- 54.4% with two or more PNGS

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

UNIVERSITÀ DI SIENA 1240

Genotypic screening of gp120 V5 sequences during Ibalizumab exposure



PNGS are underlined

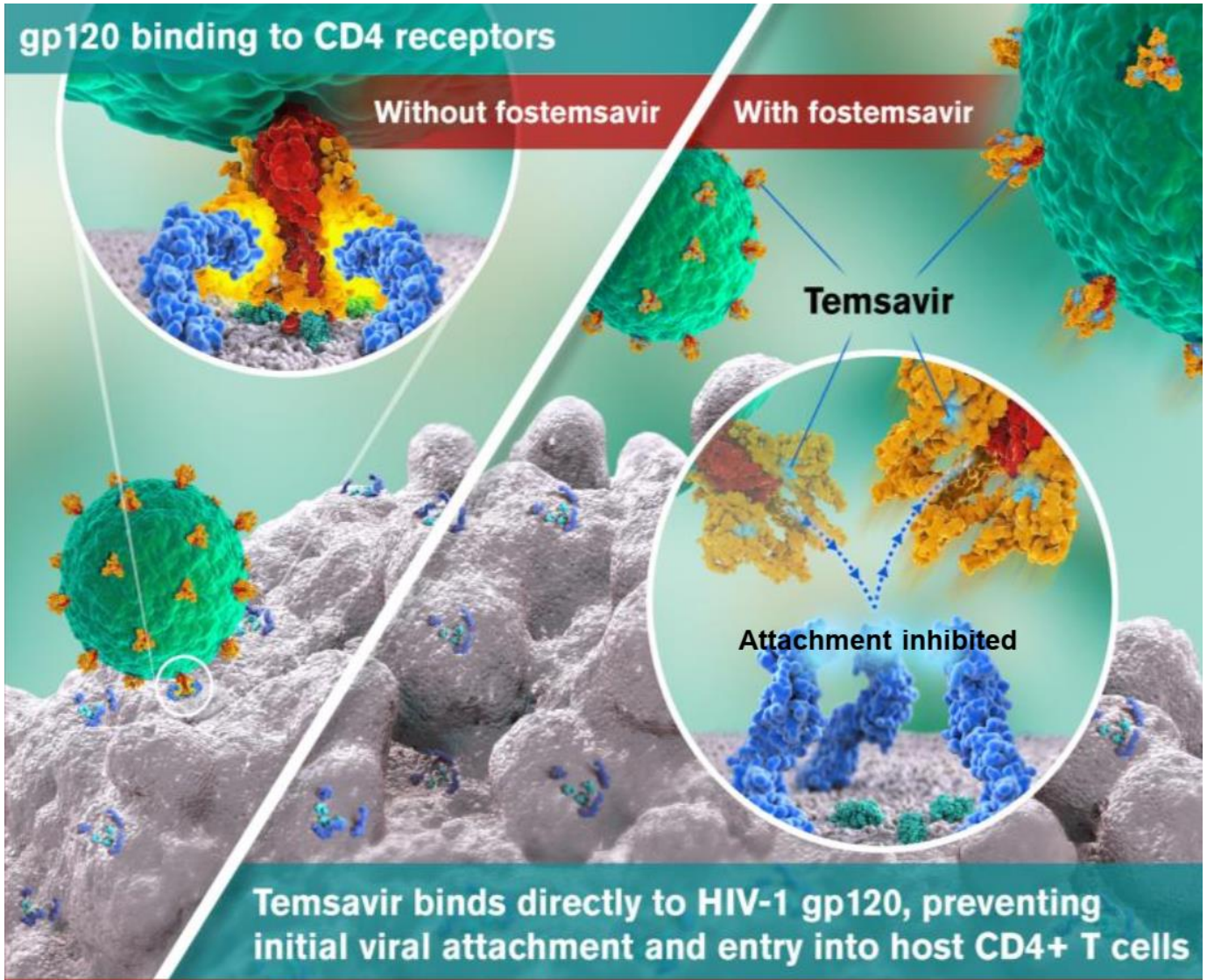
150412 = sample pre-IBA exposure
153268 = sample on-IBA treatment

Potential N-linked glycosylation site (PNGS)

Asn-any-Ser OR **Asn-any-Thr**
N – X – S OR **N – X – T**

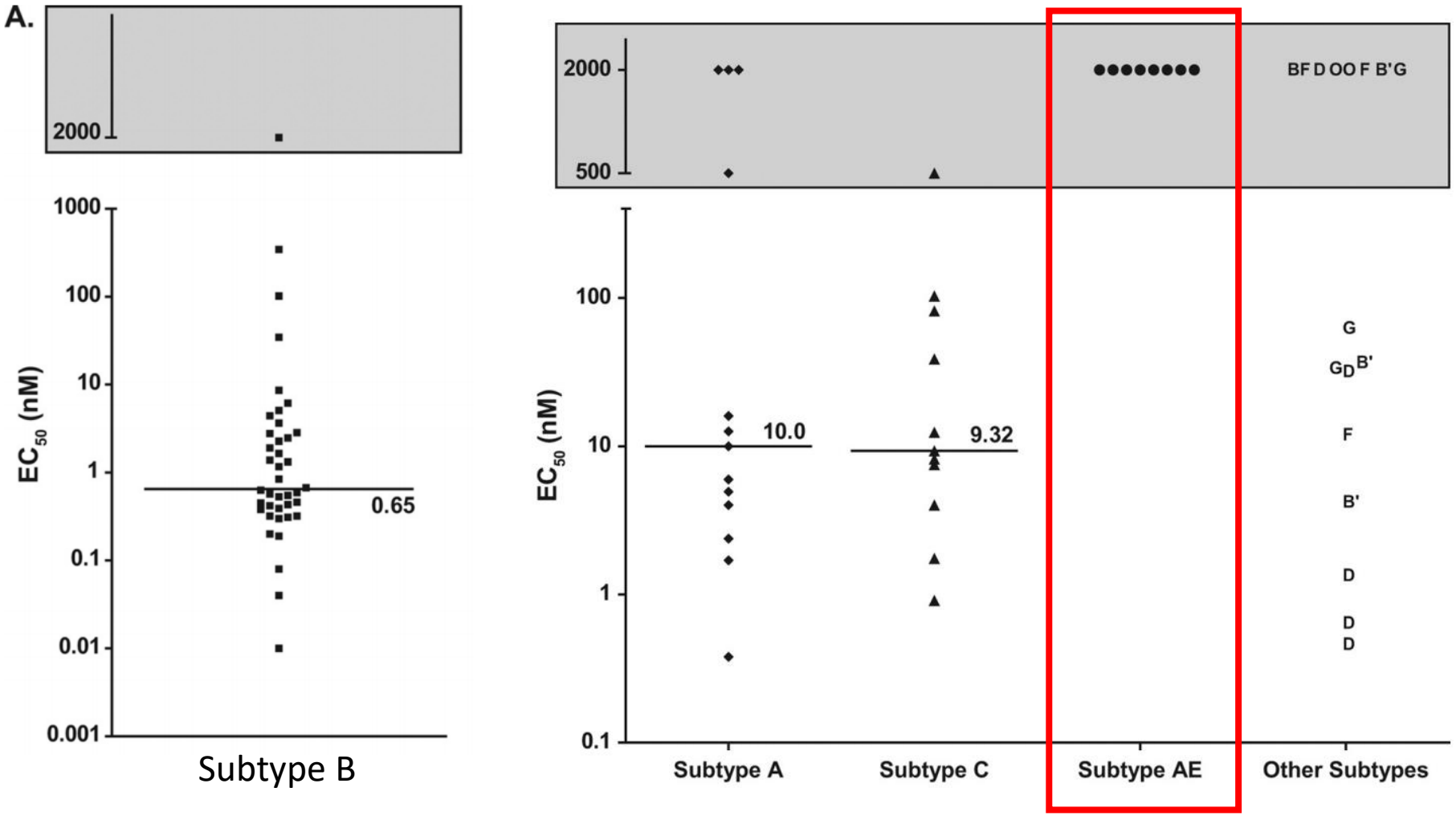
UNIVERSITÀ DI SIENA
1240

Mechanism of action of Fostemsavir



UNIVERSITÀ DI SIENA 1240

Natural susceptibility to Fostemsavir in different HIV1 subtypes

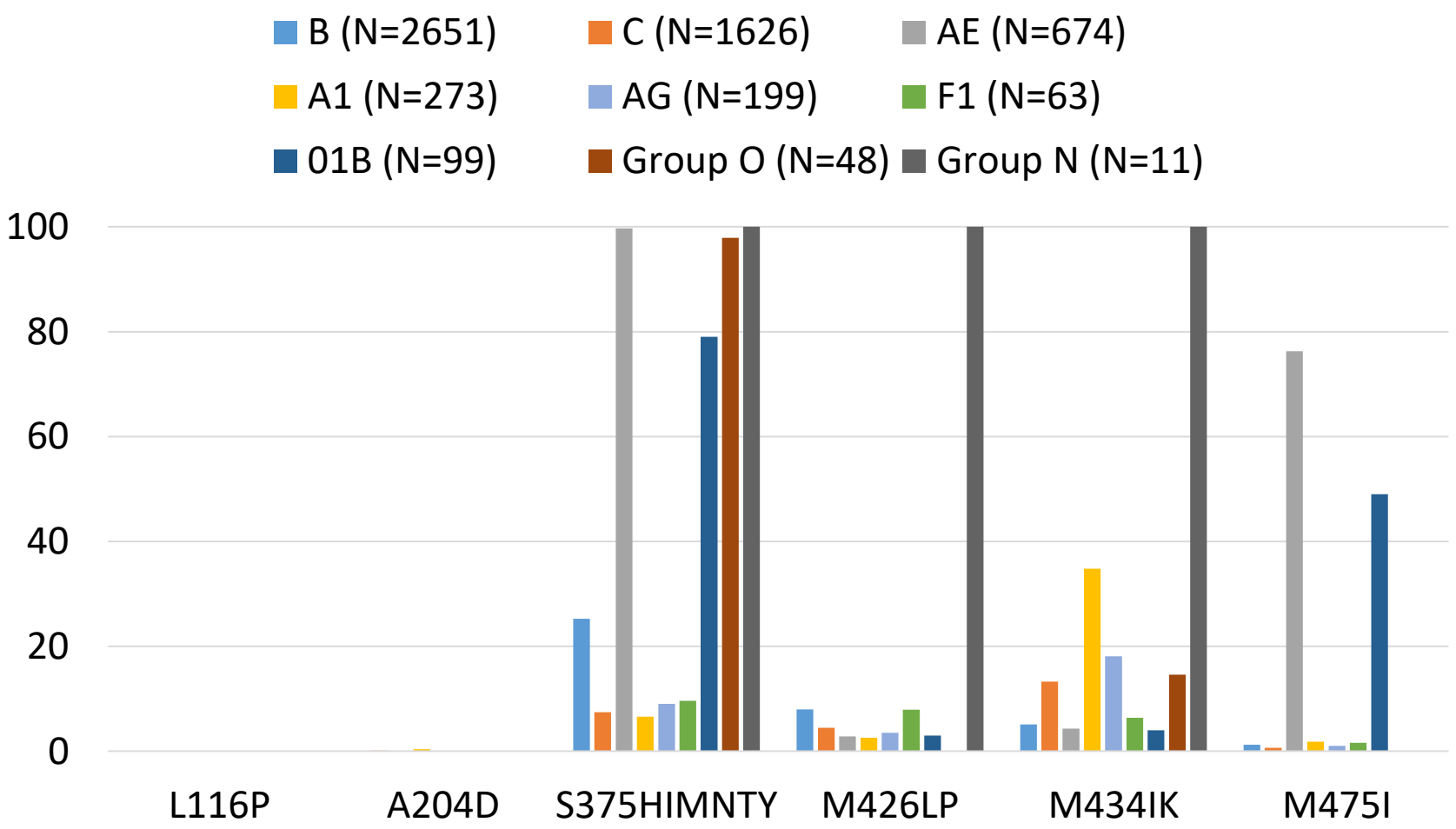


- Broad range of natural susceptibility within each subtype
- Genotypic susceptibility not fully elucidated
- Use of fostemsavir not recommended in subtype CRF01_AE isolates

Nowicka-Sanz *AAC* 2012

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: **S375H/I/M/N/T/Y, M426L/P, M434I/K, M475I**

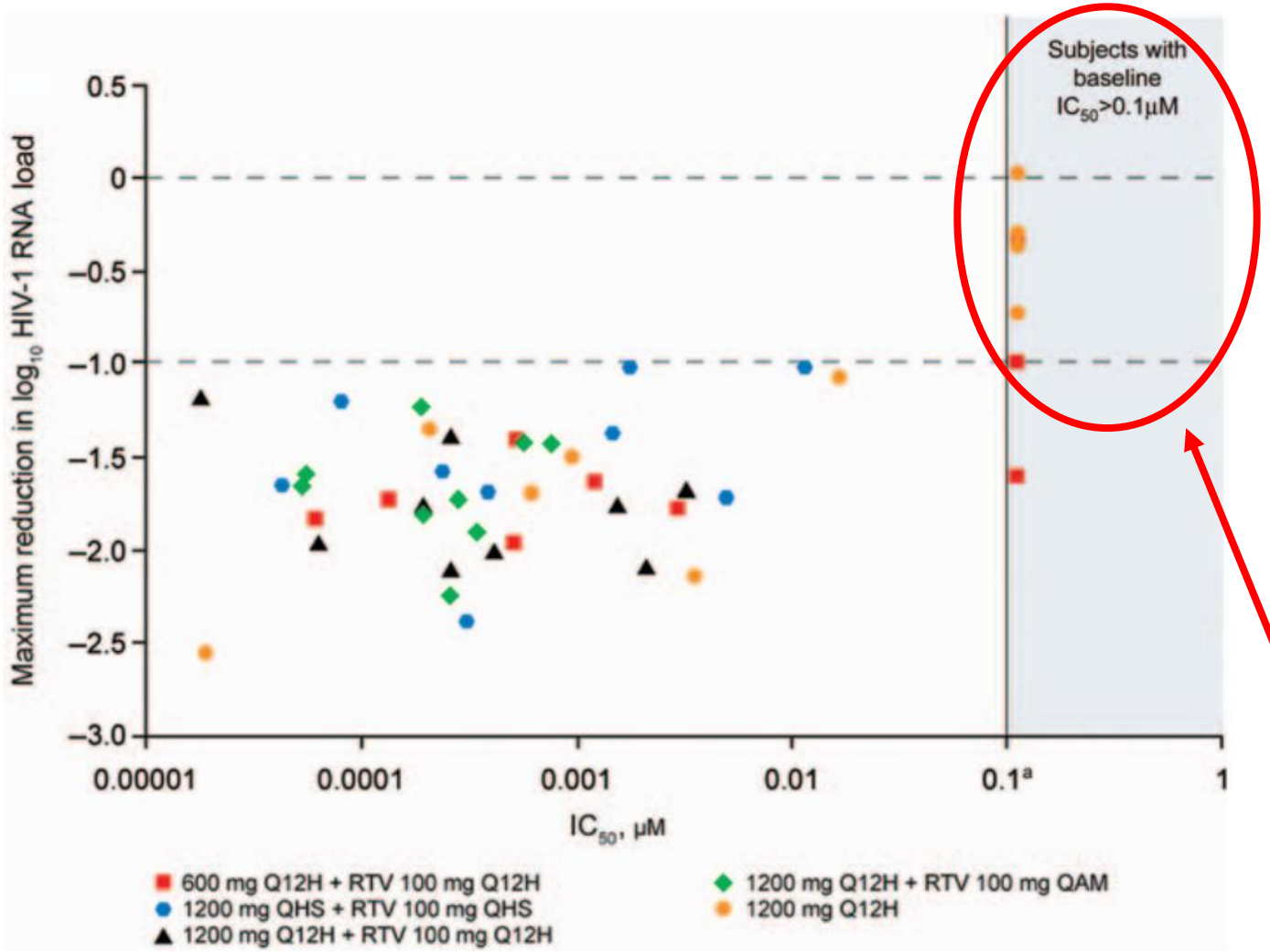
S375H and **M475I** predominant among **CRF01_AE** (S375H, 99.3%; M475I, 76.3%) and **01B** (S375H, 71.7%; M475I, 49.5%)

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

Adapted from Gartland *J Antimicrob Chemother* 2021

UNIVERSITÀ DI SIENA 1240

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**

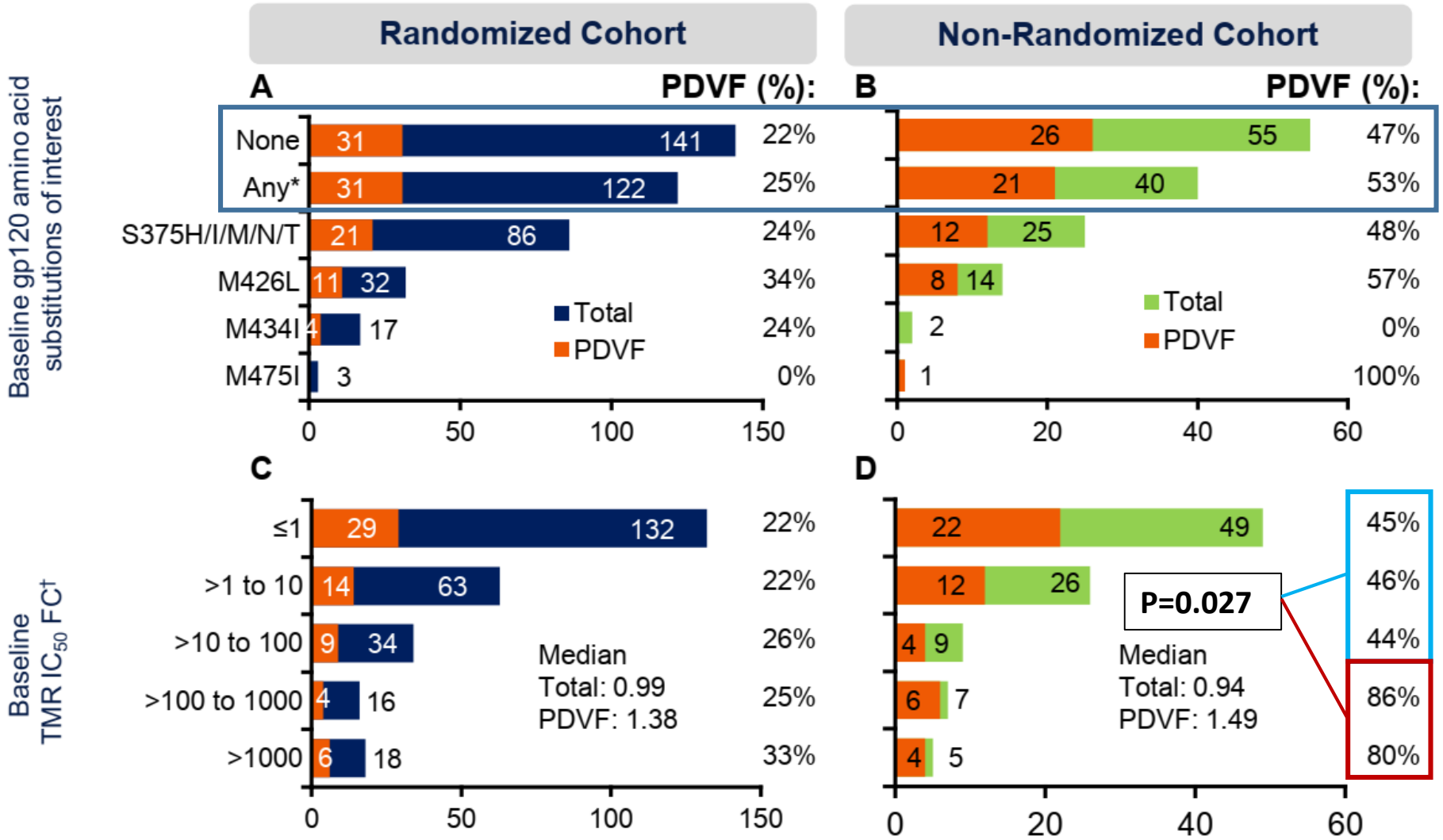
Higher **intrasubject variability** in susceptibility to BMS-626529 compared with other classes of entry inhibitors

Lower response with baseline IC_{50} >100 nM

Nettles *J Infect Dis* 2012

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)

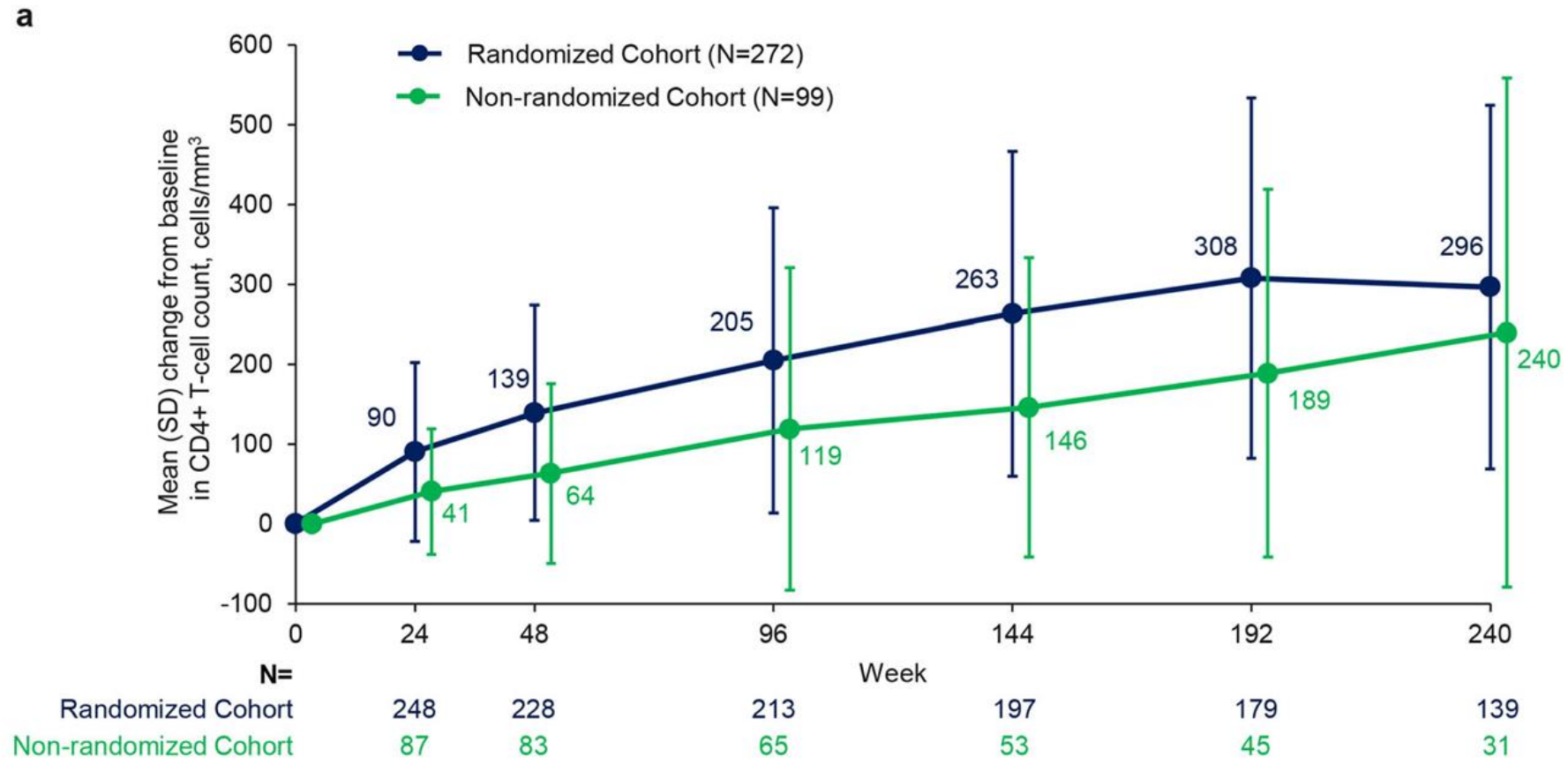
No correlation between baseline **genotypic** resistance and response

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

Gartland *AAC* 2022

UNIVERSITÀ DI SIENA
1240

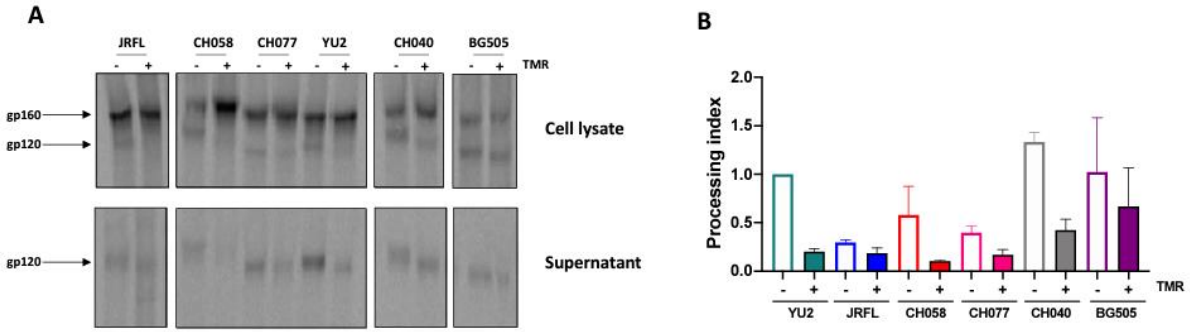
Fostemsavir in HTE individuals: the BRIGHTE study



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

Aberg *Infect Dis Ther* 2023

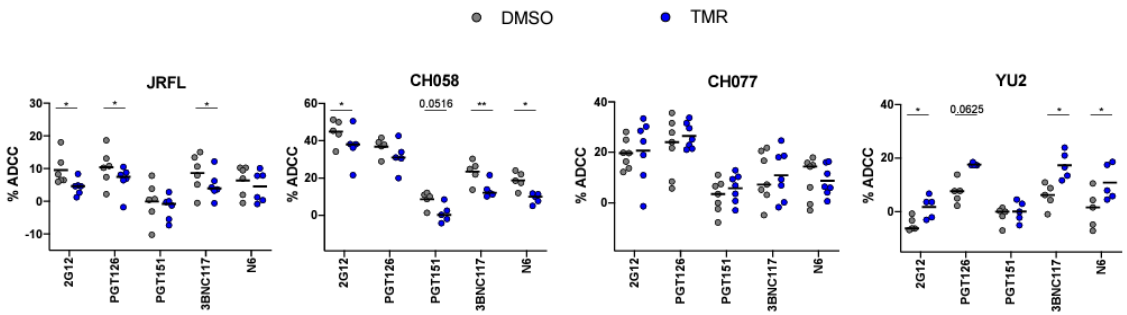
Temsavir reduces antibody-dependent cellular cytotoxicity (ADCC)

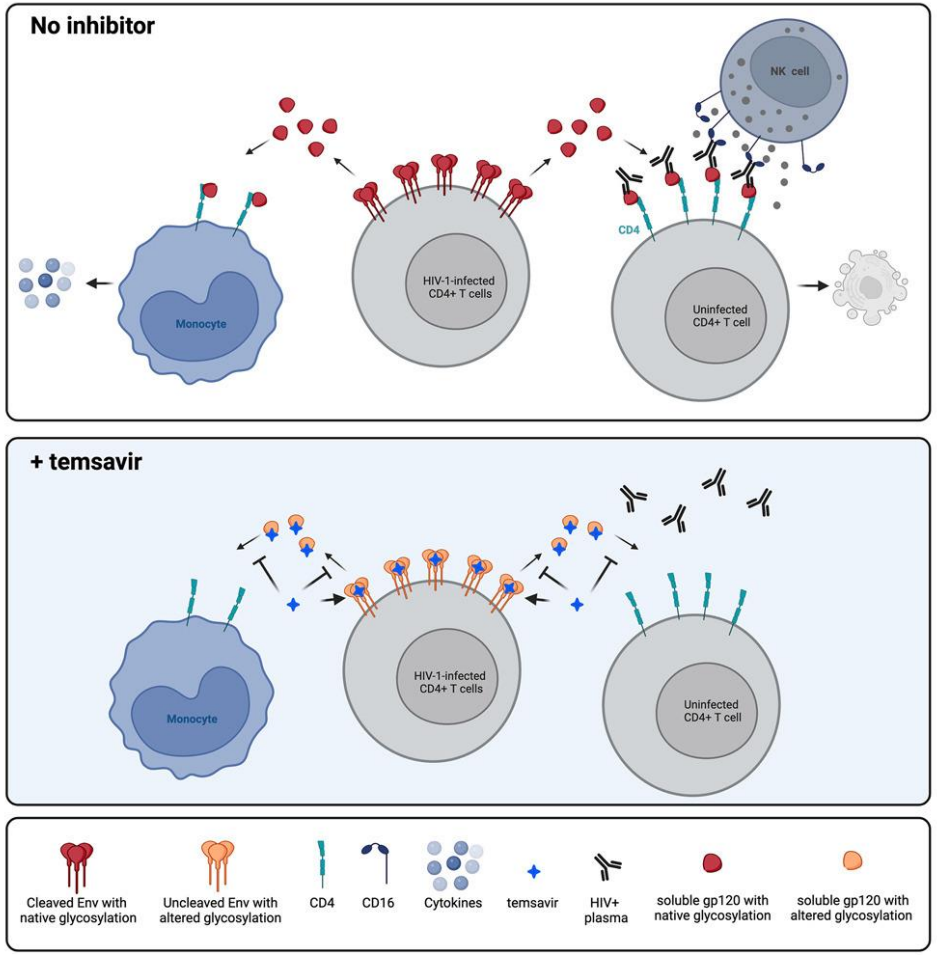


Temsavir reduces the proteolytic processing of Env

Temsavir reduces CD4+ apoptosis even in absence of viral suppression?

Temsavir reduces ADCC lowering affinity to bNAbs





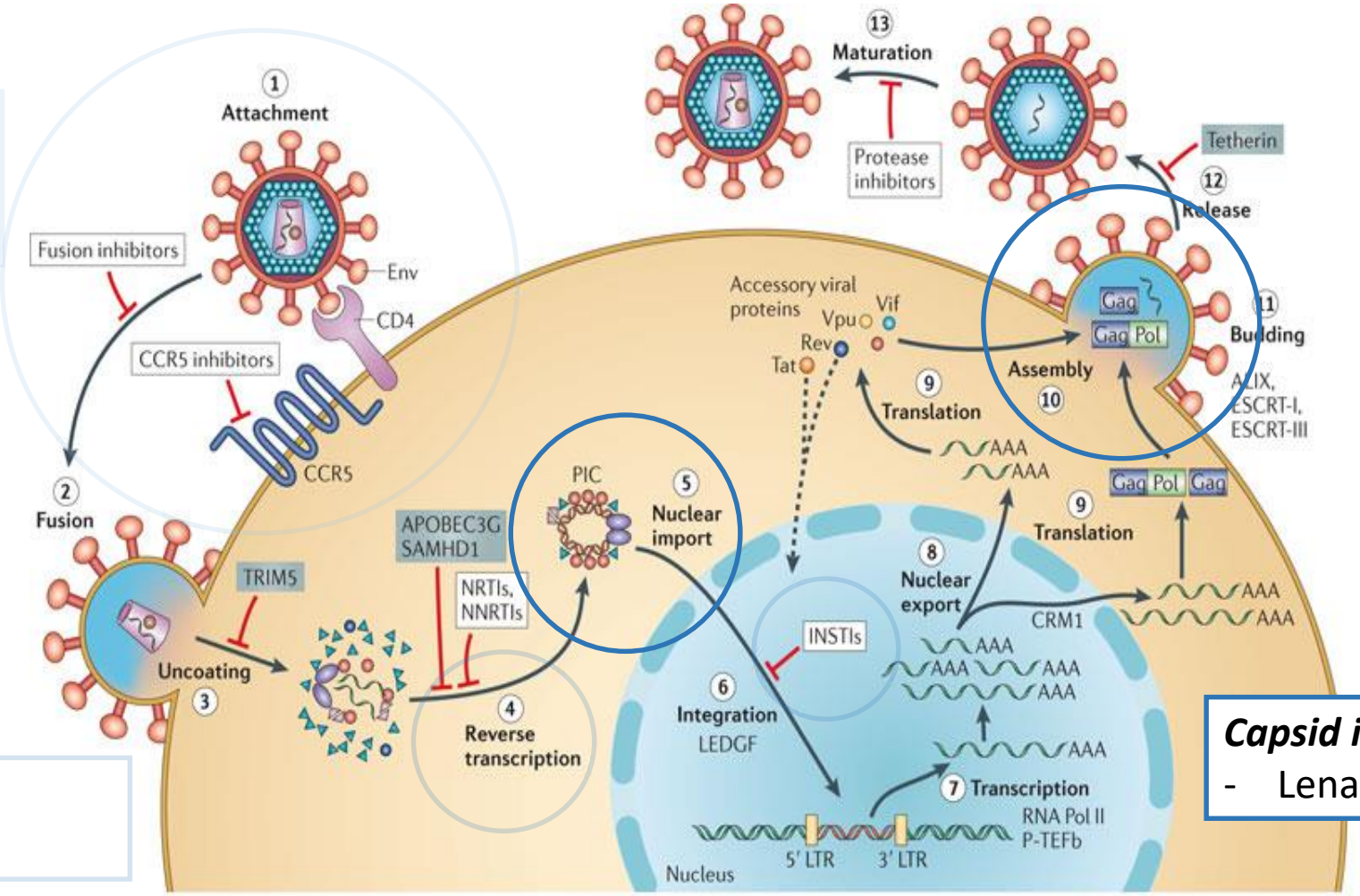
Temsavir prevents shed gp120 from interacting with uninfected bystander CD4⁺ cells, protecting them from ADCC responses and preventing a cytokine burst

Richard *Cell Chem Biol* 2023; Boutin *Viruses* 2023

UNIVERSITÀ DI SIENA
1240

Recently approved antivirals

Entry inhibitors:
- Ibalizumab
- Fostemsavir



Nature Reviews | Microbiology

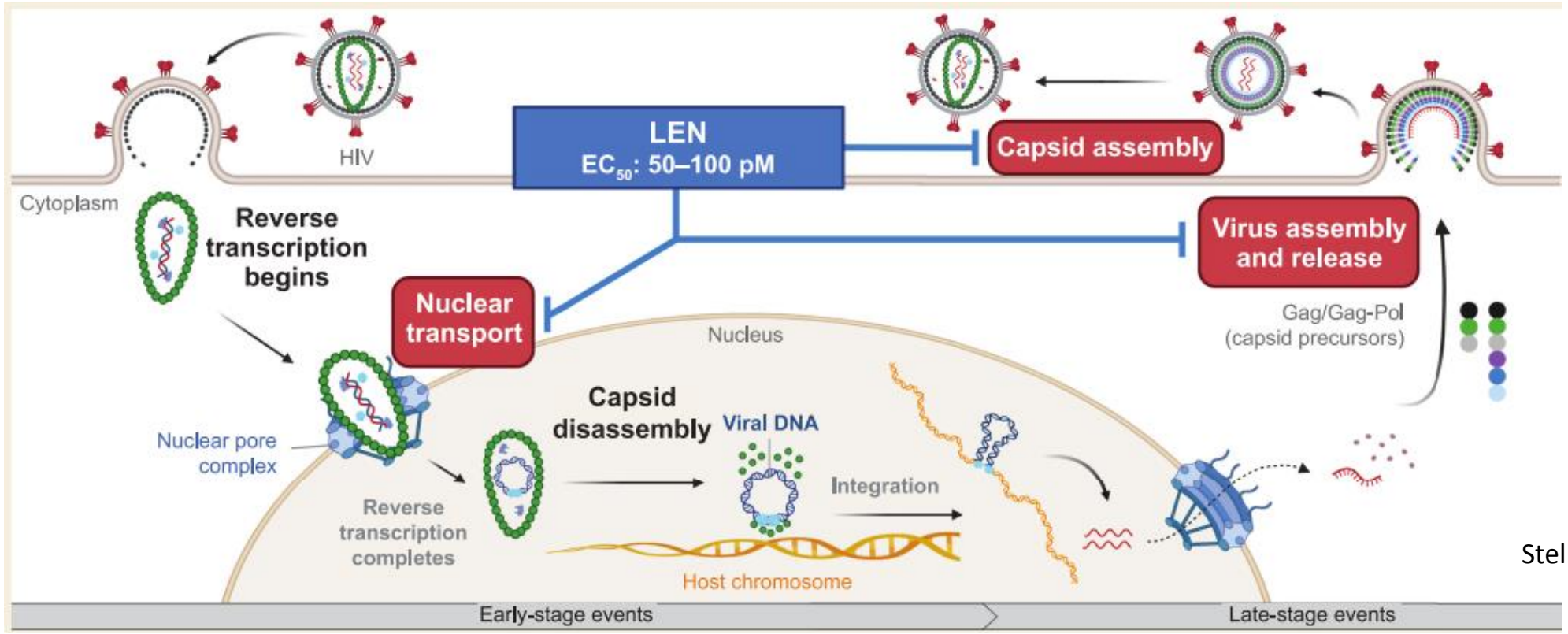
INSTIs:
- Cabotegravir (+Rilpivirine LA)

NNRTIs:
- Doravirine

Capsid inhibitor:
- Lenacapavir

UNIVERSITÀ DI SIENA
1240

Lenacapavir mechanism of action



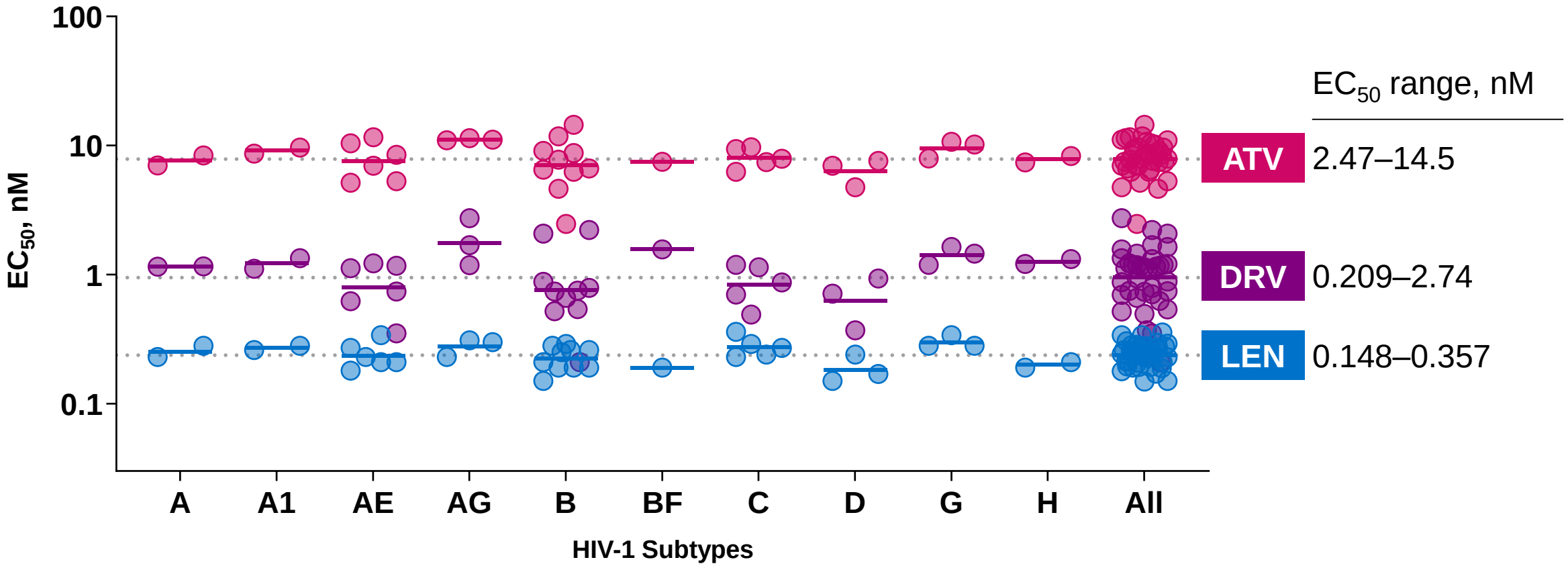
Stellbrink, EACS 2021

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, 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, 14 with emergent RAMs at week 104):
  - **M66I** ± Q67H/K/N, K70N/R/S, N74D, A105T, T107A/C
  - **Q67H+K70R** (*+2 cases in the CALIBRATE study*)
  - **K70H+A105S/T+T107N**
- Of the 14 participants who developed emergent LEN resistance, **7 were virologically resuppressed while continuing LEN** (n. 5 without OBR change)
- All mutations map to LEN binding site
- Resistance correlated with low replication capacity for most mutants

Resistance Selected by LEN

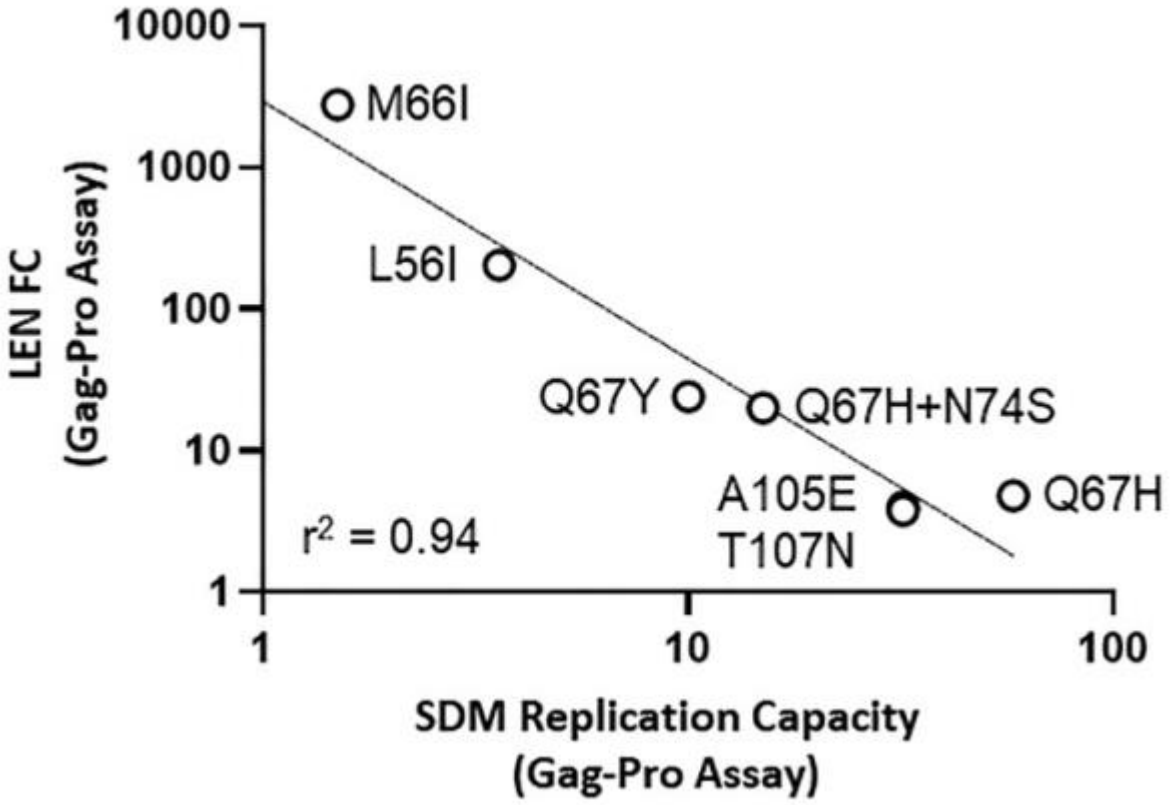
	PhenoSense Gag-Pro (single cycle)*	
HIV-1 Capsid Sequence	LEN Fold-Resistance†	Replication capacity, % WT‡
T107N	3.8	32
Q67H	4.8	58
N74D	16	ND
Q67H+N74S	20	15
Q67H+T107N	87	ND
L56I	204	3.6
Q67H+M66I	1,594	ND
Q67H+N74D	>2,700	ND
M66I	>2,700	1.5

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; Ogbuagu IDWeek 2023 Abstr. 1596

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)

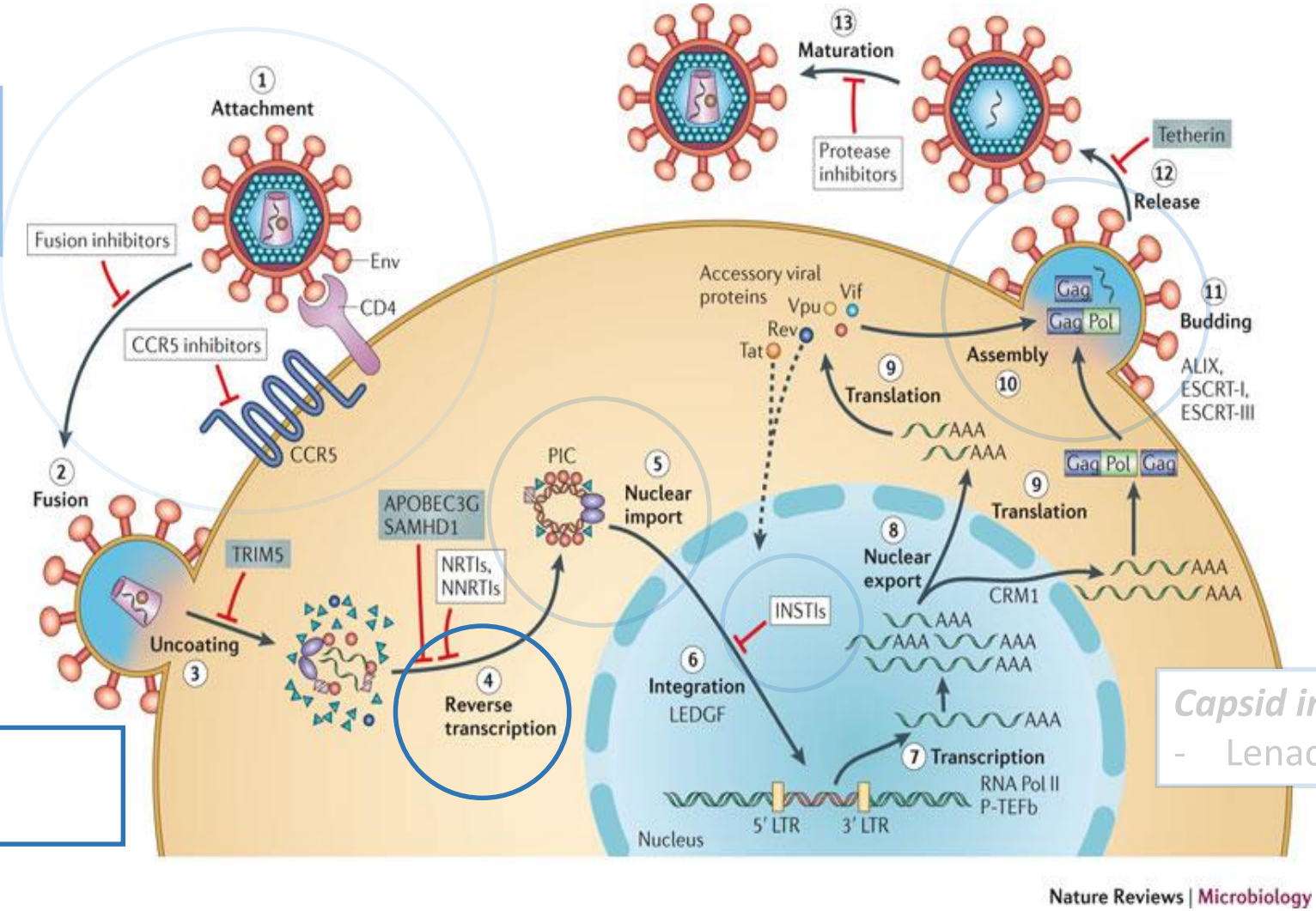
- CA mutations conferring resistance to LEN *in vitro* (**L56I, M66I, Q67H, K70N, N74D, N74S, T107N**) evaluated in 1500 patients
  - 500 ART naïve
  - 500 PI naïve ART experienced
  - 500 with history of PI failure
- **None detected**

Marcelin *J Antimicrob Chemother* 2020

UNIVERSITÀ DI SIENA
1240

Recently approved antivirals

Entry inhibitors:
- Ibalizumab
- Fostemsavir



INSTIs:
- Cabotegravir (+Rilpivirine LA)

Capsid inhibitor:
- Lenacapavir

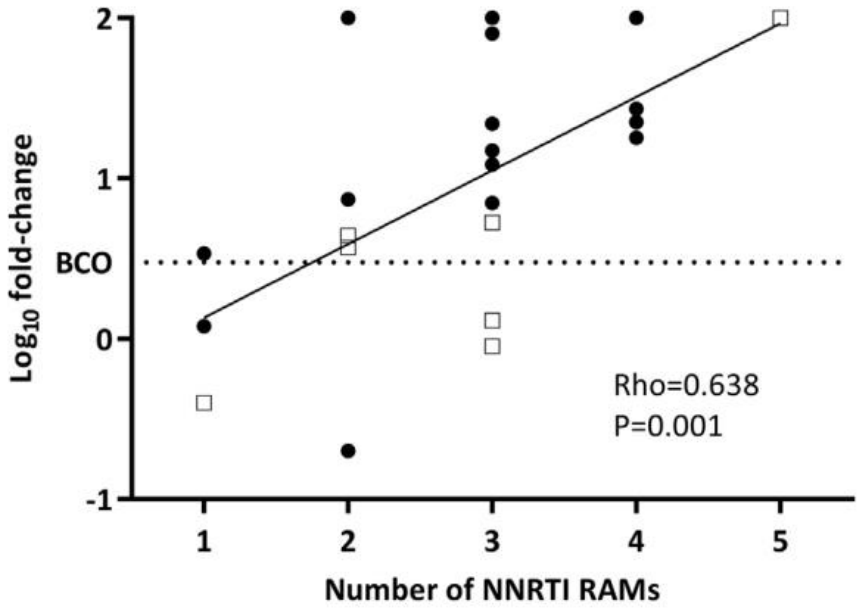
NNRTIs:
- Doravirine

UNIVERSITÀ DI SIENA 1240

Impact of HIV-1 Resistance-Associated Mutations on Susceptibility to Doravirine: Analysis of Real-World Clinical Isolates

Asante-Appiah *AAC* 2021

- DOR is active against most common single NNRTI RAMs
- Among viruses harboring single NNRTI mutations, high level resistance was observed only with Y188L or Y318F
- DOR remains susceptible in nearly half of isolates resistant to each of other NNRTI
- DOR resistant isolates retain limited susceptibility to EFV and NVP and partial susceptibility to ETR and RPV
- The higher the number of NNRTI RAMs, the higher the resistance to DOR, even in absence of specific DOR RAMs



Distribution of doravirine fold-change values calculated on 22 NNRTI resistant viruses **according to the number of major NNRTI RAMs** as defined by Stanford HIVdb.

Black circles indicate fold-change values associated with viruses harbouring doravirine RAMs.

Abbreviations: BCO, doravirine biological fold-change cut-off (= 3-fold). Black circles indicate fold-change values associated with viruses harbouring doravirine RAMs

Saladini, Int J Antimicrob Agents 2023

UNIVERSITÀ DI SIENA 1240

Impact of HIV-1 Resistance-Associated Mutations on Susceptibility to Doravirine: Analysis of Real-World Clinical Isolates

Asante-Appiah *AAC* 2021

TABLE 3 Prevalence of clinical isolates according to the number of NNRTI RAMs and respective susceptibility to doravirine

No. of RAMs	Prevalence, n (%) (N = 4,070)	Doravirine fold-change, median (IQR)	*P*-value[a]
1	953 (23.4)	0.9 (0.6–1.3)	<0.0001
2	398 (9.8)	1.3 (0.8–2.4)	<0.0001
3	219 (5.4)	2.3 (1.2–7.7)	<0.0001
4	108 (2.7)	3.0 (1.4–13.2)	<0.0001
≥5	68 (1.7)	5.6 (2.4–33.6)	<0.0001

[a]*P*-value from the Mann-Whitney U to test whether fold-change from a randomly selected group of X number of RAMs is significantly greater or less than the fold-change from a randomly selected group without any RAMs (wild-type).

IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; RAM, resistance-associated mutation.

- The higher the number of NNRTI RAMs, the higher the resistance to DOR

Isolates with FC values above the 3–fold DOR biological cutoff

- DOR remains susceptible in nearly half of isolates resistant to each of other NNRTI
- DOR resistant isolates retain limited susceptibility to EFV and NVP and partial susceptibility to ETR and RPV

TABLE 5 Proportion of NNRTI-resistant clinical isolates that are susceptible to other NNRTIs

	Percentage of resistant samples susceptible to other NNRTIs, %				
NNRTI	Doravirine-resistant[a]	Efavirenz-resistant	Etravirine-resistant	Nevirapine-resistant	Rilpivirine-resistant
Doravirine-susceptible[a]		62.2	44.7	65.8	45.0
Efavirenz-susceptible	17.1		31.0	18.7	28.1
Etravirine-susceptible	45.2	68.8		65.0	24.4
Nevirapine-susceptible	9.3	1.6	6.0		8.7
Rilpivirine-susceptible	31.9	59.4	5.6	57.4	

[a]A doravirine cutoff 3-fold was used for this comparison.

NNRTI, nonnucleoside reverse transcriptase inhibitor.

Residual phenotypic susceptibility to doravirine in multidrug-resistant HIV-1 from subjects enrolled in the PRESTIGIO Registry

RV ID	NNRTI RAMs	NNRTI exposure at sample collection	Previous exposure to NNRTI	FC values			Stanford HIVdb predicted susceptibility		
				DOR	ETR	RPV	DOR	ETR	RPV
1	A98G, K103N, Y181C, P225H	ETR	EFV, NVP	17.9	100	100	R	I	R
2	L100I, K103N, V179VI, K238N	ETR	EFV	12.2	37.8	100	I	I	R
3	K103N, Y181V	ETR	NVP	7.4	100	100	LLR	R	R
4	K103KNRS, Y181C, **G190S**, H221HY	ETR	none	22.5	48.0	100	R	R	R
5	E138Q, V179E, Y181C	none	EFV, NVP	0.9	26.0	12.2	PLLR	I	R
6	V108I, Y181C	none	EFV, ETR, NVP	3.7	3.2	30.6	I	I	I
7	V106I, **Y188L**, K238N	none	EFV	100	2.9	100	R	LLR	R
8	Y181C, H221Y, M230I	RPV	ETR	80.1	100	100	I	I	R
9	A98G	none	NVP	3.4	0.5	3.7	LLR	PLLR	LLR
10	L100I, E138R, V179L	ETR	None	21.9	100	100	LLR	I	R
11	K103N, Y181I	none	EFV, ETR, NVP	0.2	0.4	3.9	LLR	R	R
12	K103N, E138A, P225H, **M230L**	none	ETR	100	14.1	100	R	I	R
13	K101E, Y181C, G190A	none	EFV, ETR	5.3	22.7	100	I	R	R
14	L100V, K101H, V179F, Y181C, G190A	ETR	NVP, RPV	100	100	100	I	R	R
15	K101E, Y181C, G190A	ETR	None	1.3	2.2	14.4	I	R	R
16	E138K, **G190E**	ETR	EFV, RPV	100	100	100	R	I	R
17	K103N	none	EFV, ETR, NVP	0.4	0.2	0.6	S	S	S
18	V108I, E138A, Y181V	ETR	EFV, NVP	14.9	100	100	I	R	R
19	Y181I	ETR	None	1.2	100	100	LLR	R	R
20	L100I, K103N, E138G	none	EFV, ETR	7.0	100	100	I	I	R
21	A98G, L100I, K103N, E138Q	none	ETR	27.1	100	100	I	I	R
22	K103N, Y181C	none	ETR	4.4	4.0	19.1	LLR	I	I
		Median FC (IQR)		**9.8 (2.9-40.4)**	**42.9 (3.1-100)**	**100 (17.9-100)**			

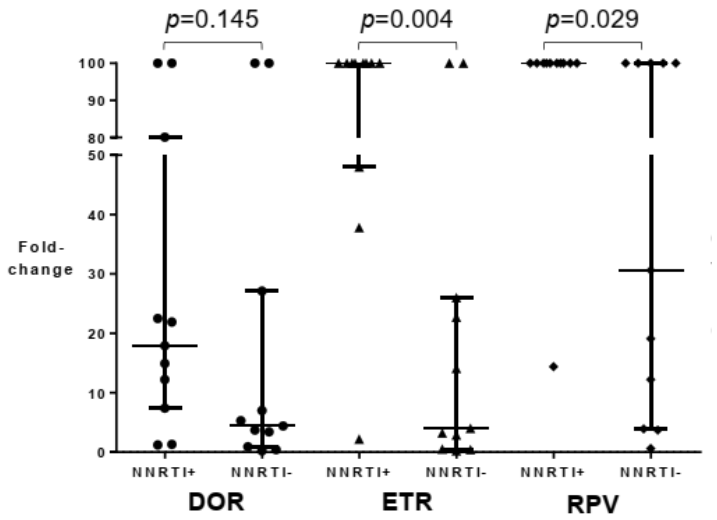
Susceptible | Intermediate resistance | High-level resistance

Fold-change (FC) cut-off values associated with reduced susceptibility:

- DOR >3 (biological)
- RPV >2.5 (biological)
- ETR 2.9 (lower) – 10 (upper) (clinical)

Stanford HIVDB DOR RAMs in bold, IAS-USA DOR RAMs underlined

- 5/22 (23%) isolates are full susceptible to DOR
- Median DOR FC values were higher in samples collected in individuals exposed to NNRTI at sample collection



Saladini, Int J Antimicrob Agents 2023

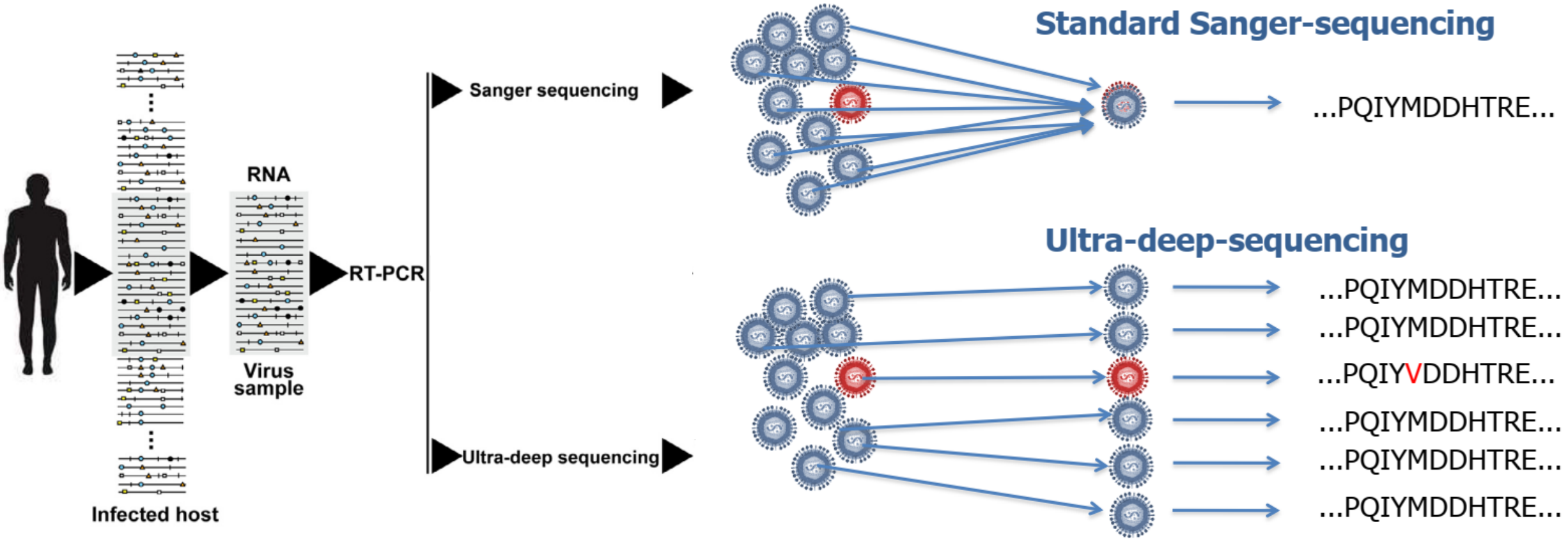
UNIVERSITÀ DI SIENA 1240

Towards undetectability in MDR PLWH: How to manage old ARVs?

- **Genotype on viral RNA** provide a **snapshot** of currently circulating viral populations
- **Historical RNA genotype** provides additional information on the **cumulative past resistance that might re-emerge** and and may help to predict virological failure (Zaccarelli *Antivir Ther* 2009, Garcia *Antivir Ther* 2011)
- **Phenotypic testing might be helpful to identify ARVs with residual activity** when intermediate/high-level resistance is predicted by genotype

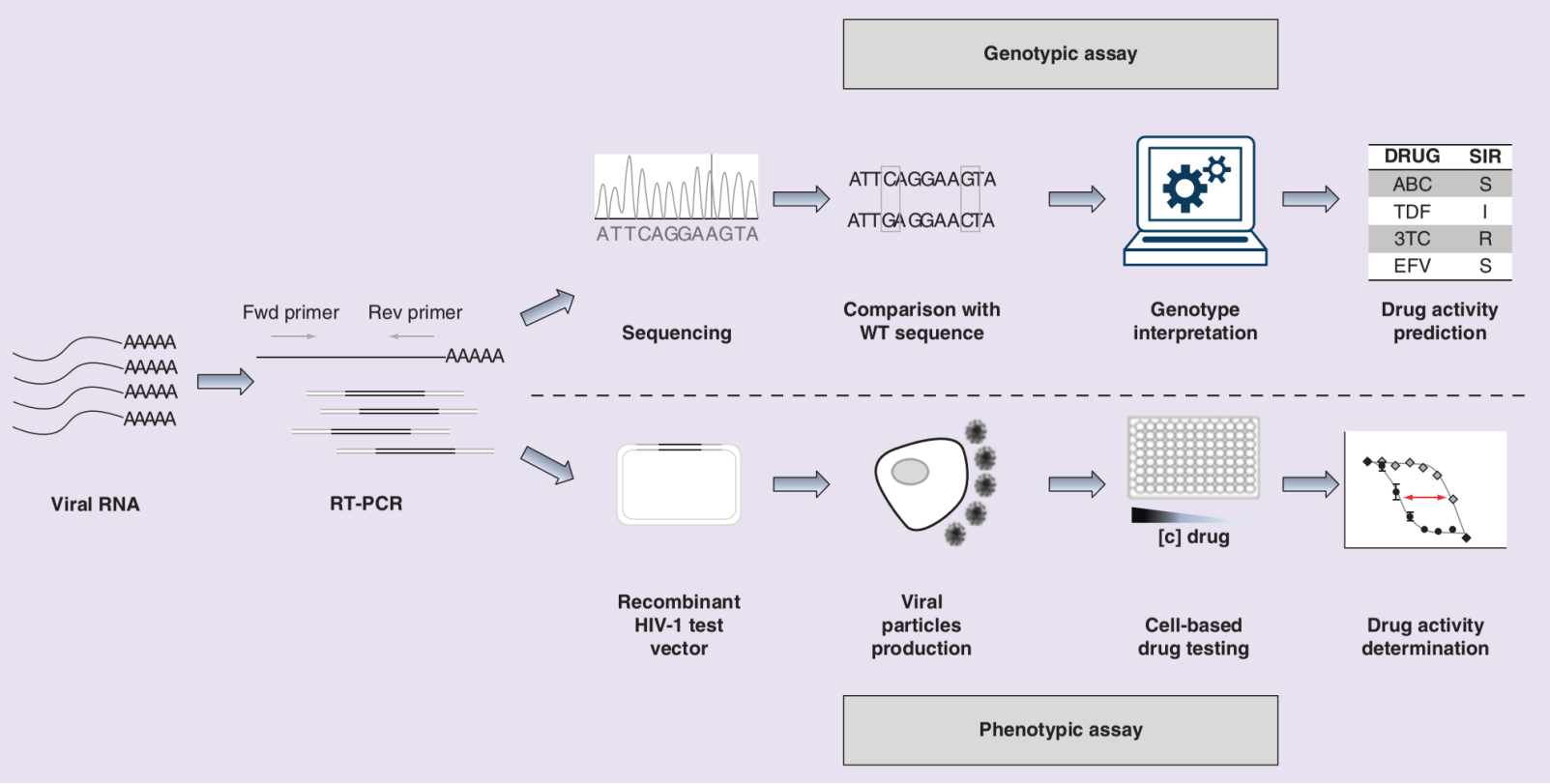
UNIVERSITÀ DI SIENA 1240

Towards undetectability in MDR PLWH: Next-generation sequencing



UNIVERSITÀ DI SIENA 1240

Differences between genotypic and phenotypic testing



FEATURE	GENOTYPE	PHENOTYPE
Execution time	1-3 weeks	4-8 weeks
Costs	Medium	High
Technical complexity	Medium (diagnostic kits available)	High (advanced lab training, BSL3 facilities…)
Result	**Prediction**	**Direct measurement**
Sensitivity	Species >20% (Sanger)/5% (NGS) of total population	Species >20% of total population (reported 10% or lower in some cases)
Inter-laboratory reproducibility	Fair to good depending on lab experience	Limited data

Saladini and Vicenti, Future Virol 2016

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 fostemsavir
- **Phenotyping may help to evaluate drug activity and facilitate the choice of drugs of the OBR**
  - To be considered in deep salvage with complex MDR
- **Building active and durable regimens in HTE/MDR patients remains a challenge requiring a multidisciplinary approach**