UNIVERSITÀ
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1240

"OTTIMIZZAZIONE TERAPEUTICA DELLE PERSONE CHE VIVONO CON HIV:
TERAPIE DUPLICE, TRIPLICE E NUOVE OPZIONI PER HTE" – Milano, 16/05/2025

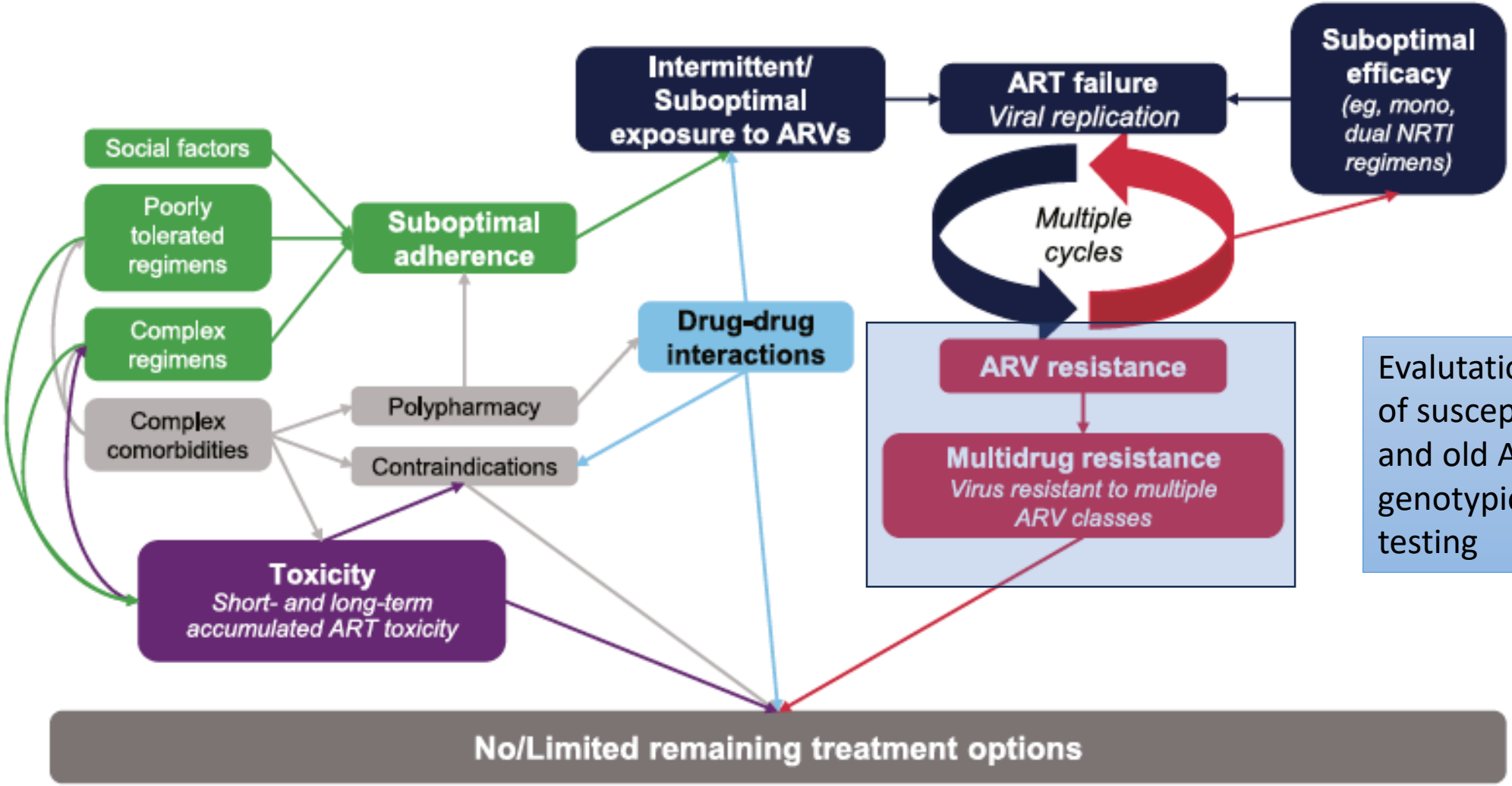
Il ruolo del virologo nell'ottimizzazione del soggetto HTE

Francesco Saladini, PhD

Conflicts of interest

- Dr. Francesco Saladini has received personal fees for consultancy from MSD

Clinical challenges to optimal ARV treatment in heavily treatment-experienced (HTE) PWH

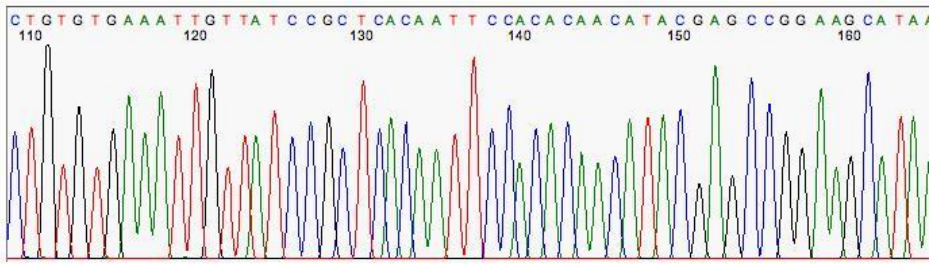


Evalutation/determination of susceptibility of new and old ARVs through genotypic and phenotypic testing

Aberg *Antivir Ther* 2023

Genotype vs. Phenotype

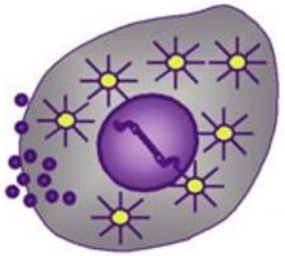
- **Genotypic Assay**: Sequencing of viral genes involved in drug resistance

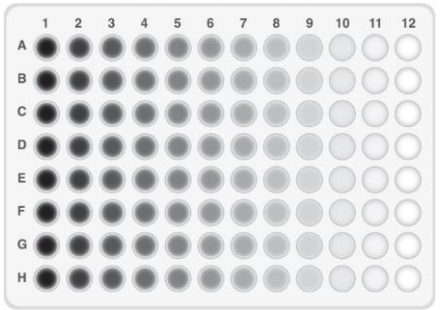


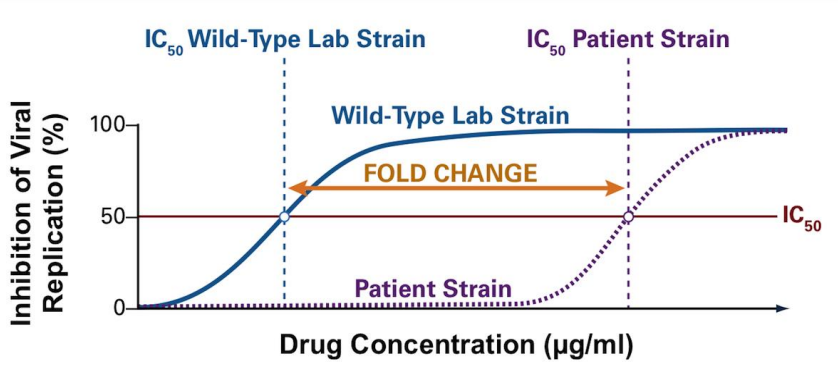
INSTI Major Mutations:	G140S · Q148H
INSTI Accessory Mutations:	None
IN Other Mutations:	K7Q · E11D · V31I · M50L · I72V · L101I · T124A · I135V · I200L · V201I · I220L

Integrase Strand Transfer Inhibitors	
bictegravir (BIC)	Intermediate Resistance
cabotegravir (CAB)	High-Level Resistance
dolutegravir (DTG)	Intermediate Resistance
elvitegravir (EVG)	High-Level Resistance
raltegravir (RAL)	High-Level Resistance

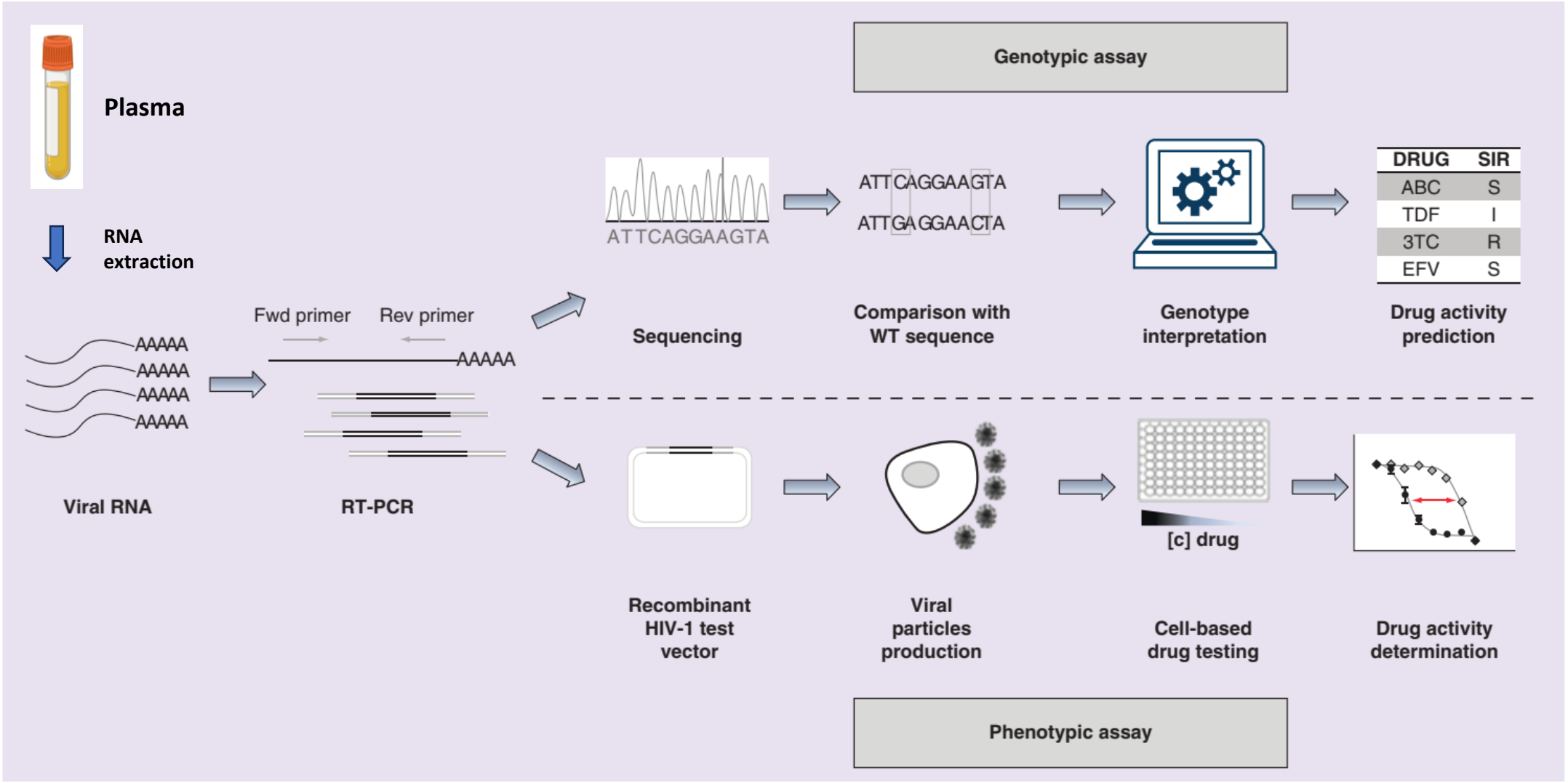
- **Phenotypic Assay**: *in vitro* cell-based system evaluating the replication of HIV in presence of serial dilutions of antivirals





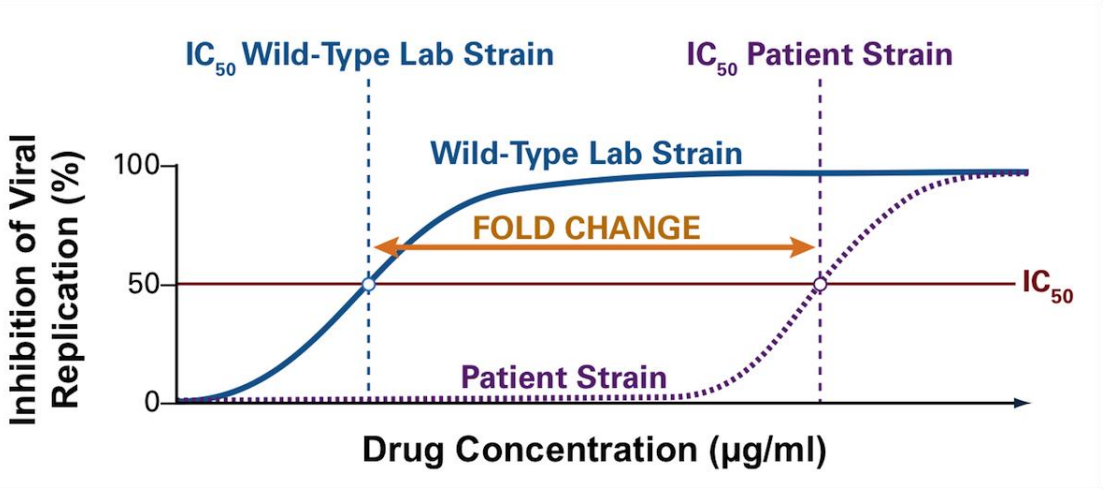


Genotype vs. Phenotype



Adapted from Saladini & Vicenti, *Future Virol.* 2016

Fold-change values as a parameter to assess drug susceptibility



$$\text{Fold change (FC)} = \frac{IC_{50}\ \text{Recombinant Strain}}{IC_{50}\ \text{WT Strain}}$$

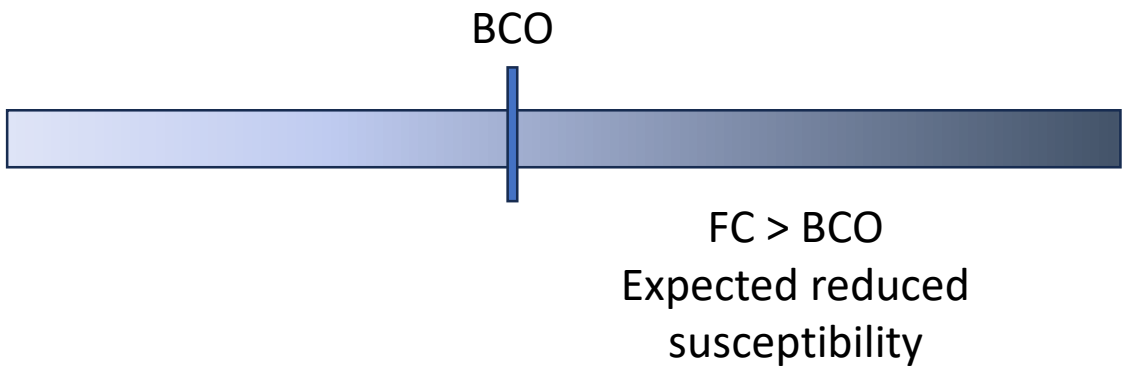
Hypersusceptibility

Expected full susceptibility

Reduced susceptibility – partial activity

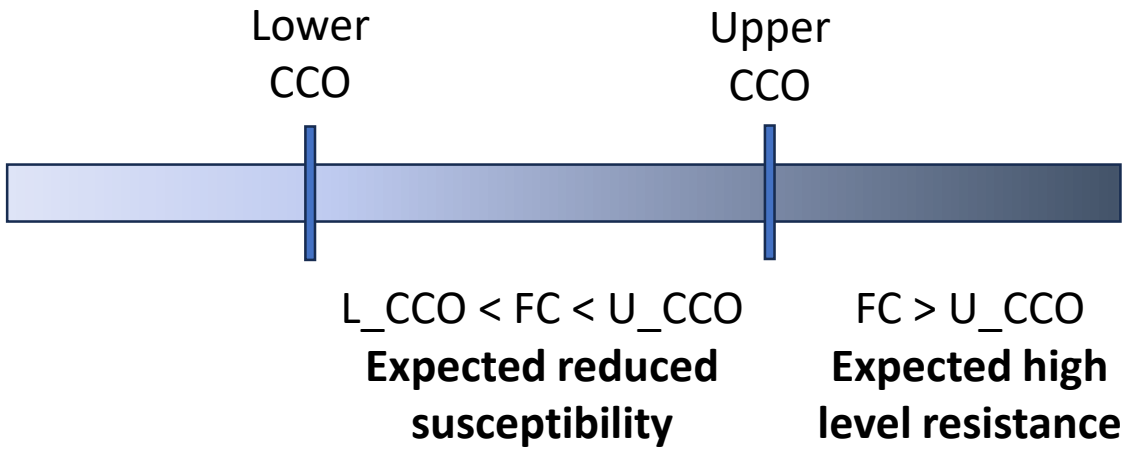
High level resistance – no residual activity

Fold-change cut-off values as a threshold for estimating drug susceptibility



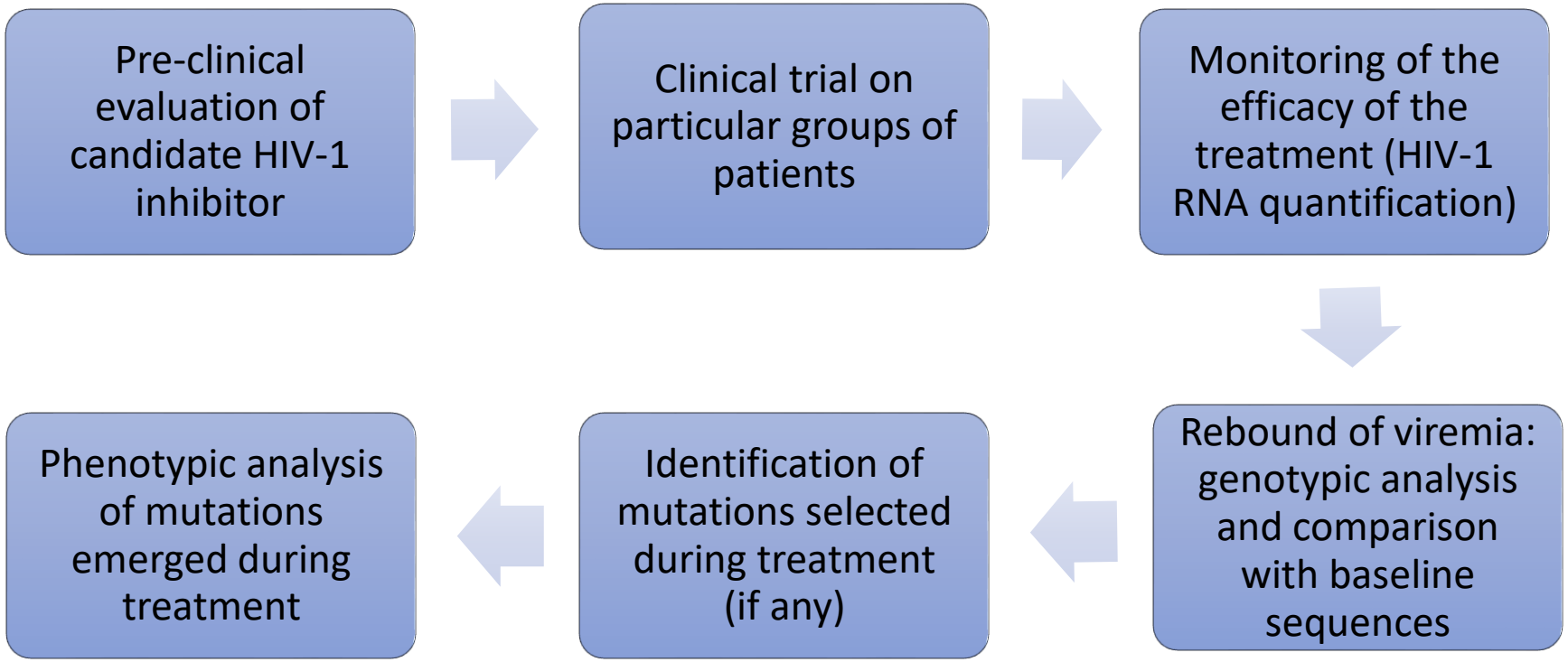
Biological Cut-Off (BCO): mean FC observed with samples from treatment-naive PWH, plus two standard deviations.

No information on the expected response to the treatment *in vivo*



Clinical Cut-Off (CCO): based on virological response from clinical studies.
FC > Lower CCO (L_CCO) indicates a reduced virological response and associated with partial activity of a drug
FC > Upper CCO (U_CCO) indicates lack of virological response (e.g., reduction of viral load <0.5 log copies HIV-1 RNA/mL) and associated with high-level resistance

Fold-change cut-off values as a threshold for estimating drug susceptibility



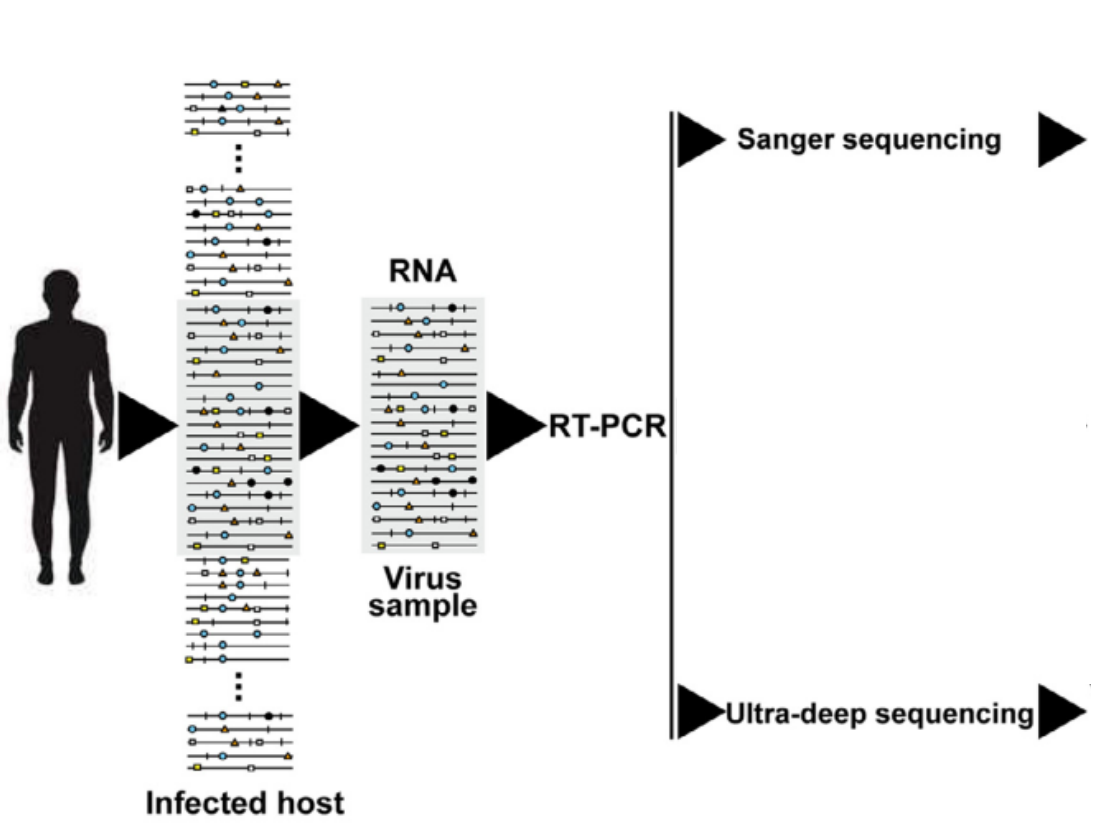
- **Major** resistance mutations: confers resistance on its own, may often decrease fitness
- **Minor** resistance mutations: does not confer resistance on its own but may modulate resistance and/or (partially) restore fitness which was decreased by a major mutation

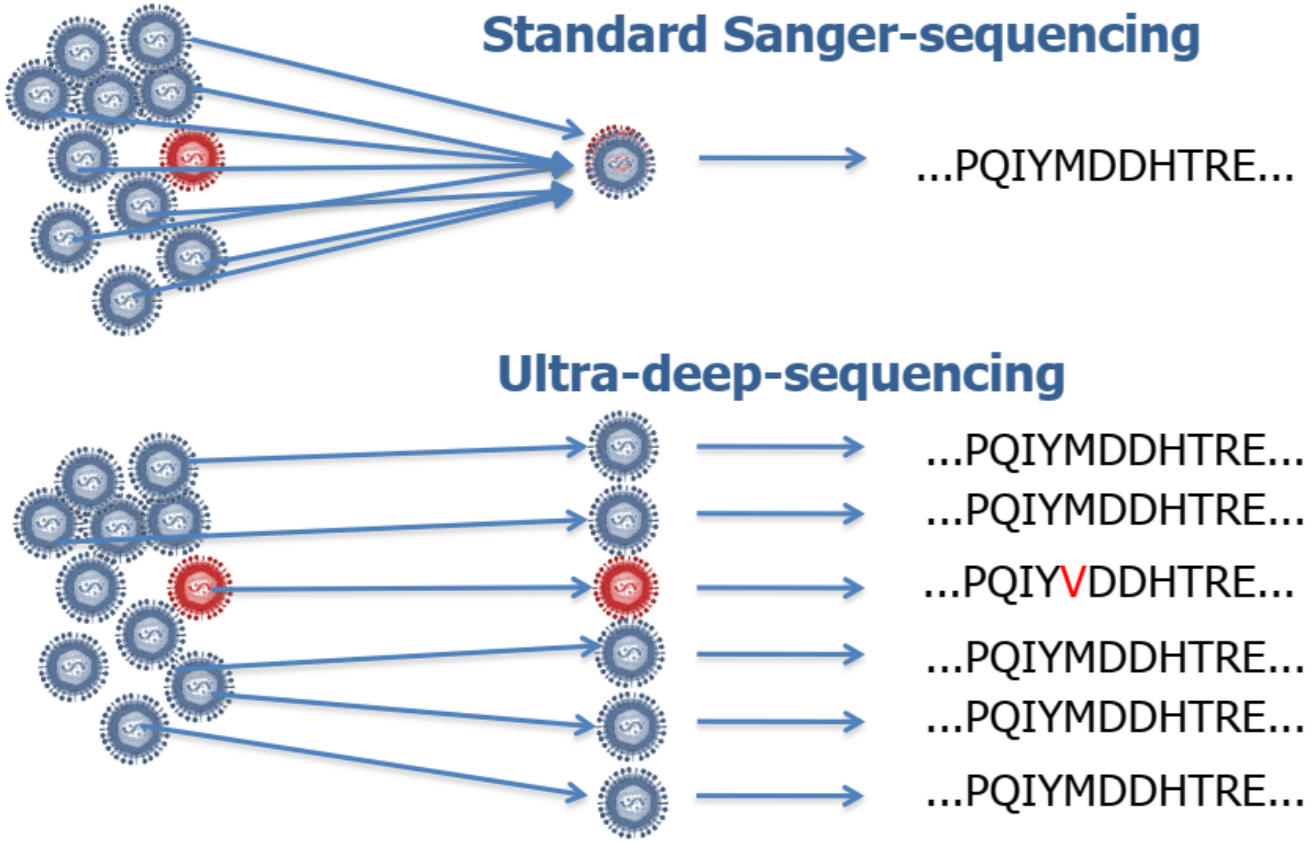
Genotype vs. Phenotype

FEATURE	GENOTYPE	PHENOTYPE
Execution time	1-2 weeks	6-8 weeks
Costs	Medium	High
Technical complexity	Medium (diagnostic kits available)	High (advanced lab training, BSL3 facilities…)
Result	Prediction	Direct measurement
Sensitivity	• Sanger seq: species >20% of total viral population • NGS: species >5% of total viral 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
Off-target mutations	Detection possible	Only as site-directed mutants

From Sanger to Next-Generation Sequencing

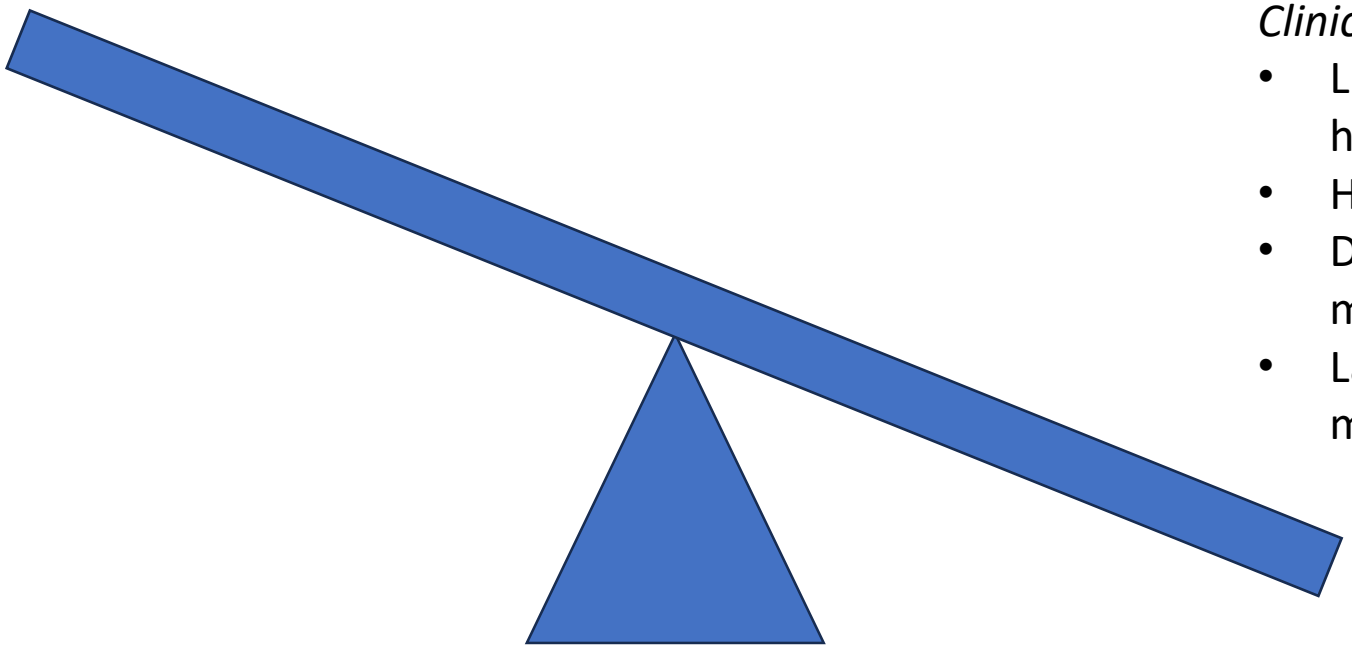
Sanger sequencing can detect viral populations which have a frequency >15-25% (Leitner *Biotechniques* 1993; Schuurman *AHRH* 2002; Palmer *JCM* 2005)





PROs and CONs of HIV-1 drug resistance testing using Next-Generation Sequencing approaches

- CE-IVD kits already available for both drug target regions and whole genome sequencing
- Detection of minority RAMs with frequency as low as 1%
- NGS on HIV-DNA **more concordant with cumulative genotype** than NGS on HIV-RNA (Armenia, IJAA 2022)



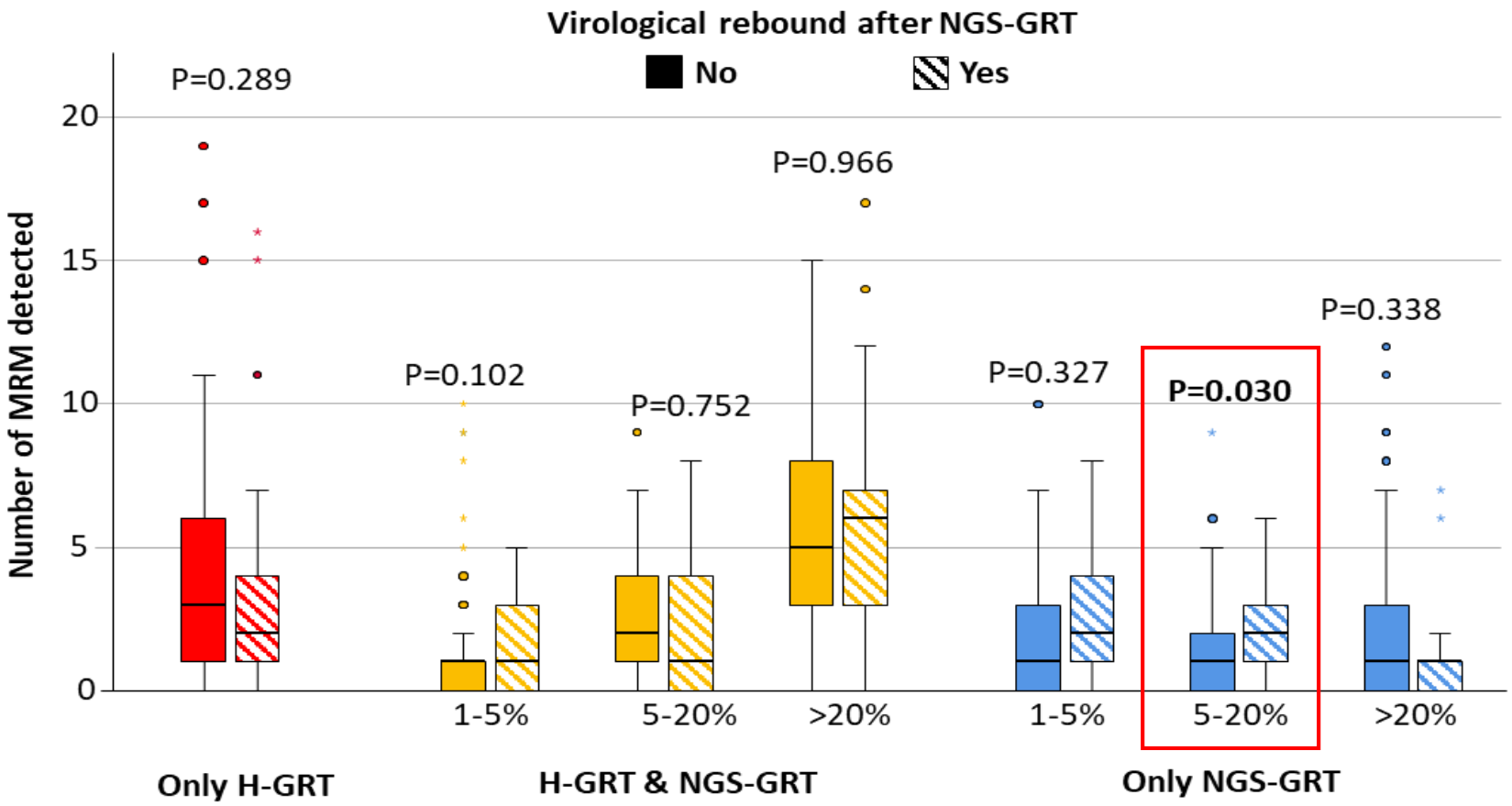
Methodology issues

- Low-abundance DRMs were overrepresented at thresholds <3%: ***artifacts due to sequencing errors***? (Balakrishna, JAC 2023)
- Quality assurance programs required
- Need for standardized reporting → consensus shared between labs and clinics

Clinical relevance

- Limited impact on first-line therapy, particularly with high-genetic barrier drugs
- Higher impact for salvage therapy
- Depends on drug class (or specific drugs) and mutational load
- Lack of a threshold to identify clinically relevant minority RAMs

Evaluation of HIV-1 DNA resistance burden through NGS in highly treatment-experienced multi-resistant individuals under virological control enrolled in the PRESTIGIO Registry



Differences in number of major resistance mutations (MRM) according were evaluated with Mann-Whitney test. P value<0.05 were indicated in boldface.

21/91 virologically suppressed HTE experiencing **virological rebound** in the PRESTIGIO registry, with baseline **NGS DNA genotype** and **historical RNA genotype (H-GRT)** data

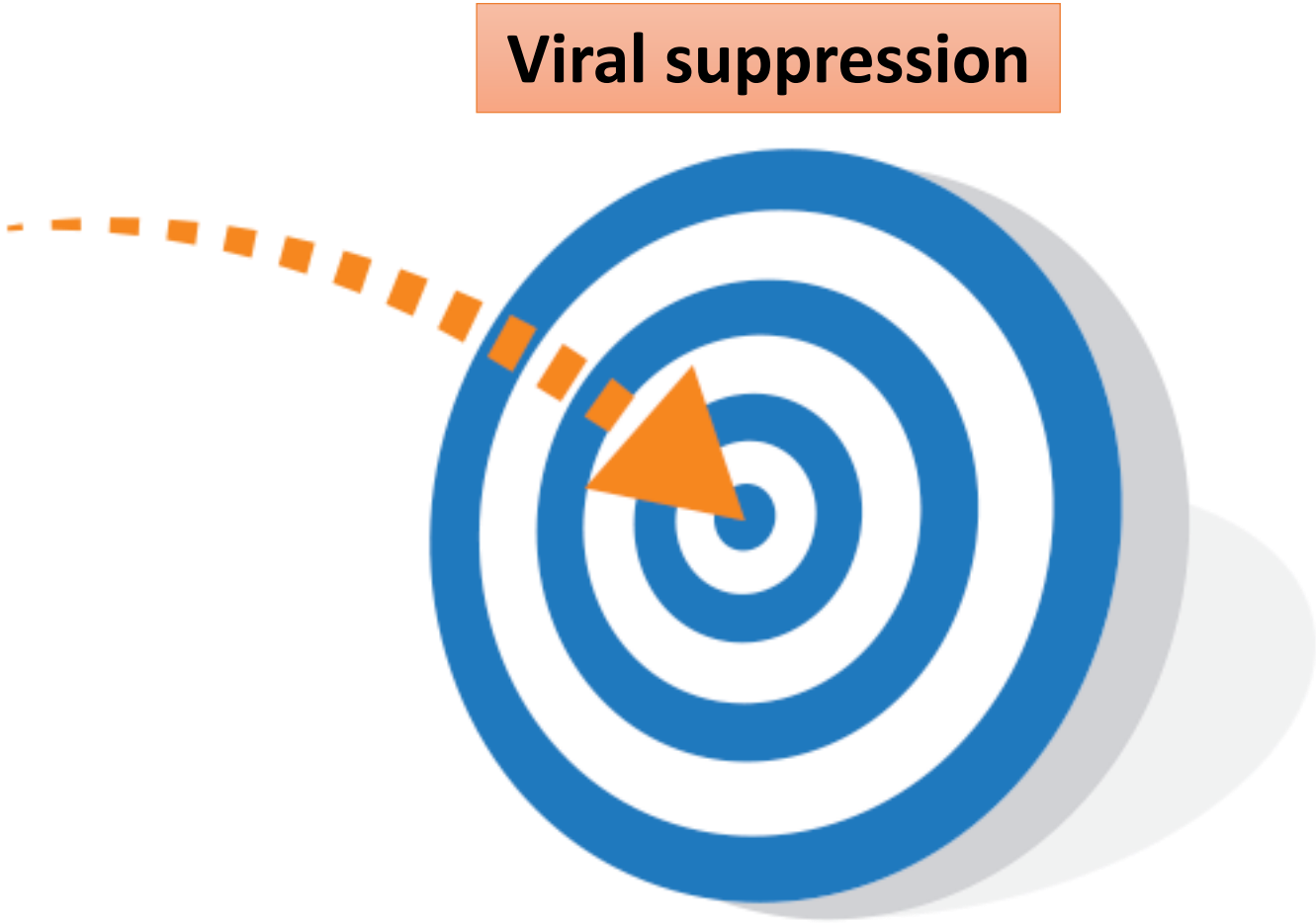
A higher number of baseline MRMs as detected by **NGS at 5-20% threshold** was associated with virological rebound

Armenia, JAC 2024

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



Towards undetectability in viremic MDR PLWH

Last licensed ARVs

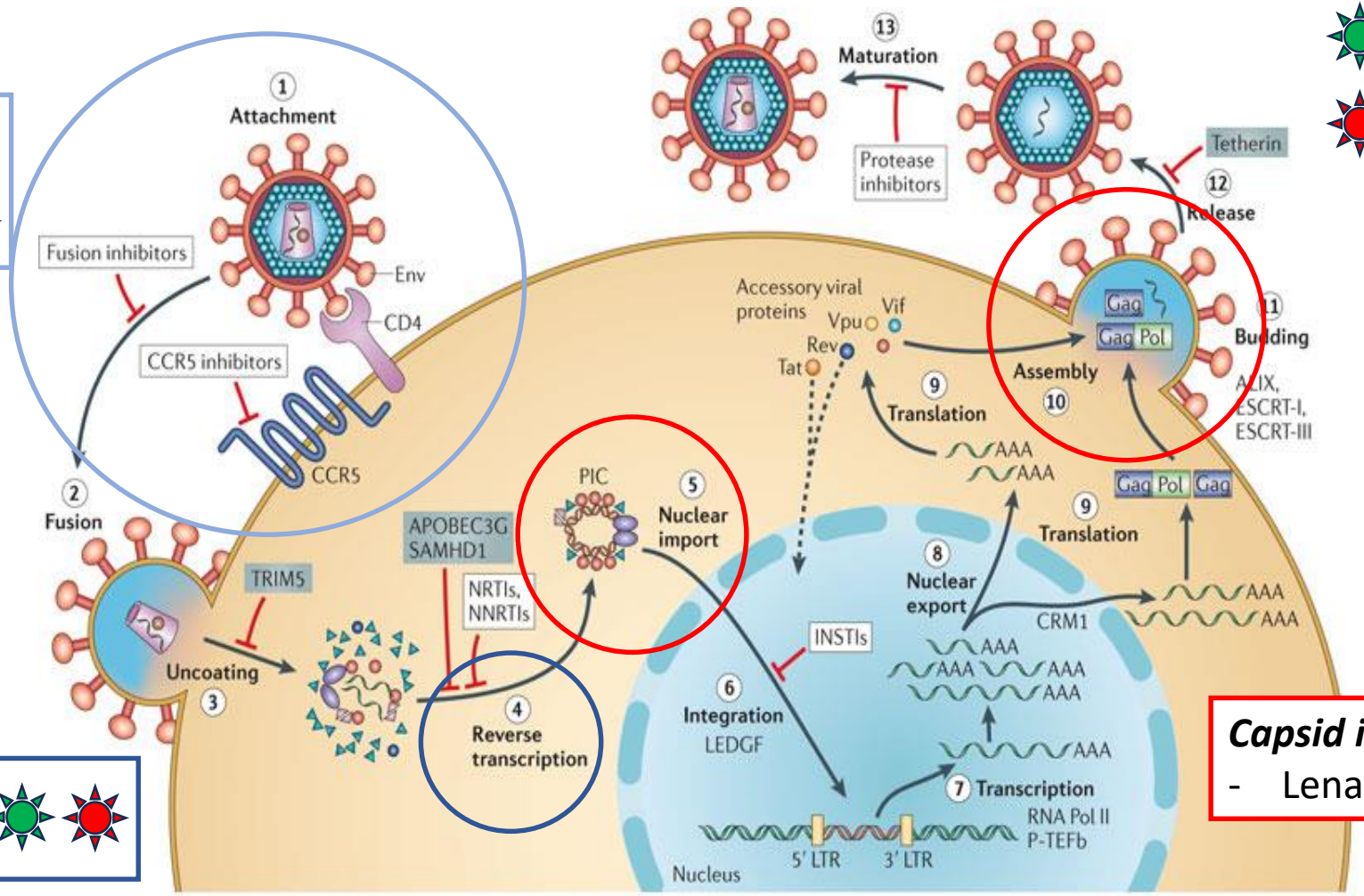
First-line therapy

Maintenance therapy

Salvage therapy

Entry inhibitors:
- (Ibalizumab)*
- Fostemsavir

* EU marketing withdrawal from January 1, 2023



NNRTIs:
- Doravirine

Capsid inhibitor:
- Lenacapavir

Towards undetectability in viremic MDR PLWH

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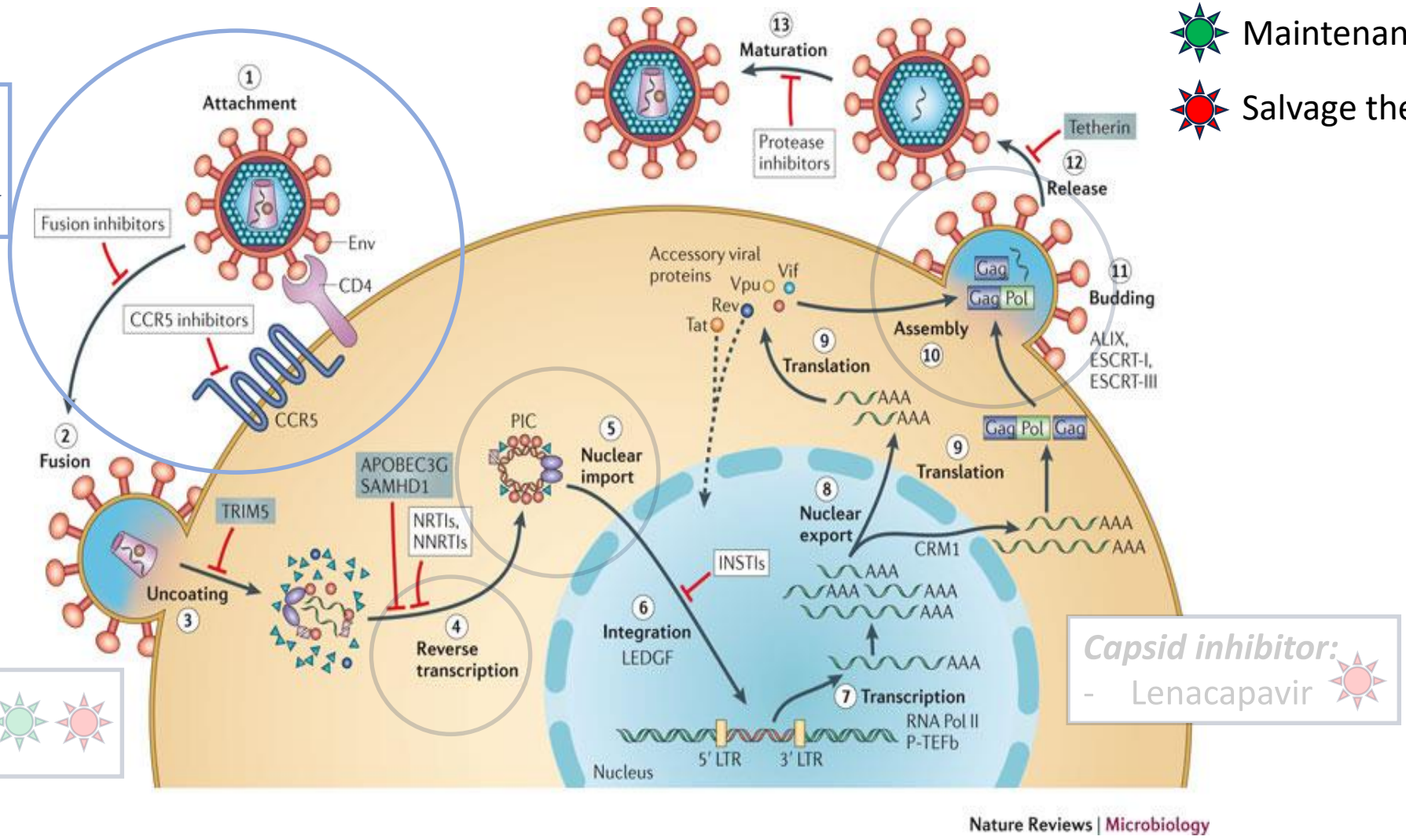
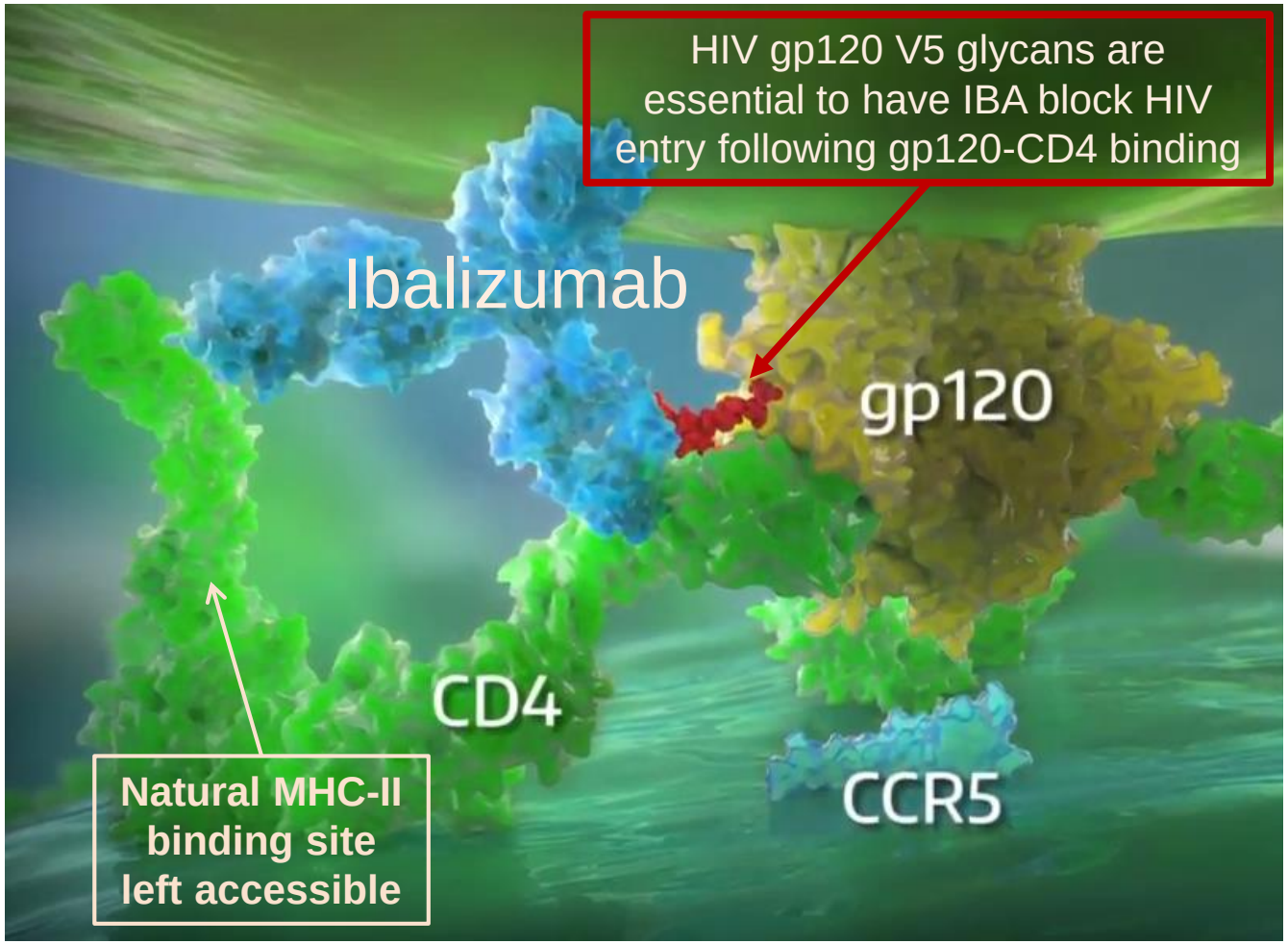


NNRTIs:
- Doravirine

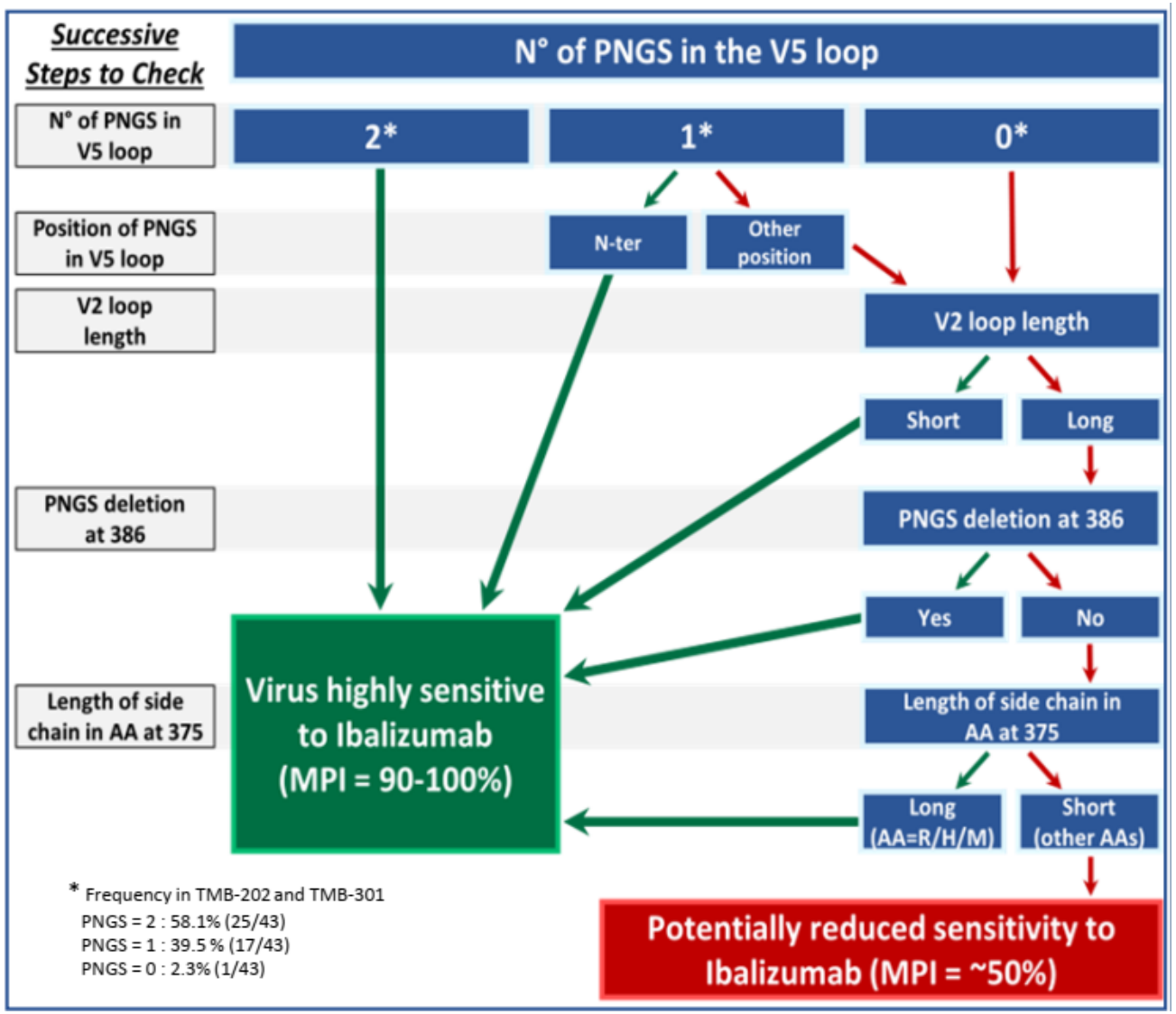
Capsid inhibitor:
- Lenacapavir

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

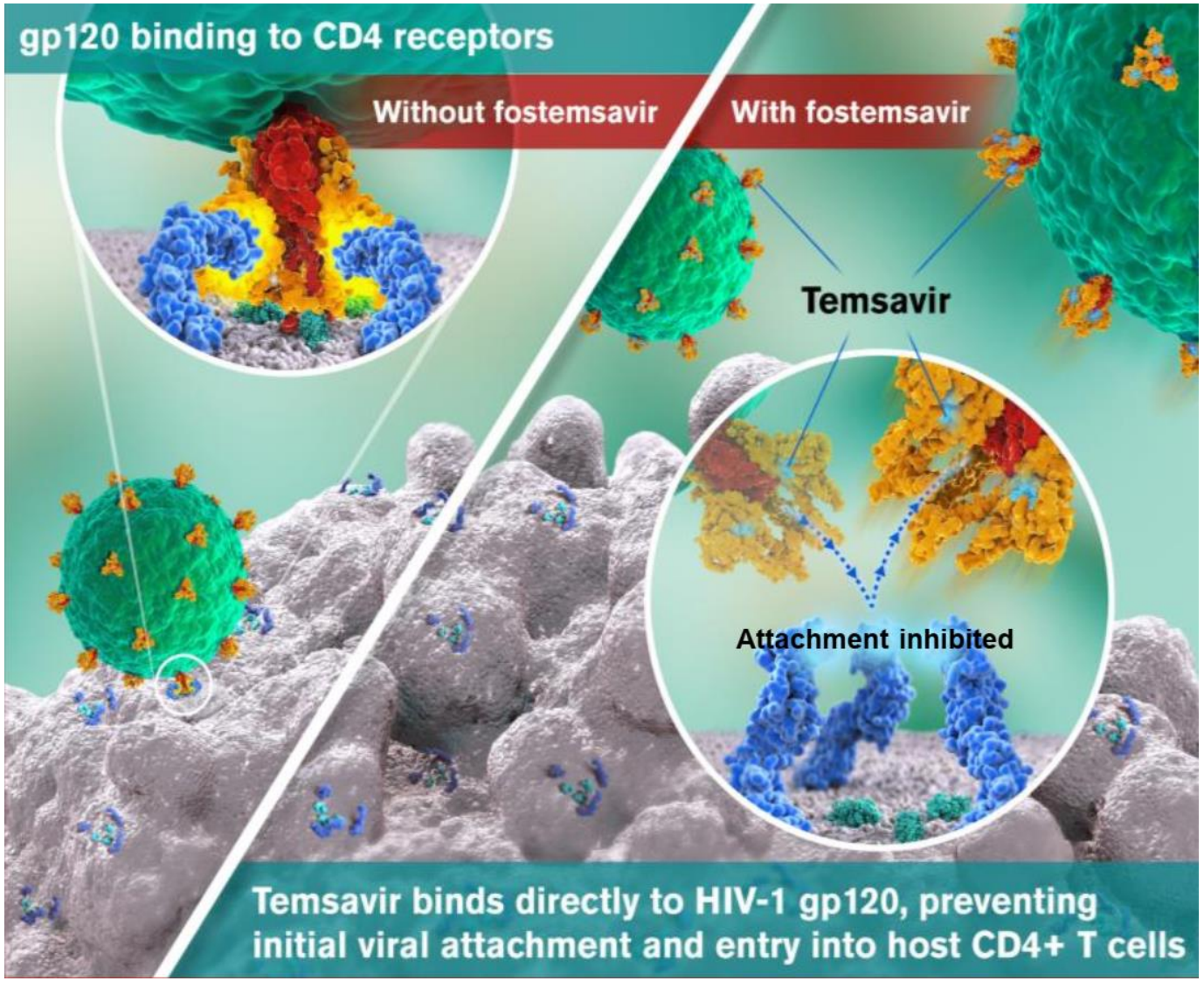
Assessment of genotypic patterns associated with HIV-1 sensitivity to ibalizumab



- 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

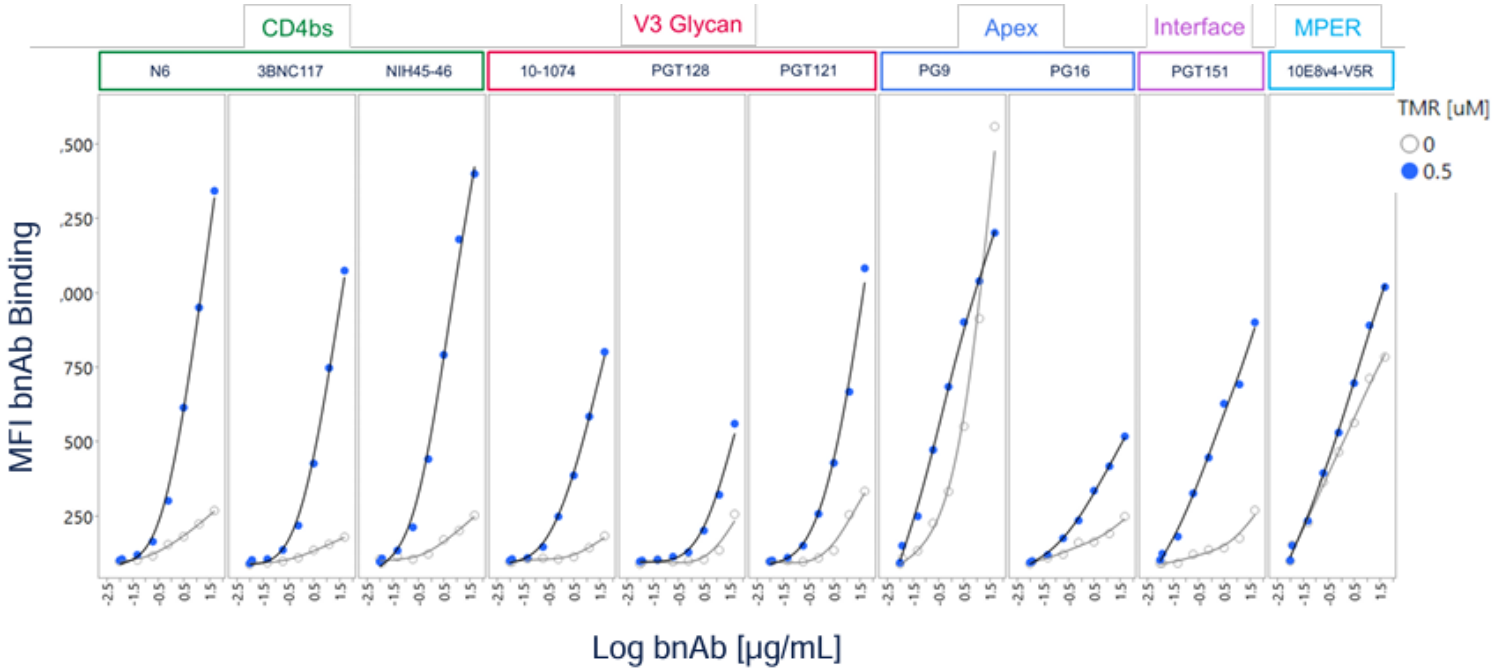
Mechanism of action of fostemsavir



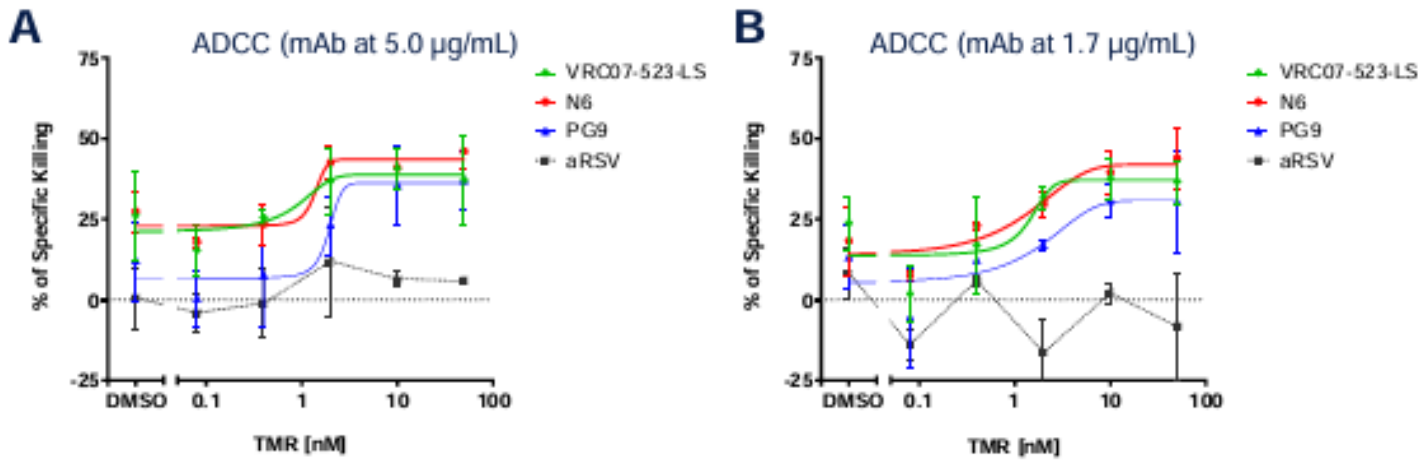
- Fostemsavir is a pro-drug of the attachment inhibitor temsavir
- Broad range of natural susceptibility within each subtype, while CRF01_AE is naturally resistant[1]
- Signature mutations identified, although other sites in the close CD4 binding site are involved[2]
- No correlation between baseline **genotypic** resistance and response, although baseline **phenotypic** resistance (>100 FC IC_{50}) correlated with limited response in the non-randomized cohort of the BRIGHTE study[3]
- Similar recovery of CD4+ T-cell count in both viremic and aviremic PWH on BRIGHTE study at week 240[4]

1)Nowicka-Sanz *AAC* 2012; 2) Prevost *Nat Commun* 2023; 3) Gartland *AAC* 2022; 4) Aberg *Infect Dis Ther* 2023

Temsavir enhances the neutralizing activity of bNAbs



- Temsavir (TMR) enhanced the binding of most bNAb classes (excluding MPER) to HIV-1 infected cells expressing CD4 (CD4+p24+) post TMR treatment.



- TMR treatment dose-responsively enhances antibody-dependent cellular cytotoxicity (ADCC) of bnAbs against CD4+p24+ cells. Increased antibody concentrations promote max level of killing

Ferris CROI 2025

Towards undetectability in viremic MDR PLWH

Last licensed ARVs

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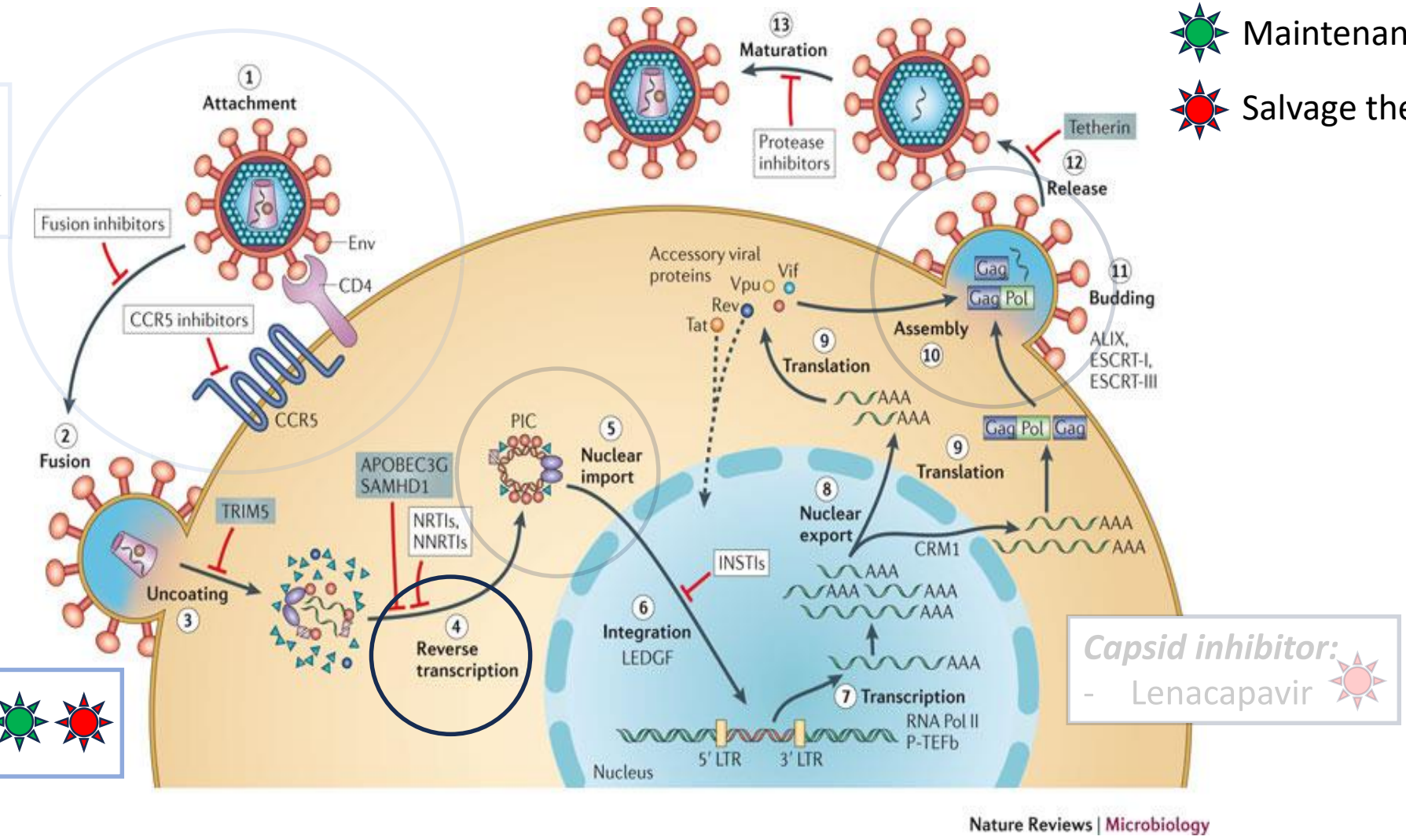
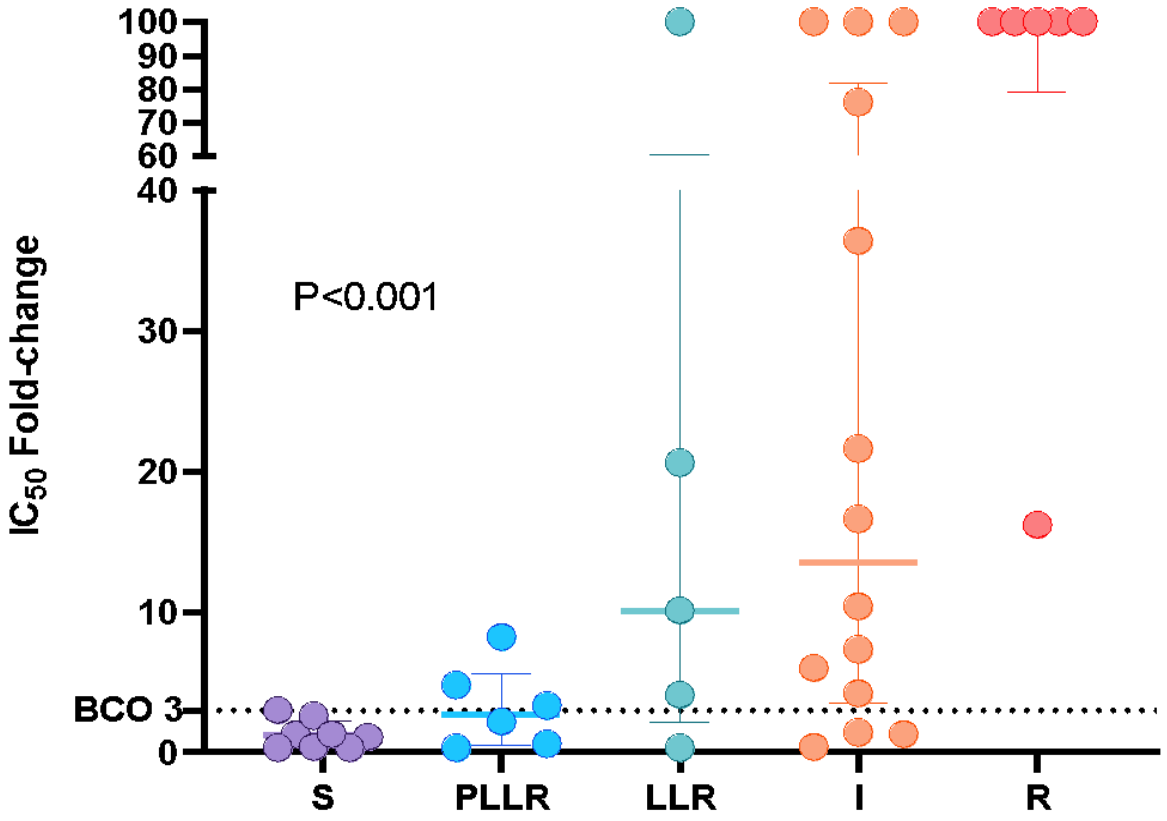


NNRTIs:
Doravirine

Capsid inhibitor:
- Lenacapavir

Genotypic and phenotypic susceptibility to doravirine



N	8	6	5	14	5
Median	1.2	2.8	10.1	13.5	100
IQR	0.3-2.3	0.5-5.7	2.2-60.3	3.5-82	79-100

- Doravirine (DOR) has a partially overlapping resistance profile with other NNRTI and remains susceptible in nearly half of isolates resistant to each of other NNRTI[1]
- DOR is active against most common single NNRTI RAMs excluding Y188L and Y318F[1]
- The higher the number of NNRTI RAMs, the higher the resistance to DOR, even in absence of specific DOR RAMs[1,2]
- According to the 3-fold biological FC cut-off (BCO), 15/39 (38.5 %) NNRTI resistant viruses were fully susceptible to DOR[3]
- The distribution of FC values strongly correlated with the levels of predicted susceptibility to doravirine as determined by the HIVdb algorithm[3]

1) Asante-Appiah *AAC* 2021; 2) Saladini *Int J Antimicrob Agents* 2023; 3) Giammarino *Antiviral Res* 2025

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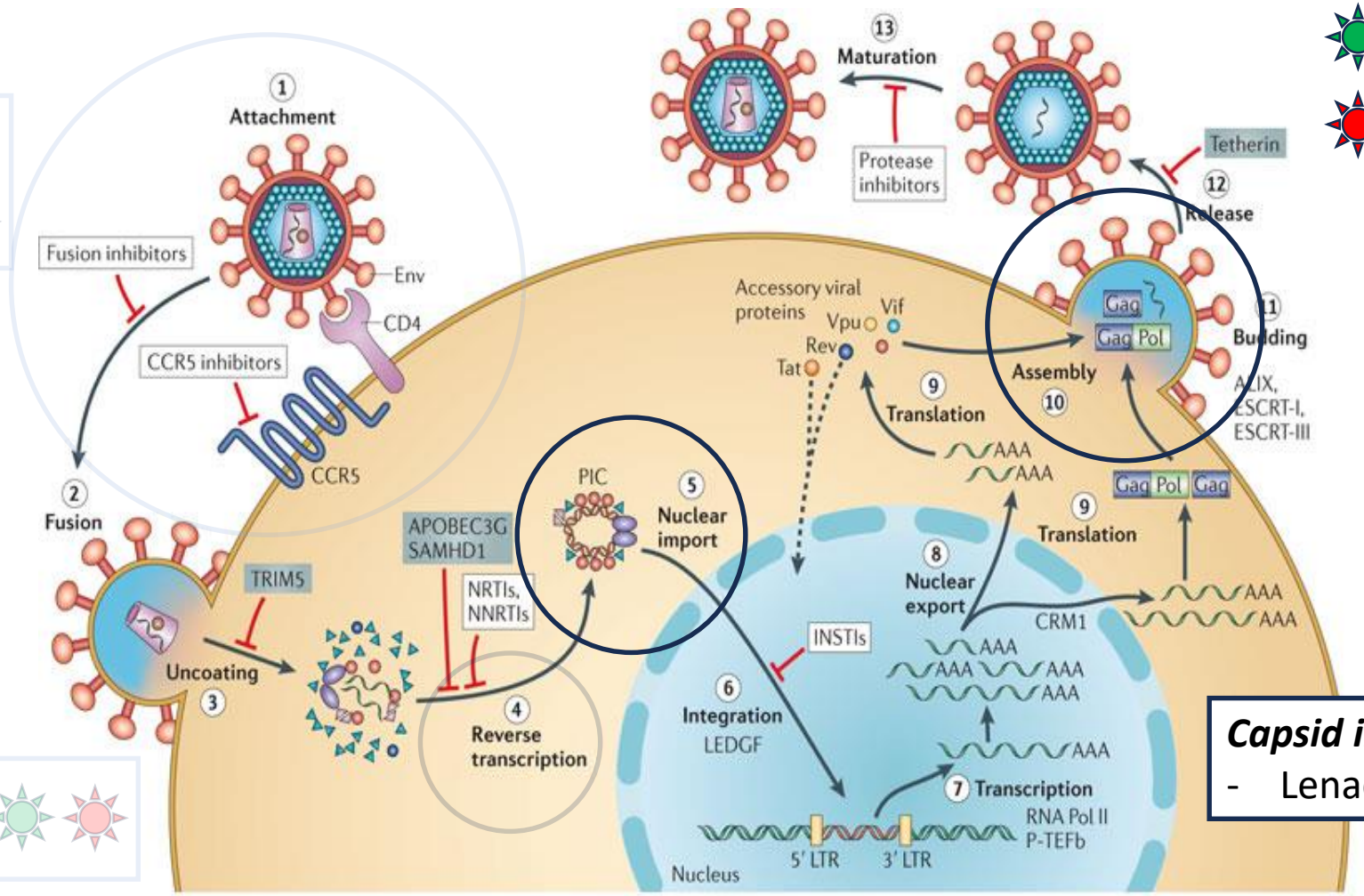
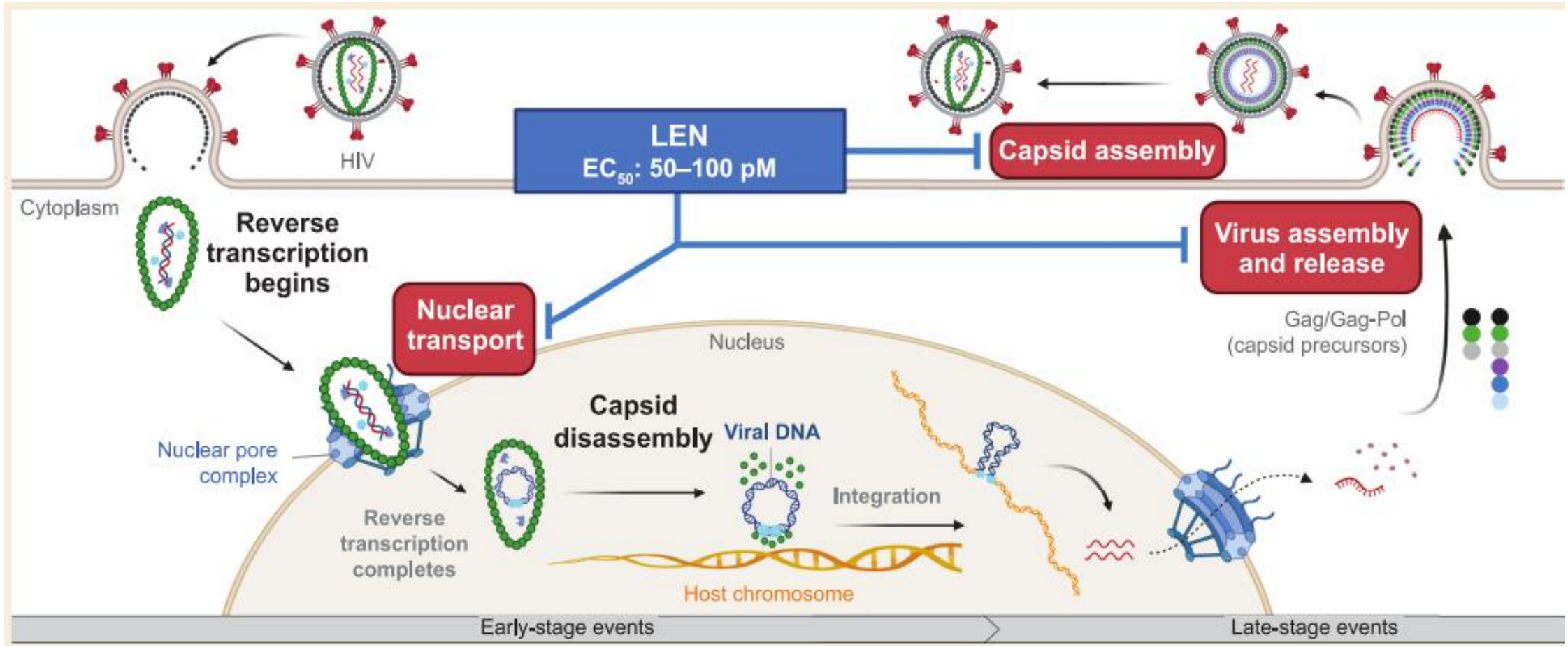


NNRTIs:
Doravirine

Capsid inhibitor:
- Lenacapavir

Mechanism of action of lenacapavir



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

Resistance to lenacapavir: results from the CAPELLA study

- 2-cohort phase II/III trial

Persons living with HIV-1 RNA ≥400 c/mL, resistance to ≥2 agents from 3 of 4 main ARV classes, and ≤2 fully active agents from 4 main ARV classes (N = 72)

Repeat HIV-1 RNA at screening

Randomized
Decline of <0.5 $\log_{10}$ c/mL (vs screening) or ≥400 c/mL

Nonrandomized
Decline of ≥0.5 $\log_{10}$ c/mL (vs screening) or <400 c/mL

	Functional Monotherapy	*Maintenance Therapy*
Randomized	**Oral LEN + Failing Regimen** (n = 24)	**SC LEN Q6M + OBR**
Randomized	**Placebo + Failing Regimen** (n = 12)	**Oral LEN → SC LEN Q6M + OBR**
Nonrandomized	**Oral LEN + OBR** (n = 36)	**SC LEN Q6M + OBR**

Oral LEN administered as 600 mg on Days 1 and 2, 300 mg on Day 8; SC LEN administered as 927 mg (2 x 1.5 mL) in the abdomen on Day 15 and Q6M thereafter.

- **Endpoints:** efficacy (FDA snapshot), resistance emergence, and safety at Wk 156

Ogbuagu. IDWeek 2023. Abstr 1596. Segal-Maurer. NEJM. 2022;386:1793.

Resistance to lenacapavir: results from the CAPELLA study

- **14/72 (19.4%)** participants developed emergent LEN resistance (9 participants by Wk 52, 5 participants between Wk 52 and 104, none between Wk 104 and 156)
  - **All mutations map to LEN binding site and are not natural polymorphisms or selected by other ARVs: M66I; Q67H/K/N; K70H/N/R/S; N74D/H/K; A105S/T; T107A/C/N/S**
  - 2 participants with earlier resistance developed additional mutations
    - 1 participant with emergent K70R+T107N with existing Q67H: LEN susceptibility reduced from 4.5- to 85-fold of WT
    - 1 participant with emergent T107T/N with existing K70N + N74K resulting: no LEN susceptibility data for triple mutant
  - All participants with no fully active drugs in OBR or inadequate OBR adherence
  - Median change in CD4+ cell count change: 82 cells/mm³ (IQR: 48-399 cells/mm³)

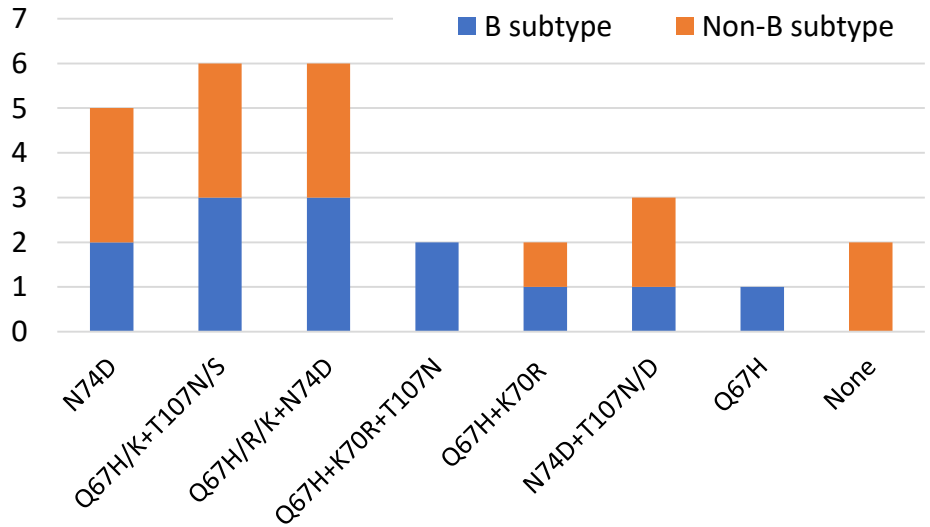
Characteristic, n	Total Population (N = 72)
OBR	
▪ No fully active agents in OBR	4
▪ Inadequate adherence to OBR*	10
Resuppressed after LEN resistance emergence while continuing LEN	5
▪ With OBR change	2
▪ Without OBR change	3
Not resuppressed after LEN resistance emergence	9
▪ Continued LEN†	6
▪ Discontinued LEN for reasons unrelated to efficacy (death, nonadherence, LTFU)	3

Ogbuagu. IDWeek 2024. Abstr 155.

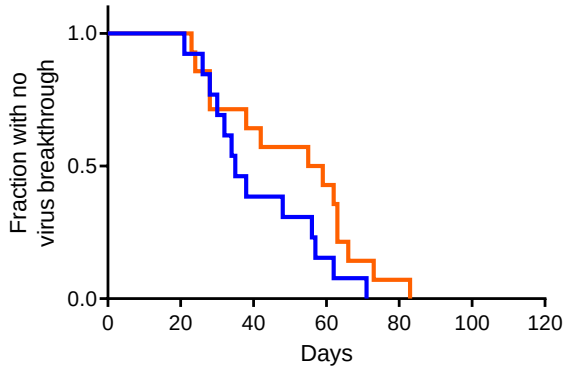
Impact of the HIV-1 genetic variability on the barrier to resistance to lenacapavir

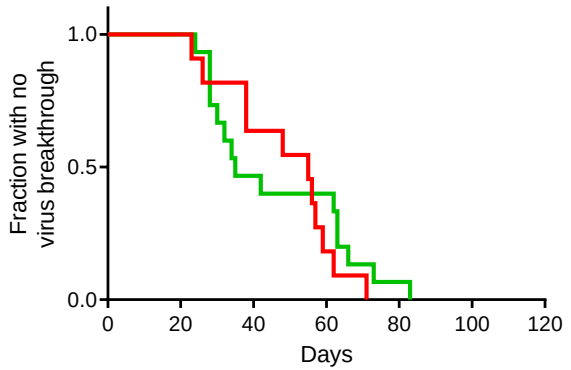
- In vitro study including 26 gag-PR recombinant viruses using samples from therapy naïve (TN, n. 15) or heavily treatment-experienced (HTE, n. 11) PWH from the PRESTIGIO Registry

- 13 non-B subtype (CRF02_AG and F1 in three cases each, while A1, C, D, G, CRF01_AE, CRF06_cpx, B/F URF in one case each)

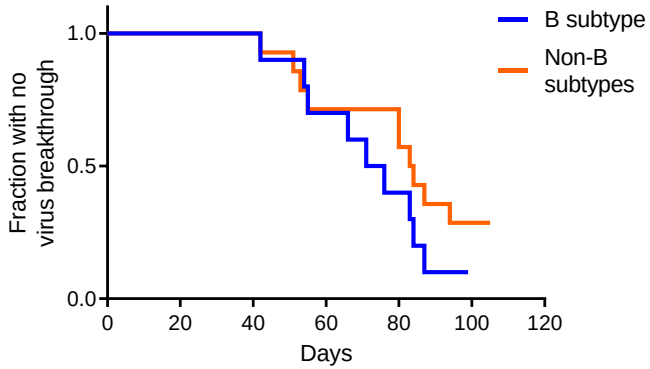


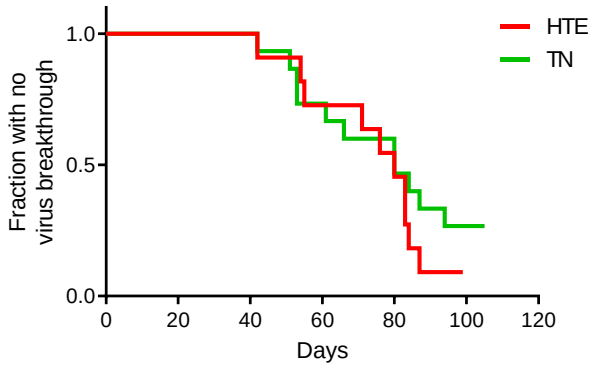
LEN 10X IC_{50}





LEN 100X IC_{50}





- Phenotypic baseline susceptibility and time to viral breakthrough was comparable among B vs. non-B subtypes and HTE vs. TN PWH
- No difference in the selection of LEN RAMs between B and non-B subtypes

Paletti oral communication ICAR 2024

Resistance to IBA, FTR, LEN - Summary

Drug	Natural resistance	Cross resistance	Genetic barrier to resistance
IBA	Isolates with no PNGS in gp120 V5 (2-8% depending on subtype)	• None among the three new classes • None with old classes	Very low
FTR	CRF01_AE, occasional isolates of other subtypes	• None among the three new classes • None with old classes	Very low
LEN	Virtually none	• None among the three new classes • None with old classes	Low

- No need of genotypic/phenotypic screening before treatment, but…
- …**genotypic and phenotypic testing might be helpful for IBA and FTR at failure** to evaluate emergent mutations and possible loss of susceptibility as compared to the pre-therapy sample
- **LEN RAMs already characterized**, but data from real-life use should be carefully monitored

Towards undetectability in viremic MDR PLWH

How to manage old drugs?

- **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

Different susceptibility levels to TAF according to either genotype or phenotype

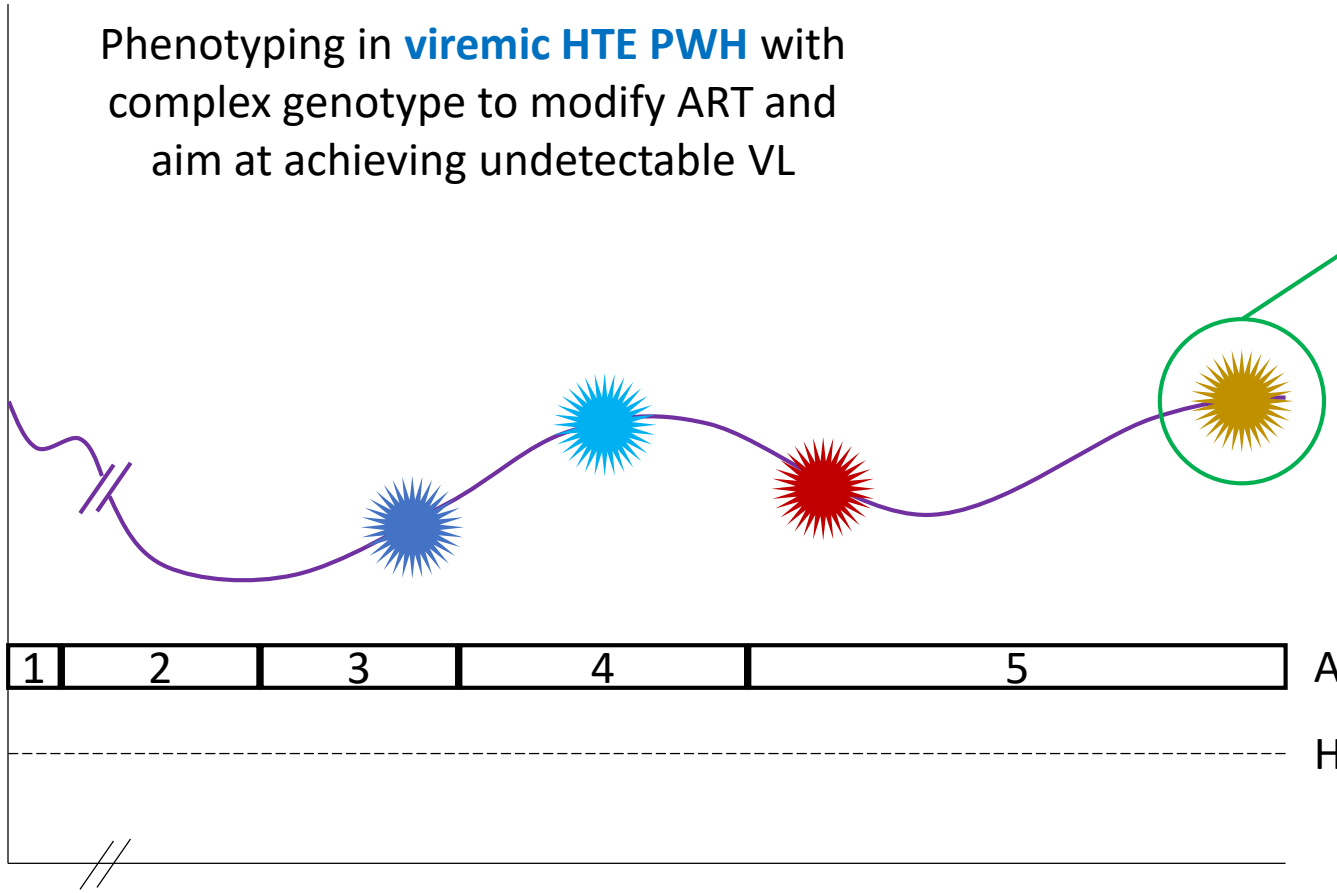
NRTI RAMs	NNRTI RAMs	IC_{50} Fold Change		Predicted susceptibility	Phenotypic susceptibility
		TAF	ISL	TAF	TAF
M41L, M184V, L210W, T215Y	none	2.7	6.8	I	I
M41L, D67G, S68G, K70R, L74I, M184V, T215Y, K219E	A98G, K103N, Y181C, P225H	3.9	7.3	I	I
K70Q, M184MV, T215F	E138Q, V179E, Y181C	1.0	3.9	LLR	S
M41L, M184V, T215Y	V106I, Y188L, K238N	2.4	22.6	LLR	I
K65R, Y115F, M184V	Y181C, H221Y, M230I	7.7	2.4	R	R
D67N, K70R, M184V, T215F, K219Q	A98G	2.6	13.7	I	I
M41L, E44D, L74V, M184V, L210W, T215Y, K219N	L100I, E138R, V179L	1.6	3.8	R	I
K65R, D67G, M184V, K219Q	none	4.9	3.4	I	R
M41L, E44D, D67N, T69D, M184V, L210W, T215Y, K219KR	K103N, Y181I	4.9	8.0	R	R
M41L, E44D, D67N, K70Q, V75M, F77L, M184I, L210W, T215Y, K219R	E138A, G190A	4.2	37.8	R	R
M41L, A62AV, D67N, K70G, V75I, M184MV, L210W, T215Y, K219Q	K101E, Y181C, G190A	3.3	2.3	R	I
D67G, S68G, K70R, M184V, T215F, K219E	Y188L	0.5	8.9	I	S
M184V	none	1.2	9.0	S	S
M41L, S68G, M184V, L210W, T215C, K219E	Y181I	0.5	5.5	LLR	S
M41L, M184V, L210W, T215Y, K219E	K101E, E138A, G190Q	4.5	9.8	I	R

- Tenofovir FC cut-offs: L_CCO=1.4, U_CCO= 4.0
- Agreement genotype/phenotype in 8/15 (53%) cases, underestimation of drug activity by genotype in 5/15 (33%) cases

Paletti et al., European Meeting on HIV and Hepatitis, 22-24 May 2024, Barcellona (Spain)

Support for treatment decisions in PWH with MDR HIV-1

Phenotyping in **viremic HTE PWH** with complex genotype to modify ART and aim at achieving undetectable VL

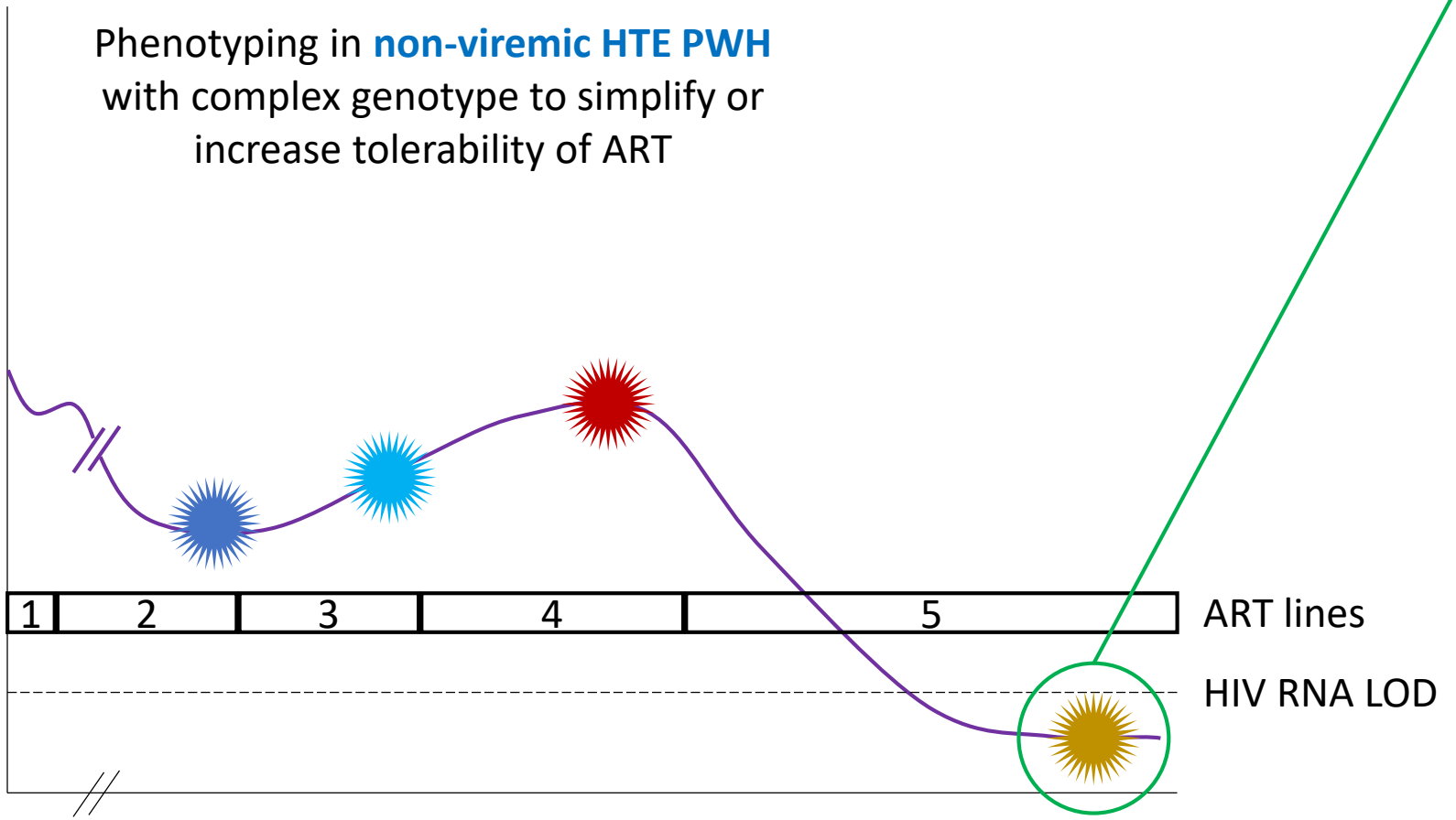


Phenotypic analysis on HIV **RNA** to determine what drugs are active against the **replicating** virus and what drugs are not

- You are phenotyping an individual time point!
- Do not forget past resistance data!
- Reasonable choice, if you need it

Support for treatment decisions in PWH with MDR HIV-1

Phenotyping in **non-viremic HTE PWH** with complex genotype to simplify or increase tolerability of ART



Phenotypic analysis on HIV **DNA** to determine what drugs are active against the **suppressed** virus and what drugs are not

- You are phenotyping an individual time point!
- Do not forget past resistance data!
- You are phenotyping dormant virus, including relics
  - Viral RNA from induced CD4+ in vitro might be an option, but labor intensive
- Too many caveats?