The Use of Phenotypic vs. Genotypic Assays

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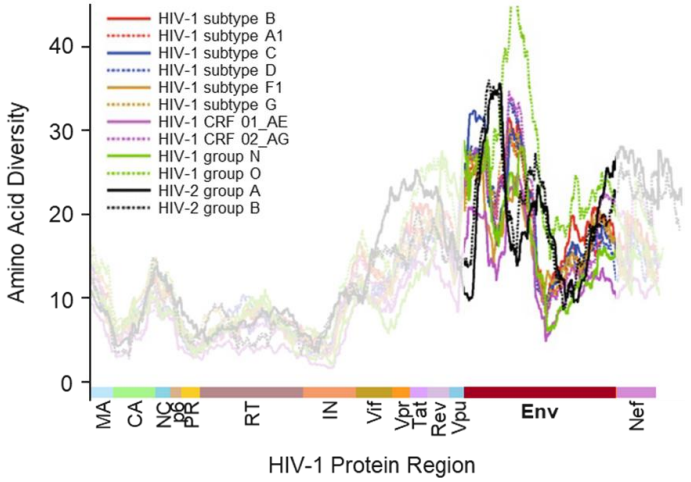
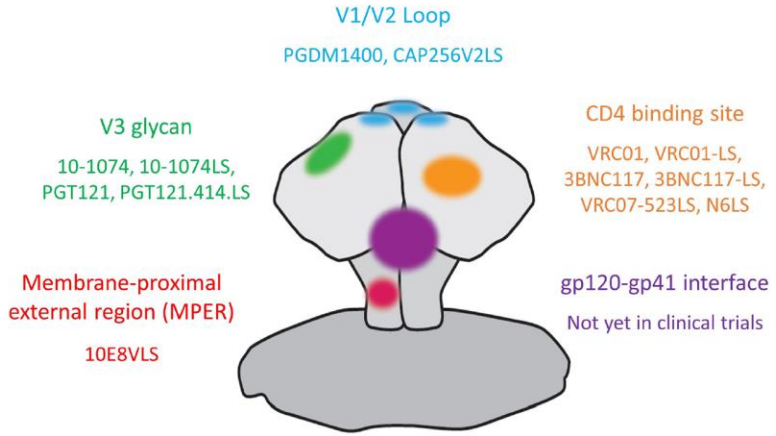
HIV envelope diversity presents clinical challenge for bNAbs



Adapted from *Li G, et al. Retrovirology 2015;12:18*

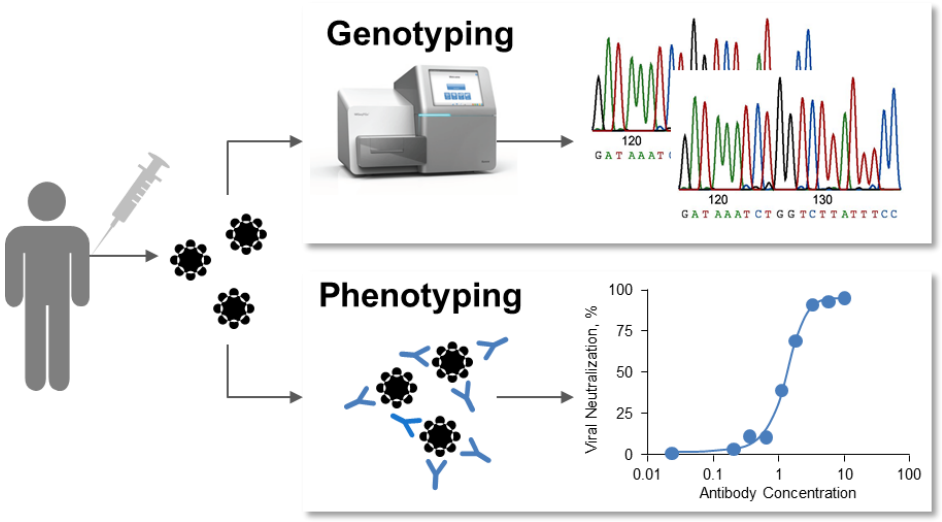


Hsu, D. et al. Front Immunol 2021, 12, 710044

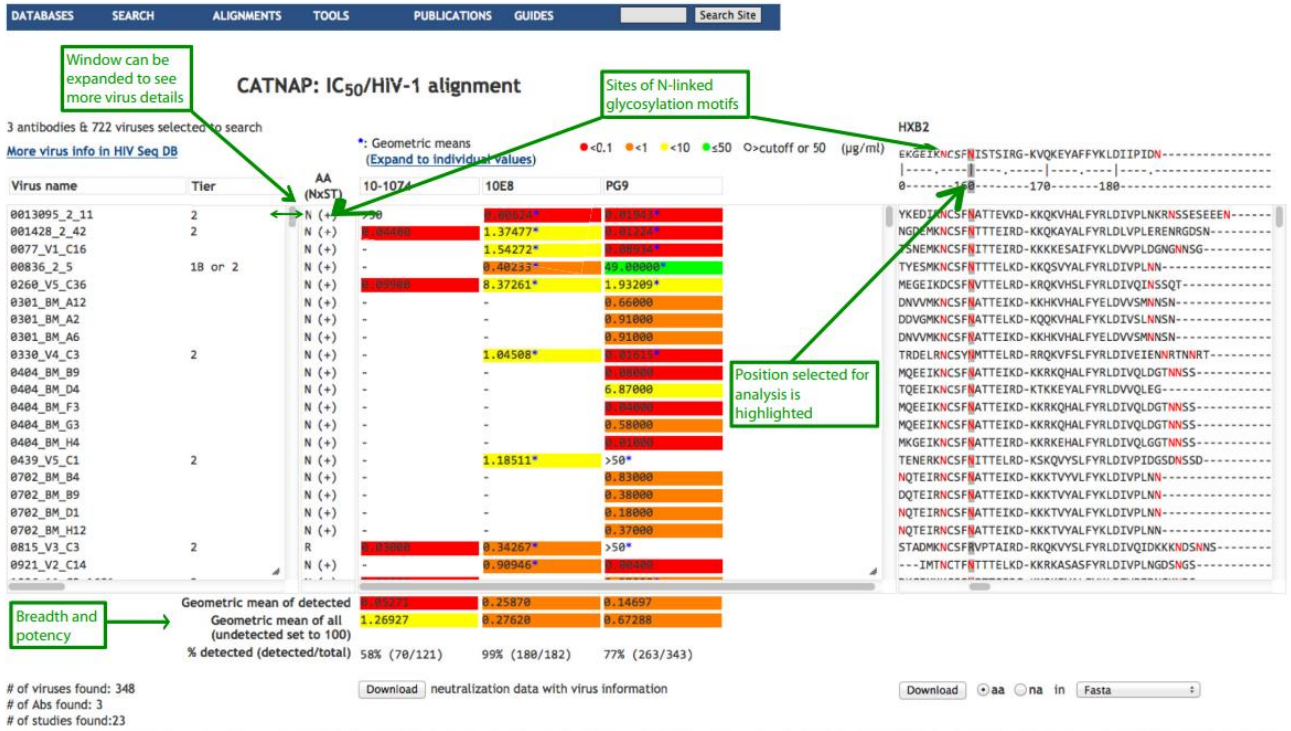
Select bNAbs	Description
Elipovimab	GS-9722, PGT121 derivative
10-1074	
Zinlirvimab	ZAB, GS-2872, 10-1074-LS
3BNC117	
Teropavimab	TAB, GS-5423, 3BNC117-LS

- Classical approach of resistance testing is challenging for bNAbs:
  - Diversity of HIV envelope target
  - Multiple protein-protein interactions, conformational changes

Correlating genotypic and phenotypic data



CATNAP



Identification of genotypic signatures to predict bNAb susceptibility

- Neutralization data combined with virus sequence information* to identify HIV env amino acid positions important for susceptibility to elipovimab and 3BNC117

Elipovimab

Amino acid positions	PPV (%)	Sensitivity (%)	Prevalence (%)
No signature	62	100	100
N332	75	98	82
N332/D325	80	87	68
N332/D325/H330	83	78	58
N332/D325/H330/T63	91	67	45
N332/D325/H330/T63/T320	93	61	41
N332/D325/H330/T63/T320/L179	97	49	32

3BNC117

Amino acid positions	PPV (%)	Sensitivity (%)	Prevalence (%)
No signature	75	100	100
I201	78	91	87
I201/F353	84	79	71
I108/I201/F353	86	75	65
I108/I201/A281/F353	91	61	50
E102/I108/I201/A281/F353	92	51	42
E102/I108/I201/A281/Y318/F353	93	47	38

N332, N332 glycan N332-NotP333-S/T334; HXB2 numbering used for HIV env amino acid positions; No signature indicates all viruses included.
Analysis based on 203 subtype B viruses for elipovimab and 234 subtype B viruses for 3BNC117;
Positive Predictive Value (PPV), probability that a virus with a given signature is susceptible; Sensitivity, probability of the signature being present in susceptible viruses
*Los Alamos National Laboratory CATNAP database, Yoon H, et al. Nucleic Acids Res. 2015 Jul 1;43(W1):W213-9

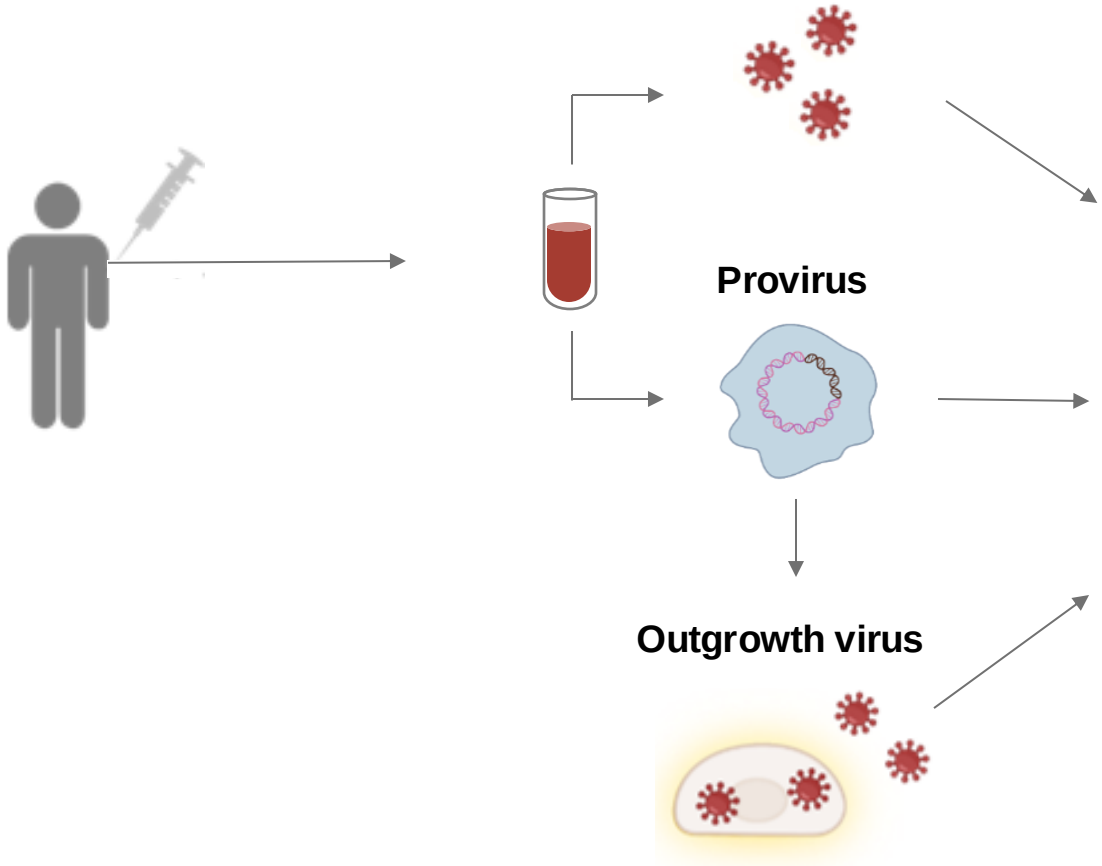
Moldt et al. JAIDS 2021, 88(1):61-69

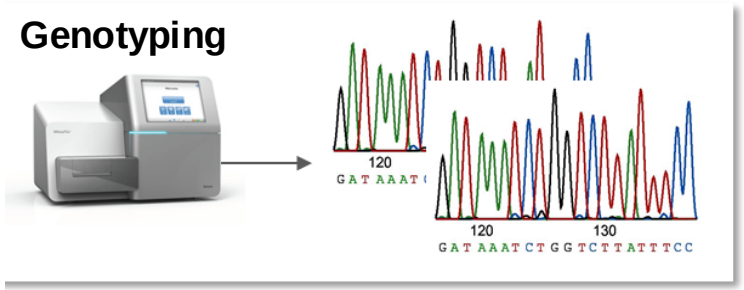
Assessing viral susceptibility to bNAbs

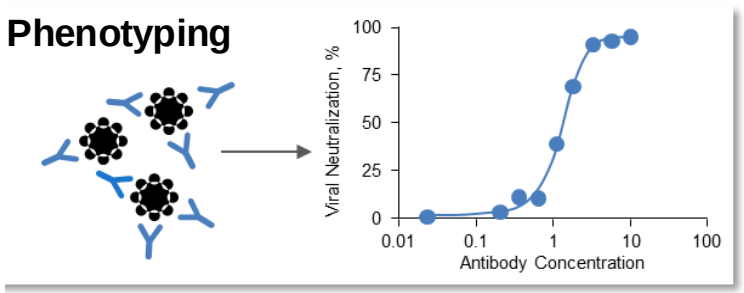
1 Patient characteristics

2 Sample type

3 Modality



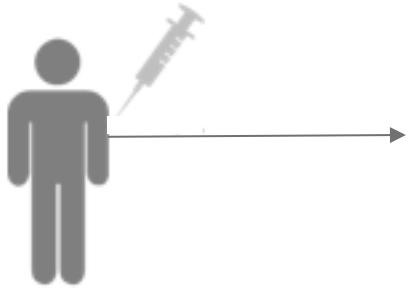




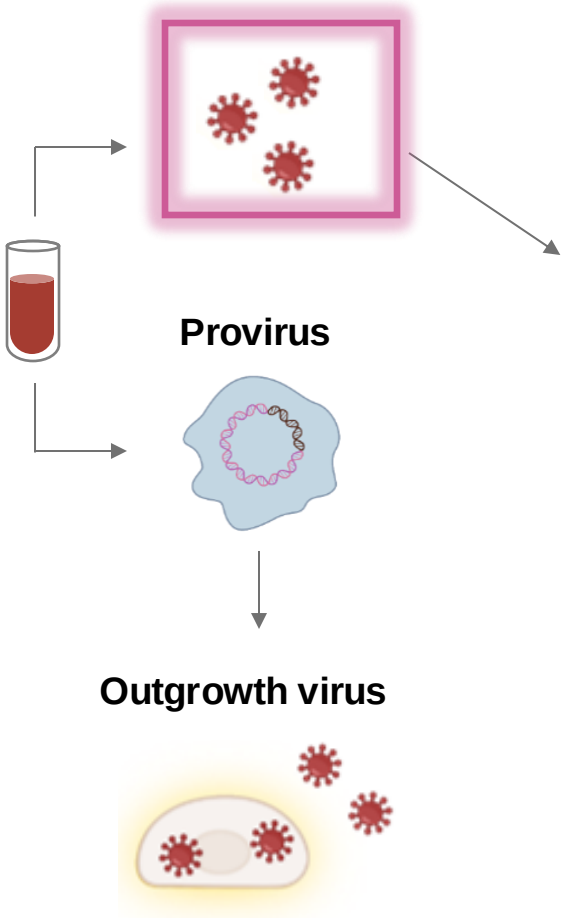
Approach 1: Confirm signature performance

1 Patient characteristics

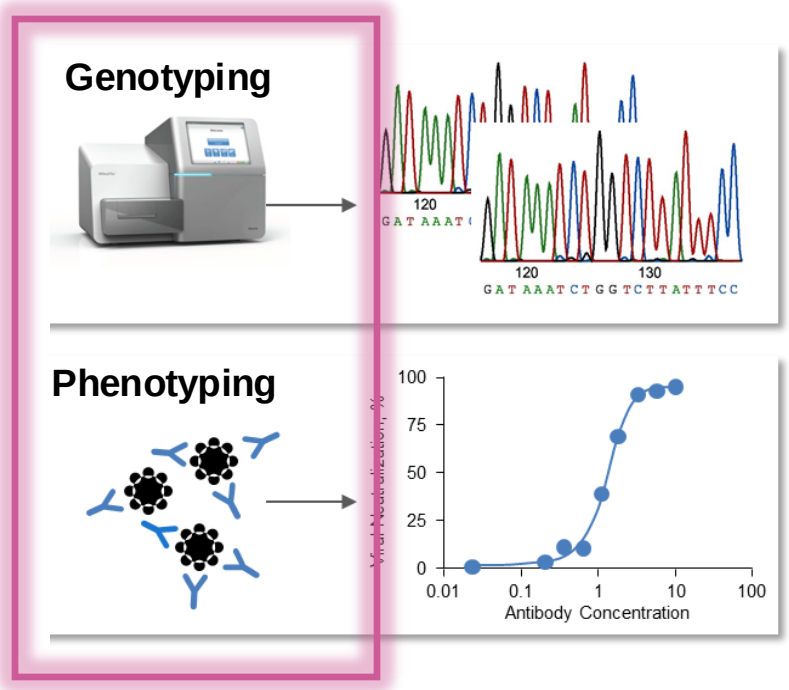
- Acute infection



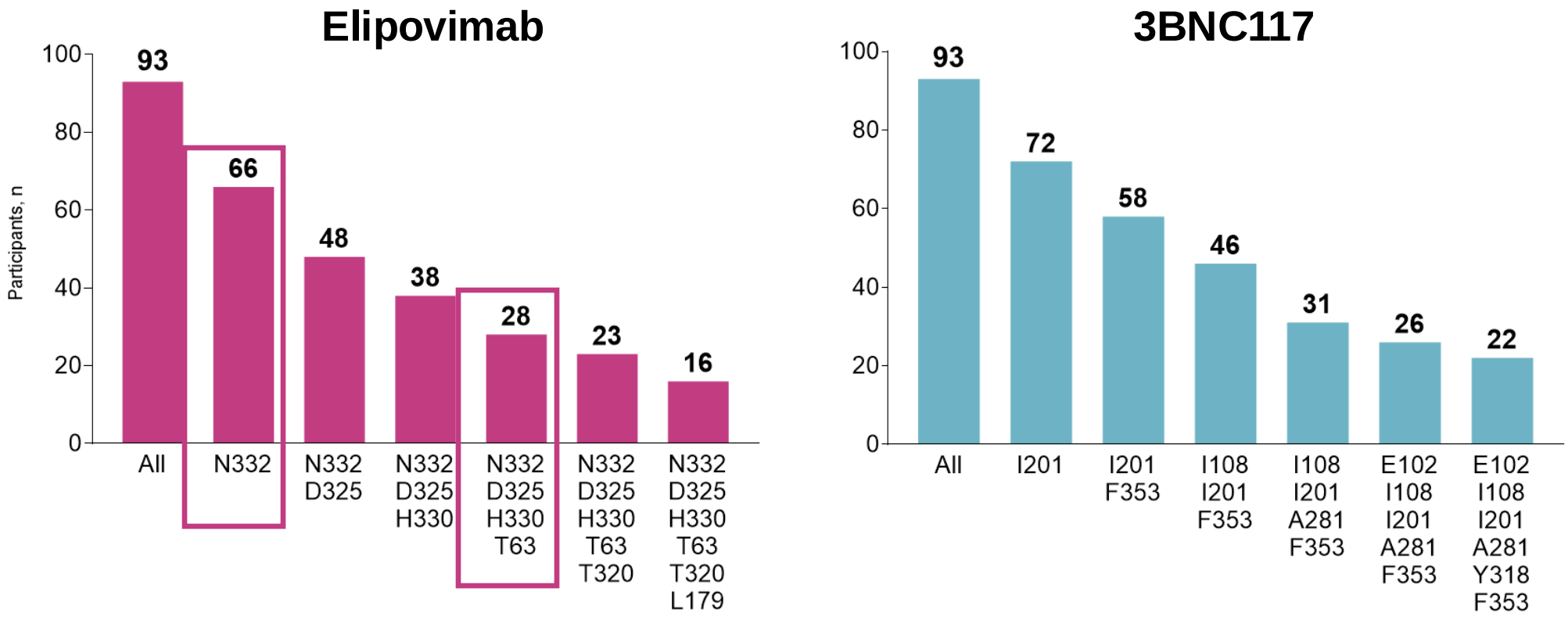
2 Sample type



3 Modality



Prevalence of HIV env signatures by genotypic assessment



- Pre-ART plasma virus from Zurich Primary HIV Infection (ZPHI) study participants was genotyped* and analyzed for the presence of the specified HIV env signatures
- No sequence variability was permitted in the positions to qualify for a given HIV env signature

*GenoSure HIV envelope RNA Assay (Monogram); N332, N332 glycan N-X-S/T

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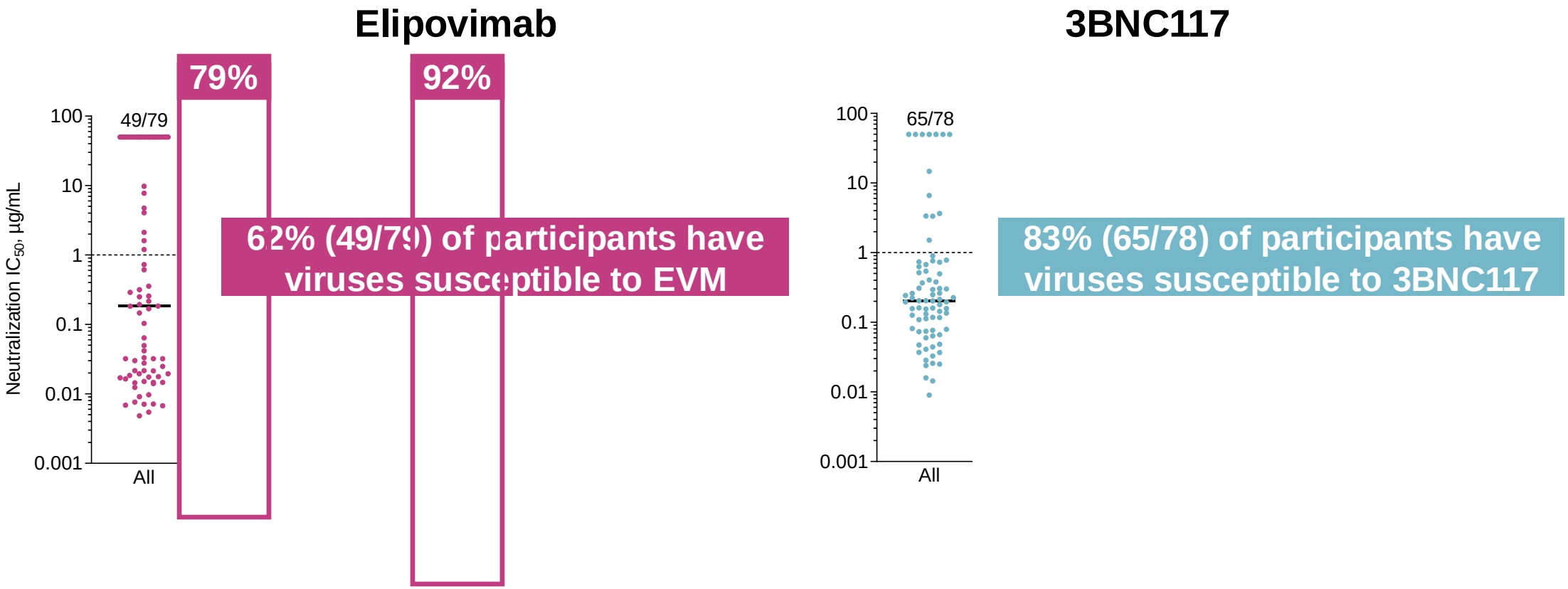
Phenotypic assessment of bNAb susceptibility by viral neutralization



- Pre-ART plasma virus from ZPHI participants was phenotyped for sensitivity to elipovimab and 3BNC117
- ZPHI participants were categorized based on HIV env signatures

PhenoSense mAb Assay (Monogram); IC_{50}, 50% inhibitory concentration; Viruses considered sensitive with IC_{50} <1 µg/mL; N332, N332 glycan N-X-S/T Moldt et al. JAIDS 2021, 88(1):61-69

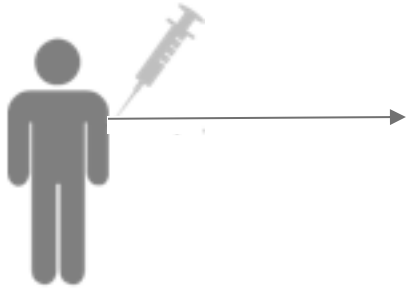
Approach 2: bNAb susceptibility of PBMC virus vs. replication-competent virus

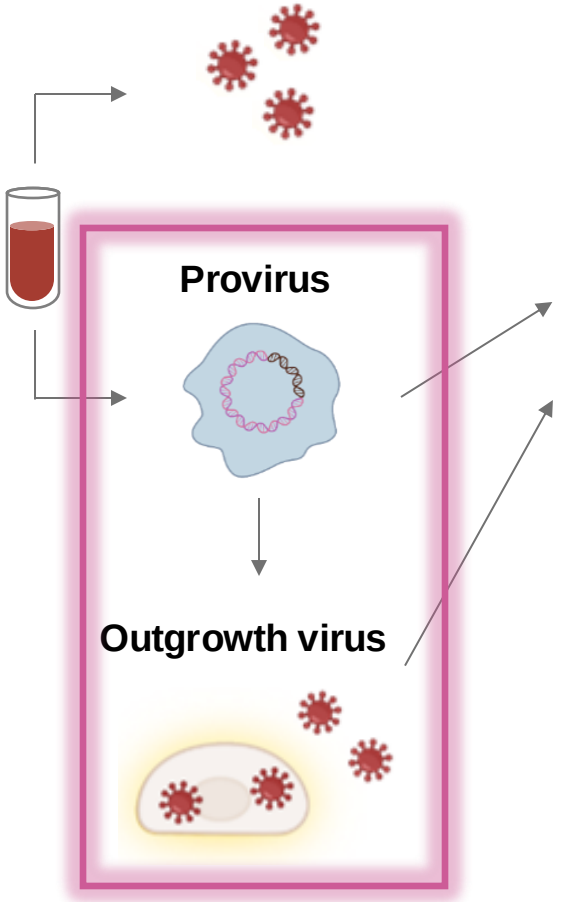
1 Patient characteristics

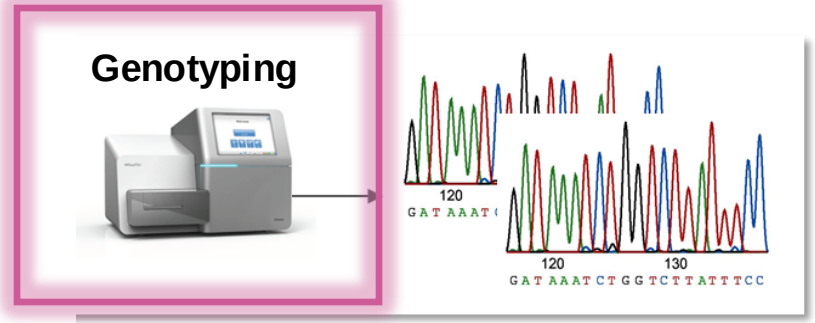
- Acute infection

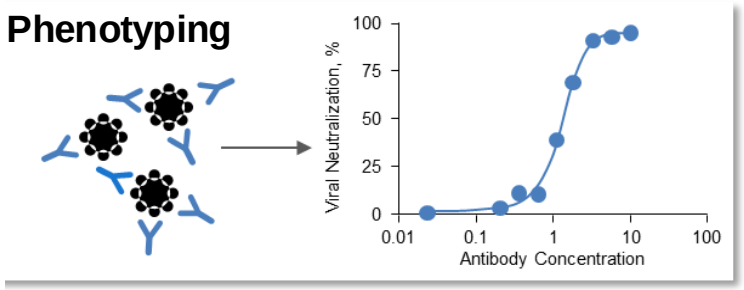
2 Sample type

3 Modality







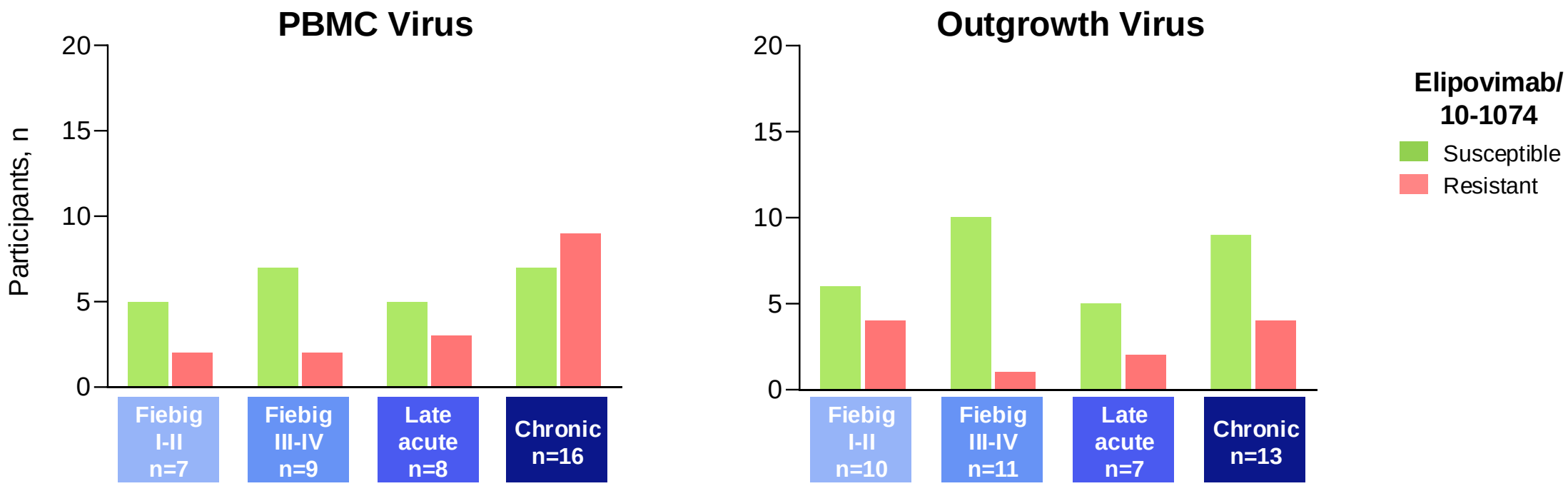


Susceptibility to bNAbs during acute infection

HIV+ ART initiated during acute/chronic infection
n=15/cohort

1. Initiated ART during Fiebig I-II*
2. Initiated ART during Fiebig III-IV*
3. Initiated ART after seroconversion but 3 months within HIV– test: "Late acute"
4. Initiated ART >6 months after estimated date of infection: "Chronic"

Leukapheresis



Envelope sequencing by Illumina MiSeq next-generation sequencing (proviral DNA/PBMCs and RNA from outgrowth virus); Viral susceptibility to elipovimab and 10-1074 based on the presence of N332 glycan and D325 residue in the HIV envelope

Moldt et al. AIDS 2021, 36(2):205-214

bNAb susceptibility in PBMC virus and outgrowth virus

	Number of participants with bNAb susceptible/resistant PBMC virus and outgrowth virus			
	PBMC virus: S Outgrowth virus: S	PBMC virus: R Outgrowth virus: R	PBMC virus: S Outgrowth virus: R	PBMC virus: R Outgrowth virus: S
Fiebig I-II, n=5	**3**	**2**	0	0
Fiebig III-IV, n=8	**7**	0	0	**1**
Late acute, n=5	**4**	**1**	0	0
Chronic, n=13	**4**	**4**	0	**5**

- bNAb susceptibility was generally in agreement between PBMC virus and outgrowth virus for participants that initiated ART during acute infection (Fiebig I-II, III-IV and Late acute)
- Five individuals that initiated ART during chronic infection (Chronic) had a discrepancy as the PBMC virus was resistant and the outgrowth virus was susceptible

Only participants with data from both PBMC virus and from outgrowth virus included in analysis (n=31)

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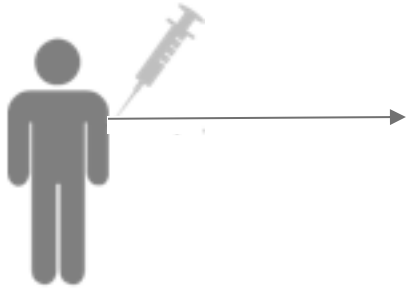
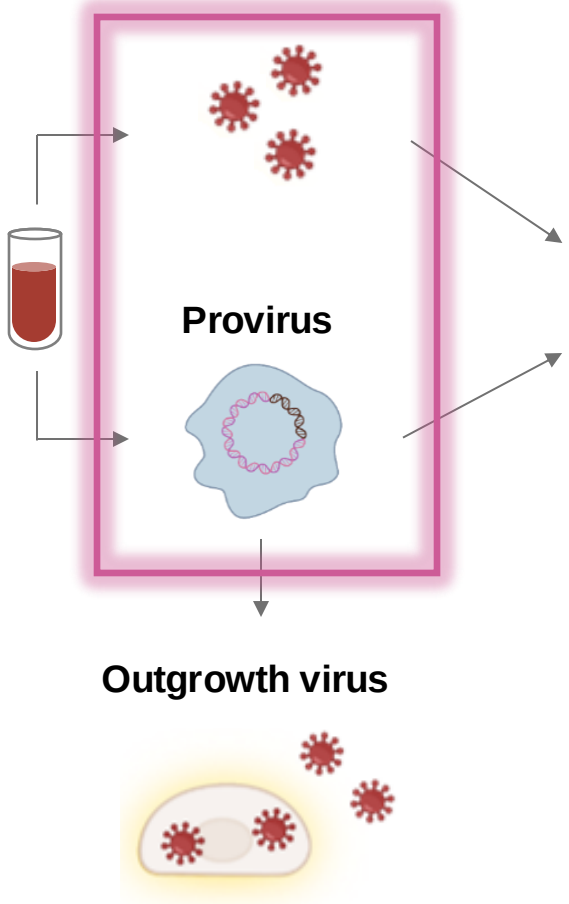
Approach 3: Assess evolution of bNAb susceptibility during ART

1 Patient characteristics

- Acute infection

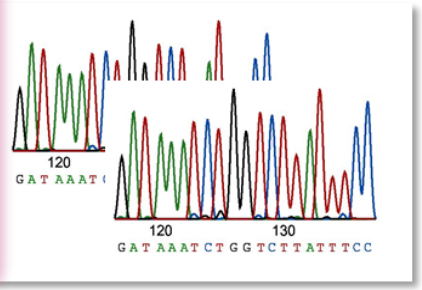


2 Sample type

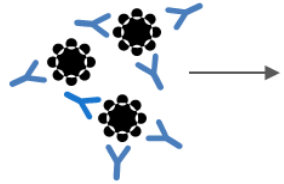


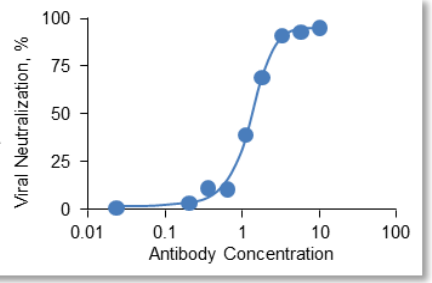
3 Modality

Genotyping

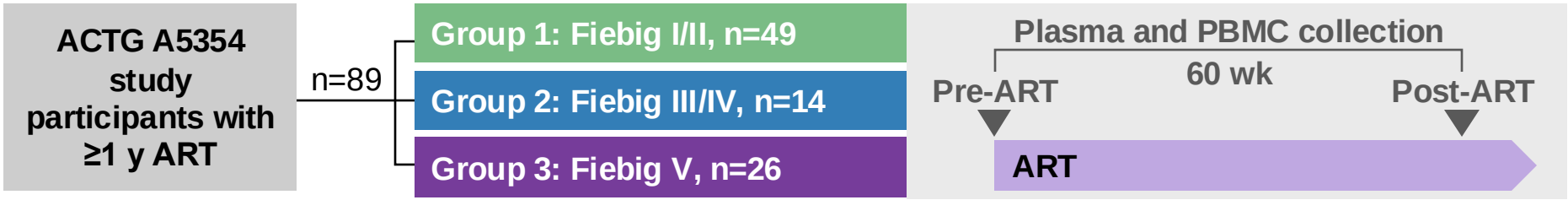


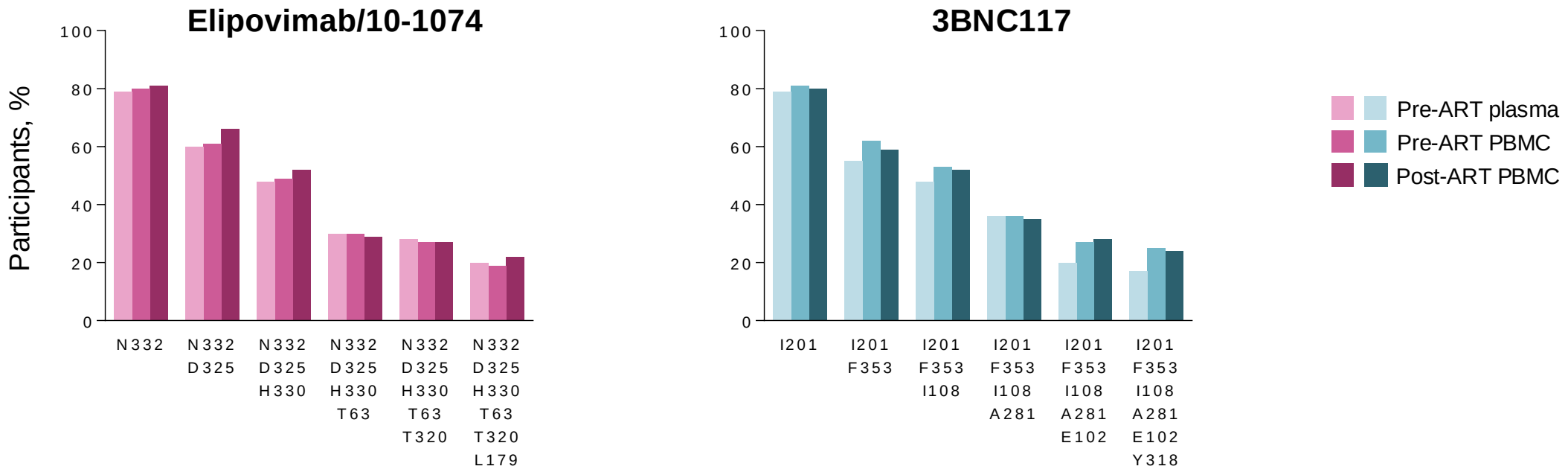
Phenotyping





Presence of genotypic signatures for bNAb susceptibility





- Proportion of signatures similar in pre-ART plasma, pre-ART PBMC, and post- ART PBMC

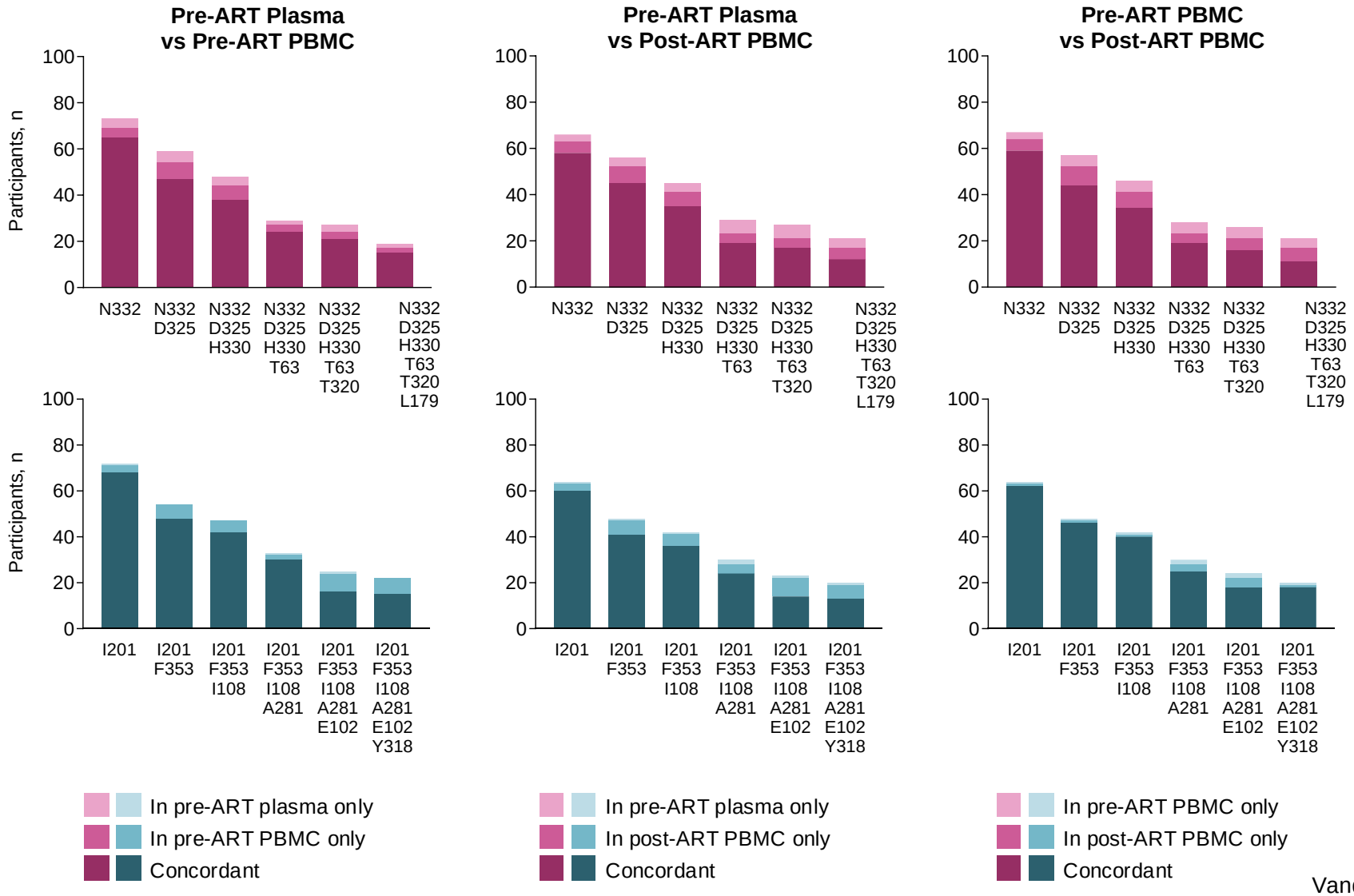
Envelope sequencing by Illumina MiSeq next-generation sequencing

VanderVeen, L et al. CROI 2022

Concordance of susceptibility signatures across sample types and time points

Elipovimab/ 10-1074

3BNC117



VanderVeen, L. et al. AIDS, *in press*

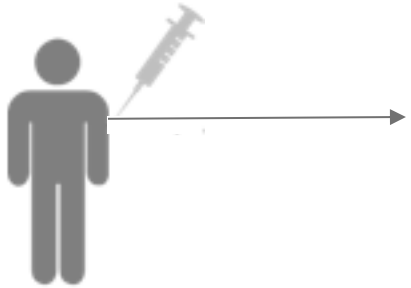
Approach 4: Implement and compare assays in clinical study setting

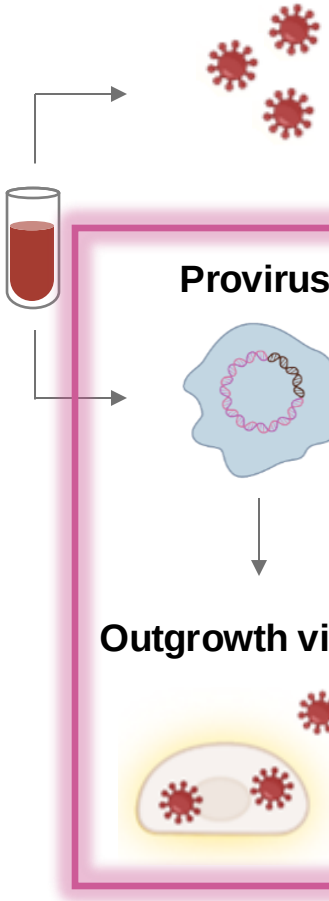
1 Patient characteristics

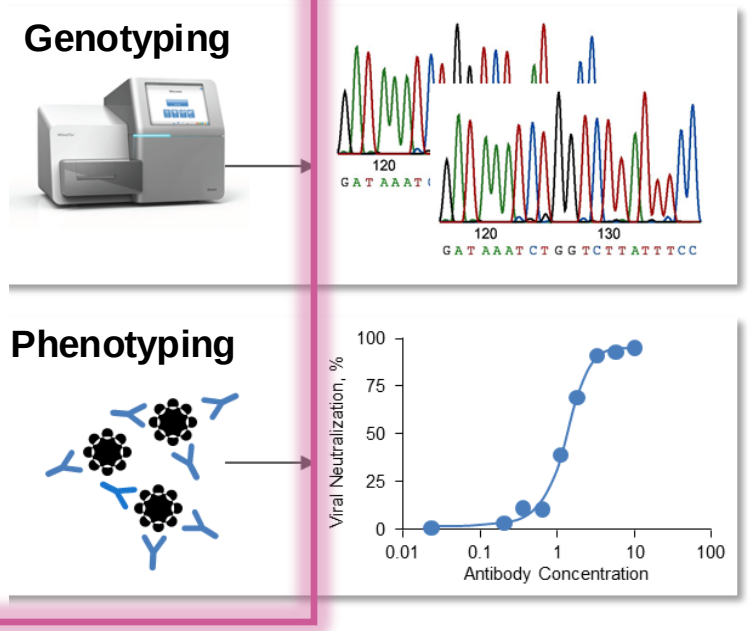
- Chronic infection

2 Sample type

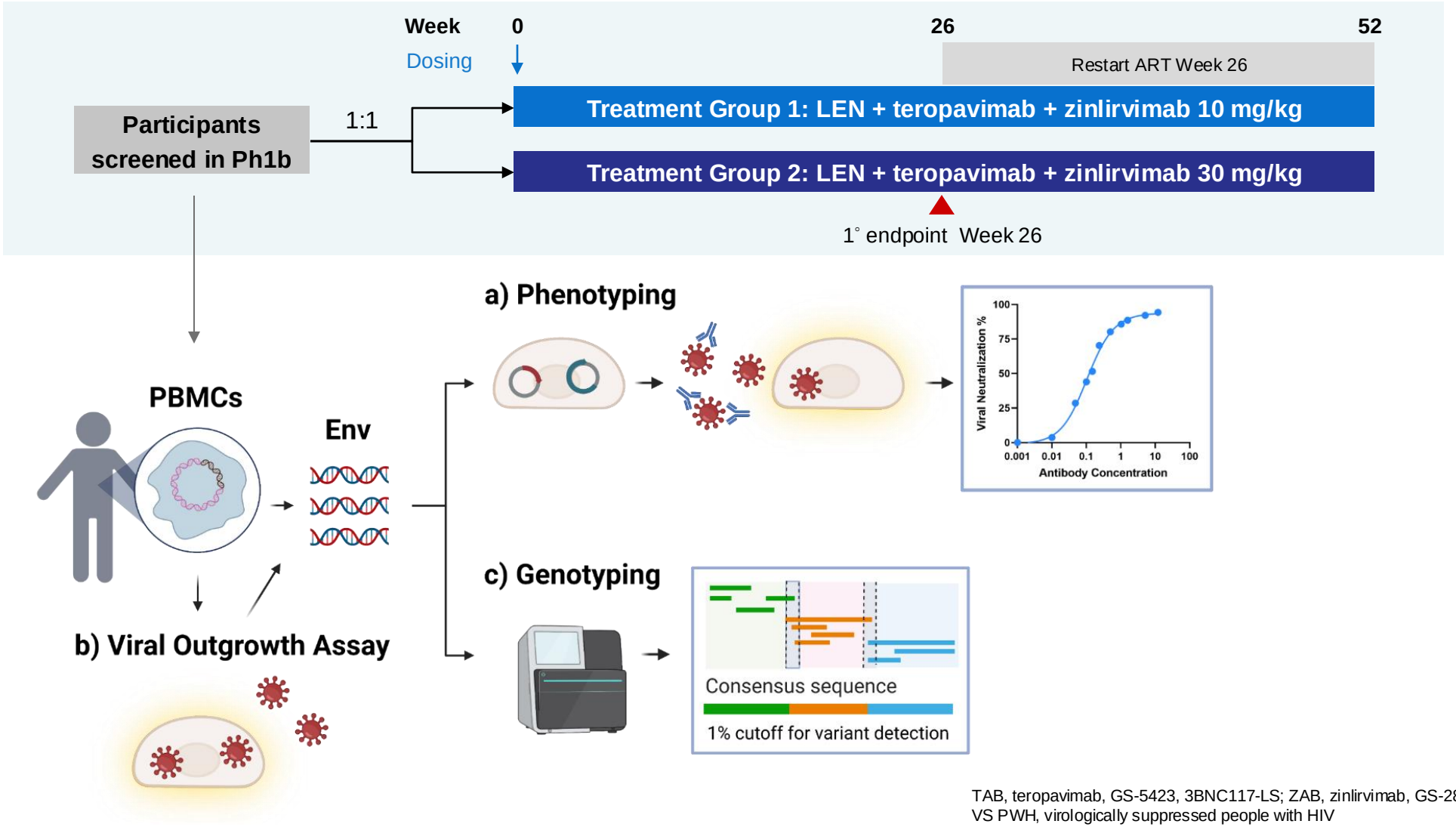
3 Modality





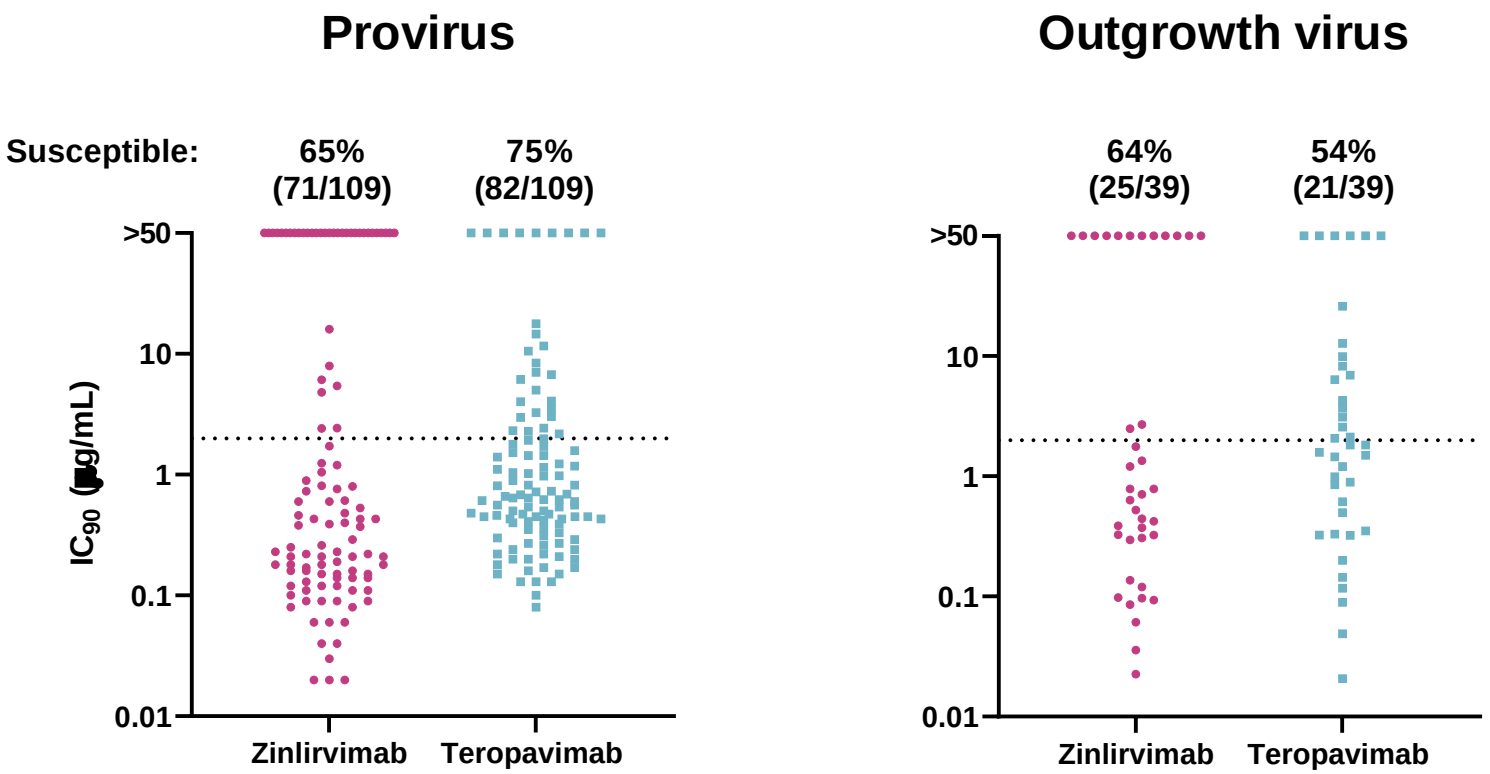


Ph1b study of combination of lenacapavir + TAB + ZAB in VS PWH



TAB, teropavimab, GS-5423, 3BNC117-LS; ZAB, zinlirvimab, GS-2872, 10-174-LS
VS PWH, virologically suppressed people with HIV

PhenoSense mAb IC_{90} values in participants screened for study

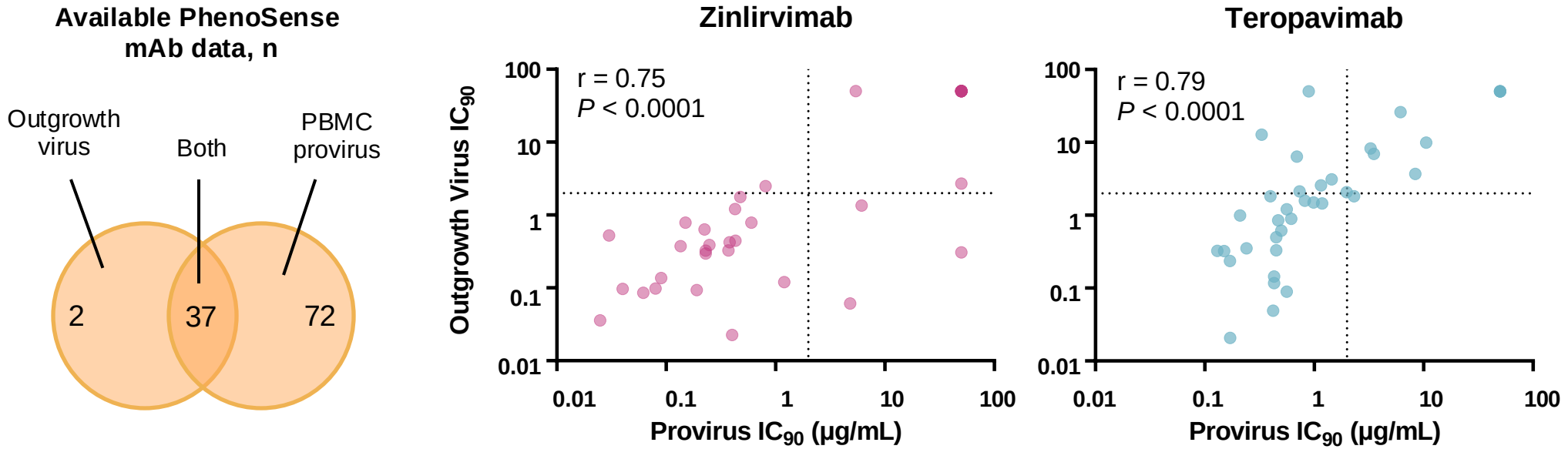


- 109 of 124 participant proviral DNA samples were successfully phenotyped using PhenoSense mAb DNA assay

Zinlirvimab, 10-1074-LS; teropavimab, 3BNC117-LS.
Proviral phenotyping by PhenoSense mAb DNA assay; Outgrowth virus phenotyping by PhenoSense mAb RNA assay
Susceptibility defined as $IC_{90} \leq 2$ µg/mL.

Selzer, L. et al. CROI 2023

Correlation of IC_{90} values for provirus and outgrowth virus



- IC_{90} values for proviruses and outgrowth viruses were well correlated
- bNAb susceptibility for provirus and outgrowth virus was in agreement for 75% and 89% of participants for teropavimab or zinlirvimab, respectively

Zinlirvimab, 10-1074-LS; teropavimab, 3BNC117-LS.
Susceptibility defined as $IC_{90} \leq 2$ µg/mL.

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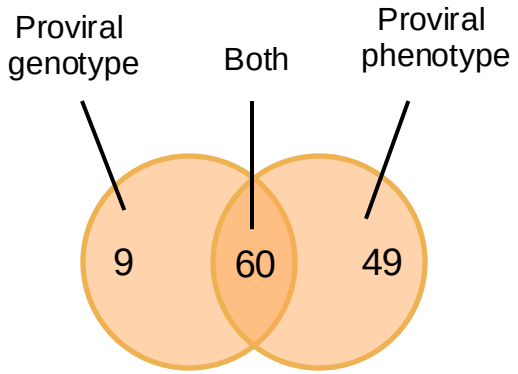
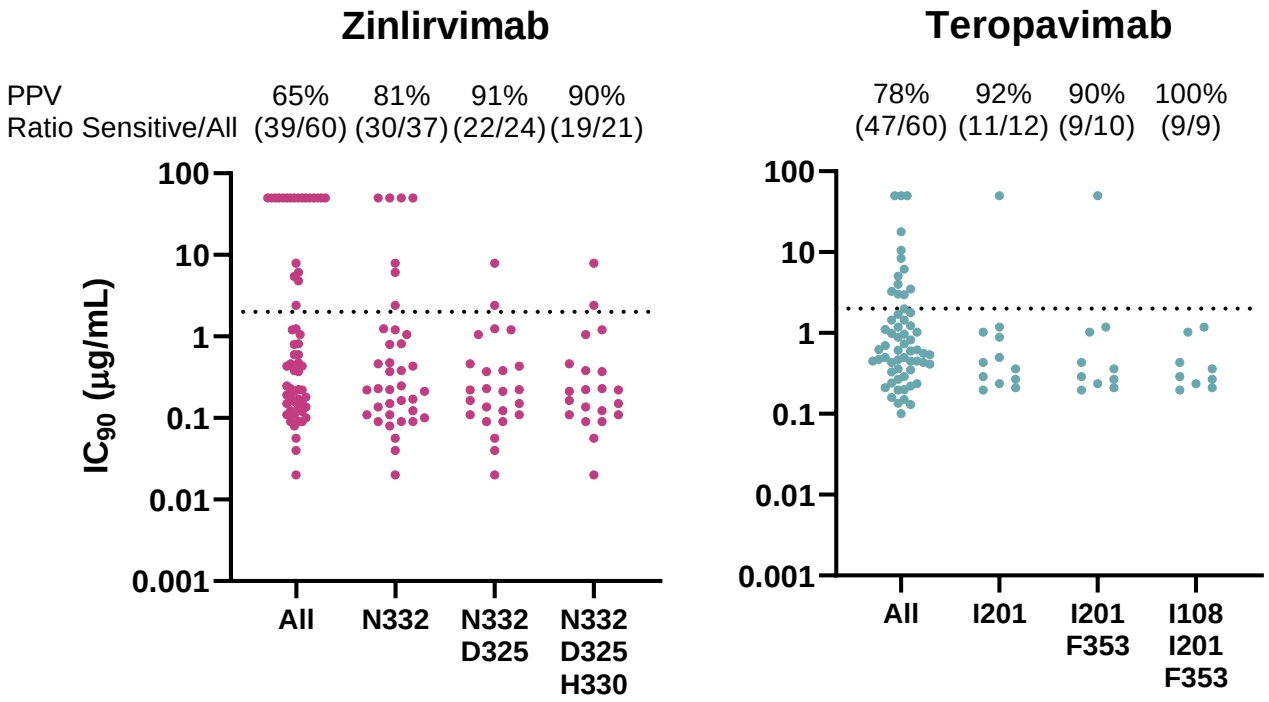
Genotypic prediction of phenotypic susceptibility

Available Genotyping and PhenoSense mAb Data, n





- Proviral genotypic signatures predicted phenotypic susceptibility of proviruses with high PPV and specificity (93% to 100% teropavimab, 71% to 96% zinlirvimab), but low sensitivity

Zinlirvimab, 10-1074-LS; teropavimab, 3BNC117-LS. Susceptibility defined as $IC_{90} \leq 2$ µg/mL.
PPV = positive predictive value.

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Where are we now?

- Gilead has operationalized bNAb susceptibility testing as part of clinical studies with teropavimab and zinlirvimab
  - Similar approaches have been implemented by others[1]
- Phenotypic assessment provides a direct measurement of virus susceptibility to bNAbs
- Genotypic signatures predict virus susceptibility to bNAbs with high specificity, but some susceptible variants may be missed
- Although data is accumulating, more data on the clinical use of bNAbs is needed to guide correlation of susceptibility testing with clinical outcomes

1. Caskey et al 2015; Scheid et al 2016; Schoofs et al 2016; Caskey et al 2017; Mendoza et al 2018; Sneller et al 2022; Gaebler et al 2022; Tebas et al 2022; Gunst et al 2023

Acknowledgments

Gilead Sciences Inc, USA:

Lisa Selzer
Brian Moldt
PC Parvangada
Jiani Li
Ross Martin
Craig Pace
Mini Balakrishnan
Nathan Thomsen
Sean Collins
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Study participants and clinical study teams

GS-US-420-3903 Study:

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ACTG A5354 Study:

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GS-US-536-5816 Study:

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Zurich Primary HIV Infection Study:

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