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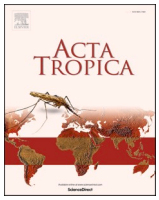

Neutralizing activity of African lineage Zika virus immune sera towards Asian lineage

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ABSTRACT

Genetic and phylogenetic studies indicated that Zika virus (ZIKV) has evolved into 2 major lineages, the African and Asian. However, ZIKV has been described as a single serotype. This study aimed at assessing the cross-neutralization between ZIKV African and Asian lineages strains.

Sixthy-five samples collected in 2007 and 30 samples collected from the same subjects in 2011/2012 in West Africa and positive to neutralizing antibody against ZIKV MR-766 strain (African lineage) were tested against ZIKV H/PF/2013 strain (Asian lineage) by microneutralization assay.

All samples showing neutralizing antibodies against MR-766 strain showed also neutralizing activity against H/PF/2013 strain, although with lower titers. This is consistent with about 120 amino acid differences between the two strains. Despite differences in the magnitude of neutralizing activity against different ZIKV strains, all samples showed neutralizing antibody titers considered to be protective.

1. Introduction

Zika virus (ZIKV) is a *Flavivirus* transmitted to humans by *Aedes* mosquitoes. ZIKV was first isolated in Uganda in 1947 but gained new attention after its spread in the Pacific and then to the Americas, causing an epidemic of more than 700,000 cases (Musso and Gubler, 2016; Musso et al., 2019).

Genetic and phylogenetic studies indicated that ZIKV has evolved into 2 major lineages, the African and the Asian, which differ by <5% at the amino acid level (Haddow et al., 2012). The African lineage includes the historical MR-766 strain originally identified in 1947, whereas the Asian lineage have been implicated in the outbreaks in Yap Island in 2007, French Polynesia in 2013, and the Americas (Dowd et al., 2016). Within this cluster, a new American lineage has emerged and includes strains from the 2015/2016 outbreak in the Americas (Gubler et al., 2017).

Antibody cross-protection against different ZIKV lineages has been evaluated after ZIKV inoculation in mice through neutralization assay by incubating each virus strain and its homologous or heterologous hyperimmune serum from mice. Antibodies produced against both strains were able to neutralize ZIKV infection from both, homologous and heterologous incubation, with higher titers observed with homologous strain. Cross-protective antibody neutralization was found to be efficient for both viruses and infection with any of the strains conferred long-lasting immunity against the homologous strain (Esposito et al., 2018).

Moreover, immunity elicited by African lineage has shown to protect rhesus macaques against subsequent infection with Asian lineage (Aliota et al., 2016), demonstrating that protective immunity resulting from natural ZIKV infection confers protection against detectable viremia following rechallenge with a heterologous genotype of the virus (African lineage followed by Asian lineage). This evidence is further supported by a study demonstrating that ZIKV likely circulated as a single serotype (Dowd et al., 2016) and by a study that demonstrated protection with a heterologous strain of the virus (Osuna et al., 2016).

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Understanding how variation among ZIKV strains impacts antibody recognition is of particular importance to vaccine development. In fact, desirable ZIKV vaccine candidates should provide equivalent protection against both Asian and African lineages (Dowd et al., 2016).

In a previous study (Marchi et al., 2020), we tested human serum samples collected in West Africa in 2007 and 2011/2012 for the presence of neutralizing antibodies against MR-766 ZIKV strain (African lineage), with a total of 95 samples found positive. Here we assess the neutralization activity of this panel of MR-766 positive samples against H/PF/2013 ZIKV strain (Asian lineage).

2. Materials and methods

Human serum samples were collected by individuals enrolled in clinical trial performed within the framework of the Meningitis Vaccine Project (MVP) (LaForce et al., 2007). The clinical trial was performed in 2007 in Mali, The Gambia, and Senegal to evaluate the safety and immunogenicity of MenAfriVac® vaccine (Serum Institute of India, Pune, India) in 2–29-year-old participants (Sow et al., 2011). To evaluate antibody persistence to MenAfriVac® vaccine (Serum Institute of India, Pune, India), blood samples from the same study participants were collected between 2011 and 2012 (Diallo et al., 2015). The study was conducted under the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (ICH-GCP) with identifier ISRCTN87739946.

The present study was approved by Ethic Committees in Mali, Senegal and The Gambia (Senegal: 0-000124, released 14 September 2017; Mali: 240/CVD-Mali/CNAM, released 12 September 2017; Gambia: SCC1574v1.1, released 16 October 2017).

Samples positive to MR-766 strain were collected from 65 subjects in 2007. Out of these 65 subjects, 30 had a second sample collected in 2011/2012 and positive to MR-766. These samples were tested by microneutralization (MN) assay against the ZIKV strains UVE/ZIKV/1947/UG/MR766 (African lineage), obtained from the European Virus Archive (EVAg, Genbank reference DQ859059), and H/FP/2013 (Asian lineage), kindly provided by the Istituto Superiore di Sanità (Rome, Italy) (Genbank reference KJ776791). Briefly, serum dilutions from 1:10 to 1:5120 were incubated with 100$TCID_{50}$ of ZIKV in infection medium (Dulbecco's Modified Eagle's Medium supplemented with 1% fetal bovine serum and 1% Penicillin/Streptomycin). After 1 hour incubation at 37 °C, serum-virus mixture was added to a 96-well plate containing Vero E6 cells (ATCC® CRL-1586™) seeded at 3×10^3 cells/ml and incubated for 1 hour at 37 °C, subsequently, additional infection medium was added. After 5 days of incubation at 37 °C, 5% CO_2, the presence/absence of cytopathic effect (CPE) was observed by microscopy. The highest sample dilution able to completely inhibit viral growth, in terms of CPE, was regarded as the neutralization titer.

Median MN titers were calculated along with their interquartile range (IQR). The results were evaluated for normal distribution by D'Agostino and Pearson, Shapiro-Wilk and Kolmogorov-Smirnov normality tests. MN titers were log-transformed (base 10) and statistically significant differences in MN titers for H/PF/2013 strain with respect to the MR-766 strain were determined by Wilcoxon signed-rank test, as they were not normally distributed even after log transformation. Statistical significance was set at $p < 0.05$, two tailed. Correlation between MN titers for the two ZIKV strains as well as for the two sampling time points of the same subject was determined by Spearman's nonparametric correlation coefficient rho (r). All statistical analyses were performed using GraphPad Prism version 9 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

Multiple sequence alignments were performed using BLAST server (National Center for Biotechnology Information, 2020). Polyprotein and envelope (E) protein sequences of MR-766 and H/FP/2013 strains were compared, and the difference/similarity of the sequences was expressed as percentages.

3. Results

All of 65 samples showing neutralizing antibodies against MR-766 strain showed also neutralizing activity against H/PF/2013 strain, although with lower titers ($p < 0.0001$) (Fig. 1a). The median MN titer for MR-766 was 640.0 (IQR 320.0–2560.0), while median MN titer for H/PF/2013 was 80.0 (40.0–480.0). MN titers were 8.0-fold higher against the MR-766 strain than against the H/PF/2013 strain.

Out of 65 subjects, 30 had a second available sample positive to MR-766. Also in this case all samples positive to MR-766 were also positive to H/PF/2013 with lower titers ($p < 0.0001$) (Fig. 1b). The median MN titer for MR-766 was 960.0 (IQR 320.0–2560.0), while median MN titer for H/PF/2013 was 160.0 (IQR 40.0–640.0). MN titers were 6.0-fold higher against the MR-766 strain than against the H/PF/2013 strain.

The comparison between the MN titers for the 30 subjects of which a second sample was available showed a strong correlation between the two ZIKV strains, both for the sample collected in 2007 ($r = 0.8687$) and the one collected in 2011/2012 ($r = 0.8494$) (Fig. 2a,b).

For these 30 subjects, persistence of antibodies showed that 19 subjects maintained the same MN titer against MR-766 strain in the second available sample. For seven subjects an increase in the MN titer was observed, while for 4 subjects a decrease occurred. The increase/decrease in MN titers for H/PF/2013 strain was consistent with that observed for MR-766, and in none of the cases this was significant within the same strain. The comparison between MN titers for these subjects showed a correlation between the two sampling time points, both for MR-766 strain ($r = 0.6651$) and H/PF/2013 strain ($r = 0.7181$) (Fig. 2c, d).

Alignment of the sequence from MR-766 strain with the H/PF/2013 strain showed about 11% nucleotide change. A pairwise alignment of the polyprotein amino acid sequence for H/PF/2013 revealed 120 amino acid differences from the MR-766 strain polyprotein. The two strains exhibited 96.8% conserved amino acids across the E protein, with 10 amino acid substitutions, of which 7 in the domain III of the E protein.

4. Discussion

In this study, 65 samples collected in 2007 and 30 samples collected from the same subjects in 2011/2012 in West Africa and positive to neutralizing antibody against ZIKV MR-766 strain were tested against ZIKV H/PF/2013 strain to investigate cross-neutralization activity between ZIKV strains.

We found that all samples showing neutralizing antibodies against MR-766 showed also neutralizing activity against H/PF/2013 strain, although with lower titers. These results show that there is a significant strain specificity in neutralizing antibodies induced by ZIKV infection. Strain-specific antigenic variability has been already described for other flaviviruses (Barr, 2012; Brault et al., 2011; Mann et al., 2013) and characterization of monoclonal antibodies (mAbs) to ZIKV have documented strain-specific neutralization of ZIKV isolates (Barr et al., 2020; Balmaseda et al., 2017). Moreover, neutralization assays with homologous and heterologous ZIKV hyperimmune serum from mice showed higher neutralizing titers with the homologous strain (Esposito et al., 2018).

These differences in neutralizing activity toward ZIKV may be associated with conformational differences due to changes in amino acid sequences (Barba-Spaeth et al., 2016; Stettler et al., 2016). In fact, ZIKV MR-766 and other isolates belonging to the African lineage have been shown significant differences in amino acid sequence compared to strains belonging to Asian and American lineages (Austin et al., 2012; Lanciotti et al., 2016; Dick et al., 1952).

To investigate the differences in neutralizing antibody responses, the amino acid sequences of polyprotein and E protein have been compared between the two strains. The polyprotein amino acid sequence of H/PF/2013 differed from the MR-766 strain polyprotein of 120 amino acid,

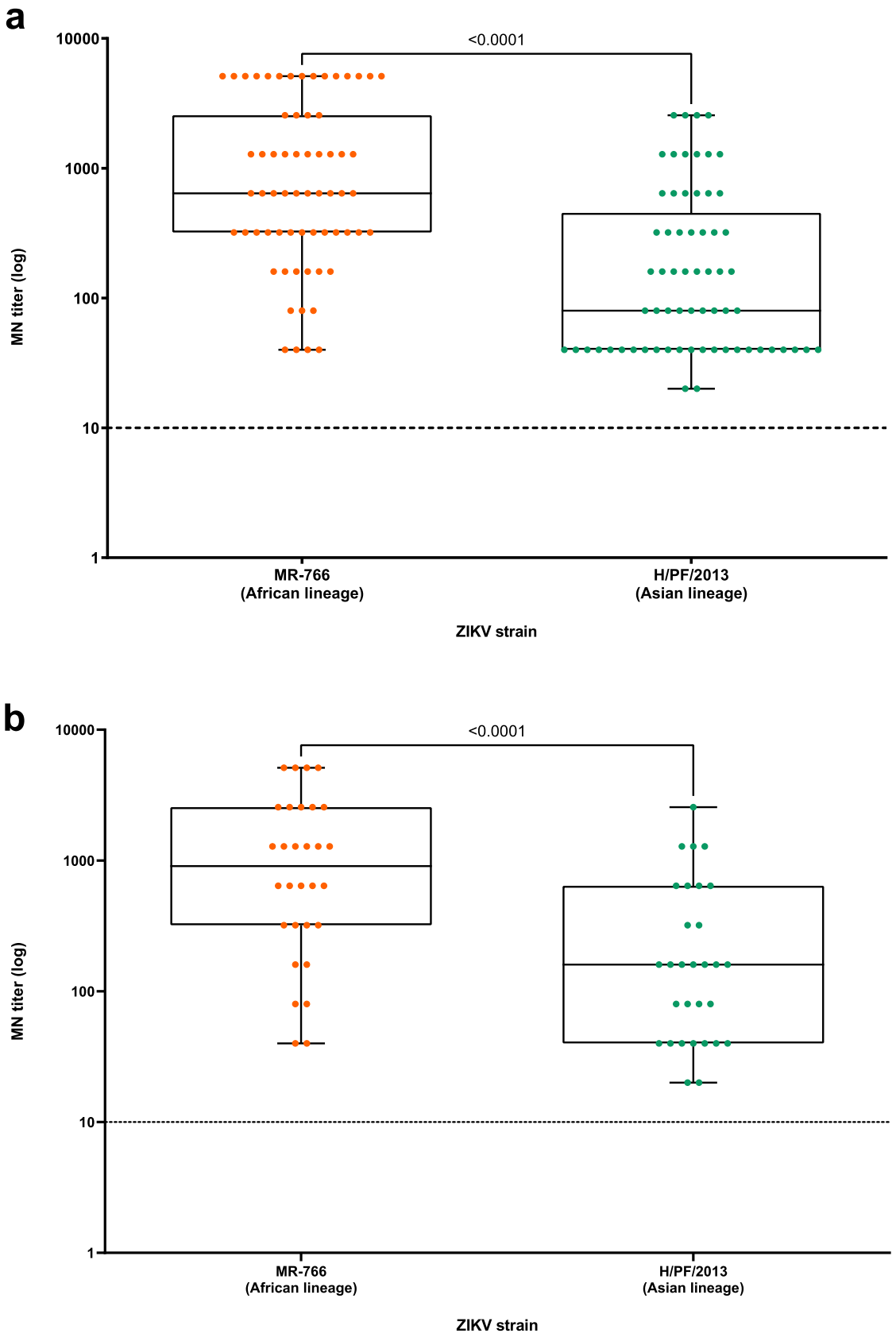

Fig. 1. Neutralizing antibody titers to Zika virus (ZIKV) MR-766 (African lineage) and H/PF/2013 (Asian lineage) strains in samples collected in 2007 (panel a) and 2011/2012 (panel b). Boxplots show individual values (dots), medians (middle line), third and first quartiles (boxes), while the whiskers display the minimum and maximum values. Horizontal dashed line represents the Lower Limit of Quantification (LLOQ) of microneutralization (MN) assay. MN titers were log-transformed (base 10). Statistically significant differences in MN titers for H/PF/2013 strain with respect to the MR-766 strain were determined by Wilcoxon signed-rank test ($p < 0.05$).

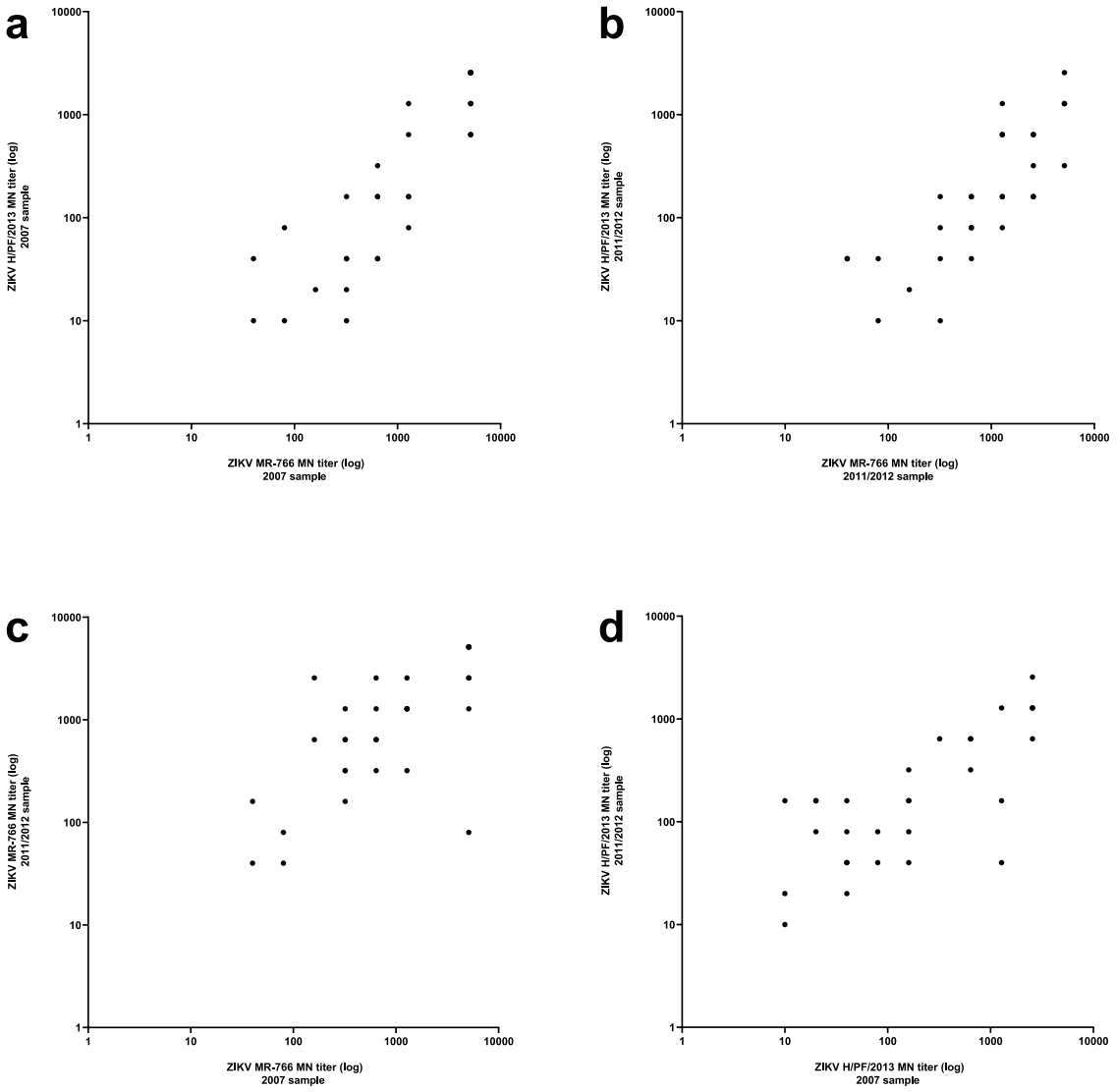

Fig. 2. Scatter plots showing correlation between neutralizing antibody titers to Zika virus (ZIKV) MR-766 (African lineage) and H/PF/2013 (Asian lineage) strains in samples collected in 2007 (panel a) and 2011/2012 (panel b). Scatter plots showing correlation between neutralizing antibody titers in samples collected in 2007 and 2011/2012 from the same subject to ZIKV MR-766 (panel c) and H/PF/2013 (panel d) strains. Microneutralization (MN) titers were log-transformed (base 10).

consistent with about 11% of nucleotide change between MR-766 and H/PF/2013 sequences. The two E protein amino acid sequences exhibited 96.8% of homology, with 10 amino acid substitution, 4 of which are predicted to be exposed on the virion surface and located in the target regions for human ZIKV neutralizing mAbs (Nix et al., 2020).

The E protein is the primary target of neutralizing antibodies and considered to be responsible for long-term protection against reinfection (Heinz and Stiasny, 2017). The E protein is highly conserved among ZIKV strains and phylogenetic analyses designated ZIKV as a single serotype. However, Nix et al. (2020) highlight that for dengue virus (DENV), a flavivirus close to ZIKV, significant differences in neutralizing titers have been explained by two amino acid substitutions in the E protein (VanBlargan et al., 2013), and that the amount of amino acid variability required to produce a distinct DENV serotype is difficult to predict (Katzelnick et al., 2015). Given the high homology between DENV and ZIKV, the possibility of a similar behavior cannot be excluded.

Despite differences in the magnitude of neutralizing activity against different ZIKV strains, it is important to note that all samples showed at least a MN titer of 10 against the H/PF/2013 strain. Larocca et al. (2016) noted that a microneutralization titer of 10 was sufficient to protect mice against challenge. The lower limit of detection for the MN assay used in this study was a 1:10 dilution of serum sample. It is therefore plausible that MN titers observed against H/PF/2013 strain, although low, are still protective.

This study has some limitation. Only two ZIKV strains have been investigated and none of the strains belonging to the American lineage has been included in the study. Moreover, it would have been of interest to determine the potential lineage/serotype behavior in recent African strains compared to MR-766 strain.

After the massive epidemic in the Americas, since 2017, the incidence of ZIKV infections has considerably decreased. The most common hypothesis to explain the decline of the circulation of ZIKV is a high herd immunity of the population (Masmejan et al., 2020). However, evidence of decline in ZIKV neutralizing antibody over time after natural infection has been reported (Henderson et al., 2020). Understanding the impact of different ZIKV strains on antibody protection is of utmost importance for evaluating of the potential of ZIKV vaccines currently under development to protect against likely future ZIKV strains and lineages.

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CRediT authorship contribution statement

Serena Marchi: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Filippo Dragoni:** Investigation, Writing – review & editing. **Adele Boccuto:** Investigation, Writing – review & editing. **Olubukola T. Idoko:** Resources, Writing – review & editing. **Maurizio Zazzi:** Methodology, Writing – review & editing. **Samba Sow:** Resources, Writing – review & editing. **Aldiouma Diallo:** Resources, Writing – review & editing. **Simonetta Viviani:** Writing – review & editing. **Emanuele Montomoli:** Resources, Writing – review & editing. **Ilaria Vicenti:** Methodology, Writing – review & editing. **Claudia Maria Trombetta:** Methodology, Project administration, Writing – review & editing.

Declaration of Competing Interest

M.Z. reports consultancy for ViiV Healthcare, Gilead Sciences, GlaxoSmithKline, Janssen-Cilag, Theratechnologies and Merck Sharp and Dohme and grants for his institution from ViiV Healthcare, Theratechnologies and Gilead Sciences outside the submitted work. E.M. is founder and Chief Scientific Officer of VisMederi srl.

Data Availability

Data will be made available on request.

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