Conclusions: Although slightly higher among those at elevated risk, PrEP use overall was very low. Challenges in improving awareness of PrEP remain, especially in Indonesia. COVID-19 had disrupted access for some. It is critical that PrEP be scaled up consistently across Asia and steps taken to address sexuality-related stigma in healthcare settings.

PEC307

HIV recent infection test-based incidence as a counter-factual for new PrEP trials

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Background: Clinical trials of new PrEP agents are challenging because it is not ethical to include a placebo-only group. Innovative ways to evaluate new PrEP modalities are needed without impractically large sample sizes (SS) required for non-inferiority trials. HIV recent infection testing algorithms (RITAs) such as the limiting antigen avidity assay (LAg) plus viral load (VL) could be used to derive a "counter-factual" incidence estimate (CFIE) using specimens from untreated, HIV-positive people identified during screening, to which on-PrEP incidence can be compared. The feasibility of this approach is partly dependent on the SS needed to ensure adequate power, which is impacted by RITA performance, the number of recent infections identified, the expected efficacy of the intervention, and other factors.

Methods: SS (number of persons screened) required to support detection of an 80% reduction in incidence (null hypothesis: 50% reduction) were calculated based on a test statistic of log incidence ratio (https://github.com/feigao1/samplesize_RA) in different populations, and assuming: 4th generation Ab/Ag testing to identify HIV-positives, 90% enrollment, 90% recency testing success, two years of follow-up on PrEP, significance level 0.05 and power 0.8. Subtype-specific mean durations of recent infection and false recent ratios (FRR) for the LAg + VL RITA were derived from pooled calibration data.

Results: Required SS for three key populations were modeled: women aged 14-17 years or >18 years in South Africa (subtype C), and men who have sex with men in the USA (subtype B). SS for these three populations were 2882, 5463, and 2327, respectively. These SS are comparable to the number of participants in recent phase 3 PrEP trials.

Conclusions: CFIEs based on recent infection testing can facilitate next-generation PrEP trials, at least in high incidence populations for which RITAs have been calibrated, and where the efficacy of the intervention is expected to be very high. SS may not be feasible in populations with lower incidence, where the FRR is higher (e.g. subtype D), or if PrEP efficacy is expected to be lower. Despite these limitations, generation of a CFIE based on recency assays appears to be feasible, offers high statistical power, and is nearly contemporaneous with the on-PrEP population.

Microbicides (including vaginal and rectal microbicides)

PEC308

Castanea Sativa Mill. bark extract (ENC®) inhibits R5 and X4 HIV-1 strains infectivity *in vitro*

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Background: The development of alternative strategies in Pre-Exposure Prophylaxis (PrEP), such as topical microbicides, might be crucial to prevent or reduce HIV transmission at level of genital and rectal mucosa. We analysed the antiviral activity of the partially purified *Castanea Sativa Mill. bark extract* (ENC®), a natural molecules consisted of over 78% hydrolysable tannins, in cell cultures infected with different HIV-1 strains.

Methods: Attachment, pre-attachment and post-attachment assays were performed to investigate ENC® related antiviral mechanisms *in vitro*, using HIV-1 strains with different tropism.
HIV-1_{Bal}, HIV-1_{Ada}, HIV-1_{J} (R5 strains), HIV-1_{C} (R5 strain isolated from HIV-1 positive cART naïve patient) and HIV-1_{IIIb} (X4 strain) (5ng/ml HIV-1 gag p24), were pre-incubated with scalar concentrations (20, 10, 5 µg/ml) of ENC®, then added to activated PBMCs. ENC® antiviral effect was determined measuring HIV-1 gag p24 in cell supernatant at day 7 post-infection (pi) using an ELISA kit (Biomerieux) and was compared with untreated control. In addition, in a dilution assay, the compound was pre-incubated with viral strains and diluted 50-fold to reduce ENC® concentration below the level capable of preventing HIV infection. Moreover, ENC® cytotoxicity was evaluated by analysis of the lactate dehydrogenase (LDH) levels.

Results: In the first set of experiments, the antiviral activity of ENC® on HIV-1 replication was evaluated. In the attachment assay, ENC® (20, 10, 5 µg/ml) significantly ($p<0.05$; Two-tailed Student test) decreased HIV-1 gag p24 content in cellular supernatant at day 7 pi respect to untreated control, irrespective of the HIV-1 strain employed.
Pre and post-attachment assay were performed to determine the stage of the viral replication cycle at which ENC® interferes with the infection. No inhibition was observed by these experimental approaches. These results suggesting that the antiviral effect might be related to a direct interaction between virus and compound during extracellular phase. Finally, ENC® was not cytotoxic at the concentrations tested.

Conclusions: ENC® shows a significant antiviral activity against all HIV-1 strains tested, it is safe and free of side effects in vitro. Accordingly, could be an attractive candidate microbicide against HIV-1 infection and may be interesting to examine its antiviral mechanism using a human cervicovaginal histocultures model.