

Poster presentation

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P04-18. Comparison of HIV neutralization assays for use in vaccine research and clinical trials, phase II: results from the NeutNet working group

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Background

In vitro assessment of HIV neutralization for pathogenesis or vaccine efficacy studies is a complex task, attributable to several confounding variables surrounding virus, antibodies and host cells employed. NeutNet, a collaboration involving 18 independent laboratories from 12 countries, showed during the first phase clear differences in neutralization assay sensitivity that were dependent on both the antibody (TriMab, 4E10, sCD4) and the virus used (<http://www.PlosOne.org>). The second phase of NeutNet focused on testing polyclonal reagents against a panel of viruses with 17 different assays.

Methods

Each laboratory evaluated TriMab, 8 HIV-positive and one seronegative sera at a given range of dilutions against 8 viruses representing different subtypes and phenotypes with 17 different assays. Assays utilized uncloned virus supernatant (virus infectivity assays-VIA) or Env-pseudotyped viruses (PSV assays). Target cells included PBMCs

and engineered cell lines in a single- or multiple-cycle infection format. A range of read-outs were used, which included intra- and extra-cellular p24 detection, and luciferase or beta-galactosidase reporter gene expression.

Results

Neutralization with TriMab showed variation for both PSV and VI assays when comparing results of phase I and II. Negative serum gave sporadic neutralization in both types of assays only when the inhibitory concentration (IC) 50 was considered. IC50 showed more variation than IC 75 and 90 for PBMC-based VIA. PSV assays were in general not more sensitive than VIA. Variation was dependent on both sera and viruses used. Specific assay to assay comparison showed impact of the target cell used.

Conclusion

In agreement with our phase I observations, we here observed that also for polyclonal agents, the assay conditions seem to influence the outcome of HIV-1 neutraliza-

tion *in vitro*. Since protective neutralizing immunity *in vivo* is not yet defined, no single assay can be recommended to achieve optimal information on the neutralization potential of a serum or agent.

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