Development of Novel breastmilk TF SHIVs to advance bnAb strategies in pediatric HIV-1 infection.

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Background: Pediatric HIV continues to be an important public health concern in resource-limited areas, and in 2025, ~40,000 children acquired HIV via breastmilk (BM) transmission. Broadly neutralizing antibodies (bnAbs), are promising candidates for HIV prevention, treatment, and cure in pediatric HIV and warrant evaluation in nonhuman primate (NHP) models. Here, we generated a panel of novel breastmilk transmitted/founder Simian-Human Immunodeficiency Viruses (TF SHIVs) for use in NHP bnAb transmission studies.

Methods: 14 HIV envelopes (Envs) from two pediatric cohorts (ZEBS and CHAVI 009; infants who tested negative at birth and subsequently acquired HIV during breastfeeding) were synthesized to characterized neutralization phenotypes against a panel of clinically relevant bnAbs. Select Envs were mutagenized at the corresponding HXB2 375 amino acid position (F, H, M, S, T, W, Y) and cloned into a SHIV backbone. Rhesus CD4- and CCR5-expressing ZB5 cells were infected with 375-point mutant SHIVs to compare viral entry. Primary NHP CD4 T cells were infected with 375-point mutant SHIVs, and viral replication was monitored by SIVp27. Infant NHPs were intravenously inoculated with combine challenge stock of all point mutations (200ng/375 mutant) to confirm *in vivo* replicative capacity and identify the 375-mutants with highest replication fitness.

Results: Neutralization assays revealed that the BM TF Envs display a range of neutralization sensitivities. Two SHIVs were characterized for entry, *in vitro* replication kinetics, and NHP *in vivo* infections. For both candidate Envs, the 375W mutants showed greater entry into ZB5 cells and higher production of SIVp27 on NHP CD4 T cells. Infant NHP studies are ongoing and aim to confirm BM TF SHIVs are replication competent and that *in vitro* results are predictive of *in vivo* fitness in models of pediatric HIV infection.

Conclusions: We have generated a panel of novel, breastmilk TF SHIVs with diverse bnAb sensitivities for innovative studies in an infant NHP model. These BM TF SHIVs provide a unique opportunity to model pediatric infection with primary HIV TF Envs, to evaluate efficacy of bnAb passive immunization in a pediatric setting and provide foundational pre-clinical data to compliment future clinical trials for management and treatment of pediatric HIV.