Viral adaptation to a CD4bs bnAb N49-P9.6 in primary, participant-derived HIV Envelopes in a humanized mouse model.

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Introduction: Broadly neutralizing antibodies (bnAbs) counteract HIV by targeting Env epitopes and are promising components of therapies for HIV prevention, treatment and cure. However, HIV rapidly adapts to bnAb pressure through *env* mutations. Here, we used infectious molecular clones (IMCs) with participant-derived Envs from CD4 binding site (CD4bs) bnAb clinical trials to identify patterns of viral adaptation to treatment with a next generation CD4bs bnAb.

Methods: PBMC-engrafted humanized mice (HIS-PBMC mice) were infected with one of three HIV IMCs expressing *env*s identified from participants of clinical trials: KX587226 (A5340 study; n=4), B.006.ABJ.H6 (BEAT2.0 study, n=4), or 14.00.H1 (VRC607/A5378 study, n=3). HIS-PBMC mice were viremic for 6 weeks and administered a single 500 mg dose of N49-P9.6-FR3.LS (N49-P9.6) and monitored for viral load and antibody concentration for 2 weeks. Using single genome sequencing (SGS), plasma HIV *env* genomes were compared for mutations between treated and untreated HIS-PBMC mice.

Results: Plasma N49-P.6 concentration was maintained between 1-10ug/mL for one week (mean = 8.6ug/mL) and gradually decreased over the second week. One-week post-N49-P9.6, KX587226- and B.006.ABJ.H6-infected mice showed a 0.8 and 1.1 log drop in viral load, respectively, while 14.00.H1-infected mice did not exhibit change in viral load. All mice rebounded to pre-bnAb viral load after two weeks. We generated an average of 32 *env* sequences per IMC group. Mice infected with the N49-P.6-sensitive IMCs (KX587226 and B.006.ABJ.H6) showed distinct patterns of shared mutations across treated mice unique to each virus. The B.006.ABJ.H6-infected mice demonstrated a consistent pattern dominated by a single mutation in the V3 region, while the KX587226-infected mice showed a more complex pattern with mutations across V2, V3 and C4 with several shifts potential N-linked glycosylation sites. Mice infected with the N49-P.6-resistant 14.00.H1 IMC showed no observable difference between mice with and without N49-P9.6.

Conclusions: Our results suggest sensitive viruses underwent selection with N49-P9.6 pressure with virus context-dependent patterns of escape, while the resistant virus did not show acquisition of mutations. The phenotypic costs of these mutations are being studied now. These studies provide insight into viral escape from N49-P9.6 and inform future investigation into combination bnAb approaches.