Combination Immunotherapy with Species-matched Broadly Neutralizing Antibodies Delivered via mRNA-lipid nanoparticles

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One promising strategy for HIV-1 prevention and cure is broadly neutralizing antibody (bnAb) immunotherapy. Given HIV-1's genetic diversity and propensity for rapid antibody escape, clinical studies have demonstrated that combination therapy with two or more bnAbs will be necessary. Two barriers to the development of bnAbs are the cost associated with recombinant protein production and confounded preclinical evaluation in nonhuman primates caused by cross-species anti-drug antibodies (ADAs).

To address these issues, we designed mRNA encoding mAbs that display either human (Hu) or rhesus (Rh) constant regions and tested their expression and function when administered alone or in combination. mRNA encoding human or rhesus versions of HIV-1 bnAbs PGDM1400 and PGT121, SARS-CoV-2 bnAb DH1047, and influenza bnAb CH65 were synthesized and encapsulated in lipid nanoparticles (LNPs). Heavy and light chains were encoded either as separate mRNAs (HC/LC) delivered in an equimolar ratio or as a single bicistronic mRNA with an intervening self-cleaving P2A site.

Expression of all mRNA constructs by Expi293F cells was confirmed by western blot. Following injection of 30 μg of Hu.PGDM1400 mRNA-LNP, mouse sera harbored human IgG at similar, high titers regardless of mRNA format (HC/LC vs. bicistronic, day 1: 763-1272 μg/ml) as measured by ELISA. These sera neutralized 6/8 heterologous HIV-1 strains with 50% inhibitory dilutions (ID50) ranging from 1:306 to >1:218,700 and displayed identical neutralization patterns to purified PGDM1400. Neutralization did not significantly differ based on mRNA format (HC/LC vs. bicistronic, Wilcoxon rank sum, $p=0.1049$) or species-matching of constant regions (human vs. rhesus $p=0.4609$). Co-delivery of Rh.PGDM1400 and Rh.PGT121 mRNA-LNPs resulted in neutralization patterns consistent with expression of both bnAbs. Following single and combination administration, proper formation of Rh.PGDM1400 and Rh.PGT121 Fabs was further validated with anti-idiotype ELISA. Combination injection of mRNA-LNPs encoding PGDM1400, DH1047, and CH65 resulted in sera that neutralized HIV-1, SARS-CoV-2, and influenza.

These data demonstrate that functional broadly neutralizing antibodies can be generated *in vivo* from multiple mRNA formats and with species-matched constant regions, even when multiple mAb-encoding mRNAs are delivered simultaneously. mRNA-LNPs may ultimately offer an accelerated, tailorable toolkit for the preclinical evaluation of antibody immunotherapy.