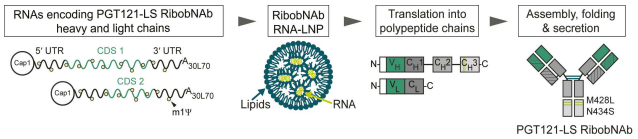
RNA-Encoded bNAb PGT121-LS: Preclinical Characterization and Pharmacokinetics in Non-Human Primates

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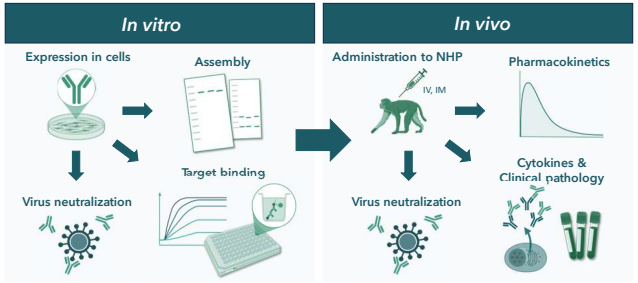
The HIV-1-targeting, RNA-encoded PGT121-LS (RibobNAb) showed high binding affinity and antiviral activity *in vitro*. Following intravenous administration to non-human primates, PGT121-LS RibobNAb was well-tolerated and maintained the extended half-life and neutralizing potency of recombinant PGT121-LS.

1. Platform for PGT121-LS RibobNAb expression



PGT121-LS RibobNAb is an RNA-encoded HIV-1 bNAb with half-life extending LS mutations designed for *in vivo* delivery via LNP.

2. Preclinical characterization of PGT121-LS RibobNAb



3. Results

RibobNAb assembly

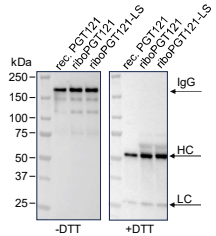


Figure 1. Western blot analysis of PGT121-LS RibobNAb assembly. HEK-293T-17 cells were transfected with RNAs encoding PGT121-LS RibobNAb. Cell culture supernatants were analyzed under non-reducing and reducing conditions. Recombinant PGT121 served as positive control.

HIV-1 Envelope binding

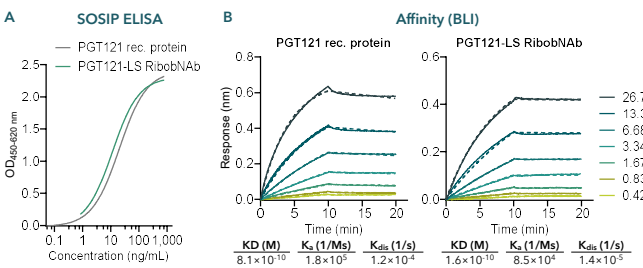


Figure 2. PGT121-LS RibobNAb binding to HIV-1 Envelope (SOSIP). (A) ELISA comparing the binding of PGT121-LS RibobNAb and recombinant PGT121 to SOSIP. (B) BLI sensorgrams showing the binding kinetics of recombinant PGT121 and PGT121-LS RibobNAb to SOSIP. Recombinant PGT121 or PGT121-LS RibobNAbs were immobilized on anti-hIgG Fc (AHC) biosensors. Binding affinities were determined using multi-cycle kinetics and decreasing SOSIP concentrations (26.71 – 0.42 nM, 2-fold dilutions).

Neutralization activity

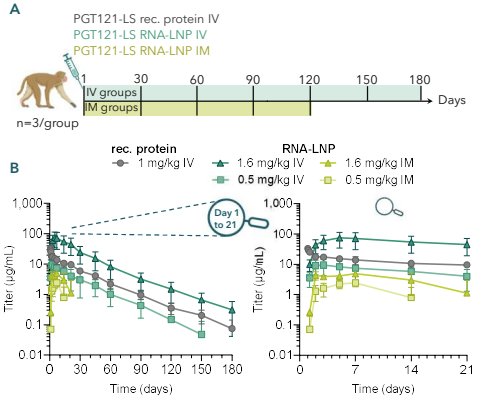
pVNT

Virus strain	Clade	Recombinant PGT121 IC_{80}	PGT121-LS RibobNAb IC_{80}	PGT121-LS RibobNAb FC
X2008_c9	G	0.011	0.008	0.71
6535.3	B	0.018	0.009	0.50
CAAN5342.A2	B	0.039	0.020	0.53
Du156.12	C	0.018	0.021	1.17
Q23.17	A	0.017	0.010	0.63
7030102001E5 (Rev-)	C (T/F)	0.036	0.024	0.66
MuLV (Neg. Ctrl)	NA	>0.300	>0.330	NA

IC_{80} (µg/mL) <0.01 | 0.01–0.05

Figure 3. Neutralization activity of *in vitro*-expressed PGT121-LS RibobNAb. TZM-bl pseudovirus neutralization assay against a cross-clade panel of HIV-1 Env pseudoviruses using a dilution series of PGT121-LS RibobNAb and recombinant PGT121 to determine IC_{80} values. Fold-change (FC) values compare PGT121-LS RibobNAb potency (IC_{80}) over recombinant PGT121 potency.

Pharmacokinetic characteristics in NHPs



C

Test item	Dose (mg/kg)	Route	C_{max} (µg/mL)	AUC_{0-inf} (d×µg/mL)	$t_{1/2}$ (d)
rec. protein	1.0	IV	33.1	546.1	27.5
RNA-LNP	0.5	IV	9.6	258.1	22.7
RNA-LNP	1.6	IV	75.7	2,315.9	23.9
RNA-LNP	0.5	IM	2.4	NA	NA
RNA-LNP	1.6	IM	5.2	NA	NA

Figure 4. Pharmacokinetic profile and characteristics of PGT121-LS RibobNAb in NHPs. (A) The PK of PGT121-LS RibobNAb was assessed in male rhesus monkeys (n = 3/group) following a single IV or IM RNA-LNP injection at doses of 0.5 and 1.6 mg/kg. IM- and IV-treated animals were monitored for 120 and 180 days, respectively. PGT121-LS recombinant protein was administered IV at 1 mg/kg. (B) RibobNAb titers were determined by Binding Antibody Multiplex Assay. Data are presented as mean ± SD. (C) Summary of PK parameters determined by non-compartmental analysis.

Neutralization activity after *in vivo* expression

A

Virus strain	Clade	PGT121-LS rec. protein 1.0 mg/kg IV IC_{50}	PGT121-LS RNA-LNP 0.5 mg/kg IV IC_{50}	0.5 mg/kg IV FC	PGT121-LS RNA-LNP 1.6 mg/kg IV IC_{50}	1.6 mg/kg IV FC
RHPA4259.7	B	0.010	0.015	1.50	0.011	1.10
Du422.1	C	0.028	0.028	1.00	0.031	1.11
0260.v5.c36	A	0.041	0.035	0.85	0.040	0.98
X1254_c3	G	0.026	0.036	1.39	0.024	0.92
MuLV (Neg. Ctrl)	NA	/	/	NA	/	NA

IC_{50} (µg/mL) <0.01 | 0.01–0.05

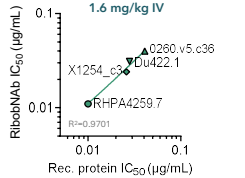


Figure 5. Neutralization activity of *in vivo*-expressed PGT121-LS RibobNAb. (A) TZM-bl pseudovirus neutralization assay from NHP serum samples taken one day post-administration. Serum samples were tested against the indicated HIV-1 Env pseudoviruses. Mean IC_{50} values of three animals are shown. FC values compare RibobNAb potency over recombinant protein potency. (B) Correlation plot comparing neutralization potency (IC_{50}) between recombinant PGT121-LS and PGT121-LS RibobNAb administered at 1.6 mg/kg RNA-LNP IV.

Cytokines and clinical pathology in NHP

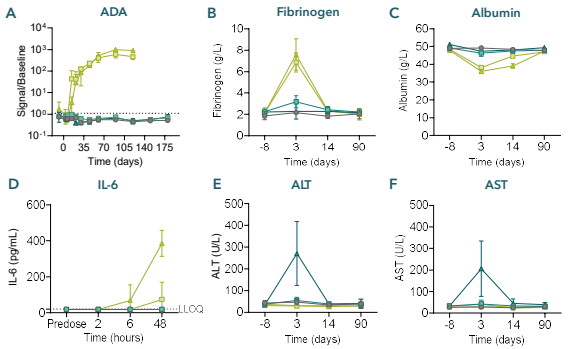


Figure 6. Clinical chemistry and cytokine changes in NHPs post IM and IV interventions. (A) Biotin/Sulfo-Tag-based bridging ADA screening assay of serum samples. Samples above signal/baseline threshold (dotted line) are considered ADA-positive (tier1) (B,C) Acute phase proteins: fibrinogen and albumin levels in peripheral blood from NHPs at Days –8, 3, 14 and 90 post-injection. (D) Serum concentrations of the pro-inflammatory cytokine IL-6 were quantified predose and 2, 6 and 48 h post-administration in peripheral blood of NHPs via multiplex assay. (E,F) Liver enzymes: ALT and AST levels in peripheral blood from NHPs at Days -8, 3, 14 and 90 post-injection.

Rec. protein
1 mg/kg IV

RNA-LNP
1.6 mg/kg IV
0.5 mg/kg IV
1.6 mg/kg IM
0.5 mg/kg IM

4. Conclusions

- *In vitro*: PGT121-LS RibobNAb assembled correctly and retained binding affinity and neutralization activity of recombinant PGT121
- NHPs, IV: PGT121-LS RibobNAb was well-tolerated without ADA elicitation; half-life and neutralizing activity comparable to recombinant PGT121-LS
- NHPs, IM: Functional RibobNAbs were expressed but with lower bioavailability compared to IV administration, correlating with pro-inflammatory response and ADA development

Acknowledgements: Additional authors from the PGT121 RibobNAb Study Group: Maximilian Kullmann for PK analysis, Imke Gerhard and Candice Morin for technical assistance, Uğur Şahin for scientific advice; Charles River Laboratories for NHP handling and Non-clinical Safety Assessment, Georgia Tomaras for PK analysis, Margaret E. Ackerman for ADA analysis and Acuitas Therapeutics for LNPs preparation. Illustrations were created with BioRender.com. This study was funded by BioNTech SE and the Gates Foundation.

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Disclosures: F.T., J.N., R.J., J.C., A.C., L.F., A.M., S.K., J.P.B., A.K., U.E., C.S. and V.L.D. are employees at BioNTech SE and may hold stock options.
V.L.D., F.T. and J.N. are inventors on International Patent Application related to WO2023218431A1, WO2025027585A1.

Abbreviations: ADA = anti-drug antibody; ALT = alanine aminotransferase; AST = aspartate aminotransferase; bNAb = broadly neutralizing antibody; BLI = biolayer interferometry; CDS = coding sequence; DPBS = Dulbecco's phosphate-buffered saline; DTT = dithiothreitol; ELISA = enzyme-linked immunosorbent assay; Env = envelope protein; Fc = fragment crystallizable; FC = fold-change (h)FcRn = (human) neonatal Fc receptor; HC = heavy chain; HEK-293T-17 = HEK-293T-17 production cell line; HIV = human immunodeficiency virus; IC_{50} = half maximal inhibitory concentration; IC_{80} = 80% inhibitory concentration; (h)IgG = (human) immunoglobulin G; IL-6 = interleukin-6; IM = intramuscular, IV = intravenous; LC = light chain; LLOQ = lower limit of quantification; LNP = lipid nanoparticle; LS = half-life-extending mutations (M428L, N434S); NHP = non-human primates; NSG = NOD SCID gamma; ox = oxidation; pVNT = pseudovirus neutralization test; PK = pharmacokinetics; rec. = recombinant; RibobNAb = RNA-encoded bNAb, RNA/mRNA = messenger ribonucleic acid; SOSIP = stabilized soluble HIV-1 Env Trimer; TZM-bl = TZM-bl reporter cells.