Development of BNT351: A Potent HIV-1-Neutralizing Antibody with Enhanced Pharmacokinetics

524

Sven Kratochvil[1], Maximilian Kullmann[1], Henning Gruell[2], Sophie Sayettat[2], Natasa Vukovic[1], Uğur Şahin[1,3], Philipp Schommers[4], Penny Moore[5,6], Michael S. Seaman[7], Florian Klein[2,4], Valentin Le Douce[1], the BNT351 Study Group

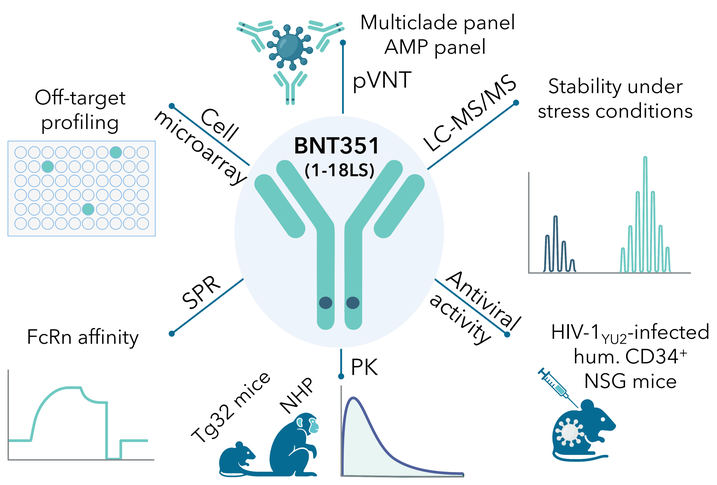
[1]BioNTech SE, Mainz, Germany, [2]Institute of Virology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [3]HI-TRON - Helmholtz Institute for Translational Oncology Mainz by DKFZ, Mainz, Germany, [4]Department I of Internal Medicine, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany, [5]SA MRC Antibody Immunity Research Unit, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa, [6]National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa, [7]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Recombinant bNAb BNT351 showed an extended half-life in animal models, while maintaining the antiviral activity of its parental bNAb 1-18. With favorable developability and no off-target binding, BNT351 has entered a Phase 1 trial in 2026 (NCT07392372).

1. BNT351: half-life-extended bNAb 1-18

- bNAb 1-18, targeted against the CD4 binding site on the HIV-1 envelope protein, is a potentially promising tool for shaping future therapeutic and cure-focused clinical strategies against HIV-1[1]
- To enhance 1-18's clinical potential, we introduced half-life-extending LS mutations and evaluated the resulting bNAb, BNT351

2. Preclinical characterization of BNT351



3. Results

No off-target binding to ~6,500 human proteins

	BNT351 binding strength	Comment
gp140	+	BNT351 positive control
FCGR1A	+++	Expected binding to Fc receptors
FCGR2A	+++	
FCGR2B	+	
FCGR3A	++	
FCGR3A + CD247	+	
FCGR3A + FCER1G	++	
FCGRT + B2M	+	
IGHG1	+++	Secondary antibody binding
IGHG2	+++	
IGHG3	+++	
IGHG4	+++	
PGC	+	Not confirmed via flow cytometry
SPNS2	+	Non-specific binding (observed for both BNT351 and rituximab)
CXCXL12	+	
IGF1	+	
MFSD2B	+	
SLC15A + SLC15B	+	
CD20	-	Rituximab positive control
EGFR	-	Negative control

+ weak ++ medium +++ strong

Figure 1. Off-target binding assessed by Retrogenix® cell microarray. Table shows interactions (mostly expected [FcγRs and IgGs] or non-specific) identified during the library screen with ~6,500 human proteins. Interaction with pepsinogen C (PGC) that appeared to be specific was re-tested by flow cytometry with HEK293 cells transfected with PGC.

Potent neutralization activity

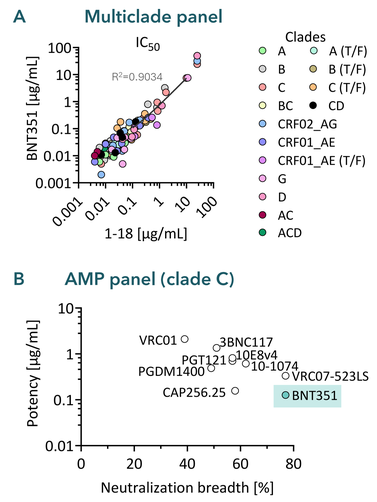


Figure 2. pVNT with TZM-bl reporter cells against (A) the multiclade panel (n=119 strains) and (B) an AMP panel of clade C (n=106 strains). For 1-18 and other bNAbs, historical data from CATNAP database are shown. Neutralization breadth was calculated using a threshold of IC_{50} < 1 µg/mL.

Extended half-life

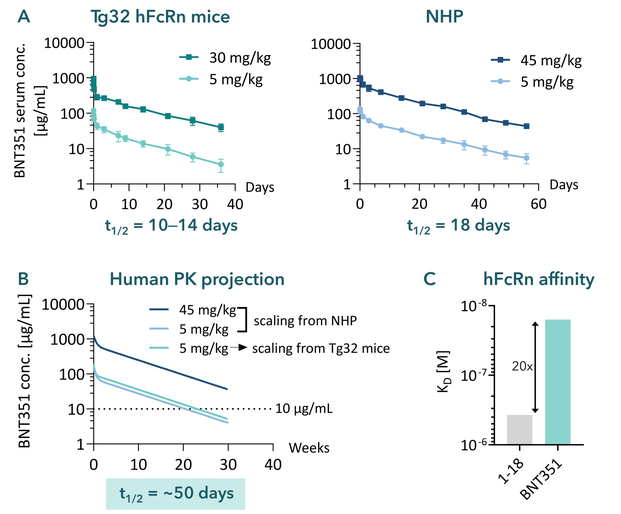


Figure 3. PK and FcRn affinity. (A) BNT351 serum concentration in Tg32 mice, which express hFcRn, (n=4) and in cynomolgus macaques (n=3) injected i.v. with indicated doses of BNT351 on Day 0. (B) Human PK projection using allometric scaling of PK parameters from Tg32 mice and NHP. (C) The binding affinity of BNT351 and 1-18 to hFcRn by SPR.

In vivo viremia suppression without escape variants

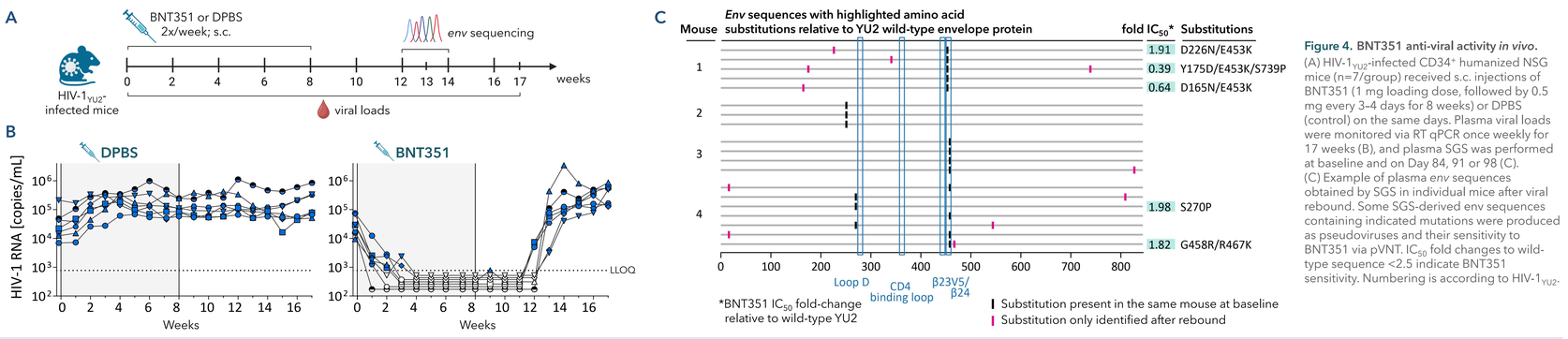


Figure 4. BNT351 anti-viral activity *in vivo*. (A) HIV-1_{YU2}-infected CD34+ humanized NSG mice (n=7/group) received s.c. injections of BNT351 (1 mg loading dose, followed by 0.5 mg every 3–4 days for 8 weeks) or DPBS (control) on the same days. Plasma viral loads were monitored via RT qPCR once weekly for 17 weeks (B), and plasma SGS was performed at baseline and on Day 84, 91 or 98 (C). (C) Example of plasma *env* sequences obtained by SGS in individual mice after viral rebound. Some SGS-derived env sequences containing indicated mutations were produced as pseudoviruses and their sensitivity to BNT351 via pVNT. IC_{50} fold changes to wild-type sequence <2.5 indicate BNT351 sensitivity. Numbering is according to HIV-1_{YU2}.

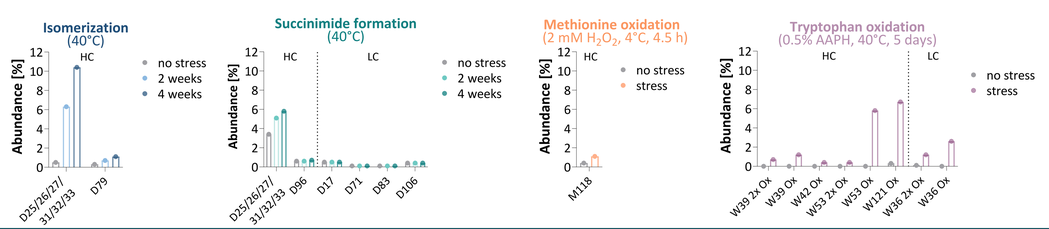
Low degradation under stress conditions

A

BNT351 light chain (LC)
EVVLTQSPAILSVSPGDRVILSCRASQGLDSSHLAWYRFKRGQIPTLVIFGTSNRARGTPDRFSGSGSG
ADFTLTISRVEPEDFATYYCQRYGGTPITFGGGTTLDKK
BNT351 heavy chain (HC)
QGRLFQSGAEVKRPGASVRISCRADDDPYTDDDTFTKYWTHWIRQAPGQRPEWLGVISPHFARPIYS
YKFRDRLTLTRDSSLTAVYLELKGLQPDDSGIYFCARDPFGDRAPHYNYHMDVWGGGTAVIVSS

Figure 5. Developability. BNT351 was exposed to indicated stress conditions, digested with trypsin, and the obtained peptides were analyzed with LC-MS/MS. (A) BNT351 amino acid sequence of BNT351 variable LC and HC domains with CDRs underlined. Residues analyzed for degradation are marked according to color scheme in B. (B) Abundance of degradation product at BNT351 residues indicated on x-axes. BNT351 sample not exposed to stress conditions was used as reference (no stress).

B



4. Conclusions

- LS mutations increased BNT351's hFcRn affinity, resulting in a long half-life, without affecting its antiviral activity
- No off-target binding → no cross-reactivity toxicities expected
- Low viral escape risk *in vivo*
- Favorable developability → suitable for large-scale production

Acknowledgements: Additional authors from the BNT351 Study Group: C-H. Tsai, C. Janaitis, C. Lindemann, S. Praßl, R. Stumpf, J. Knüfer, F. Tolksdorf, J. Nelke, A. Malz, S. Bhebhe, and N. Mkhize; A. Plaschke, A. Trapp, S. Krapp, and L. Fischer for assay development; R. Shetge, R. Wagner-Uhler from BioNTech CMC unit for overseeing antibody production; Biaffin GmbH & Co KG for SPR analyses; Charles River Laboratories for off-target profiling; Natural and Medical Sciences Institute (NMI, Germany) for stability studies; Evotec, Charles River Laboratories, and BioAgilytix for PK studies; the staff of the Animal Care Facility Weyertal at the University of Cologne for humanized mouse experiment. Illustrations were created with BioRender.com. This study was funded by BioNTech SE.

Author contact information:
Sven.Kratochvil@biontech.de
Valentin.LeDouce@biontech.de

Disclosures: S.K. and V.L. are employees at BioNTech group and may hold stock options. S.K. and V.L. are inventors on International Patent Application related to BNT351.

Abbreviations: AAPH = 2,2'-azobis(2-amidinopropane) dihydrochloride; AMP = antibody-mediated prevention; bNAb = broadly neutralizing antibody; CDR = complementarity-determining region; DPBS = Dulbecco's phosphate-buffered saline; env = envelope protein; (h)FcRn = (human) neonatal Fc receptor; HC = heavy chain; HIV = human immunodeficiency virus; IC50 = half maximal inhibitory concentration; IC80 = 80% inhibitory concentration; IgG = immunoglobulin G; LC = light chain; LC-MS = liquid chromatography-mass spectrometry; LLOQ = lower limit of quantification; LS = half-life-extending mutations; NHP = non-human primates; NSG = NOD SCID gamma; ox = oxidation; pVNT = pseudovirus neutralization test; PK = pharmacokinetics; RT qPCR = real-time quantitative polymerase chain reaction; s.c. = subcutaneous; SGS = single genome sequencing; SPR = surface plasmon resonance; TZM-bl = TZM-bl reporter cells.

References: 1. Schommers et al., 2020, Cell