RNA-encoded IL-7 and IL-2 with extended half-lives synergize to modulate T cell responses and enhance anti-tumor efficacy

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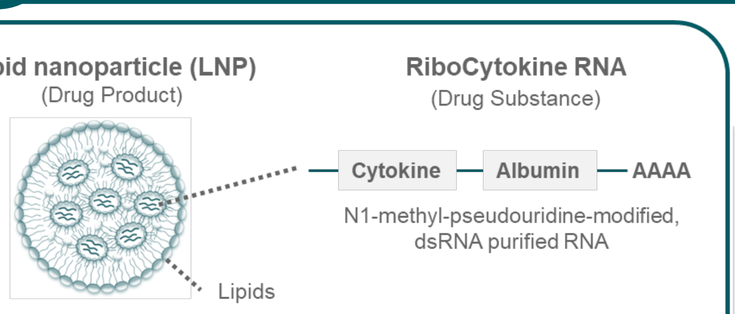
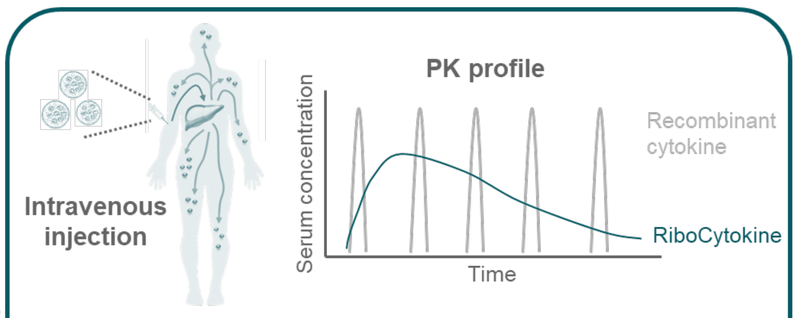
The Ribocytokines IL-2 (BNT153) and IL-7 (BNT152)



LNP
Protects RNA, facilitates intravenous injection, mediates delivery to hepatocytes

Nucleoside-modified mRNA
Non-immunogenic, high and prolonged translation of fusion protein

Albumin
Prolongs protein half-life, leads to accumulation in the tumor and lymph nodes



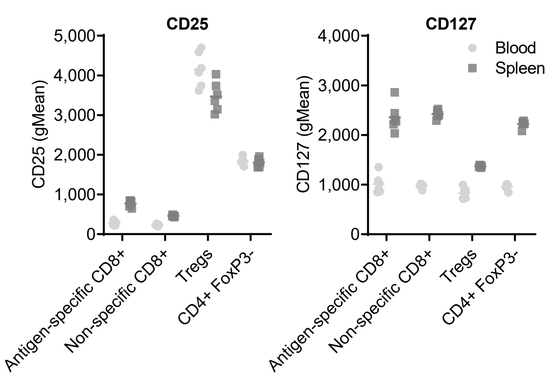
Sustained production of translated protein in the liver

Systemic availability of translated cytokine (detectable up to day 7)

Reduced application frequency required compared to recombinant protein

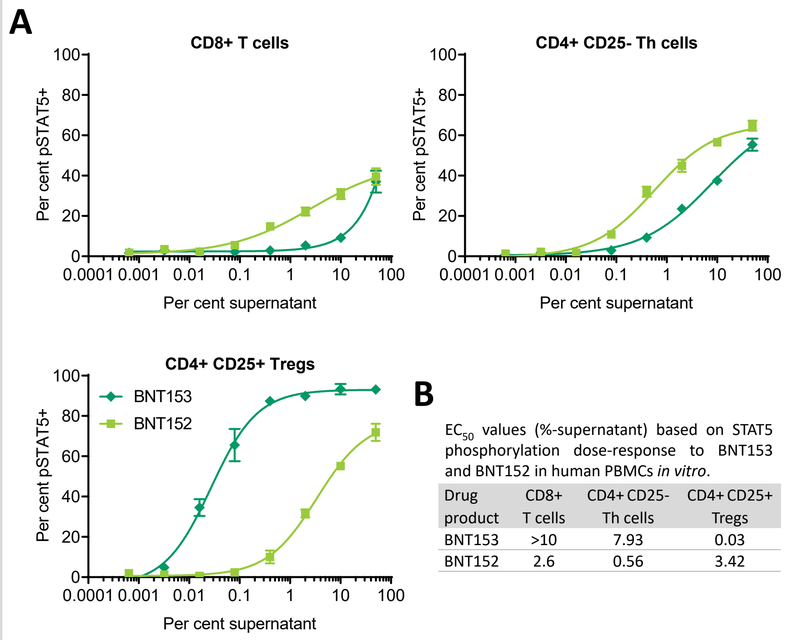
Improved safety profile by avoiding high peak doses compared to recombinant protein

CD25 and CD127 Expression on T Cell Subsets



CD25 and CD127 expression on T cell subsets *in vivo*. Strength of expression within receptor-positive subsets of CD25 (IL-2Rα) and CD127 (IL-7Rα) in splenic lymphocyte subsets seven days after vaccination of C57BL/6 (n=6) with OVA RNA-LPX vaccine. Mean±SEM.

in vitro and *in vivo* Characterization

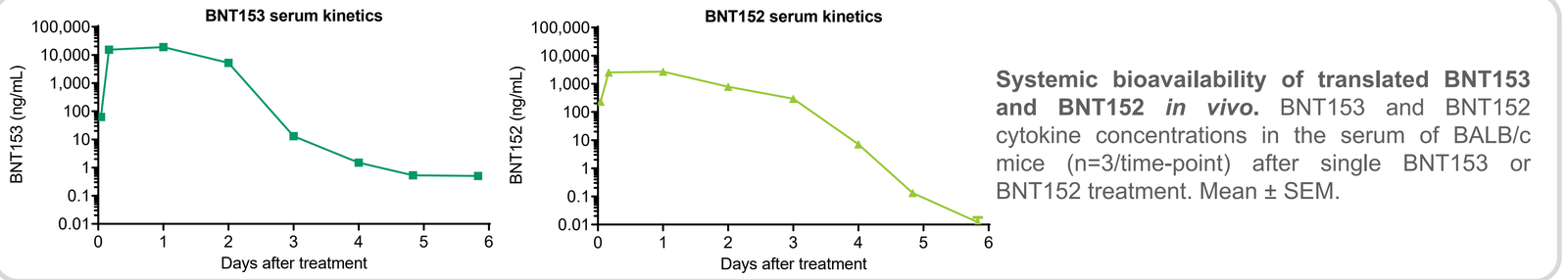


B

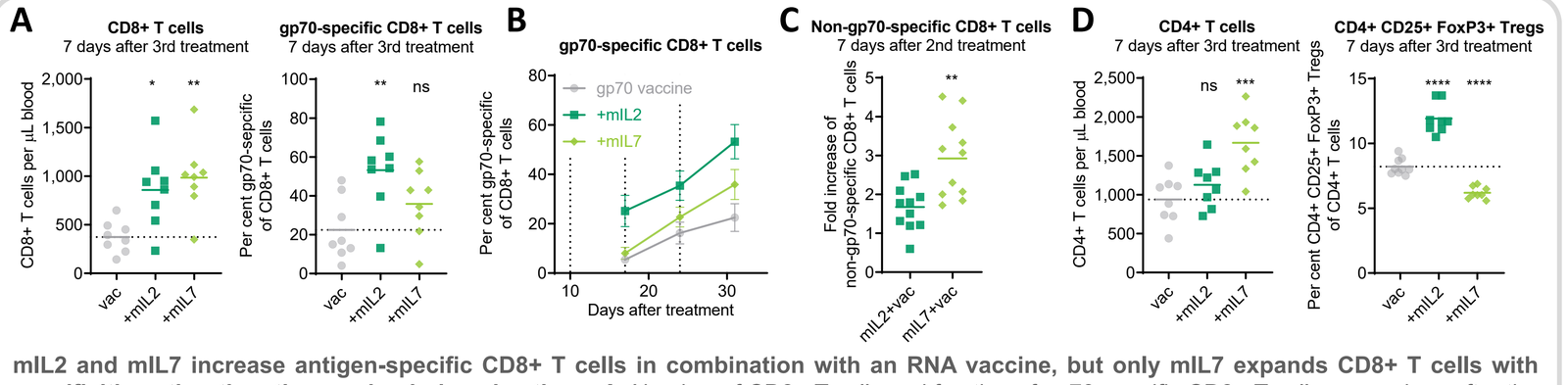
EC_{50} values (%-supernatant) based on STAT5 phosphorylation dose-response to BNT153 and BNT152 in human PBMCs *in vitro*.

Drug product	CD8+ T cells	CD4+ CD25- Th cells	CD4+ CD25+ Tregs
BNT153	>10	7.93	0.03
BNT152	2.6	0.56	3.42

Biological activity of BNT153 and BNT152 in human PBMCs measured by CD25 and CD127 activation mediated phosphorylation of STAT5. Serial dilution of supernatants containing translated BNT153 and BNT152 and phosphorylation of STAT5 in human PBMCs (n=2 technical replicates) **(A)**, and EC_{50} values calculated by four parameter logarithmic fit **(B)**. Mean±SD.

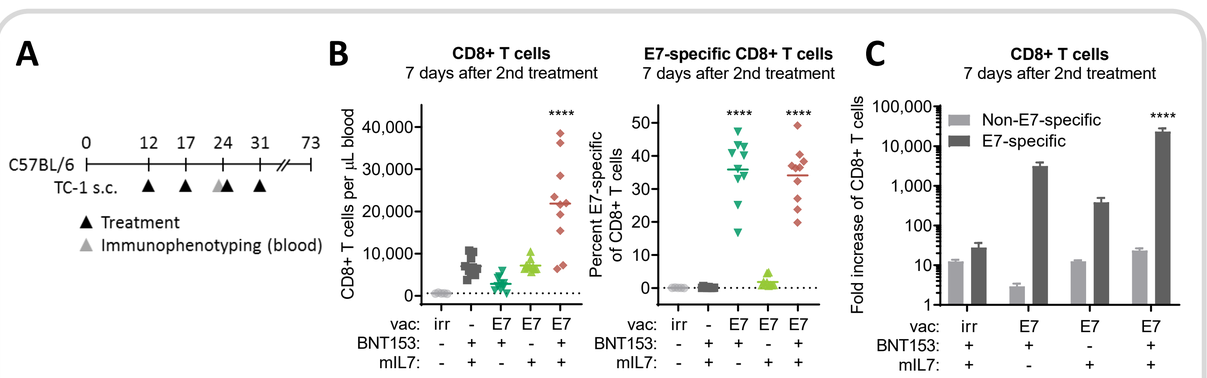


Systemic bioavailability of translated BNT153 and BNT152 *in vivo*. BNT153 and BNT152 cytokine concentrations in the serum of BALB/c mice (n=3/time-point) after single BNT153 or BNT152 treatment. Mean ± SEM.



mIL2 and mIL7 increase antigen-specific CD8+ T cells in combination with an RNA vaccine, but only mIL7 expands CD8+ T cells with specificities other than the vaccine-induced antigen. A, Number of CD8+ T cells and fraction of gp70-specific CD8+ T cells seven days after the third treatment or **B,** seven days after each treatment, and **C,** fold increase of individual cell numbers of non-gp70-specific CD8+ T cells in cytokine-treated groups over the median cell number of non-gp70-specific CD8+ T cells in the vaccine only group seven days after the second treatment of CT26 tumor bearing BALB/c mice (n=8-11/group) with mIL2 or mIL7, gp70 RNA-LPX vaccine and anti-PD-L1. **mIL2 strongly expands, while mIL7 reduces Tregs in combination with an RNA vaccine. D,** Number of CD4+ T cells and fraction of Tregs seven days after the third treatment of CT26 tumor bearing BALB/c mice (n=8/group) with mIL2 or mIL7, gp70 RNA-LPX vaccine anti-PD-L1. Horizontal dotted lines indicate mean of the vaccine only group. Vertical dotted lines indicate days of treatment. One-way ANOVA and Dunnett's multiple comparisons test (A, D), unpaired, two-tailed Student's t test (C).

BNT152 Boosts Therapeutic Antitumoral Activity of BNT153



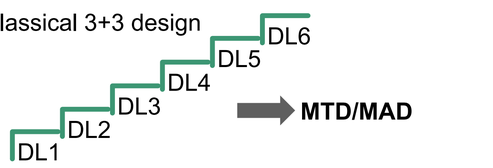
mIL7 expands CD8+ T cells, increases the antigen-specific CD8+ T cell to Treg ratio and enhances antitumoral activity of BNT153 in combination with an RNA vaccine in the TC-1 model. C57BL/6 mice (n=10-15/group) were inoculated s.c. into the right flank with TC-1 tumor cells and treated either with E7 RNA-LPX vaccine alone or in combination with BNT153, mIL7 or both. Groups receiving an irrelevant (irr; non-antigen coding) RNA-LPX vaccine with or without both cytokines served as controls. **A,** Experimental design. **B,** Number of CD8+ T cells and fraction of E7-specific CD8+ T cells. **C,** Fold increase of individual cell numbers of E7-specific and non-E7-specific CD8+ T cells over the median cell number of corresponding subsets in the irr vaccine only group. **D,** Number of CD4+ CD25+ FoxP3+ Tregs. **E,** E7-specific CD8+ T cell to Treg ratio based on cell numbers. **F,** Survival. Horizontal dotted lines indicate mean of the irrelevant vaccine only group.

BNT152-01C: BNT152+153 FIH Ph1 clinical trial in patients with solid tumors

Part 1: Parallel dose escalation of BNT152 and BNT153

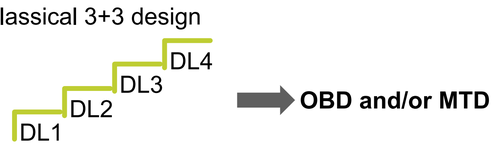
Group A: BNT153 monotherapy dose escalation

Classical 3+3 design



Group B: BNT152 monotherapy dose escalation*

Classical 3+3 design



*Enrollment in group B strats after activation of group A at sponsor's decision

For both groups, DLT observation period: 1 cycle = 21 days

Part 2: Dose escalation of BNT152 and BNT153 in combination

Classical 3+3 design

DL	BNT152 dose	BNT153 dose
1	OBD	MTD-2/MAD-2
2	OBD	MTD-1/MAD-1

MTD: Highest tolerated dose where less than 1 of 3 patients experiences a DLT
MAD: Highest dose administered if MTD not reached
OBD: Lowest safe dose associated with biological efficacy
RP2D: Recommended Ph2 dose for the BNT152+153 combination based on safety, tolerability, clinical benefit, PJ, and selected pharmacodynamics markers, for all DLs tested

BNT152-01C First-in-human dose escalation trial design. The Phase 1 trial is currently ongoing and the combination dose finding is expected to start in 2022. NCT04710043. This trial is funded by BioNTech SE. DL, Dose Level; DLT, Dose limiting toxicities.

The combination of BNT153 and BNT152 boosted the number of antigen-specific CD8+ T cells beyond the levels of BNT153 alone and further improved the ratio of antigen-specific CD8+ T cells to Tregs, translating into superior antitumoral activity. Based on the complementary preclinical mechanism of action, clinical evaluation is pursued in an open-label, phase 1 first-in-human trial.

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