# An RNA-encoded IL-2 variant with an extended half-life mediates synergistic anti-tumor activity when combined with immune checkpoint blockade

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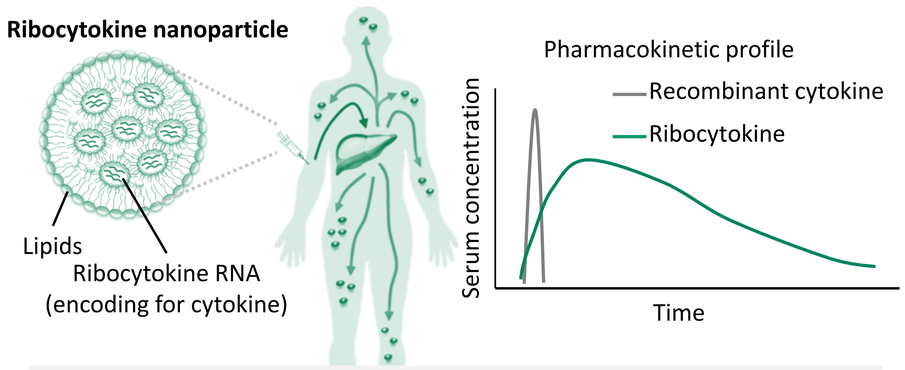
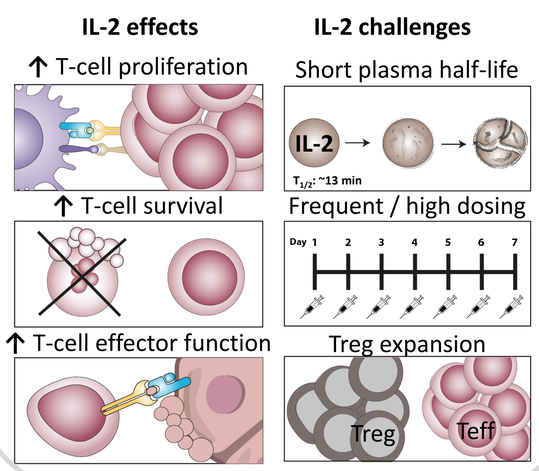
## Introduction: The RiboCytokine IL-2 Variant BNT151

### BioNTech's RiboCytokine® Platform

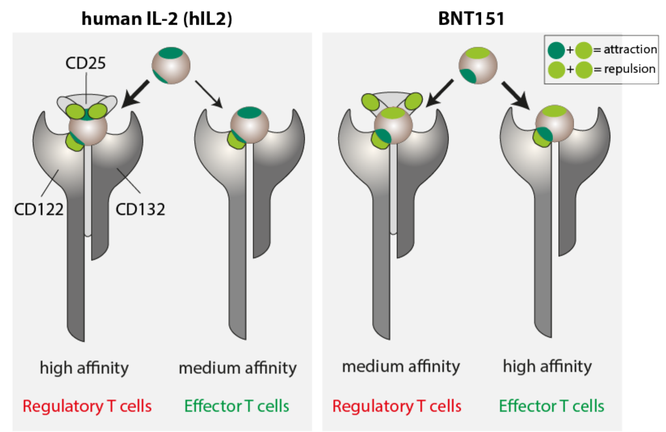



- Cytokine encoding RNA modified for increased and prolonged translation
- Protein sequence optimization for extended cytokine half-life
- i.v. application → translation in liver → systemic availability of cytokine

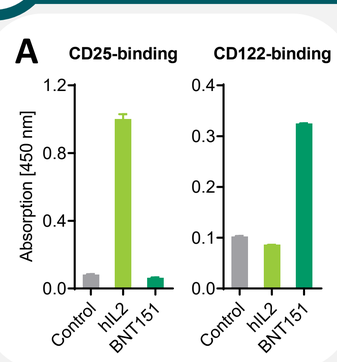
### IL-2 Effects and Challenges

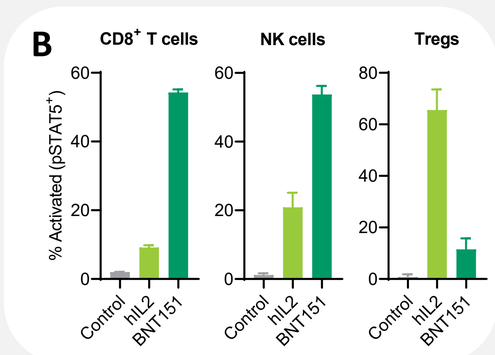



### BNT151 Design

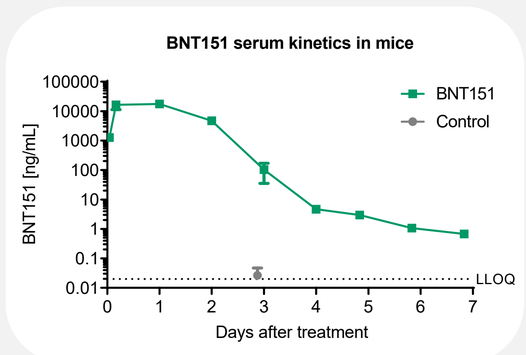



## *In vitro* and *in vivo* Characterization

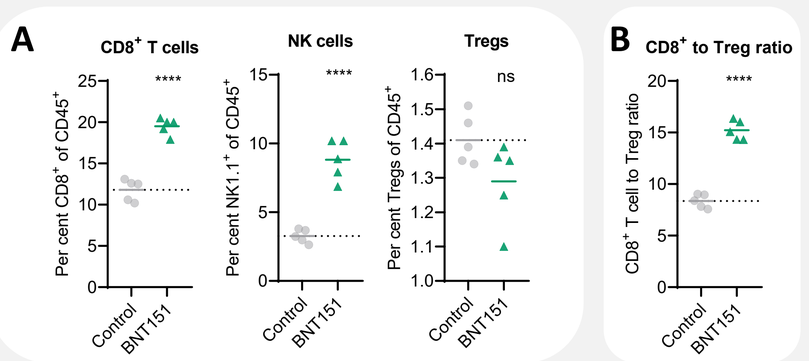



**BNT151 demonstrates reduced CD25 and improved CD122 specificity resulting in increased T and NK cell activation while reducing Treg stimulation. (A)** Human CD25- and CD122-binding of hIL2 and BNT151 measured by ELISA. Mean ± SD of two technical replicates shown. **(B)** Activity of hIL2 and BNT151 on immune cell subsets in human PBMC measured by STAT5 phosphorylation.

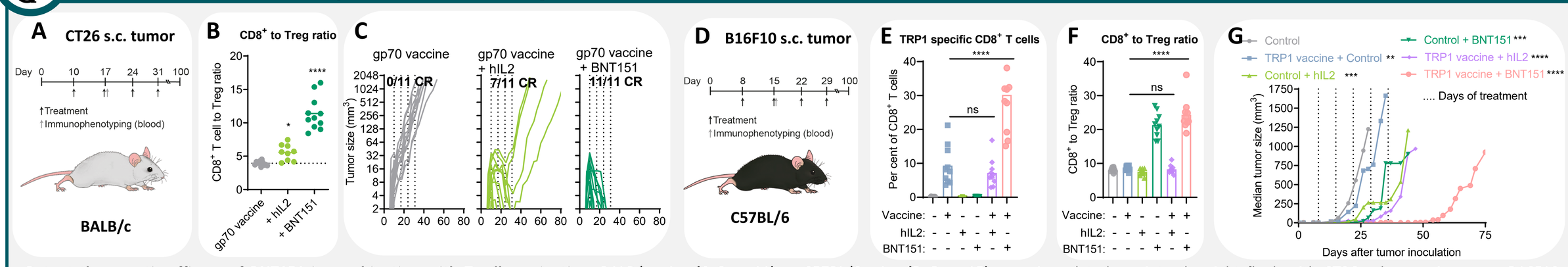



**Systemic bioavailability of translated BNT151 *in vivo*.** BNT151 cytokine concentrations in serum of BALB/c mice (n=3/time-point) after single BNT151 treatment. Mean ± SD is shown.



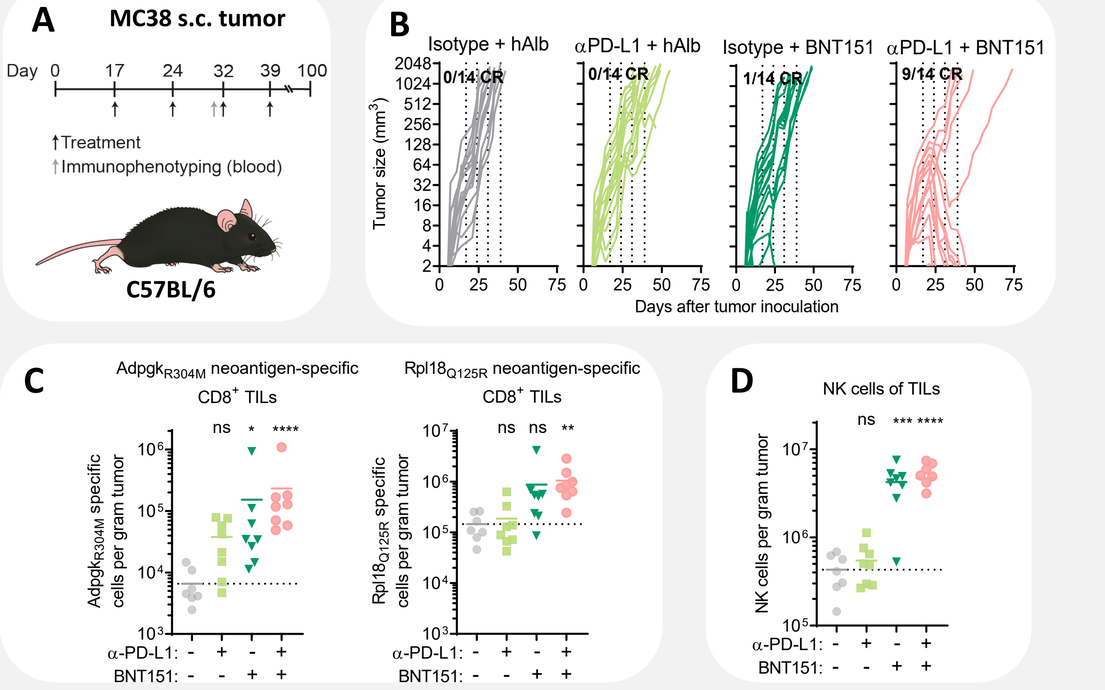

**BNT151 mediates potent increase of the effector to Treg ratio.** Flow cytometry analysis of blood from C57BL/6 mice (n=5) seven days after BNT151 treatment. **(A)** Frequencies of CD8+ T cells, NK cells and CD25+ Foxp3+ CD4+ T cells (Tregs) within CD45+ cells. **(B)** CD8+ T cell to Treg ratio. Mean and individual values are shown. Horizontal dotted lines indicate control levels.

## Therapeutic Activity of BNT151 in Combination with T-cell Vaccination



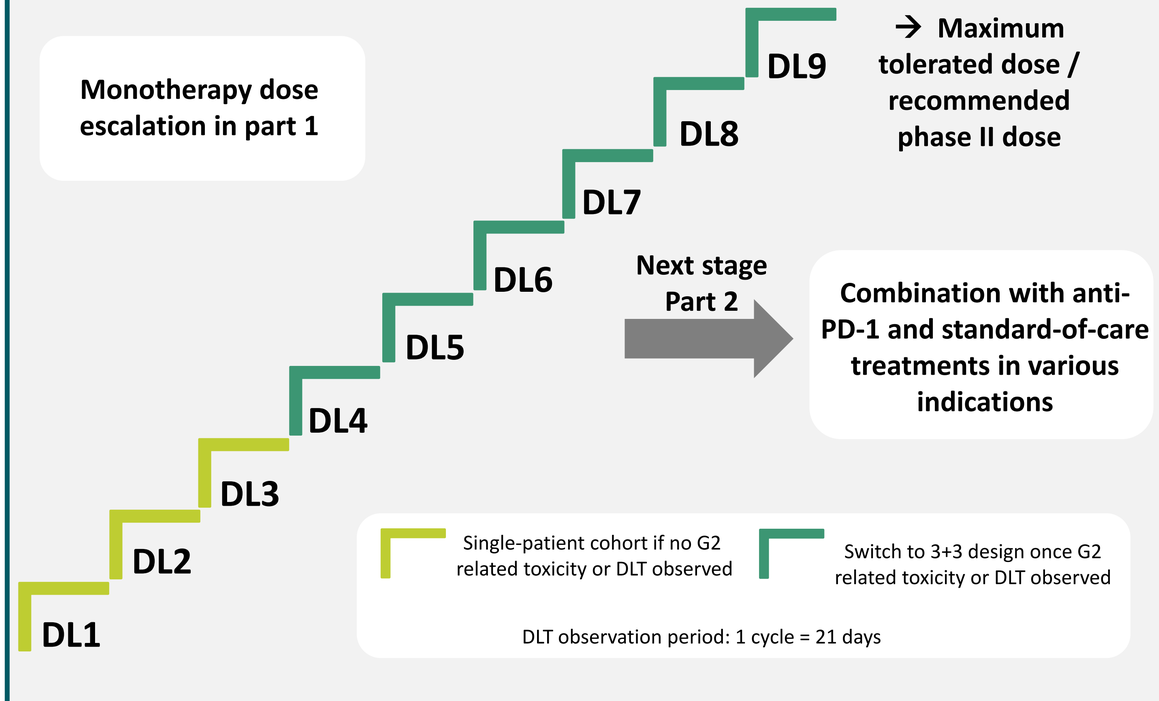

**Potent therapeutic efficacy of BNT151 in combination with T-cell vaccination.** BALB/c mice **(A-C,** n=11**)** or C57BL/6 mice **(D-G,** n=15**)** were inoculated s.c. into the right flank with CT26 colon carcinoma or B16F10 melanoma tumors, respectively. Mice were treated either with RNA-lipoplex T-cell vaccines alone or in combination with hIL2 Ribocytokine or BNT151 as depicted in **A** and **D.** The CD8+ to Treg ratios **(B)** were analyzed in blood 17 days after tumor inoculation. Mean and individual values are shown. Horizontal dotted lines indicate control levels. **(C)** Tumor growth curves and fractions of complete responses (CR). Dashed vertical lines indicate time point of treatment. **(E)** TRP1 (shared differentiation tumor antigen) specific CD8+ T-cell expansion as measured by flow cytometry in blood on day 15. **(F)** CD8+ T cell to Treg ratio in blood on day 15**. (G)** Median tumor growth curves. Vertical dotted lines highlight days of treatment. Asterisks highlight the significance level in comparison to the control group as measured by two-way ANOVA and Dunnett's test.

## BNT151 + PD-L1 Checkpoint Blockade




**Synergistic anti-tumor efficacy of BNT151 and anti-PD-L1 checkpoint blockade in an advanced mouse tumor model.** MC38 tumor bearing C57BL/6 mice (n=14) were treated with anti-PD-L1, BNT151 or the combination of both as indicated in **A**. **(B)** Tumor growth curves and fractions of complete responses (CR). Dashed lines indicate time point of treatment. **(C-D)** Quantification of Adpgk$_{R304M}$ and Rpl18$_{Q125R}$ neoantigen-specific CD8+ T cells **(C)** and NK cells **(D)** within tumor infiltrating lymphocytes (TILs) five days after BNT151 and anti-PD-L1 treatment (n=8). Statistical significance was determined by Kruskal-Wallis and Dunn's test **(C)** or by two-way ANOVA and Dunnett's test **(D)**.

## Phase I/II, first-in-human trial (BNT151-01)




**Trial design of BNT151-01 (NCT04455620).** Part 1 of the ongoing phase I/II, first-in-human trial BNT151-01 (NCT04455620) investigating clinical safety and efficacy of BNT151 as monotherapy. Included are patients with solid tumor, either metastatic (stage IV) or unresectable with no available standard therapy likely to confer clinical benefit. Part 2 of the trial will evaluate the safety and efficacy of BNT151 in combination with other anti-cancer agents such as anti-PD-1 checkpoint blockade (not shown). DLT, Dose limiting toxicities.

**Conclusions:** The RNA encoded, nanoparticle formulated IL-2 variant BNT151 mediates substantial anti-tumor efficacy in monotherapy and in combination with T-cell vaccination or anti-PD-L1 checkpoint blockade. An open-label, Phase I/II, first-in-human trial in patients with advanced solid tumors was initiated to further explore the clinical safety and efficacy of BNT151 as monotherapy or in combination with other anti-cancer agents (NCT04455620)