Inducing Anti-Tumor T Cell Response with INT-1B3, a Synthetic miR-193a-3p Mimic

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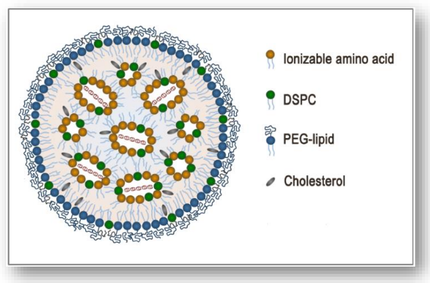
ABSTRACT

MicroRNAs are a family of non-coding RNAs that exert biological effects by concurrently regulating the expression of multiple target genes, leading to attractive potential for oncology therapeutic intervention. We are developing a novel synthetic miRNA-193a-3p mimic (1B3) which targets key processes involved in cancer initiation and progression, including immune surveillance. Systemic administrations of LNP-formulated 1B3 (INT-1B3) demonstrated a marked anti-tumor activity in several experimental tumor models. The impact of 1B3 on immuno-oncology parameters was explored in vitro, showing an increased vulnerability of 1B3-transfected tumor cells to human PBMC cytotoxicity. Furthermore, in the mouse 4T1 orthotopic breast cancer model, tumor immune cell profiling of INT-1B3-treated animals revealed a transition from immunosuppressive to immunostimulatory tumor microenvironment, illustrated by a time-dependent effect on Teff/Treg ratio. Consistent with these results, distant metastasis development was inhibited in the INT-1B3-treated group and surviving animals were fully protected against 4T1-cell challenge. Interestingly, T-cell depletion of INT-1B3-treated animals was shown to eliminate such a protection, and in parallel adoptive T-cell transfer study demonstrated protection of naïve animals against challenge with 4T1-tumor cells. Taken together, these results indicate a strong immuno-oncology potential for INT-1B3 as monotherapy with involvement of long-term T-cell mediated immune response memory against tumor antigens.

INT-1B3

Synthetic double stranded chemically-modified miR-193a-3p mimic

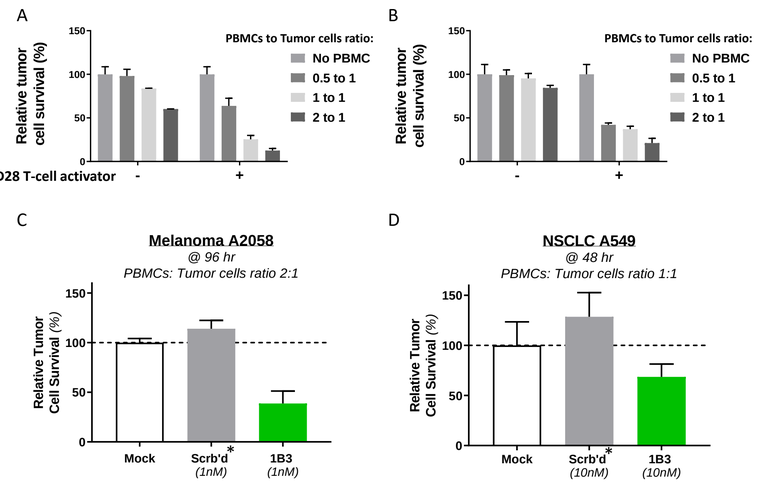


INT-1B3 contains a XL amino (cationic)-lipid, a helper lipid, a PEG lipid, and a hydrophobic small molecule

3′- **TTUUGACCGGAUGUUUCAGGGU** -5′ *Sense (passenger)*

5′- **AACUGGCCUACAAAGUCCCAGU** -3′ *Antisense (guide)*

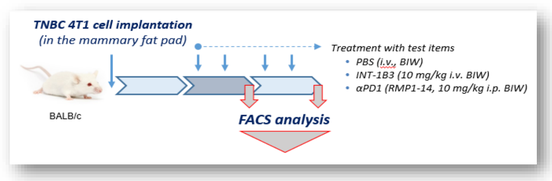
1B3 – *IN VITRO*

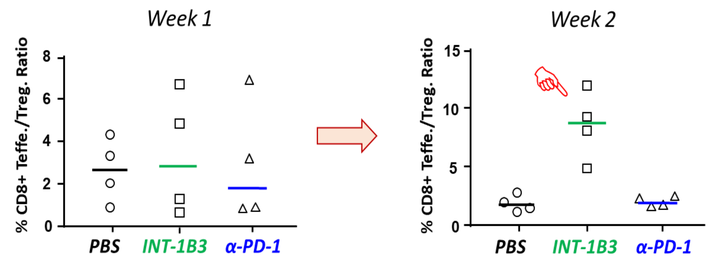


A and B: *Effect of human Peripheral Blood Mononuclear Cells (hPBMCs) on (A) human melanoma A2058 and (B) NSCLC A549 tumor cells* ***C and D:*** *Effect of hPBMCs on A2058 (C) and A549 (D) tumor cells upon tumor cell transfection with 1B3.*

**"Scrb'd" is the mirVana™ negative control #1, a miRNA mimic with a scrambled random sequence designed by Ambion for use as a negative control for experiments with miRNAs.*

INT-1B3 – MODULATION OF TUMOR MICROENVIRONMENT COMPONENTS

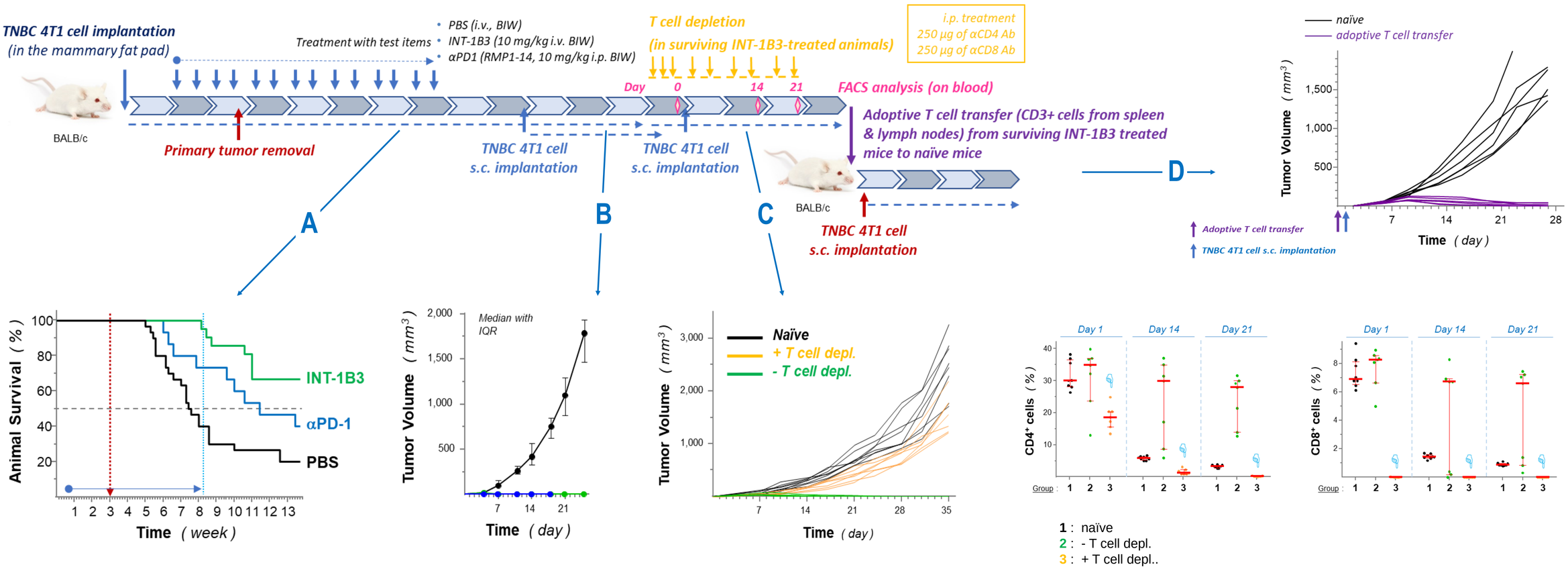




- ✓ *Increased IL-2 and IFN-γ expression*
- ✓ *Increased CD8⁺ CTLs as well as NK(T)-cell*
- ✓ *Increased expression of Granzyme B*
- ✓ *Decreased expression of immunosuppressive regulatory T-cells or Tregs (CD3⁺/FOXP3⁺)*
- ✓ *Decreased expression of immunosuppressive CD4⁺/LAG3⁺ and monocytic MDSC (CD11b⁺ GR1⁺ᵈⁱᵐ) markers*

INT-1B3 causes a transition from immune-suppressive to immune-stimulatory tumor microenvironment

INT-1B3 - ANIMAL SURVIVAL, LONG-TERM AND TRANSFERABLE T-CELL MEDIATED IMMUNE RESPONSE



A: *Pronounced increase in animal survival (inhibition of metastasis formation) in INT-1B3-treated group*

B: *Complete protection of surviving INT-1B3-treated animals against challenge with 4T1 cells*

C: *Protection against 4T1 cell challenge was markedly hampered upon in vivo CD4+/CD8+ T-cell depletion*

- FACS analysis shows a Time-dependent effective T-cell depletion (complete CD8+ and time-dependent CD4+ cell depletion from Day 1)

D: *Adoptive T-cell transfer from surviving tumor-bearing INT-1B3-treated animals to naïve animals markedly protects these animals against further homologous tumor cell challenge*

CONCLUDING REMARKS

Consistent with the target engagement profile, and in addition to the pronounced effect on tumor cell survival/cycle, apoptosis/senescence, and migration/invasion (data not shown), we demonstrated that

- ➢ 1B3 increases vulnerability of transfected tumor cells to human PBMC-mediated cytotoxicity *in vitro*.
- ➢ upon systemic treatment of TNBC 4T1 tumor-bearing syngeneic mice, INT-1B3 is able to…
  - ✓ modulate the immune tumor microenvironment turning 'cold' (immuno-suppressed) tumors into 'hot' (immuno-stimulated) tumors via, e.g. …
    - *attracting immune cells by upregulation of relevant cytokines (e.g., IL-2 and IFN-γ)*
    - *decreasing the immunosuppressive cells (e.g., CD4⁺/LAG3⁺ and CD3⁺/FOXP3⁺)*
  - ✓ trigger a long-term and transferable T-cell mediated immune response against challenge with autologous 4T1 cells (and HCC H22 cells, *data not shown*)

We conclude that treatment with INT-1B3 represents a novel and promising therapeutic approach in the immuno-oncology armamentarium (as monotherapy, and/or in combination with immuno-checkpoint inhibitors)