

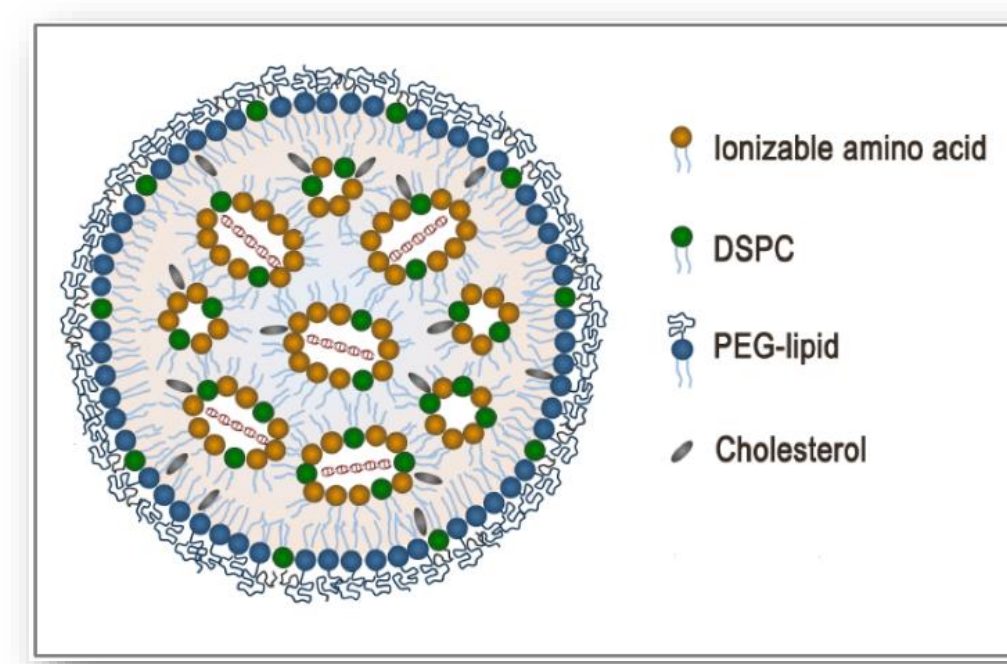
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ABSTRACT

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 T_H1/T_H2 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

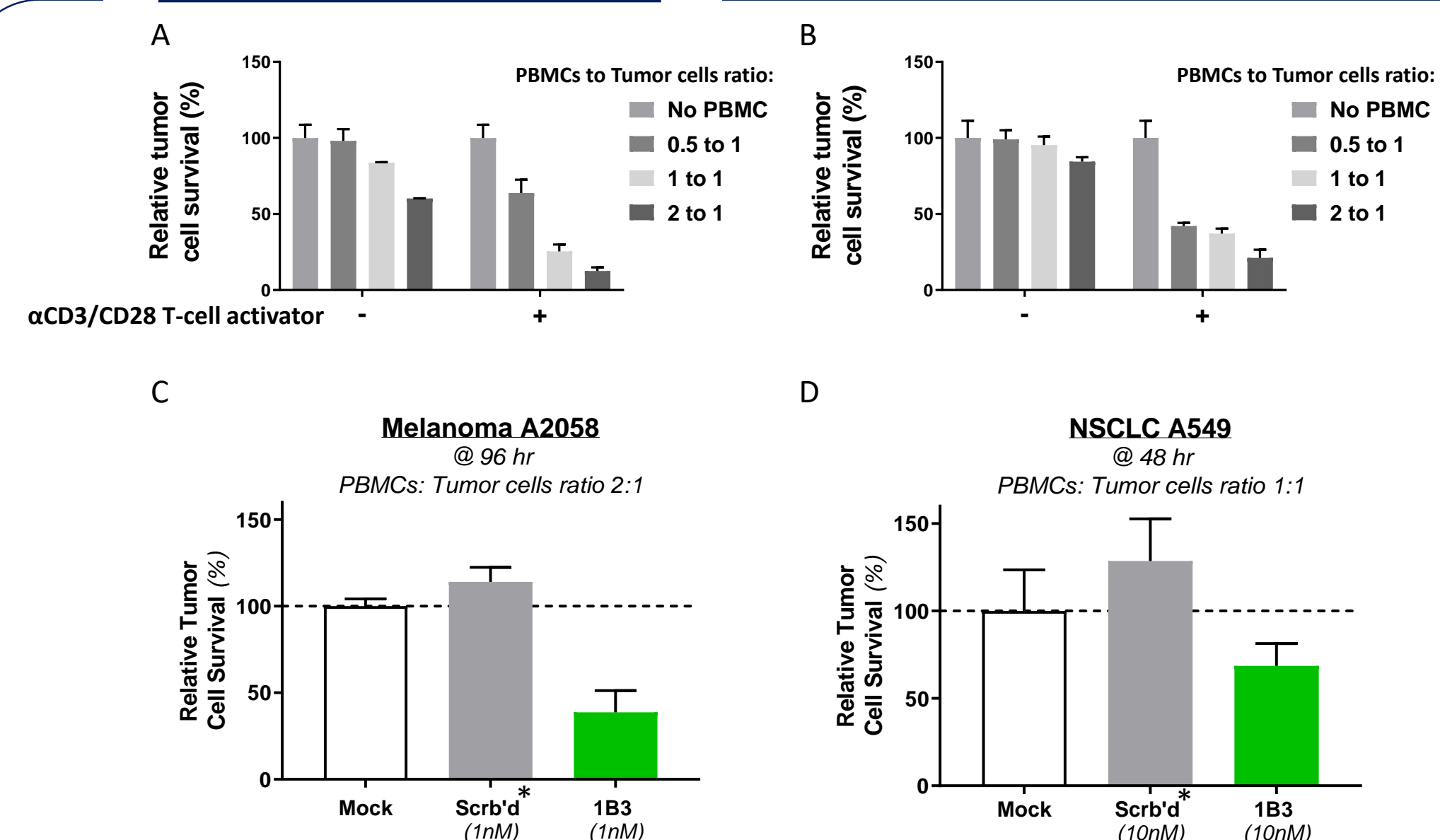
Synthetic double stranded chemically-modified miR-193a-3p mimic



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' - **AACUGGCCUACAAAGUCCAGU** -3' Antisense (guide)

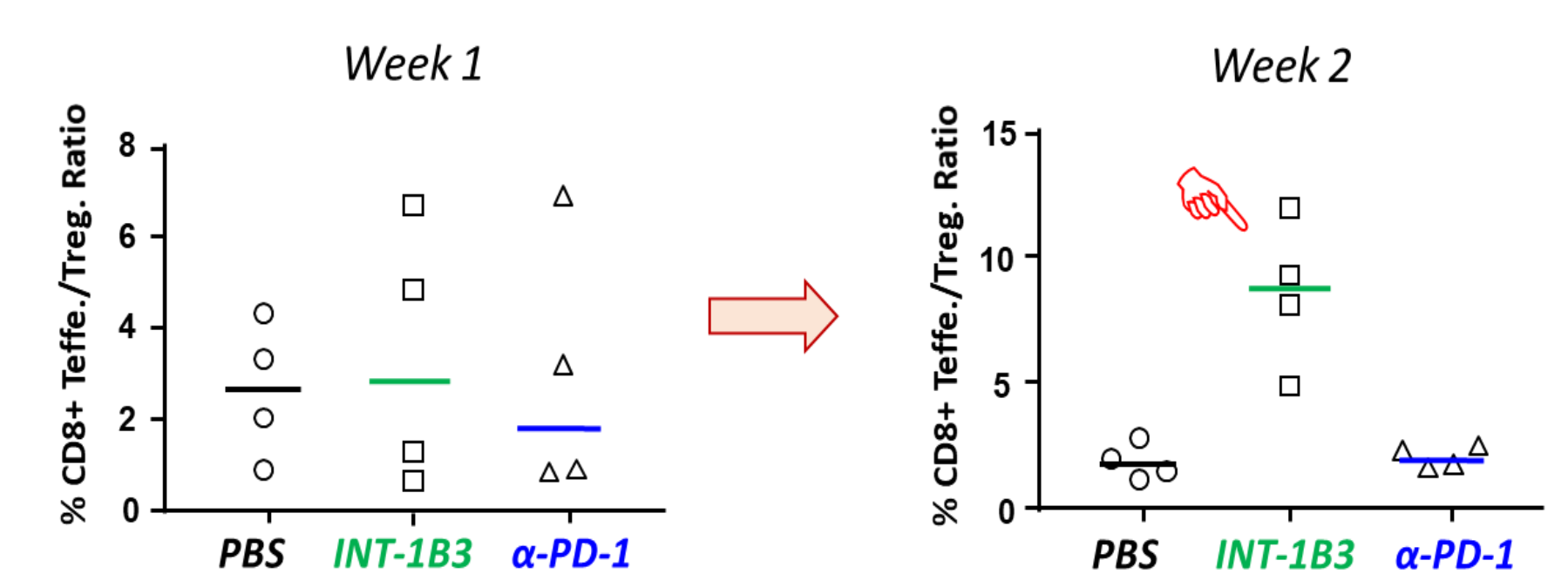
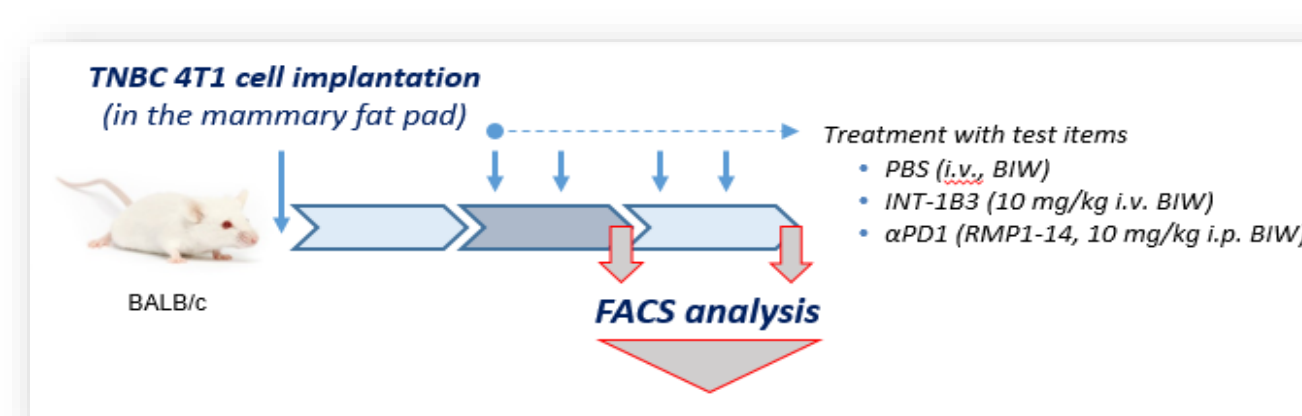
1B3 - IN VITRO



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.

*"Scrbd'" 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.

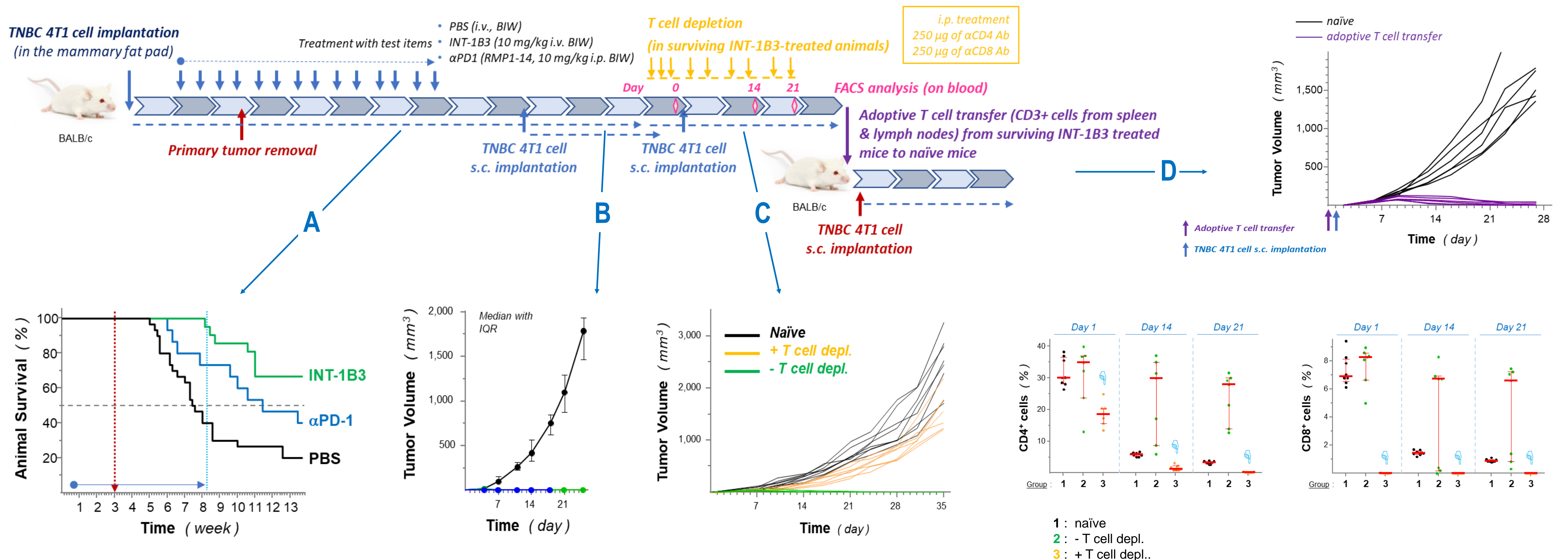
INT-1B3 - MODULATION OF TUMOR MICROENVIRONMENT COMPONENTS



- ✓ 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)