Direct and indirect anti-leukemic properties of activity-on-target interferons for the treatment of T-cell acute lymphoblastic leukemia

Steven Goossens,[1,2,3,*] Anje Cauwels,[1,3,4,5,*] Tim Pieters,[1,3] Renate De Smedt,[1,3] Sara T'Sas,[1,3] André Almeida,[1,3] Willem Daneels,[1,6] Pieter Van Vlierberghe[1,3,#] and Jan Tavernier[1,3,4,5,#]

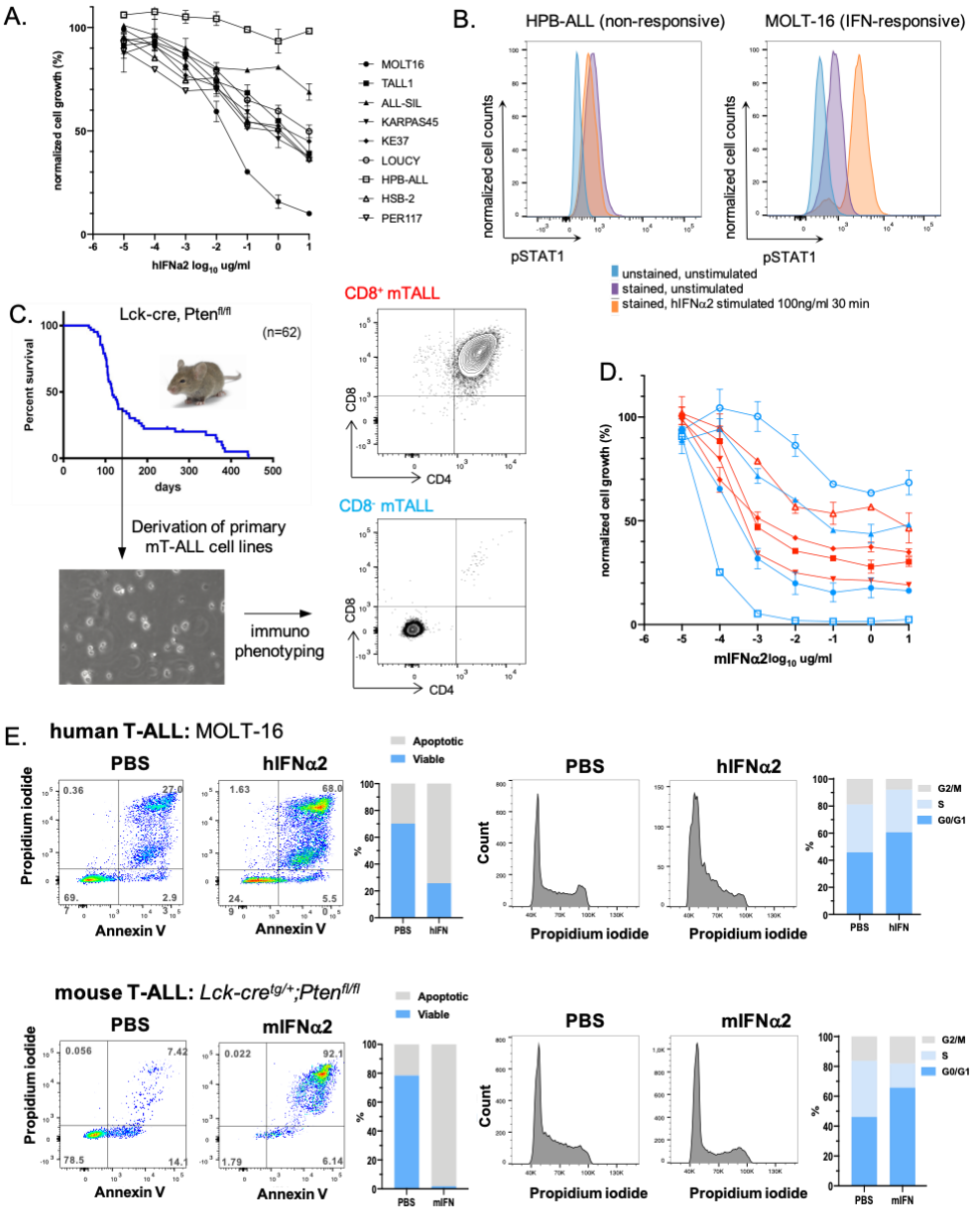
[1]Cancer Research Institute Ghent (CRIG), Ghent University; [2]Department of Diagnostic Sciences, Ghent University; [3]Department of Biomolecular Medicine, Ghent University; [4]VIB-UGent Center for Medical Biotechnology; [5]Orionis Biosciences BV and [6]Department of Hematology, Ghent University Hospital, Ghent, Belgium
**SG and AC contributed equally as co-first authors.*
[#]PVV and JT contributed equally as co-last authors.

Correspondence:
PIETER VAN VLIERBERGHE - pieter.vanvlierberghe@ugent.be
https://doi.org/10.3324/haematol.2021.278913

Direct and indirect anti-leukemic properties of Activity-on-Target interferons for the treatment of T-ALL

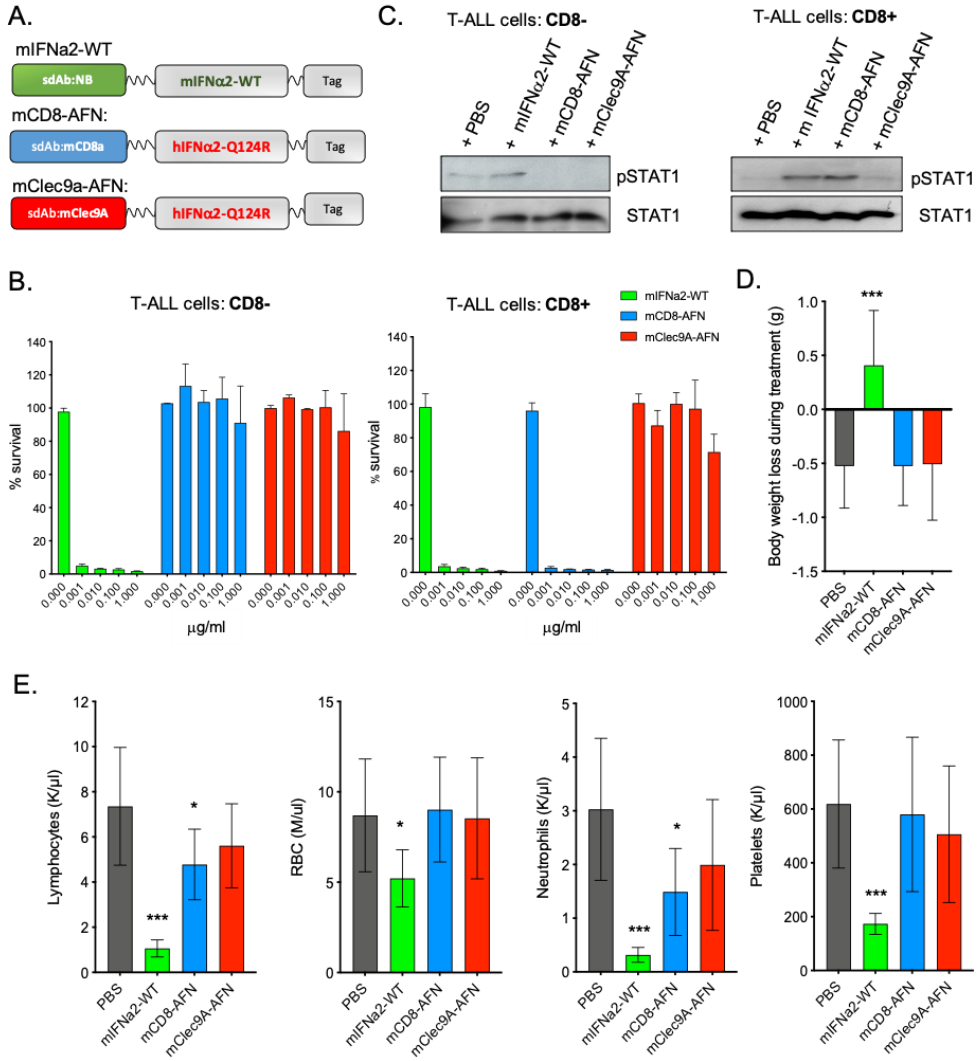
Steven Goossens[1,2,3,*], Anje Cauwels[1,3,4,5*], Tim Pieters[1,3], Renate De Smedt[1,3], Sara T'Sas[1,3], André Almeida[1,3], Willem Daneels[1,6], Pieter Van Vlierberghe[1,3,§] and Jan Tavernier[1,3,4,5,§]

Supplemental Figure S1:



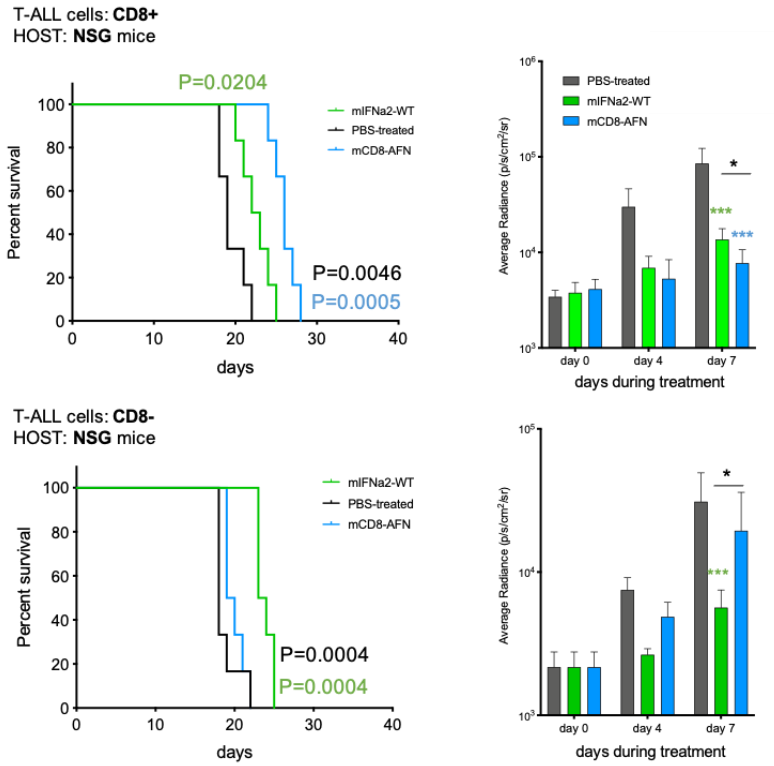
Supplemental Figure S1: Direct anti-leukemic effects of interferon alpha-2 on the progression of T-ALL *in vitro*. (A) Effect of increasing concentration of hIFNα2 administration on the *in vitro* growth of 9 human T-ALL cell lines using the CellTiter-Glo Luminescent Cell Viability Assay. Results were normalized against the non-treated control. **(B)** Flow cytometric analysis of phospho-STAT1 (pSTAT1) levels after *in vitro* stimulation with 100 ng/ml hIFNα2 of a responsive (MOLT-16) and non-responsive (HPB-ALL) T-ALL cell line. **(C)** Derivation of 8 primary murine T-ALL cell lines and immunophenotypic characterization via flow cytometric analysis, including four CD8$^+$ (red) and four CD8$^-$ (blue) cell lines. FACS immune profiling of a representative Thy1$^+$ (pre-gated) CD4$^+$CD8$^+$ and a Thy1$^+$ (pre-gated) CD4$^-$CD8$^-$ murine T-ALL primary cell lines are shown **(D)** Effect of increasing concentrations of recombinant mIFNα2 administration on the *in vitro* growth of 8 mouse T-ALL cell lines, including four CD8$^+$ (red) and four CD8$^-$ (blue) cell lines, using the CellTiter-Glo Luminescent Cell Viability Assay. Results were normalized against the non-treated control. Experiments were performed twice, in duplicate. **(E)** Increase in percentage of Propidium Iodide$^+$/AnnexinV$^+$ apoptotic cells and mild increase in live G0/G1 cells are seen in MOLT-16 cultures treated for 72 hours with 1µg/ml hIFNα2 and in primary cultures of *Lck-cre$^{tg/+}$;Pten$^{fl/fl}$* mouse T-ALL treated for 48 hours with 100 ng/ml mIFNα2.

Supplemental Figure S2:



Supplemental Figure S2: Generation of cell-type specific Activity-on-Target interferons, AcTaferons (AFN), with improved therapeutic index compared to wild type IFNα2. (A) Overview of immunocytokines that are generated and used in this study; the wild type mIFNα2 was fused to a non-binding (NB) single-domain antibody (sdAb) targeting Bcl10, an epitope that is absent in the mouse (mIFNα2-WT); the mutant hIFNα2^{Q124R} was fused to sdAb targeting the murine CD8a (mCD8-AFN) or the murine Clec9A (mClec9A-AFN). **(B)** Direct *in vitro* anti-leukemic properties of mCD8-AFN and mClec9A-AFN versus mIFNα2-WT. Inhibitory effect of AFNs on growth of CD8$^-$ and CD8$^+$ mouse T-ALL cell lines, normalized against the non-treated control. Experiment was performed twice, in duplicate. **(C)** Immunoblot analysis of phospho-STAT1 (pSTAT1) and total STAT1 levels in murine CD8$^-$ and CD8$^+$ T-ALL cell lines before and after 30 min stimulation with 100 ng/ml mIFNα2-WT, mCD8-AFN or mClec9A-AFN. **(D)** body weight loss and **(E)** automated blood analysis (Hemavet) after 7-day treatment regime with either mIFNα2-WT, mCD8-AFN, or mClec9A-AFN in non-leukemic C57BL/6 mice.

Supplemental Figure S3:



Supplemental Figure S3: mCD8-AFN is more efficient as compared to wild type mIFNα2 for the direct treatment of murine T-ALL. Direct anti-leukemic effect of mCD8-AFN in comparison to mIFNα2-WT treatment (7 consecutive days: 30μg/mouse) versus vehicle control on progression of $CD8^-$ and $CD8^+$ murine T-ALL cell lines transplanted in immunodeficient NSG mice. Leukemic burden was quantified via *in vivo* bioluminescence imaging during 7-day treatment regime (right) and Kaplan-Meier survival curve (left). Statistical analysis is included for mCD8-AFN treated versus control group (blue), mIFNα2 versus control (green) and mCD8-AFN versus mIFNα2 treated groups (black)