Teaching an old dog new tricks: Activity-on-Target Interferon to treat T-cell acute lymphoblastic leukemia

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INTRODUCTION

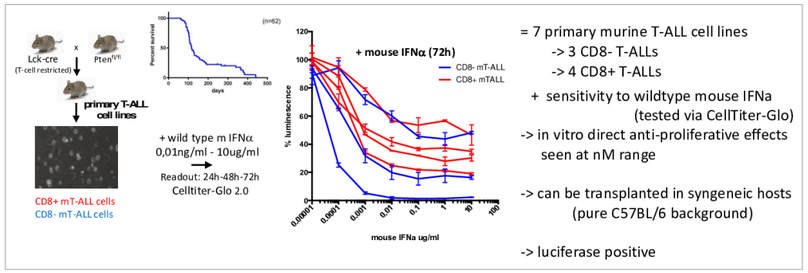
Type 1 interferon (IFN) has a long history in the treatment of cancer, including hematological malignancies. The anti-cancer effects induced by IFN result from a combination of **1) direct cancer cell growth inhibition** by cell cycle arrest, apoptosis, or differentiation and **2) the activation of the immune system** involving antigen presentation by Clec9A+ dendritic cells and priming of cytotoxic CD8+ T-cells.
However, IFN therapy experienced variable and unpredictable success in the clinic. Its clinical application is severely impeded by a complex pattern of **adverse side-effects**, due to the multifaceted activity pattern of IFN.
Therefore, safe exploitation of the anti-cancer potential of IFN requires strategies to direct their activity to selected target cells, avoiding systemic toxicity.

OBJECTIVE AND METHODS

Safe exploitation of the anti-cancer potential of IFN requires strategies to direct their activity to selected target cells, avoiding systemic toxicity.

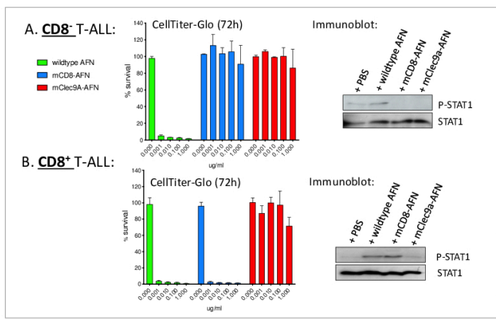
To improve the therapeutic index of IFN, we have developed **AcTaferons (Activity-on-Target Interferon), optimized (mutant) immunocytokines**. Mutated IFNa2Q124R, with a strongly reduced affinity for its receptor complex, was fused to single domain antibodies targeting cell-specific domains, which selectively restores the AcTaferon (AFN) activity in a cell-type specific manner. As such, mCD8-AFN and mClec9A-AFN were generated which selectively target either mouse CD8+ T(-ALL) cells or Clec9A+ dendritic cells.

To test the anti-leukemic properties of these AcTaferons we generated syngeneic T-ALL cell llines:



= 7 primary murine T-ALL cell lines
-> 3 CD8- T-ALLs
-> 4 CD8+ T-ALLs
+ sensitivity to wildtype mouse IFNa (tested via CellTiter-Glo)
-> in vitro direct anti-proliferative effects seen at nM range
-> can be transplanted in syngeneic hosts (pure C57BL/6 background)
-> luciferase positive

The specificity of the Actaferons was evaluated in vitro:



-> wildtype IFNa: direct effect confirmed on CD8- and CD8+ T-ALLs

-> mCD8-AFN: only direct in vitro effects seen on CD8+ and not on CD8- T-ALLs

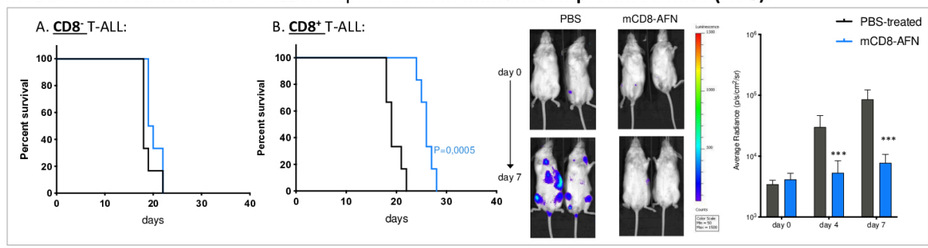
-> mClec9A-AFN: no direct in vitro effects seen

= associated with activation of dowstream JAK/STAT1 pathway

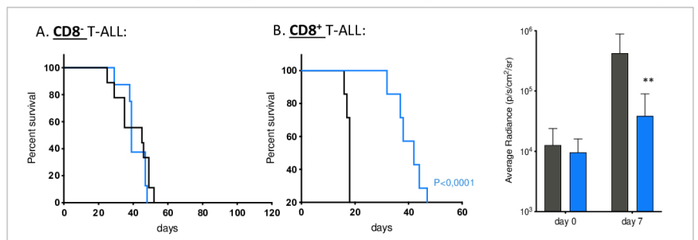
= **confirmation of selective activity of Activity-on-Target Interferons (AcTaferons)**

mCD8-AFN has direct anti-leukemic properties

mCD8-AFN treatment after T-ALL transplantation in **immunocompromised mice (NSG)**:



mCD8-AFN treatment (30ug/d) after T-ALL transplantation in **immunocompetent C57BL/6 mice**:



Treatment:
- 7 mice/group
- Started when evidence of leukemia
- 7 consecutive days: 30 ug AFN/d
- Controls: PBS

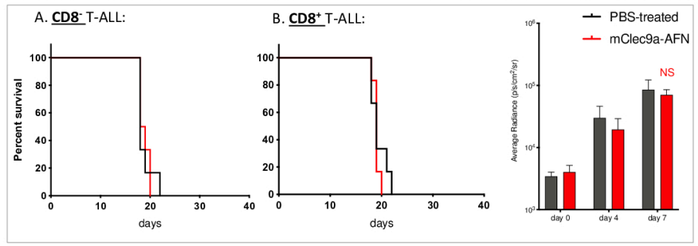
Readout:
- IVIS bioluminisence
- survival

Conclusion: mCD8-AFN

-> affects in vivo growth of CD8+ T-ALL
-> no effect on CD8- T-ALL
-> independent of immune system

mClec9A-AFN has indirect anti-leukemic properties

mClec9a-AFN after T-ALL transplantation in **immunocompromised mice (NSG)**:



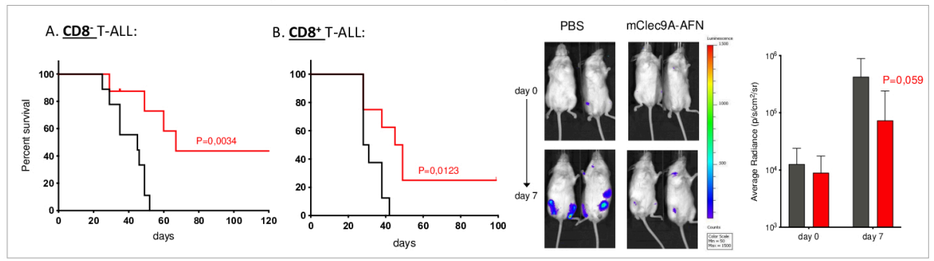
Treatment:
- 7 mice/group
- Started when evidence of leukemia
- 7 consecutive days: 30 ug AFN/d
- Controls: PBS

Readout:
- IVIS bioluminesence
- survival

Conclusion: mClec9a-AFN

-> affects growth of all T-ALLs
-> dependent of immune system
-> few longterm survivors

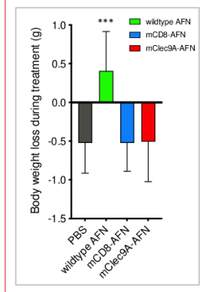
mClec9a-AFN after T-ALL transplantation in **immunocompetent C57BL/6 mice**:



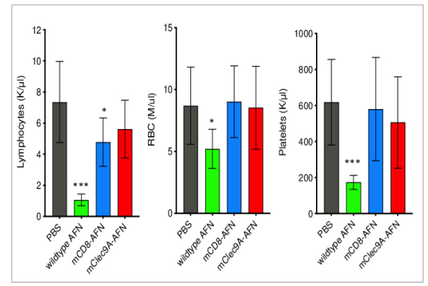
AFNs have less adverse side-effects

Adverse side-effects were evaluated in leukemia-free mice in comparison to wildtype interferon

Loss of Body weight:



Hemavet 5-diff. automated blood analysis:



CONCLUSIONS

- **Activity-on-Target Interferons** (AcTaferons; AFN) **were generated with selective activity** on CD8+ and Clec9a+ cells
- **mouse CD8-AcTaferon (CD8-AFN) induces a direct and highly selective anti-proliferative/leukemic effect** only on CD8+ acute T-cell lymphoblastic leukemia (T-ALL) cells; **both *in vitro* and *in vivo*.**
- **mouse Clec9A-Actaferon** (mClec9a-AFN) induces an **indirect anti-leukemic effect on mouse T-ALLs by activation of the immune system.**
- Due to the selective cell targeting, a drastic **reduction in toxic side-effects** was observed with AcTaferons compared to the treatments with the wild type IFNa.

CONTACT INFORMATION

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