Preclinical characterization of GT-00A x IL15: A novel IL-15-based immunocytokine with unique tumor targeting properties

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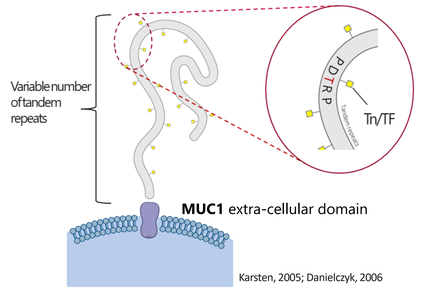
Introduction

- IL-15 has a huge potential to activate both innate and adaptive anti-tumor immunity
- Several IL-15-based immunocytokines are currently in clinical development but all of them act preferentially in the periphery and not locally within the tumor

We have developed **GT-00A x IL15, an immunocytokine targeting a tumor-associated, glycosylated epitope of MUC-1 (TA-MUC1).** GT-00A x IL15 was designed to induce anti-tumor responses directly within the tumor microenvironment for the treatment of solid tumors. .

TA-MUC1 (tumor-associated Mucin-1)



Karsten, 2005; Danielczyk, 2006

Tumor-specific combined carbohydrate and peptide epitope consisting of a **Tn** (GalNAcα1-O-) or **TF** (Galβ1-3GalNAcα1-O-) and PD**T**RP sequence of MUC1 tandem repeats.

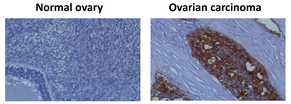
High Expression Levels
Expressed in ~ 80 -100% of all cases of
main indications: ovarian, lung and breast
Other possible indications include: urothelial, endometrial, gastrointestinal, kidney, colon a.o. cancers

High therapeutic potential
Present on carcinomas & metastasis

Potential to induce long lasting responses
Present on cancer stem cells

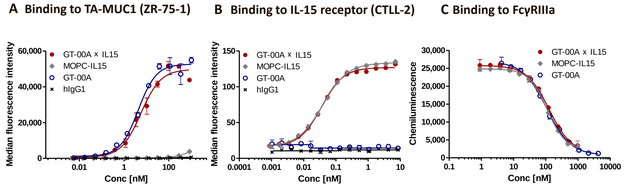
Excellent safety profile
Virtually absent on normal cells

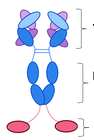
Exemplary IHC TA-MUC1:



Results: *In vitro* target binding

***In vitro* target binding:** Binding of GT-00A x IL15 to **A)** cellular TA-MUC1, **B)** IL-15R and **C)** FcγRIIIa was analyzed by flow cytometry (A+B) or AlphaScreen® technology (C) and compared to MOPC-IL15 (untargeted control construct), the parental antibody GT-00A and hIgG1.



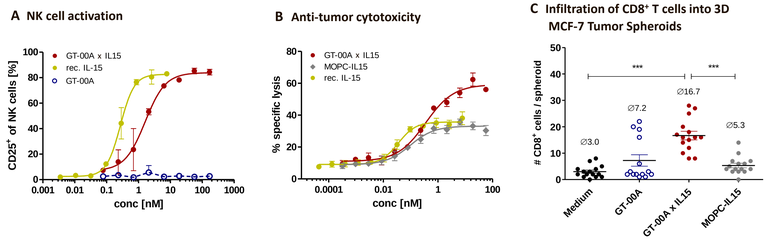


➢ **Dose dependent and specific target binding of GT-00A x IL15 to TA-MUC1, IL-15R and FcγRIIIa**

GT-00A x IL15 is available for partnering or co-development. For further discussion please contact business.development@glycotope.com or visit our webpage https://www.glycotope.com/contact/

Results: *In vitro* activation and cytotoxicity

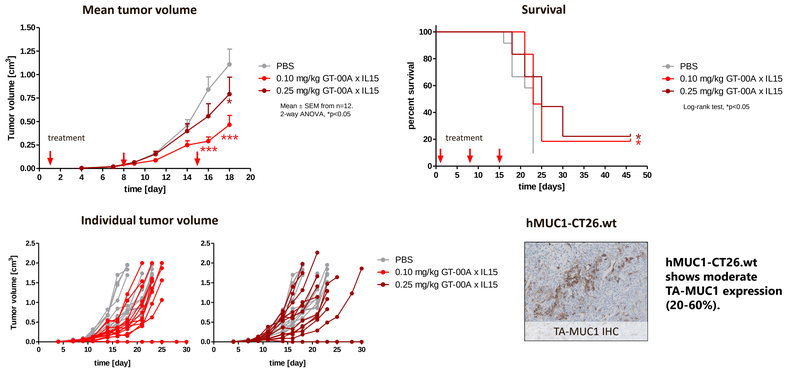
In vitro activation and cytotoxicity: A) PBMC were incubated for 5d with GT-00A x IL15, parental GT-00A or recombinant (rec.) IL-15 and expression of CD25 on NK cells was assessed by flow cytometry. **B)** PBMC were incubated with ZR-75-1 breast cancer cells in the presence of GT-00A x IL15, MOPC-IL15 or rec. IL-15. Cytotoxicity was assessed after 4h (europium release assay). **C)** MCF-7 spheroids were first treated with test items for 4 hours before washing and adding PBMC for further 48 hours. The amount of infiltrated $CD8^+$ T cells was analyzed by IHC.



➢ **GT-00A x IL15 induces dose-dependent induction of NK, NKT, CD4+, and CD8+ T cell activation and proliferation, with NK cells being the most sensitive cell population.**
➢ **Tumor cell targeted GT-00A x IL15 is superior in mediating cellular cytotoxicity compared to the untargeted control construct MOPC-IL15 and rec. IL-15.**
➢ **GT-00A x IL15 induces T cell infiltration into MCF-7 tumor spheroids in contrast to the parental antibody and the untargeted control construct MOPC-IL15. It further reduced the area of tumor spheroids (not shown).**

Results: *In vivo* efficacy

In vivo anti-tumor efficacy of GT-00A x IL15 in hMUC1-CT26.wt tumor bearing mice: Balb/c mice were inoculated s.c. with $1x10^6$ hMUC1-CT26.wt tumor cells on day 0. Mice were treated with PBS and 2 different doses of GT-00A x IL15 on day 1, 8 and 15. Animals were sacrificed if TV exceeded 1.5 cm^3.

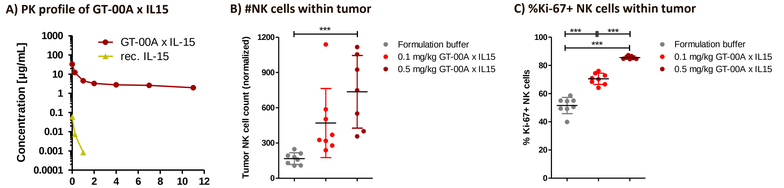


hMUC1-CT26.wt shows moderate TA-MUC1 expression (20-60%).

➢ **Treatment with GT-00A x IL15 significantly delayed tumor growth and improved survival.**
➢ **Efficacy of GT-00A x IL15 monotherapy was seen in further models (hMUC1-B16.F10, hMUC1-4T1, PBMC-humanized DU-145 xenograft model).**

Results: *In vivo* pharmacokinetic and pharmacodynamics

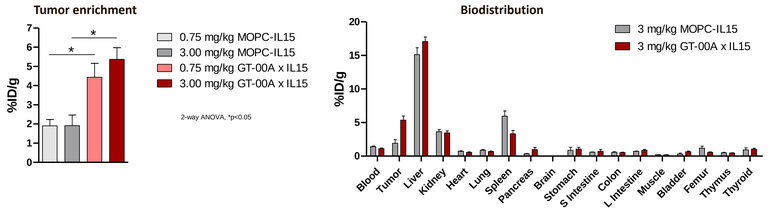
GT-00A x IL15 has a typical IgG PK profile and induces proliferation and expansion of tumor-infiltrating immune cells: A) Mice received a single i.v. bolus injection of either 2 mg/kg GT-00A x IL15 or a molar equivalent dose of 0.29 mg/kg recombinant human IL-15. Serum samples were collected and analyzed by ELISA. **B+C)** hMUC1-B16.F10 tumor bearing C57BL/6 mice were injected i.v. bolus with 0.1 or 0.5 mg/kg GT-00A x IL15. 3 days later, tumors were harvested and analyzed by flow cytometry for **B)** NK cell infiltration and **C)** Ki-67 expression.



➢ **GT-00A x IL15 shows a typical bi-phasic IgG1 PK profile with a long terminal serum half-life of 9 days i.v. which is longer compared to rec. IL-15 and competitor IL-15 immunocytokines (Hangasky et al., 2020).**
➢ **GT-00A x IL15 induces dose-dependent activation and expansion of tumor-infiltrating NK cells and CD8+ T cells (not shown here) *in vivo*.**

Results: *In vivo* biodistribution and tumor accumulation

GT-00A x IL15 shows improved tumor accumulation compared to an untargeted immunocytokine: ^{89}Zr-labelled GT-00A x IL15 and its untargeted control construct MOPC-IL15 were injected i.v. into hMUC1-B16.F10 tumor bearing mice and biodistribution of the molecules was assessed three days later.



➢ **TA-MUC1 binding significantly improves tumor accumulation of GT-00A x IL15 (5.4%) over the untargeted control construct MOPC-IL15 (1.9%).**
➢ **GT-00A x IL15 also accumulated in the liver suggesting clearance via the hepatobiliary pathway as described for other IL-15 agonists.**

Conclusion

GT-00A x IL15 – tumor-targeted IL-15 based immunocytokine:

- GT-00A x IL15 accumulates via TA-MUC1 binding in the tumor and induces local NK and T cell activation and expansion in addition to its immune stimulatory effects in the periphery
- GT-00A x IL15 shows single agent efficacy in several syngeneic tumor models (hMUC1-CT26.wt, hMUC1-B16.F10, hMUC1-4T1) but also in an DU-145 xenograft model using PBMC-humanized mice
- GT-00A x IL15 has the potential to increase the efficacy and safety of IL-15-based immunocytokines by tumor targeting and shows great promise for the treatment of TA-MUC1-positive solid tumors

Literature:
(1) Danielczyk A, Stahn R, Faulstich D, Löffler A, Märten A, Karsten U, Goletz S. PankoMab: a potent new generation anti-tumour MUC1 antibody. Cancer Immunol Immunother 2006;55(11):1337-47.
(2) Hangasky JA, Waldmann TA, Santi DV. Interleukin 15 Pharmacokinetics and Consumption by a Dynamic Cytokine Sink. Front Immunol. 2020; 13;11:1813.
(3) Karsten U, von Mensdorff-Pouilly S, Goletz S. What Makes MUC1 a Tumor Antigen?. Tumor Biology 2005;26:217-220.