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

GLYCOTOPE

Abstract 2879

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Introduction

IL-15 is a potent pro-inflammatory cytokine that enhances the differentiation, proliferation and cytolytic activity of NK cells and T cells. Due to the huge potential of IL-15 to activate both innate and adaptive anti-tumor immunity, several IL-15-based immunocytokines are currently in clinical development. Those first generation untargeted IL-15-based immunocytokines show already promising results in the clinic but 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) to**

- **improve the tumor accumulation, efficacy, safety and half-life of highly potent IL-15 compared to untargeted competitor products**
- **direct immune cells into the tumor inducing local immune cell activation & cytokine release leading to immune cell proliferation and tumor cell killing**

GT-00A x IL15 is ready for clinical development offering the potential to be at the forefront of clinical development of next generation targeted IL-15-based immunocytokines

Abundant target expression of TA-MUC1 in many indications of high medical need

Tumor type	% membrane-positive cases
Lung, bronchioalveolar carcinoma	100
Lung, adenocarcinoma	96
Breast, mucinous carcinoma	92
Ovary, endometrioid carcinoma	91
Breast, tubular carcinoma	90
Endometrium, endometrioid carcinoma	90
Ovary, serous carcinoma	88
Urothelial carcinoma, (pTa)	83
Cervix, adenocarcinoma	80
Breast, ductal carcinoma	79
Endometrium, serous carcinoma	78
Cervix, squamous cell carcinoma	75
Breast, cribriform carcinoma	74
Stomach, intestinal carcinoma	66

High Expression Level
Expressed with 80 -100% of all cases in **ovarian, lung and breast cancer**
Other indications include: urothelial, endometrial, gastrointestinal, kidney, colon a.o. cancers

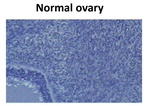
High therapeutic potential
Present on carcinomas & metastasis

Potential to induce long lasting responses
Present on cancer stem cells

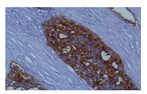
Excellent safety profile
Virtually absent on normal cells

Exemplary IHC TA-MUC1:

Normal ovary

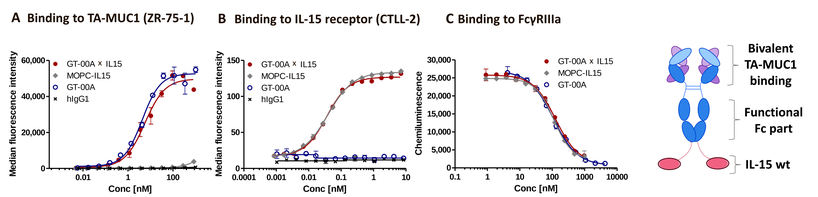


Ovarian carcinoma



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.

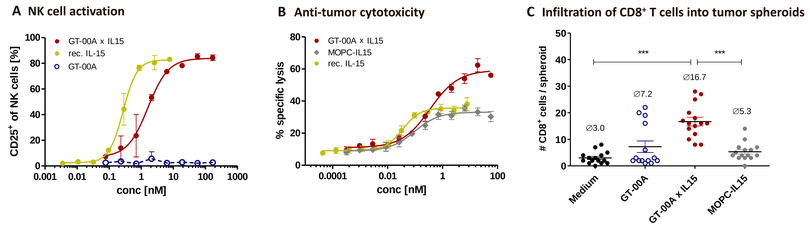


- **Dose dependent and specific target binding of GT-00A x IL15 to TA-MUC1, IL-15R and FcγRIIIa**

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/

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.

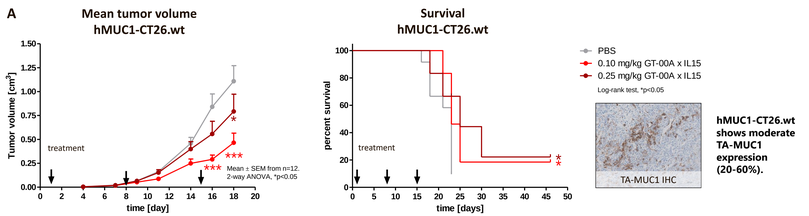


- **GT-00A x IL15 induces dose-dependent induction of NK, NKT, CD4+, and CD8+ T cell activation and proliferation, with NK cells being the most sensitive cell population.**
- **Tumor cell targeted GT-00A x IL15 is superior in mediating cellular cytotoxicity compared to the untargeted control construct 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).**

In vivo efficacy

In vivo anti-tumor efficacy of GT-00A x IL15. A) Balb/c mice were inoculated s.c. with 1x10⁶ hMUC1-CT26.wt tumor cells (CRC) 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 tumor volume exceeded 1.5 cm³. **B)** NCG mice were inoculated s.c. with a mixture of 5×10⁶ DU-145 tumor cells (prostate cancer) and 2.5×10⁶ PBMC of different donors on d0. Mice were treated with PBS and 2 different doses of GT-00A x IL15 on day 1, 8 and 15. Animals were sacrificed upon body weight loss >20%. **C)** NCG mice were inoculated s.c. with 5×10⁶ DU-145 tumor cells. When tumors reached 80-150 mm³, mice were humanized with 2.5×10⁶ PBMC. Weekly treatment (d1, d8, d15) started one day later using 2 different doses of GT-00A x IL15. Tumors were harvested on d29 and analyzed by flow cytometry for human immune cell infiltration.

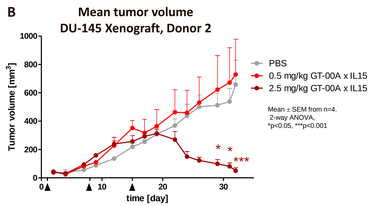
A



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

- **Treatment with GT-00A x IL15 significantly delayed tumor growth and improved survival in the hMUC1-CT26.wt model**

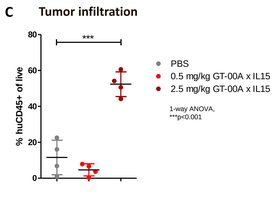
B



	tumor growth inhibition (%) at end of study	
	0.5 mg/kg	2.5 mg/kg
Donor 1	5,9	57,7
Donor 2	-10,9	92,5
Donor 3	37,2	75,5
Donor 4	-22,7	69,2

% tumor growth Inhibition = (mean(PBS)-mean(GT-00A x IL-15)) / mean(PBS) * 100%

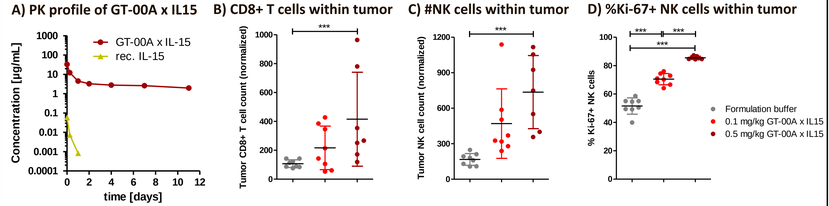
C



- **Treatment with high dose GT-00A x IL15 lead to highly significant tumor regression in different PBMC donor groups starting ~d20**
- **Tumors were highly infiltrated by human immune cells on d29 after treatment start (CD56+ cells and CD3+ T cells)**

In vivo pharmacokinetic and pharmacodynamics

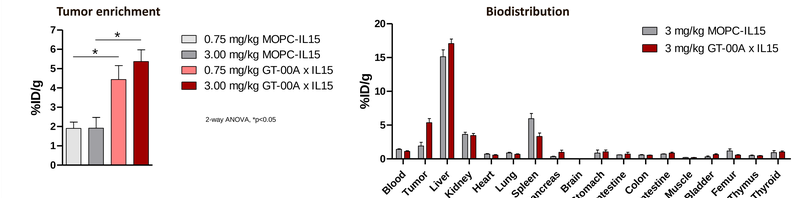
GT-00A x IL15 has a typical IgG PK profile and induces proliferation and expansion of tumor-infiltrating immune cells: A) Mice received a single i.v. bolus injection of either 2 mg/kg GT-00A x IL15 or a molar equivalent dose of 0.29 mg/kg recombinant human IL-15. Serum samples were collected and analyzed by ELISA. **B-D)** 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)** CD8+ T cell infiltration, **C)** NK cell infiltration and **D)** 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 JA, Waldmann TA, Santi DV. Interleukin 15 Pharmacokinetics and Consumption by a Dynamic Cytokine Sink. Front Immunol. 2020; 13;11:1813)
- **GT-00A x IL15 induces dose-dependent activation and expansion of tumor-infiltrating NK cells and CD8+ T cells *in vivo*.**

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 syngeneic and xenograft tumor models
- GT-00A x IL15 was well tolerated in a 3-week intravenous repeat-dose toxicity study in Wistar rats (non-GLP); final data of GLP toxicity study will be available soon (April 22)
- GT-00A x IL15 has the potential to increase the efficacy and safety of IL-15-based immunocytokines by targeting of TA-MUC1-positive solid tumors (e.g. OvCa, NSCLC, BrCa and others)
- A pre-clinical in vitro & in vivo data package is available incl. PK/PD, efficacy and toxicological assessment; first GMP run was completed successfully
- **GT-00A x IL15 has great potential to be the next generation targeted IL-15-based immunocytokine**