TCX-101, a novel antibody-drug conjugate targeting a tumor-associated carbohydrate antigen for the treatment of solid tumors

#3125

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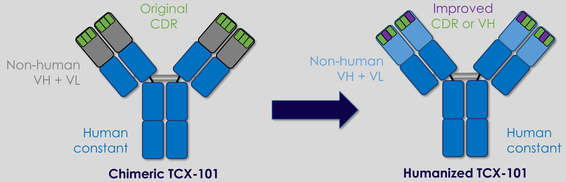
Introduction

Tumor-associated carbohydrate antigens (TACAs) are short oligosaccharides expressed on the surface of cancer cells and play key roles in cellular communication, immune evasion, apoptosis and metastasis. TACAs are therefore interesting targets for novel antibody-based therapies in solid tumors. TCX-101 is a novel first-in-class monoclonal antibody (mAb) with high affinity and specificity against a distinct TACA structure. Here we present the *in vitro* characterization of this mAb and show that an antibody-drug conjugate (ADC) using monomethyl auristatin E (MMAE) with a drug/antibody ratio (DAR) of 4 was effective in different *in vivo* xenograft models.

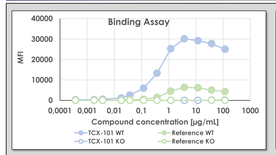
Optimization of TCX-101

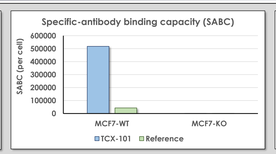
A chimeric precursor molecule of TCX-101 (left) with human IgG_1 backbone showed high affinity and binding properties to the target. These properties were maintained in an improved version (right) despite a high degree of humanization.

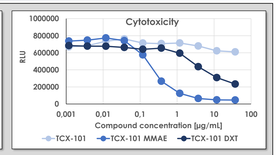


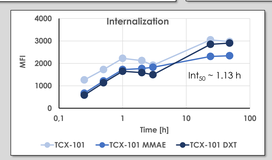
	Chimeric TCX-101	Humanized TCX-101
Affinity (KD) [mol/L]	4.29E-07	5.65E-07
K_{on} [1/ms]	1.72E+05	3.77E+04
K_{off} [1/s]	7.386E-02	2.115E-02
EC_{50} [nM]	8.13	6.27
Max. MFI signal	49150	43208
Humanization VH / VL [%]	67.0 / 62.8	85.7 / 74.2

In vitro characterization



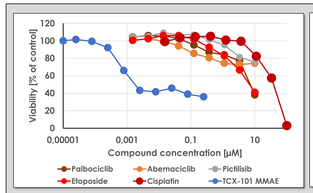






	EC_{50} [µg/mL]	max. MFI	IC_{50} [µg/mL]	% max. inhibition
Reference	0.64	30164		
TCX-101	0.38	6396	5.13	8.54
TCX-101 MMAE DAR 4	--	--	0.23	93.66
TCX-101 MMAE DAR 10	--	--	0.05	95.14
TCX-101 DXT DAR 4	--	--	3.85	65.80
TCX-101 DXT DAR 10	--	--	0.92	73.42

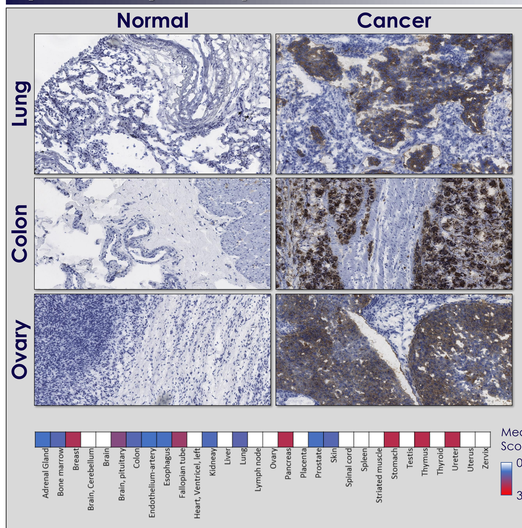
TCX-101 shows superior binding on MCF-7 WT cells compared to the reference antibody against the same target. At saturating concentrations, TCX-101 shows 518,000 bound units to the cell surface, whereas the reference antibody shows more than a 10-fold reduction, with 43,000 bound units. TCX-101 presents a high internalization rate, which was not impaired after conjugation with either MMAE or Derutxecan (DXT) toxophores. Furthermore, both antibody-drug conjugates (with drug-antibody ratios of 4 and 10) were effective against MCF-7 WT cells, with MMAE being more effective than DXT in such cells. EC_{50}: half-maximal effective concentration; MFI: median fluorescence intensity; IC_{50}: half-maximal inhibitory concentration.



Compound	IC_{50}
Palbociclib	7.8 µM
Abemaciclib	> 10 µM
Pictilisib	> 10 µM
Etoposide	6.8 µM
Cisplatin	39.3 µM (32.3 µg/mL)
Carboplatin + Etoposide (20:1)	> 10 µg/mL
Anti-HIV XTC	> 250 nM (37.5 µg/mL)
TCX-101 XTC	84.7 nM (12.7 µg/mL)
Anti-HIV MMAE	49.3 nM (7.5 µg/mL)
TCX-101 MMAE	1.6 nM (0.24 µg/mL)

TCX-101 ADCs maintained anti-tumor efficacy in a multi-resistant human breast cancer cell line (HCC1428) *in vitro*. While different standard of care compounds (left panel) did not reduce cell viability at physiologically relevant concentrations, TCX-101 MMAE (and to a lesser extend also the exatecan (XTC)-conjugated variant, middel panel) remained active and reduced cell viability by up to 60%.

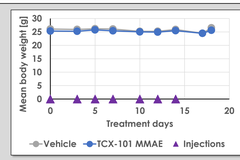
Specificity and prevalence

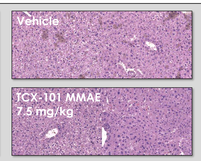


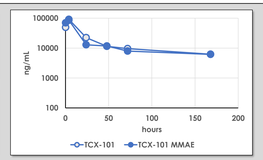
77% (23/30) of stained normal tissues show no or only weak staining (H Score < 40)

Immunohistochemistry analyses of human fresh frozen normal (FDA panel) and tumor (colon, lung and ovarian cancer) tissue microarrays showed high specificity of TCX-101 for cancer cells. Target expression on normal tissues was usually restricted to secretory cells or mucus lining on the apical side of cells whereas cancer cells also show basolateral staining.

In vivo characterization



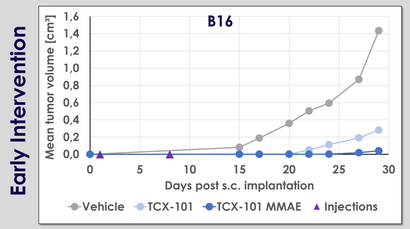


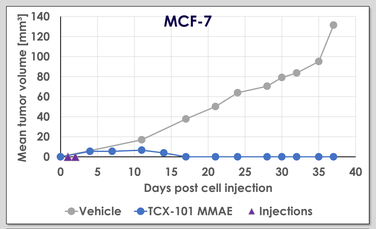


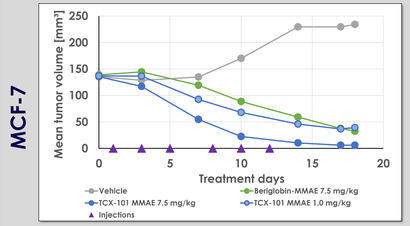
Repeated dosing of TCX-101 MMAE at 7.5 mg/kg was well tolerated in BALB/c nude mice. No body weight loss or morphological signs of toxicity were observed. H&E stainings of liver tissues did not show signs of drug-induced liver injury.

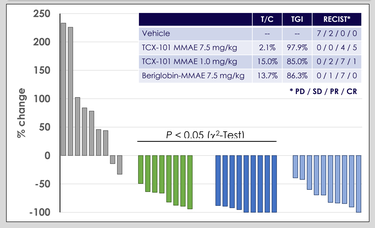
PK data after a single i.v. dose of 10 mg/kg revealed no difference in half-life in mice (167 h for TCX-101 and 175 h for TCX-101 MMAE).

In vivo efficacy



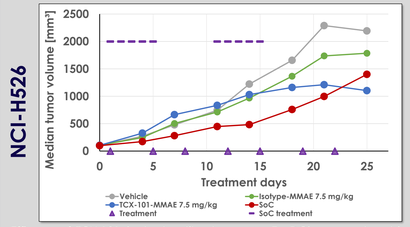


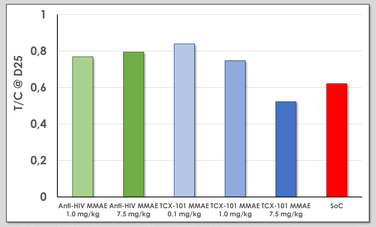




	T/C	TGI	RECIST*
Vehicle	--	--	7 / 2 / 0 / 0
TCX-101 MMAE 7.5 mg/kg	2.1%	97.9%	0 / 0 / 4 / 5
TCX-101 MMAE 1.0 mg/kg	15.0%	85.0%	0 / 2 / 7 / 1
Beriglobin-MMAE 7.5 mg/kg	13.7%	86.3%	0 / 1 / 7 / 0

* PD / SD / PR / CR





Efficacy of TCX-101 (naked antibody or MMAE-ADC) was explored in cell-line derived xenograft models. B16 murine melanoma cells were injected subcutaneoulsy whereas human MCF7 breast cancer cells were implanted into the mammary fat pad. Early treatment with either naked TCX-101 or with TCX-101 MMAE inhibited tumor cell growth.
TCX-101 MMAE caused strong tumor regression in high target-expressing MCF7 cells also in a therapeutic setting. 5/9 mice achieved complete tumor regression after repeated dosing of 7.5 mg/kg TCX-101 MMAE, while this was observed only in 1 animal treated with 1.0 mg/kg TCX-101 MMAE and in none of the animals treated with a control ADC at the same dose level and DAR.
Treatment of the aggressive and multi-resistant human small cell lung cancer cell line NCI-H526 lead to a T/C of approx. 0.5 after repeated (twice per week) dosing of TCX-101 MMAE at 7.5 mg/kg, despite only moderate to low expression of the target. Other doses (0.1 or 1.0 mg/kg) or schedules (single dose, once weekly) were less effective in this model (data not shown). Carboplatin/Etoposide was used as standard of care (SoC) for this model.

Conclusion

TCX-101 is novel highly specific antibody against a TACA which is expressed in various human cancer types. An ADC using MMAE as payload demonstrated efficacy in several models *in vitro* and *in vivo*. Further studies are ongoing to support clinical development of TCX-101.

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