Presentation 1794

Preclinical development of TGW101, a first in class click-cleavable ADC with MMAE against non-internalizing pan-carcinoma marker TAG72

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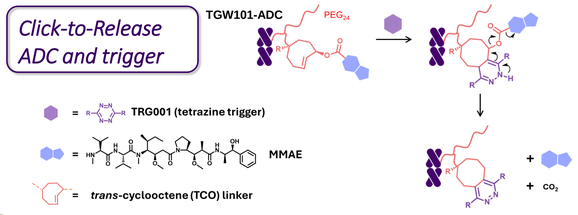
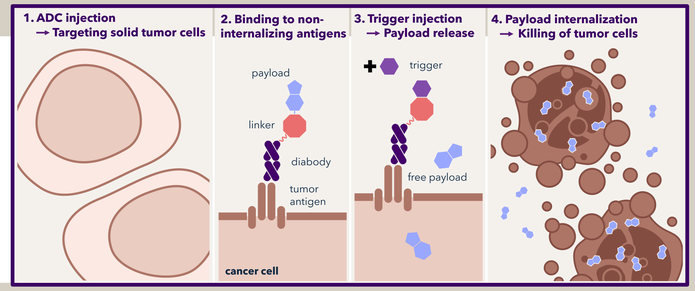
Introduction



- Current ADCs typically rely on intracellular drug release, limiting the application scope to internalizing receptors.
- To expand the ADC scope to non/slowly internalizing markers we are developing a new class of ADCs that release their payload upon on-tumor click reaction (based on Tagworks' **Click-to-Release** platform) [1] with a trigger molecule, which is administered i.v. in a second step after the ADC has sufficiently cleared from circulation [2,3].
- **Controlled payload release in the TME** and uptake in surrounding cancer cells allows maximal bystander effect.
- Here we present the preclinical development of **TGW101, a first-in-class click-cleavable ADC** combination therapy comprising TGW101-ADC, a diabody conjugated with MMAE (DAR = 4) targeting the non-internalizing pan-carcinoma marker TAG72, and trigger molecule TRG001.



Results

TGW101-ADC characterization

TGW101-ADC is an optimized derivative of 1st generation anti-TAG-72 diabody ADC (tc-ADC [3]) conjugated via 4 cysteines to MMAE via a novel TCO linker comprising PEG_{24}, and is combined with a novel tetrazine trigger. The improved TCO/trigger pair is **100-fold more reactive**, allowing orders of magnitude lower trigger doses. TGW101-ADC rapidly releases 98% MMAE upon reaction with TRG001 in PBS (<1h, 37°C) and is stable when stored at -20°C (>2yr) and plasma (max 0.25±0.01% MMAE release in mouse/rat/NHP/human plasma after 77h at 37°C).

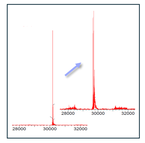


Figure 1: MS analysis of TGW101-ADC before and after TRG001 addition

TGW101 mechanism of action (MoA)

In vitro, TGW101-ADC affords dose-dependent cytotoxicity only when cells express TAG72 and TRG001 is added.

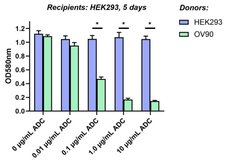


Figure 2: TAG-72+ (OV90) and TAG-72- (HEK293) "donor" cells were treated with TGW101-ADC (1h, 0°C), followed by replacement of the medium with TRG001-containing medium (10min, 37°C). The cell supernatant was then transferred to a plate containing HEK293 "recipient" cells and incubated for 5 days, followed by cell proliferation measurement. TGW101-ADC and TRG001 alone were used as controls and did not affect cell proliferation (data not shown).

TGW101-ADC biodistribution and MMAE release in mice

In mice, TGW101-ADC has fast blood clearance (Fig. 3A), high tumor uptake vs. low in non-target organs (Fig. 3B). Injection of TRG001 48h post-ADC afforded high free MMAE concentration in tumors already 3h p.i. and ≥100-fold lower in other tissues (Fig. 3C).

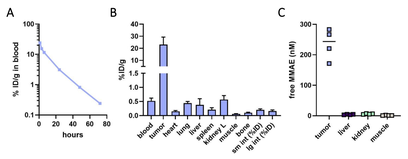


Figure 3: (A) PK and (B) biodistribution (48hrs p.i.) of ^{125}I-labeled TGW101-ADC (2 mg/kg, i.v.) in nude mice bearing TAG72+ colon cancer xenografts (LS174T); data is the mean with SD (n=3 (A) and n=5 (B)); (C) free MMAE concentration in tumor and non-target tissues when TRG001 (3.2 mg/kg, i.v.) was injected 48h post-ADC.

TGW101 on-tumor reaction efficiency

In a colorectal CDX model, a 2.5-10 mg/kg TRG001 dose was sufficient to cleave all tumor-bound ADC after a 1 and 10 mg/kg (^{125}I-labeled) TGW101-ADC dose. An ^{111}In-labeled tetrazine injected 1h after the trigger was used to detect any unreacted ADC [2].

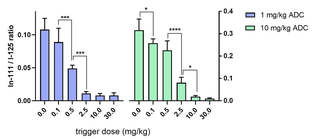


Figure 4: ^{111}In/^{125}I ratio in the LS174T xenografts of mice injected ^{125}I-labeled ADC (1 or 10 mg/kg) followed by trigger (48h post-ADC) and ^{111}In-reporter probe (1hr post trigger); low ratio signifies high on-tumor reaction between ADC and trigger (n=5; *: p<0.05; ***: p<0.0005; ****: p<0.0001).

TGW101 pharmacologic effect

Previously, 4 cycles of 1st generation ADC tc-ADC + trigger afforded pronounced therapeutic efficacy in TAG72+ ovarian cancer bearing mice (OVCAR-3), in contrast to ADC or trigger alone, or protease-cleavable analog (vc-ADC) (Fig. 6 [3]). Likewise, TGW101-ADC followed by trigger (TRG001) afforded a strong therapeutic effect in the OVCAR-3 CDX model (Fig. 7A) as well as in TAG-72+ gastric and pancreatic PDX models (Fig. 7B and 7C) at much lower trigger doses. In all studies, the administration of TGW101-ADC alone did not afford a significant reduction of tumor volume, demonstrating the MoA of TGW101 and the in vivo stability of the ADC. TGW101 was well tolerated in these mice at all doses, both with single and repeated treatments.

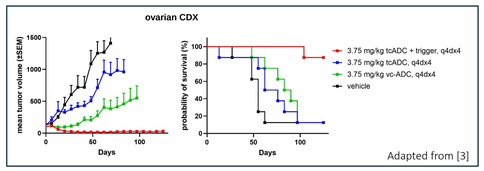


Figure 6: Therapy study in mice bearing ovarian cancer xenografts (OVCAR-3), administered i.v. with 4 cycles of anti-TAG72 diabody ADC (3.75 mg/kg; containing first generation chemically-cleavable TCO-MMAE linker-drug, DAR=4) followed 2 days later by a tetrazine trigger (0.335 mmol/kg), one cycle every 4 days [3]. Tumor volumes of euthanized mice were carried over.

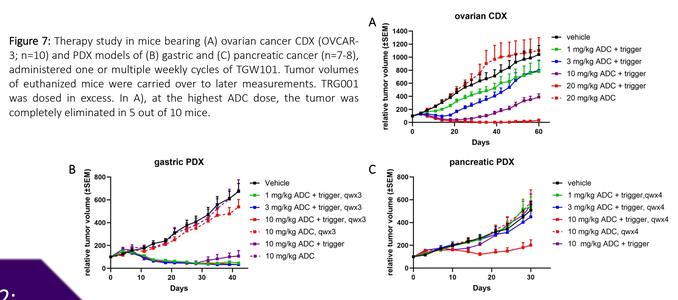


Figure 7: Therapy study in mice bearing (A) ovarian cancer CDX (OVCAR-3; n=10) and PDX models of (B) gastric and (C) pancreatic cancer (n=7-8), administered one or multiple weekly cycles of TGW101. Tumor volumes of euthanized mice were carried over to later measurements. TRG001 was dosed in excess. In A), at the highest ADC dose, the tumor was completely eliminated in 5 out of 10 mice.

TGW101 pharmacokinetics and toxicology

TGW101-ADC alone was very well tolerated in NHPs at repeated doses at 50 mg/kg (NOAEL) with a clearance half-life of 25h. Similarly, TRG001 was very well tolerated, with NOAEL of resp. 700 and 300 mg/kg in rat and NHP, with a clearance half-life of 0.6h in NHP. Based on ADC PK, a 4-day interval was used in NHP to result in >90% clearance from blood at time of trigger injection. Biweekly cycles of ADC and high dose of trigger (75 mg/kg) afforded an HNSTD of 5 mg/kg for TGW101-ADC (which equates to 12.5 mg/kg for mAb-sized ADCs), with reversible and non-adverse effects consistent with free MMAE released from residual ADC in blood upon trigger injection.

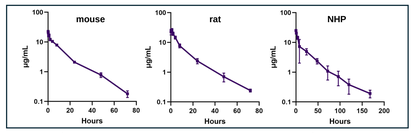


Figure 9: Plasma TGW101-ADC concentration in mouse, rat and NHP injected at 1 mg/kg. Data is the mean with SD (n=3).

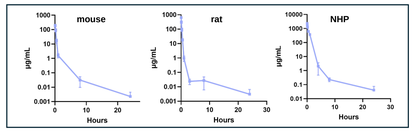


Figure 10: Plasma TRG001 concentration in mouse, rat and NHP injected at 100 mg/kg (mouse and rat; n=8) and 300 mg/kg (NHP; n=6). Data is the mean with SD.

Table 1: Summary of (GLP) toxicology studies for TRG001 in SD rat and cynomolgus monkey

	trigger	days	recovery	results
rat	50-200-700 mg/kg/dose	1-8-15-22-29	2 weeks	NOAEL = 700 mg/kg/dose
NHP	20-75-300 mg/kg/dose	1-8-15-22-29	2 weeks	NOAEL = 300 mg/kg/dose

Table 2: Summary of (GLP) toxicology studies for TGW101 and TGW101-ADC alone in cynomolgus monkey

	ADC	days	trigger	days	recovery	results
TGW101 (combo)	0.5-2-5 mg/kg/dose	1-15-29	75 mg/kg/dose	5-19-33	2 weeks	HNSTD = 5/75 mg/kg/dose
TGW101-ADC	50 mg/kg/dose	1-15-29	--	--	--	NOAEL = 50 mg/kg/dose

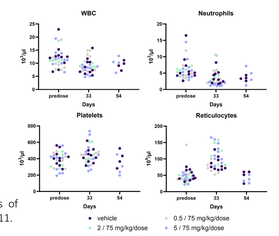
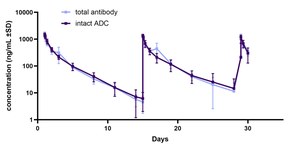


Figure 11: hematology evaluation in NHPs injected with 3 cycles of TGW101 (GLP combo toxicology study; see Table 2). Predose = Day -11.

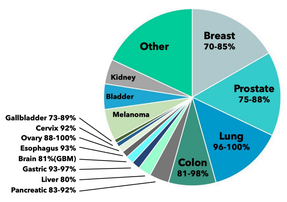


TGW101-ADC in vivo stability in cynomolgus monkeys

The curves of total antibody and intact ADC in NHP plasma can be superimposed, confirming that TGW101-ADC is highly stable in vivo.

Figure 12: Comparison between plasma concentration of total antibody and intact ADC of NHPs injected with TGW101-ADC 50 mg/kg/dose at day 1, 15 and 29. Total antibody was quantified using ELISA. Intact ADC was calculated from ADC-conjugated MMAE released in plasma samples ex vivo. Data in the mean with SD (n=6).

TAG-72 expression in solid tumors



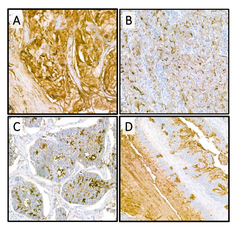


Figure 5: Staining of (A) gastric, (B) breast, (C) lung, and (D) ovarian carcinomas with TGW101 diabody

TAG-72:
A pan-carcinoma marker consisting of STn and Tn glycans presented by several mucins. It was shown to be a clean and stable, non-internalizing target in clinical radio-immunotherapy

Animal Tissue Cross-Reactivity

Based on a TCR study showing similar TGW101 diabody binding to small intestine luminal mucosa of human and NHP, the cynomolgus monkey was chosen for toxicology studies with TGW101-ADC and TGW101.

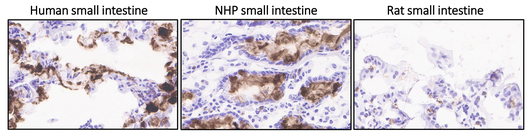


Figure 8

Conclusions and Outlook

- TGW101 demonstrated potent and specific anti-tumor activity against non-internalizing target.
- ADC / Trigger alone are well tolerated at high doses and ADC + Trigger using interval giving 90% ADC clearance prior to Trigger are better tolerated than conventional MMAE ADCs, warranting clinical development of this 1st in class ADC.
- TGW101 recently received FDA IND clearance. Clinical evaluation, an open-label, multicenter, Phase 1 dose-escalation trial, is expected to begin mid-2025 and will assess safety, tolerability, pharmacokinetics, and preliminary efficacy in patients with advanced solid tumors.

TAG WORKS

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