Abstract nr: 3486

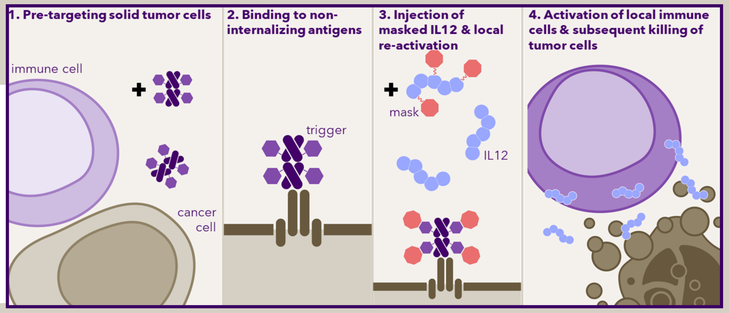
Click-to-release for controlled immune cell activation: tumor-targeted unmasking of an IL12 prodrug in vivo

Martijn H. den Brok[1], Kim E. de Roode[1,2], Luc H.M. Zijlmans[1], Laurens H.J. Kleijn[1], Marleen H.M.E. van Stevendaal[1], Ron M. Versteegen[3], Lieke W.M. Wouters[1], Raffaella Rossin[1], Marc S. Robillard[1]

1. Tagworks Pharmaceuticals, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands
2. Department of Medical Imaging, Nuclear Medicine, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands
3. SyMO-Chem, Den Dolech 2, 5612 AZ Eindhoven, The Netherlands

Introduction

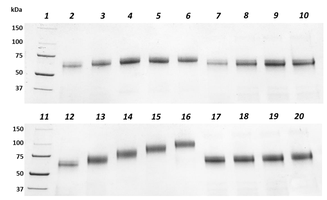
- Immunotherapy utilizing cytokines such as **Interleukin 12 (IL12)** holds great promise for the treatment of cancer, but severe toxicity after systemic administration leads to a narrow therapeutic window.
- Tagworks' **Click-to-Release platform** enables effective **on-tumor activation** of prodrugs, with the aim to increase the therapeutic index and application scope of potent anticancer drugs. We present a novel treatment strategy in which IL12 is **inactivated** by conjugation to *trans*-cyclooctene(TCO)-linked PEG masks. These can be **tracelessly released** through the *in vivo* click reaction between the TCO linker and a tetrazine (Tz) molecule (the trigger), fully restoring IL12 bioactivity.
- Here, we **pre-localize** the trigger using a *Tumor-associated glycoprotein 72* (TAG-72)-directed diabody, followed by prodrug administration and on-tumor activation.



Results

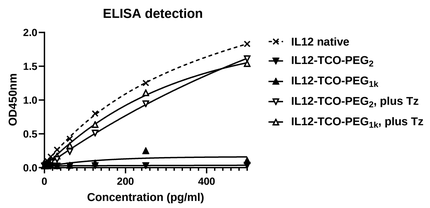
Effective masking of IL12 bioactivity

(Top) SDS-PAGE analysis of native IL12 (lanes 2 and 12), IL12-TCO-PEG$_2$ constructs conjugated by mixing with 10, 30, 50, or 100 equivalents of the TCO-PEG masks (NHS carbonate activated) relative to IL12 (lanes 3 to 6, resp.) and the same compounds after reaction with free Tz (lane 7 to 10). Lanes 13 to 16 show IL12-TCO-PEG$_{1K}$ constructs conjugated by mixing with 10, 30, 50, or 100 equivalents of the masks relative to IL12, and the same compounds after reaction with free Tz (lanes 17 to 20).

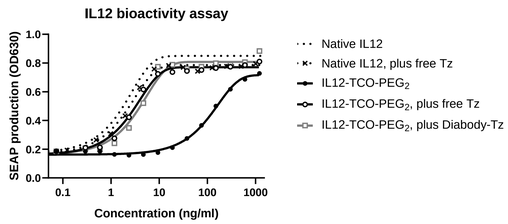


Evaluation of bioactivity and the mask to cytokine ratio showed that conjugation of 9 masks resulted in superior (>100 fold) attenuation of IL12 activity, combined with instantaneous and complete re-activation upon reaction with tetrazine (see also bottom figure).

(Middle) Detection of IL12-TCO-PEG$_2$ or IL12-TCO-PEG$_{1K}$ constructs using a hIL12-p70 ELISA assay. Shown is the inability to detect masked cytokine constructs (filled symbols), while release of the masks enables a concentration dependent detection similar to native IL12 (open symbols).

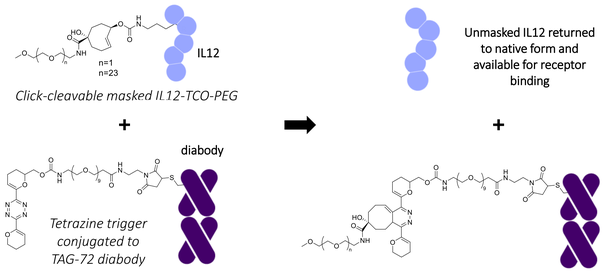


(Bottom) HEK-Blue IL12 reporter bioactivity assay using IL12-TCO-PEG$_2$ constructs, with (open symbols) and without (filled symbols) activation by free Tz, or Tz conjugated to TAG-72-targeting diabody.

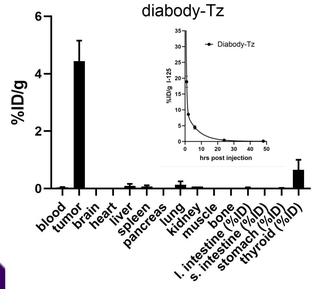


TAG-72:
A pan-carcinoma marker consisting of STn and Tn glycans presented by several mucins. It was shown to be a clean and stable, non-internalizing target in clinical radio-immunotherapy

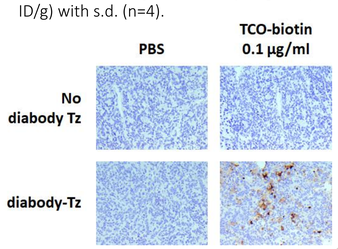
Click-to-Release designs: IL12 prodrug and trigger



Pre-localization of trigger

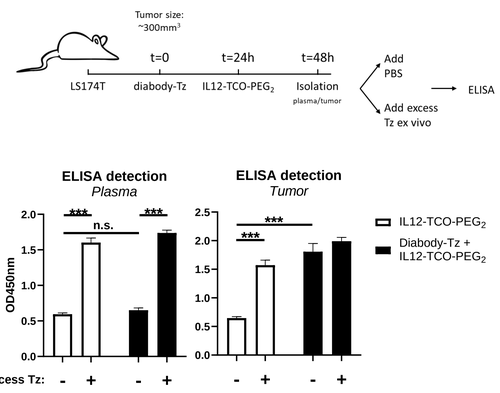


(Right) The presence of reactive Tz in the tumor was detected by TCO-PEG4-biotin on 5 µm tissue slides.



On-tumor IL12 unmasking

Diabody-Tz was injected i.v. in mice bearing LS174T tumors (40µg/mouse). 24 hrs later, when the diabody was virtually cleared from blood, TCO-masked IL12 was injected i.v. (10µg). 24 hrs later, plasma was collected, and tumors were isolated and homogenized. Directly before the ELISA, some samples received an excess of free Tz to release all unreacted masks (n=4).
Results show no IL12 unmasking in plasma *in vivo* due to low blood level of Tz at the time of IL12-TCO-PEG$_2$ injection (left panel). In contrast, the IL12 construct was locally unmasked by pre-localized Tz in the tumor (right panel).



Conclusions

- IL12 is effectively masked by conjugation to TCO-PEG (attenuation >100x).
- Employing the Click-to-Release technology, IL12 structure & bioactivity can be fully and instantaneously restored when reacted with the trigger.
- In tumor-bearing mice, a TAG-72-directed diabody conjugated to tetrazine could efficiently pre-localize trigger compound to the tumor.
- Masked IL12, injected upon systemic clearance of the diabody, was effectively reactivated locally in the tumor, but importantly, not in blood.

These results demonstrate the versatility of the Click-to-Release system. Next, *in vivo* therapy studies will showcase the therapeutic potential of our IL12 prodrug.

TAG WORKS

CONTACT
Tagworks Pharmaceuticals BV
The Netherlands
T +31 85 800 8550
www.tagworkspharma.com

REFERENCES
-den Brok et al.; Masked IL12 protein. Patent WO2023158305A1
-Robillard et al.; Compounds for fast and efficient click release, Patent WO2020256546
-Svatunek et al.; Uncovering the key role of distortion in bioorthogonal tetrazine tools that defy the reactivity/stability trade-off. J Am Chem Soc. 2022 May 11;144(18):8171-8177.

Click. Release. Treat.