Poster 1890

A solution to T-cell engager toxicity: An anti-CD3 Prodrug DARPin® (CD3-PDD) shows no toxicity, but potent anti-tumor activity in a humanized mouse model

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Introduction

- T-cell engagers (TCEs) direct cytotoxic T-cell response towards tumor cells by binding simultaneously to a tumor-associated antigen (TAA) on target cells and to CD3 on T-cells
- They are very potent anti-tumor drugs, as exemplified by blinatumomab, an α-CD19 x α-CD3 bispecific
- Development of TCEs for hematological and solid tumors has been hampered by several factors, amongst them severe toxicity, elicited by on-target / off-tumor recruitment of T-cells and cytokine release syndrome (CRS)
- Molecular Partners has developed an anti-CD3 Prodrug DARPin® (CD3-PDD), consisting of a human-mouse cross-reactive EGFR-binder and a CD3-binder, linked via a protease-cleavable linker to an anti-idiotypic DARPin domain (termed Blocker hereafter) masking the CD3 effector function
- This α-EGFR x α-CD3 x Blocker Prodrug is unable to bind and recruit T-cells in its non-cleaved state in circulation, but is designed to become activated in the tumor microenvironment (TME) upon cleavage of the linker by tumor-associated proteases

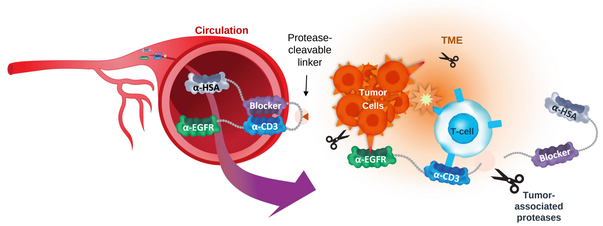


Figure 1: Cartoon illustrating the principle of an anti-CD3 Prodrug DARPin® (CD3-PDD). In circulation, CD3-PDD is inactive due to masking of the anti-CD3 binding domain by an anti-idiotypic DARPin® domain (Blocker), connected via a protease-cleavable linker. Upon cleavage of the linker in the tumor microenvironment (TME), the [Blocker x α-HSA] diffuses away, releasing active [α-EGFR x α-CD3] TCE locally. Once activated, the TCE is short-lived due to lack of half-life extension, ensuring minimal exposure of active TCE outside of the tumor.

CD3-PDD toolbox

- Half-life extension via HSA-binding DARPin® domain to provide IgG-like pharmacokinetics
- Upon activation half-life extension is lost and active TCE is quickly eliminated in circulation → **increased safety**

- Human/mouse cross-reactive EGFR binder used for this PoC study, allowing efficacy and safety assessment *in vivo*
- Tumor associated antigen (TAA)-binders can be readily exchanged → **format flexibility**

- Set of different Blockers against CD3-binder available for optimal combination
- Affinity range: from K_D = low pM to > 500 nM → **optimal blocking strength**

- Cleavable linker (CL) efficiently cut by multiple proteases which are dysregulated in many tumor indications → **local activation**

- Set of CD3-binder ready with different affinities against CD3
- Affinity-range: from K_D = single digit nM to > 100 nM → **optimal efficacy**

Results *in vitro*

CD3 binding is efficiently masked *in vitro* for CD3-PDD, whereas EGFR-binding is not impaired

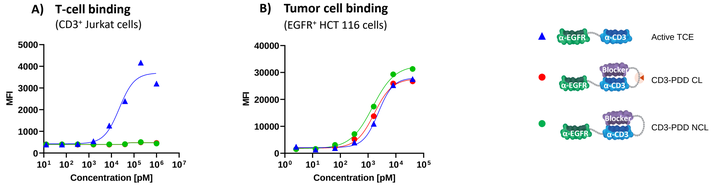


Figure 2: Cell binding of active TCE, cleavable CD3-PDD CL and non-cleavable CD3-PDD NCL to A) Jurkat cells and B) HCT 116 tumor cells. Binding was detected by fluorescently labeled anti-DARPin® mAb and measured by flow cytometry.

Efficient inhibition of T-cell activation and tumor cell killing in masked Prodrug constructs

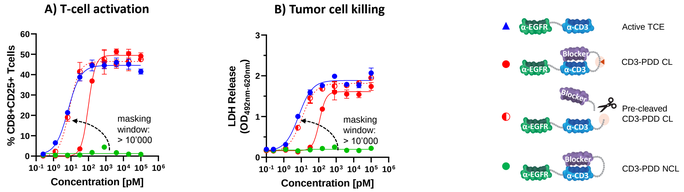


Figure 3: Masking of α-CD3 reduces T-cell activation and tumor cell killing to background if a non-cleavable linker connects Blocker and CD3-binding domain. Partial activation of T-cells and tumor cell killing of construct CD3-PDD CL (cleavable linker) are attributed to linker cleavage in the *in vitro* assay. Pre-cleavage of CD3-PDD CL fully restores its activation and killing activity. HCT 116 tumor cells were incubated with pan T-cells at a E:T ratio of 5:1 for 48h together with the test article; surface marker CD25 expression (of $CD8^+$ T-cells) was determined by flow cytometry and tumor cell killing was inferred from LDH release.

Both tumor target expression and protease cleavage are required for tumor cell killing by CD3-PDD CL

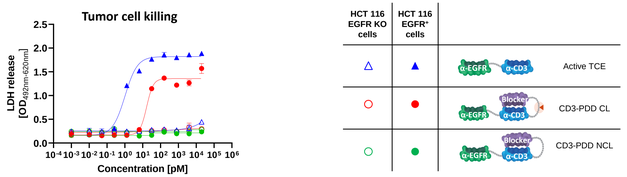
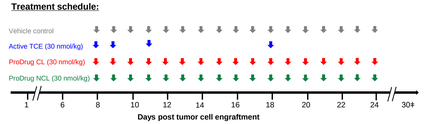


Figure 4: Killing of tumor cells HCT 116 wild-type and HCT 116 with EGFR knock-out, by active TCE, cleavable CD3-PDD CL or non-cleavable CD3-PDD NCL. For CD3-PDD, tumor cell killing is only observed in presence of EGFR and with cleavable linker activated via tumor-associated proteases. EGFR knockout in HCT 116 cells was generated via CRISPR-Cas9 and absence of EGFR was confirmed by flow cytometry.

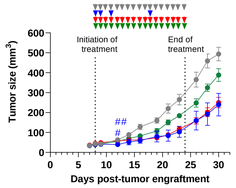
Results *in vivo*

Cleavable CD3-PDD with cross-reactive EGFR binder shows good tumor control in mice, while avoiding toxicity of the active TCE

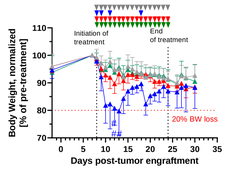
- NOD/Shi-*scid*/IL-2Rγ^{null} immunodeficient mouse strain
- Humanized with CD34$^+$ human hematopoetic stem cells (hHSC)
- N=6 per group
- HCT 116 tumor cell engraftment

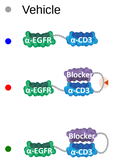


A) CD3-PDD CL demonstrates robust anti-tumor activity, similar to active, non-blocked TCE

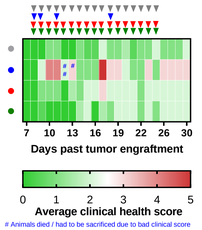


B) CD3-PDD CL can be dosed safely, but active TCE shows frank toxicity





C) CD3-PDD is well tolerated, whereas active TCE is toxic



D) CD3-PDD exhibits no cytokine release 4h after first treatment, whereas active TCE triggers cytokine release

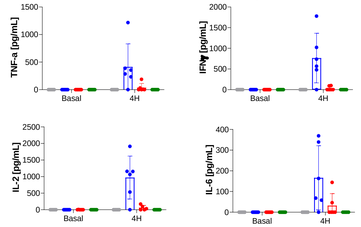


Figure 5: *In vivo* safety and efficacy study with constructs containing human-mouse cross-reactive EGFR binder in mice humanized with hematopoietic stem cells (CD34$^+$) and engrafted with HCT 116 tumor cells. Treatment is indicated by arrows for each group. Treatment of active TCE had to be suspended after the second injection due to frank toxicity. **A)** Cleavable CD3-PDD CL showed robust anti-tumor effect, comparable to active TCE over the course of treatment (D8 – D24). Error bars are SEM of n=6 (except active TCE, n=3 due to loss of 3 mice at D12 and D13). **B-C)** Daily dosing of active TCE lead to strong body weight loss and deteriorating clinical score, 3 mice died or had to be sacrificed due to frank toxicity (indicated by #) at D12 and D13. **D)** Cytokine levels before first test article injection and 4h after were determined on plasma samples by CBA human Th1/Th2/Th17 kit.

Summary and Conclusion:

- ***In vitro* and *in vivo* proof-of concept demonstrated for DARPin® protein-based protease-activatable Prodrug (CD3-PDD) platform**
- **DARPin® protein-based CD3-PDD platform is very attractive to improve the benefit/risk ratio of highly potent TCEs and enable the use of less tumor-specific targets**
- **Coming soon: Prodrug concept expanded beyond protease activation → CD3-slow release DARPin® (CD3-SRD) as a solution for reducing cytokine release syndrome (CRS) of active TCE**

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