A-340

CD3-slow release DARPin® (CD3-SRD) concept – a smart solution for reducing cytokine release syndrome while maintaining optimal anti-tumor efficacy

Julia K.J. Ahlskog[1,2] and Andreas Bosshart[1], Dieter Schiegg, Aline Eggenschwiler, Simon Fontaine, Denis Villemagne, Marco Franchini, Mirela Matzner, Waleed Ali, Martin Hänggi, Yvonne Kaufmann, Iris Schlegel, Christof Zitt, Tanja Hospodarsch, Alexandra Neculcea, Tamara Lekishvili, Heidi Poulet, Maria Paladino, Christel Herzog, Anja Schlegel, Thamar Looser and Bernd Schlereth

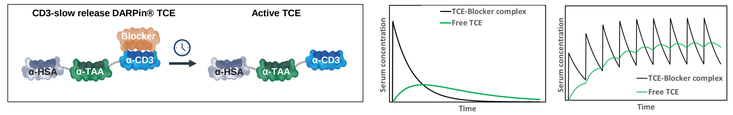
[1] These authors contributed equally to the work
[2] Corresponding author

Molecular Partners AG, Zürich, Switzerland

Introduction

Concept: Slow release of active T-cell engager (TCE) to avoid high C_{max}, continuously increase bioactivity, and ultimately reduce risk for toxicity

- T-cell engagers (TCEs) are a very potent class of drugs, but may exhibit acute toxicities, such as cytokine release syndrome (CRS) especially after the first dose, endothelial activation and neurotoxicity*
- Current mitigation strategies include pre-medication with immune-suppressive steroids, treatment with IL-6 receptor antagonists, step-dosing or continuous intravenous infusion (CIV)
- We have developed a CD3-slow release DARPin® (CD3-SRD) concept representing a solution for reducing CRS while maintaining optimal anti-tumor efficacy



Single dose
Conceptual representation

Repeat dose mimics CIV and step dosing
Conceptual representation

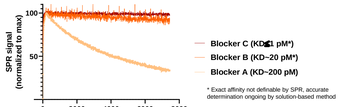
Figure 1: Slow release concept: dissociation of non-covalently bound, high affinity Blocker from TCE-Blocker complex (hereafter termed CD3-slow release DARPin® TCE) over time leads to slow activation of TCE in circulation

* Shimabukuro-Vornhagen et al. 2018, Goebeler and Bargou 2020

CD3-SRD TCE toolbox

Generation of Blockers with various affinities to explore the concept

- Off-rate (k_{off}) of Blocker from CD3-binder defines the release rate of active TCE
- A panel of Blockers with a range of high affinities was generated (KD≤1 pM to KD~200 pM)

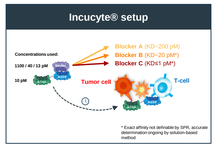


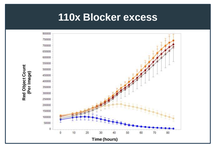
* Exact affinity not definable by SPR, accurate determination ongoing by solution-based method

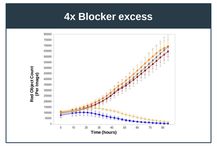
Figure 2: Off-rate (k_{off}) of Blockers determined by Surface Plasmon Resonance (SPR) measured at room temperature. Blocker C displays the slowest off-rate and the lowest equilibrium dissociation constant (KD), while Blocker A displays the fastest off-rate and the highest KD value

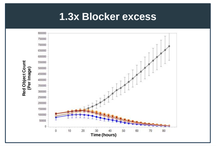
Results *in vitro*

Blocker off-rate defines release rate of TCE









- No TCE, Blocker only
- DARPin® TCE
- DARPin® TCE + Blocker A
- DARPin® TCE + Blocker B
- DARPin® TCE + Blocker C

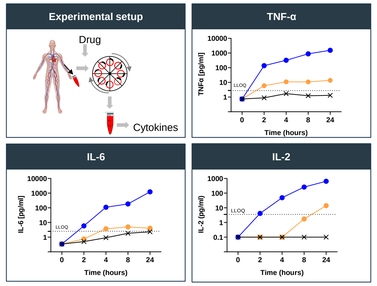
Conclusion:

- Slow release concept works *in vitro*. Blockers B and C with low pM affinity can block activity of DARPin® TCE efficiently when used at ≥4x excess

Figure 3: Incucyte® experiment comparing Blockers of various affinities for their ability to block T-cell mediated tumor growth inhibition (used as proxy for T-cell mediated tumor cell killing) of TAA-expressing tumor cells over time. Incucyte® read-out: Total red object count (corresponding to tumor cells) was determined at regular intervals. Error bars show standard error per image (36 images per well).

Results *ex vivo*

CD3-SRD TCE displays delayed and reduced cytokine release *ex vivo*



- ✕ Vehicle
- DARPin® TCE
- DARPin® TCE + Blocker A

Conclusion:

Slow release concept works *ex vivo*. Blocker A (KD~200 pM) reduces the cytokine release profile of the DARPin® TCE

Figure 5: *Ex vivo* assay with 1 nM DARPin® T-cell engager vs. 1 nM DARPin® TCE+1.2 nM Blocker A spiked into fresh human whole blood (containing target cells). Cytokine release was determined at 0h, 2h, 4h, 8h, 24h. Data is from a single experiment performed at Immuneed AB with 1 human blood donor. Cytokine release was determined by Meso Scale MULTI-ARRAY® technology.

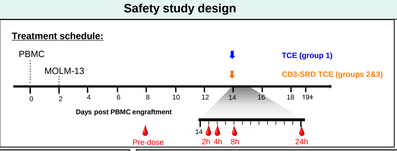
Results *in vivo*

Study aim: Evaluate impact of Blocker affinity on cytokine release *in vivo*

Safety study design

- NOG mice (immunodeficient)
- PBMC-humanized (2 donors)
- Tumor cells: MOLM-13
- **Tumor size at treatment start: ~300-800 mm³** → to have sufficient TAA expression for human-specific TCE to trigger cytokine release

Treatment schedule:



Treatment groups:

1) DARPin® TCE 1000 µg/kg
2) DARPin® TCE 1000 µg/kg + 2x molar excess Blocker B
3) DARPin® TCE 1000 µg/kg + 2x molar excess Blocker C

TCE

DARPin® TCE

CD3-slow release DARPin® TCE

DARPin® TCE + Blocker

* Exact affinity not definable by SPR, accurate determination ongoing by solution-based method

Blockers reduce cytokine release *in vivo* in an affinity-dependent manner

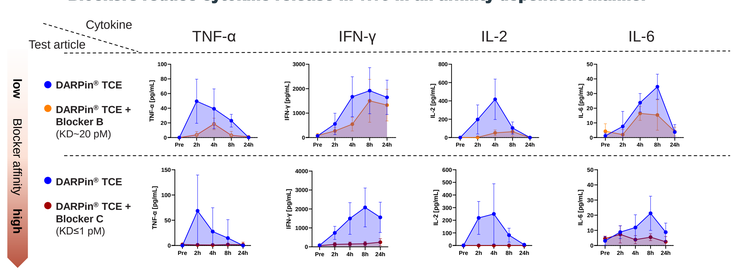


Figure 7: Cytokine levels (TNF-α, IFN-γ, IL-2 and IL-6) in blood samples taken prior or post a single dose treatment with 1000 µg/kg DARPin® TCE or 1000 µg/kg DARPin® TCE + Blocker B or C in two independent *in vivo* safety studies with established tumors (300-800 mm³). Cytokine levels are plotted as mean ± SD for n=10 mice (except for DARPin® TCE lower row, n=9) humanized with PBMCs from 2 donors (4 or 5 mice each). Cytokine levels were determined on serum samples by CBA human Th1/Th2/Th17 kit.

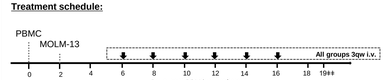
Results *in vivo*

Study aim: Evaluate impact of Blocker affinity on anti-tumor efficacy *in vivo*

Anti-tumor efficacy study design

- NOG mice (immunodeficient)
- PBMC-humanized
- Tumor cells: MOLM-13
- **Tumor size at treatment start: ~70 mm³**

Treatment schedule:



Treatment groups:

1) Vehicle
2) DARPin® TCE 200 µg/kg
3) DARPin® TCE 1000 µg/kg
4) DARPin® TCE 1000 µg/kg + 2x molar excess Blocker B
5) DARPin® TCE 1000 µg/kg + 2x molar excess Blocker C

TCE

DARPin® TCE

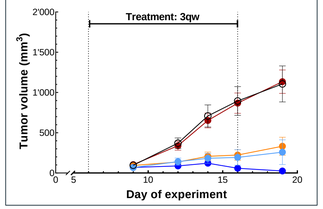
CD3-slow release DARPin® TCE

DARPin® TCE + Blocker

* Exact affinity not definable by SPR, accurate determination ongoing by solution-based method

Ideal Blocker affinity to hit the sweet spot depends on serum half-life of TCE

Donor 1 and 2



- Vehicle
- DARPin® TCE, 200 µg/kg*
- DARPin® TCE, 1000 µg/kg
- DARPin® TCE 1000 µg/kg + 2x molar excess Blocker B
- DARPin® TCE 1000 µg/kg + 2x molar excess Blocker C

** Modeling predicts similar AUC between 200 µg/kg free TCE and 1000 µg/kg transiently blocked TCE*

Conclusion:

- Slow release concept works *in vivo*:
  - Blocker B (KD~ 20 pM) shows reduced cytokine release profile and good anti-tumor activity
  - Blocker C (KD≤1 pM) shows no cytokine release but quenches anti-tumor activity (in combination with DARPin® TCE used here)
- Sweet spot in terms of Blocker affinity strongly depends on serum half-life of TCE → CD3-SRD toolbox allows to pick the best matching Blocker

Figure 6: Tumor growth curves of *in vivo efficacy* study comparing anti-tumor activity of DARPin® TCE to DARPin® TCE in complex with Blockers B or C in MOLM-13 tumor-bearing, PBMC engrafted NOG mice. Tumor growth curves are plotted as mean ± SEM for n=10 mice humanized with PBMCs from 2 donors (5 mice each). Treatment period starting on d6 and ending on d16 is indicated.

Summary and Conclusion:

- We have developed a CD3-slow release DARPin® (CD3-SRD) concept demonstrating that dissociation of a non-covalently bound, high affinity Blocker from a TCE leads to controlled release of active TCE in circulation over time
- *Ex vivo* and *in vivo* experiments demonstrate reduction of cytokine release for our transiently blocked TCEs, improving the safety of this very potent class of drugs, while maintaining good anti-tumor activity
- We claim that the generic concept of slow release is also applicable to other drug modalities and other effector functions where slower onset of drug effect is desired

Molecular Partners AG, Wagistrasse 14, 8952 Zurich-Schlieren, Switzerland – www.molecularpartners.com – info@molecularpartners.com – phone: +41 44 755 77 00