Poster 525

Novel multi-specific DARPin® T-cell engager with an improved therapeutic window to overcome dose limiting toxicities in acute myeloid leukemia (AML) therapy

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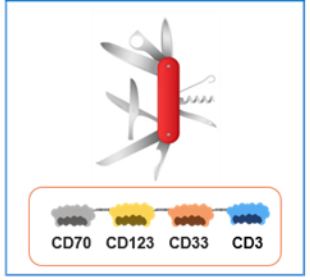
T-cell engagers in AML: promises and challenges

In AML medical need remains high. The treatment of relapsed or refractory (r/r) AML is challenging due to the heterogeneous nature of the disease and to high relapse rates with current standard-of-care[1].

Various highly potent single-targeting T-cell engager (TCE) and CAR-T therapies have entered clinical development, but are often accompanied by dose limiting toxicities (DLTs), such as cytokine release syndrome (CRS) and myelotoxicities, that exclude robust anti-tumor efficacy[1,2].

More selective therapies and rationally designed target combinations are desperately needed to allow for extended dose escalation with a more acceptable safety profile to achieve durable responses.

Multispecific DARPin® TCE for better safety and increased efficacy



[1]Daver *et al.* Blood Cancer Journal (2020) 10:107 [2]Guy *et. al.* Curr Hematol Malig Rep. (2018) 13(6): 417–425.

The DARPin® Solution: an avidity driven multi-specific DARPin® T-cell engager

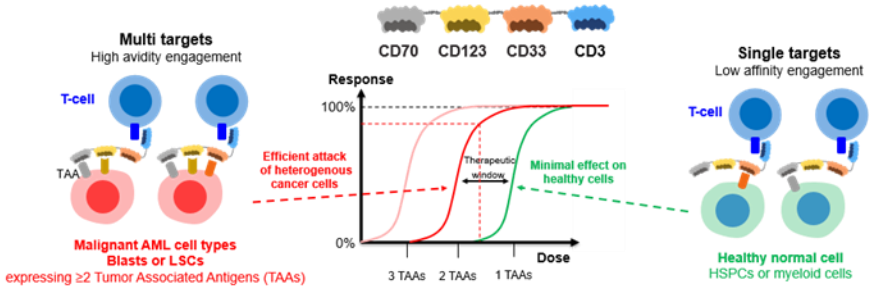


Figure 1. The concept of a multi-specific avidity driven DARPin® T-cell engager in AML

Optimizing the affinity of individual linked TAA binders utilises the avidity effect to deliver high affinity binding in the presence of ≥2 TAA targets on AML cell types e.g. blasts or leukemic stem cells (LSCs), but low affinity binding in the presence of single TAA presenting cell types e.g. hematopoietic stem and progenitor cells (HSPCs). This should reduce effects on off-target healthy cells, increasing the safety window, but still allow elimination of heterogenous malignant cells expressing 2 or 3 TAAs, thereby providing a novel AML treatment modality with an improved benefit/risk profile (middle graph).

Multiple dimension screening for optimal therapeutic window

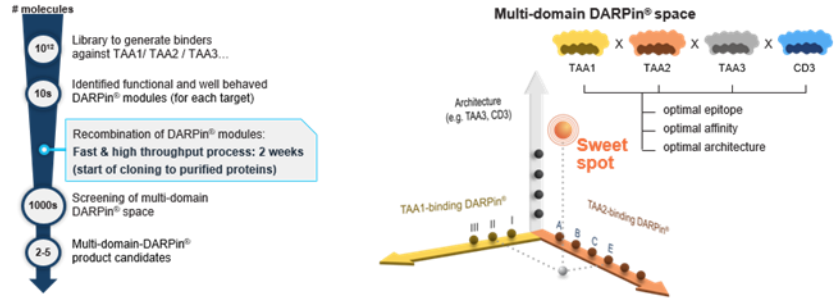


Figure 2: Leveraging our unique DARPin® platform to generate multi-specific TCE DARPin® molecules

Identification of an avidity driven multi-specific DARPin® molecule, which binds to multiple different selected TAAs and is conjugated to a CD3-binding DARPin® molecule. The multi-dimensional space based on different TAAs, affinities, epitopes and concatenation architectures was extensively sampled using random and/or rational assembly of multi-specific DARPin® molecules. Constructs were produced in 96-well format and screened for T-cell activation.

The multi-specific DARPin® T-cell engager is characterized by strong avidity gain *in vitro*

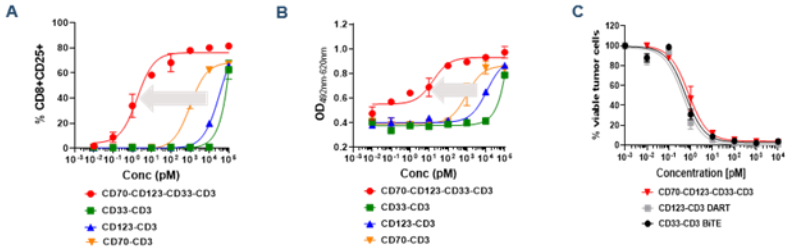


Figure 3: In vitro characterization of the multi-specific DARPin® T-cell engager

Multi-specific DARPin® T-cell engager with significant activity gain in: A) T-cell activation compared with single targeting controls (grey arrow). Molm-13 AML cells in co-culture with human T-cells (E:T 1:1, 24 h), T-cell activation analyzed by flow cytometry detecting CD25+ CD8+ T-cells; B) tumor cell killing compared with single targeting controls (grey arrow). Molm-13 AML cells in coculture with human T-cells (E:T 5:1, 48 h), tumor cell killing analyzed by LDH release, and C) showing similar potency and efficacy compared to competitor molecules currently tested in the clinic. Molm-13 AML cells in coculture with human T-cells (E:T 5:1, 48 h), tumor cell killing analyzed by absolute count of living cells by flow cytometry. DART, dual affinity re-targeting; BiTE, bispecific T-cell engager

The multi-specific DARPin® TCE counteracts tumor heterogeneity and increases selectivity

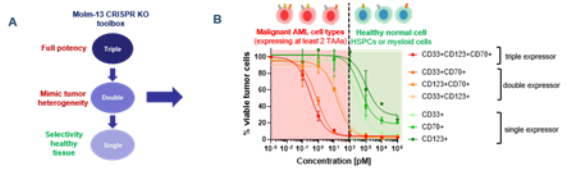


Figure 4: A multi-specific DARPin® T cell engager to potentially counteract tumor heterogeneity and increase selectivity

A) CRISPR knockout (KO) toolbox to analyse a multi-specific DARPin® TCE: Parental cell line/triple expressor (Triple) for full potency; Single KO/Double expressor (Double) for the potential to counteract tumor heterogeneity; Double KO/single expressor (Single) for selectivity towards healthy tissue.
B) multi-specific DARPin® TCE is highly active when 3 (triple expressor) or 2 targets (dual expressor) are present suggesting potential to counteract tumor heterogeneity. Multi-specific DARPin® TCE shows selectivity window in the presence of only one target (single expressor) suggesting selectivity towards healthy tissue. Molm-13 AML parental and CRISPR KO cells in coculture with human T-cells (E:T 5:1, 48 h), tumor cell killing analyzed by absolute count of living cells by flow cytometry.

The multi-specific DARPin® TCE shows a better safety profile

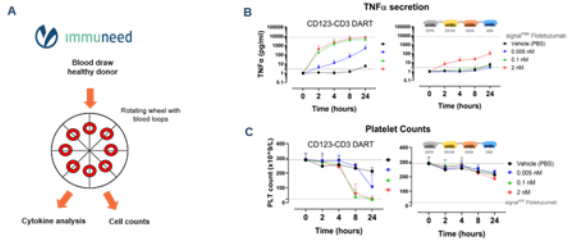


Figure 5: A multi-specific DARPin® T-cell engager to potentially counteract tumor heterogeneity and increase selectivity

A) Blood Loop Technology by Immuneed. Multi-specific DARPin® T-cell engager shows preferential safety profile of B) TNFα secretion measured by MULTI-ARRAY® technology from Meso Scale Discovery (MSD) and C) Platelet count analyzed by flow cytometry when compared to a CD123-CD3 DART molecule. DART, dual affinity re-targeting

Conclusions

Our unique modular DARPin® platform enables the efficient screening and generation of affinity tailored, multi-specific, avidity driven molecules

The optimized multi-specific DARPin® TCE shows similar *in vitro* potency as benchmarks on target cells expressing various combinations of at least 2 TAA, but preserves cells expressing 1 TAA only (e.g. healthy cells)

A whole blood assay shows a preferential safety profile of the multi-specific DARPin® TCE by cytokine secretion and platelet count, supporting the low affinity engagement of healthy single expressing cells and improved therapeutic window

Our data encourage the development of this multi-specific DARPin® TCE to ultimately tackle the dose limiting toxicities of TCEs in the clinic

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