Avidity-Engineered CD3 Engaging DARPin Targeting Three Tumor Associated Antigens Induces Strong and Specific T Cell Dependent Killing of AML Cells with Potential for Improved Safety

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Introduction

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.

[1]Daver et al. Blood Cancer J. (2020), [2]Guy et. al. Curr Hematol Malig Rep. (2018)

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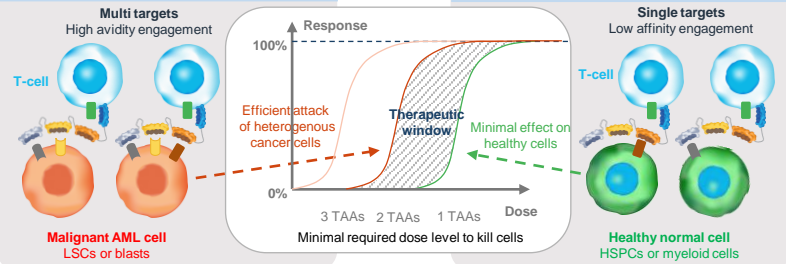

Figure 1. The concept of a multi-specific avidity driven DARPin T-cell engager in AML. Optimizing the affinity of individual linked Tumor-Associated Antigen (TAA) binders utilizes the avidity effect to deliver high affinity binding in the presence of ≥2 TAA targets on AML cell types e.g. blasts or leukemic 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.

Target selection and molecule design

The Tumor Associated Antigens (TAAs) selected for our multispecific DARPin MP0533 are **CD70**, **CD123** and **CD33**. They are clinically validated targets in AML:

- CD33 is expressed on ~80-90% of AML blasts and on LSCs[1,2]
- CD123 is expressed on ~70-80% of AML blasts and is more specific for LSCs than CD33[1,2]
- CD70 is expressed on ~86-100% of AML blasts and is highly LSC specific[3,4]

While they are also expressed on healthy cells, leading to on-target off-tumor toxicity with mono-specific TCE, their co-expression pattern on LSCs and blasts differs from healthy cells..

[1]Ehninger et al. Blood Cancer J. (2014), [2]Haubner et. al. Leukemia (2019), [3]Perna et. al. Cancer Cell (2017), [4]Riether et. al. Nat Med (2020)
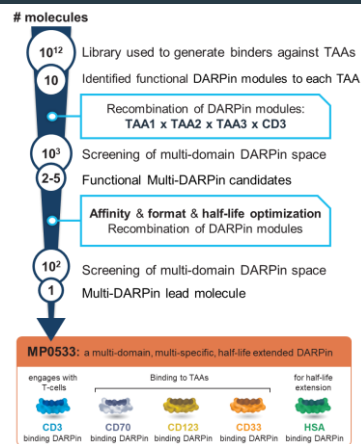
Potency and selectivity of MP0533 on AML cell line Molm-13
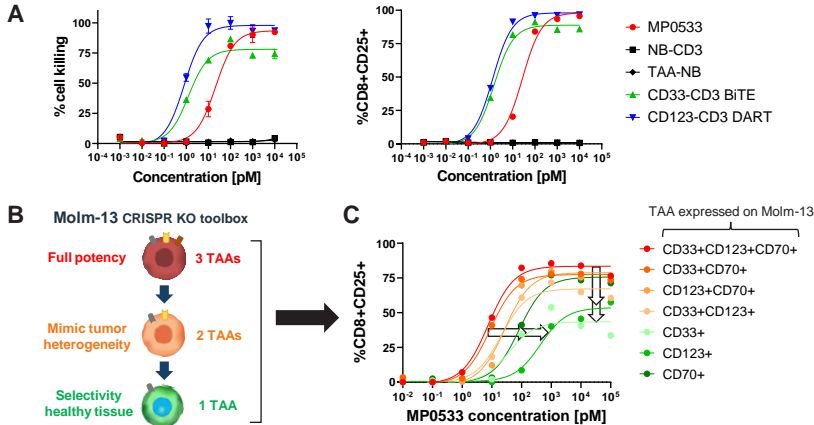

Figure 2: In vitro characterization of MP0533. A) Molm-13 AML cells in co-culture with human T-cells (E:T of 5:1, 48 h) showing normalized tumor cell killing analyzed by LDH release (left) and T-cell activation by flow cytometry detecting CD8+CD25+ T-cells (right). B) Schematic showing Molm-13 CRISPR knockout (KO) toolbox to analyze a multi-specific DARPin TCE: Parental cell line (Triple TAA) for full potency; Single KOs (Double TAA) for the potential to counteract tumor heterogeneity; Double KOs (Single TAA) for selectivity towards healthy tissue. C) MP0533 is highly active when 3 or 2 targets (red and orange, resp.) are present suggesting potential to counteract tumor heterogeneity. MP0533 shows both selectivity (horizontal arrows) and efficacy (vertical arrows) windows in the presence of only one target (green) suggesting selectivity towards healthy tissue. Molm-13 AML parental and CRISPR KO cells in co-culture with human T-cells (E:T of 5:1, 48 h), T-cell activation analyzed by flow cytometry detecting CD8+CD25+ T-cells. NB = Non-Binding control. CD33-CD3 BiTE and CD123-CD3 DART are based on AMG330 and MGD006 molecules, respectively.

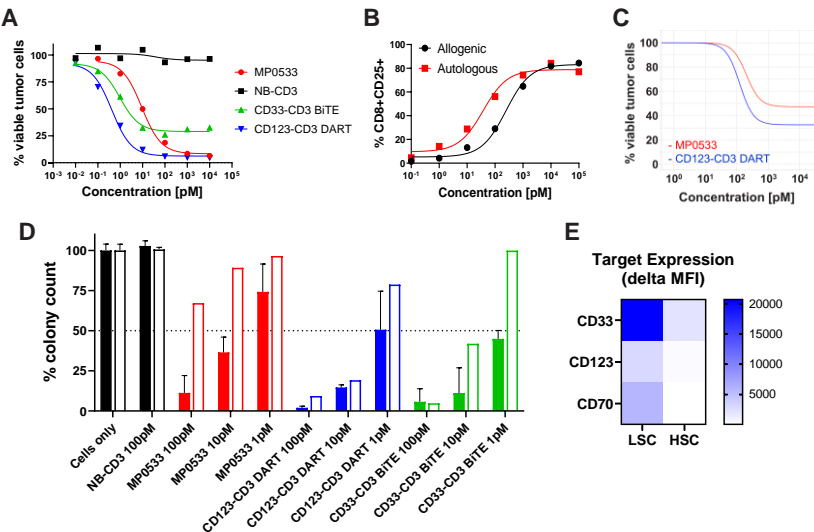## Potency and selectivity of MP0533 on primary AML cells

Figure 3: Ex vivo characterization of MP0533. BMMC from an AML patient in co-culture with allogenic human T-cells (E:T of 4:1, 48 h) showing A) normalized tumor cell killing and B) allogenic and autologous CD8+CD25+ T-cells analyzed by flow cytometry. C) Autologous tumor cell killing assay performed by Vivia Biotech showing the median fit of killing at 120 h from 5 AML BMMC samples (median E:T of 1:80). D) Colony Forming Unit (CFU) assay. CD34+ sorted cells from two AML (LSC) or one healthy donor (HSC) were incubated in co-culture with allogenic human T-cells (E:T of 1:1) for 4 to 6 allow killing, then cells were plated in semi-solid media and further cultured for 2 weeks until CFU could be counted. CFU count is normalized to untreated control (cells only) and shows preferential killing of LSC (solid bars) over HSC (empty bars). E) Target expression of samples reported in D) analyzed by flow cytometry, shown as average of delta Median Fluorescence Intensity (MFI) values. NB = Non-Binding control. CD33-CD3 BiTE and CD123-CD3 DART are based on AMG330 and MGD006 molecules, respectively.
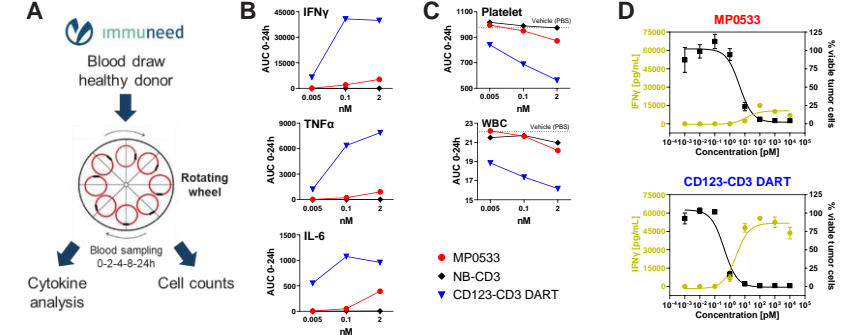
Low cytokine release profile of MP0533

Figure 4: Cytokine release and cell depletion in healthy whole blood and cytokine release in in-vitro tumor cell killing assay. A) Schematic of Immuneed's Whole Blood Loop Technology. B) Low cytokine release and C) cell depletion induced in healthy whole blood suggest preferential safety profile of MP0533. Data shown as Area Under the Curve (AUC) of cytokine levels or cell count over time (0-24 h), compared to a CD123-CD3 DART competitor molecule. Cytokines measured by MULTI-ARRAY® technology from Meso Scale Discovery (MSD), hematology analysis was performed by Hematology Analyzer Sysmex XN-L350. NB = Non-Binding control. CD123-CD3 DART is based on MGD006 molecule. D) Molm-13 AML cells in co-culture with human T-cells (E:T of 5:1, 48 h) showing normalized tumor cell killing (analyzed by flow cytometry) and IFNγ released in the corresponding supernatants (measured by Multiplex MSD).

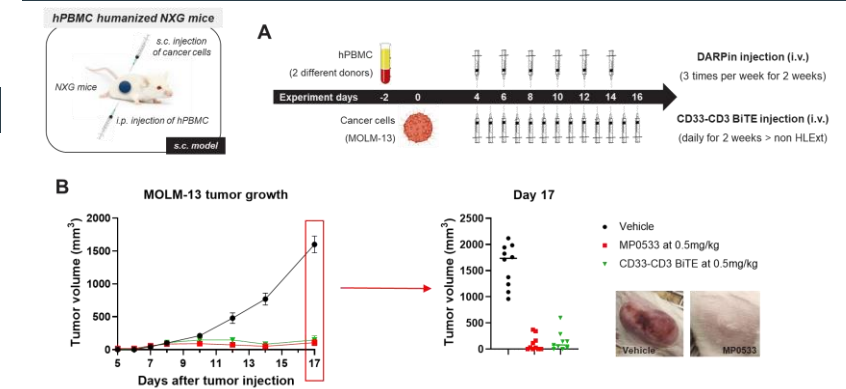## In vivo efficacy of MP0533 against Molm-13 tumor

Figure 5. MP0533 antitumor activity in a MOLM-13 xenograft mouse model. A) Mice were xenografted with hPBMC (i.p) and MOLM-13 (s.c.) as described in the protocol design. B) MOLM-13 tumor growth over time (links) and at day 17 (right), also shown in representative images. CD33-CD3 BiTE is based on AMG330 molecule.

Conclusions

- Our unique modular DARPin platform enables the generation of affinity tailored, multi-specific, avidity driven molecules
- MP0533, an avidity-driven multi-specific DARPin TCE, shows high potency on AML cell lines expressing at least 2 TAA, and both selectivity and efficacy windows towards cells expressing 1 TAA only
- MP0533 also potently kills primary AML cells, both in allogenic and autologous setting, and shows a therapeutic window by preferentially killing AML LSC over healthy HSC
- Ex-vivo whole blood and in-vivo tumor cell killing assays show a preferential safety profile of MP0533 towards cytokine secretion and cell depletion (platelet and WBC), supporting an improved therapeutic window of MP0533
- MP0533 demonstrates anti-tumor activity in human PBMC-reconstituted mice
- Our data supports the development of MP0533 as a unique therapeutic solution for the treatment of AML, potentially able to tackle the dose limiting toxicities of TCEs in the clinic