Poster
P481

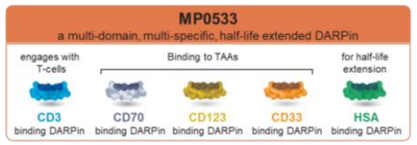
MP0533, A New Multispecific DARPin CD3 Engager Targeting Three Tumor Associated Antigens Induces Specific T-cell Activation And AML Tumor Killing *In Vivo*

Amelie Croset, Marco Franchini, Tamara Lekishvili, Yvonne Kaufmann, Alienor Auge, Martin Hänggi, Waleed Ali, Christof Zitt, Stephan Wullschleger, Mirela Matzner, Matteo Bianchi, Christian Reichen, Stefanie Fischer, Yvonne Grübler, Aline Eggenschwiler, Thamar Looser, Patricia Spitzli, Vladimir Kirkin, Daniel Steiner, Anne Goubier
[1]Molecular Partners AG, Wagistrasse 14, 8952 Zürich-Schlieren, Switzerland

Introduction

AML treatment options are generally focused on chemotherapy followed by allogeneic hematopoietic stem cell transplantation. However, many patients are ineligible for these options and often receive palliative treatments with poor long-term survival and there is an urgent need for improved therapeutic solutions. Newer therapies, including T cell engagers (TCE) and chimeric antigen receptor (CAR) T cells that target specific molecules overexpressed on leukemic blasts and stem cells are promising alternative treatment options for AML. However, achieving efficacy and low toxicity remains challenging.

MP0533 is a multi-specific, half-life extended, T cell engaging DARPin in development for treatment of patients with AML and higher risk myelodysplastic syndrome (HR-MDS). MP0533 consists of a chain of six covalently linked DARPin domains.



Three of these domains engage CD33, CD123 and CD70 on the target cells, utilizing an avidity driven approach to ensure that binding occurs preferentially when two or more TAAs are present. A fourth DARPin domain targets CD3 for T-cell engagement. Lastly two additional HSA binding DARPin domains prolong half-life in circulation.

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]. Both CD33 and CD123 are also expressed on healthy myeloid cells, and CD123 additionally on endothelial cells, potentially leading to hematological toxicity and endothelial damage observed in the clinic when targeted with potent mono-specific TCEs.

In vitro, MP0533 has demonstrated, in both allogeneic and autologous settings, single to double digit pM potency against AML cell lines and AML primary cells expressing any combination of at least 2 of the 3 targeted TAAs and low cytokine release *(data presented at ASH2021 conference – poster 1164).*

[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)

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

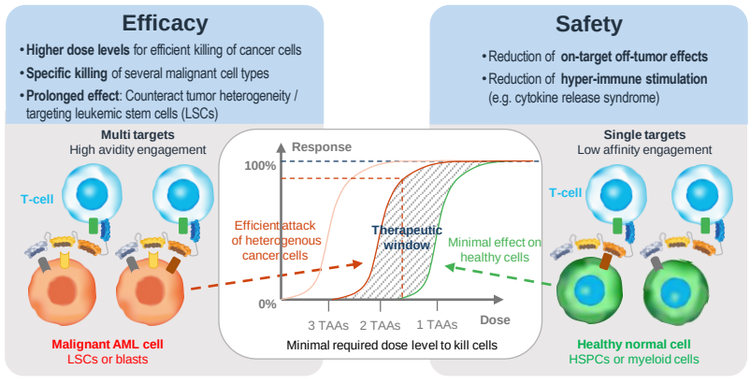


Figure 1. The concept of a multi-specific avidity driven DARPin® T-cell engager in AML. Optimizing the affinity of individual linked Tumor-Associated Antigen (TAA) binders utilizes the avidity effect to deliver high affinity binding in the presence of ≥2 TAA targets on AML cell types e.g. blasts or leukemic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

Potency of MP0533 on AML MOLM-13 tumors

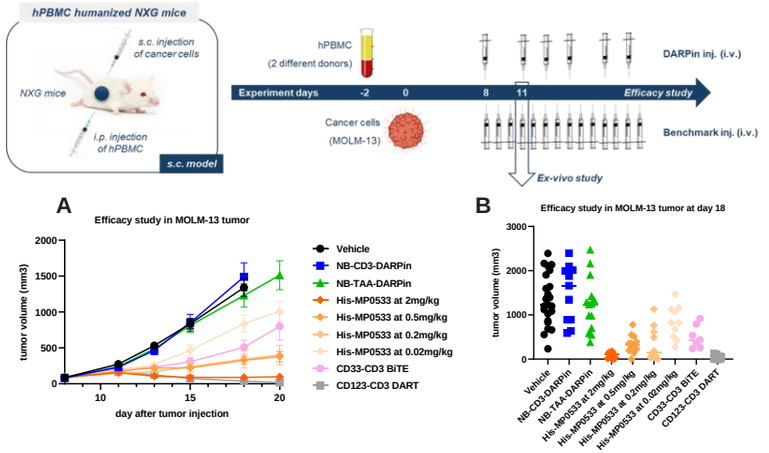
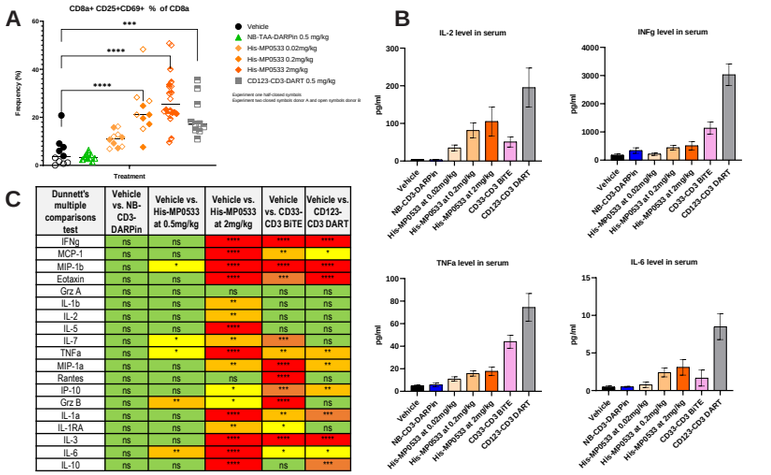


Figure 2. His-MP0533 efficacy in MOLM-13 tumors. Tumor growth over time (A) or tumor volume at day 18 (B). Mice were injected intraperitoneally with hPBMC (n=5 mice per donor / 2 to 6 hPBMC donors used depending of the treatment), xenografted subcutaneously with MOLM-13 cells and treated with vehicle, NB-CD3-DARPin at 0.5mg/kg (DARPin having only CD3 binder), NB-TAA-DARPin at 0.5mg/kg (DARPin having only TAA binders), His-MP0533 at 2mg/kg, 0.5mg/kg, 0.2mg/kg and 0.02mg/kg, CD33-CD3 BiTE at 0.5mg/kg and CD123-CD3 DART at 0.5mg/kg eight days after tumor cell injection. Treatments were administrated when tumors were established at day 8. Data are presented in average + SEM in graph A. Pulled data from three independent experiments.

MP0533 induces tumor-specific T cell activity and cytokine release

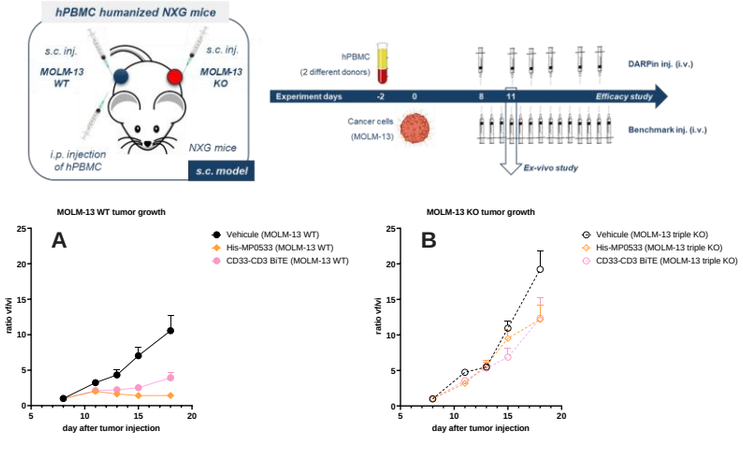


C

Dunnett's multiple comparisons test	Vehicle vs. NB-CD3-DARPin	Vehicle vs. His-MP0533 at 0.5mg/kg	Vehicle vs. His-MP0533 at 2mg/kg	Vehicle vs. CD33-CD3 BiTE	Vehicle vs. CD123-CD3 DART
IFNg	ns	ns	****	****	****
MCP-1	ns	ns	****	**	*
MIP-1b	ns	*	****	****	****
Eotaxin	ns	ns	****	***	****
Grz A	ns	ns	ns	ns	ns
IL-1b	ns	ns	**	ns	ns
IL-2	ns	ns	**	ns	ns
IL-5	ns	ns	****	ns	ns
IL-7	ns	*	**	***	ns
TNFa	ns	*	****	**	**
MIP-1a	ns	ns	**	****	**
Rantes	ns	ns	ns	****	ns
IP-10	ns	ns	*	***	**
Grz B	ns	**	*	****	ns
IL-1a	ns	ns	****	**	***
IL-1RA	ns	ns	**	*	ns
IL-3	ns	ns	****	****	****
IL-6	ns	**	****	*	*
IL-10	ns	ns	****	ns	***

Figure 3. T cell activation and cytokines/chemokines release induced by MP0533 on AML MOLM-13 tumors. Mice were injected intraperitoneally with hPBMC and xenografted subcutaneously with MOLM-13 cells. Treatments were initiated on established tumors eight days after xenograft. All molecules were injected at 0.5mg/kg. His-MP0533 was also tested at 2mg/kg, 0.2mg/kg and 0.02mg/kg. Three days after the first injection, mice were sacrified and tumors were harvested and dissociated. Tumor cell suspension were analyzed by FACS to check T cell activation (A) and tumor supernatants were analyzed by Luminex/MSD to check cytokines and chemokines release in tumors (C). Four hours after the first injection, mice were bleed and mice serum was analyzed by Luminex/MSD to check cytokines and chemokines release in mice serum (B). Data are presented in average + SEM in graph B. Pulled data from three independent experiments.

MP0533 does not induce MOLM-13 Triple KO tumor killing



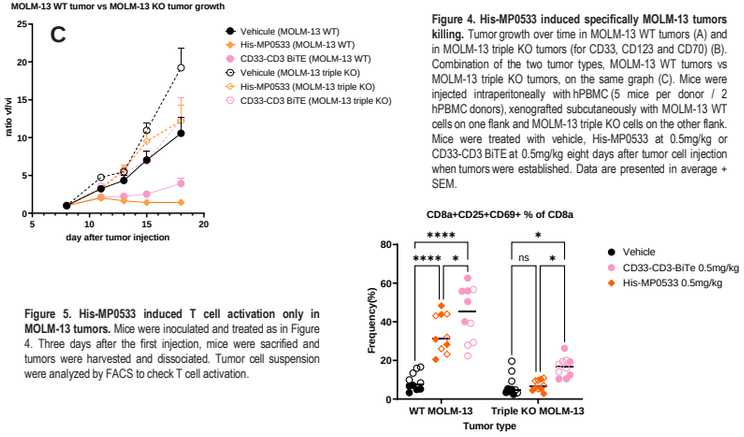


Figure 4. His-MP0533 induced specifically MOLM-13 tumors killing. Tumor growth over time in MOLM-13 WT tumors (A) and in MOLM-13 triple KO tumors (for CD33, CD123 and CD70) (B). Combination of the two tumor types, MOLM-13 WT tumors vs MOLM-13 triple KO tumors, on the same graph (C). Mice were injected intraperitoneally with hPBMC (5 mice per donor / 2 hPBMC donors), xenografted subcutaneously with MOLM-13 WT cells on one flank and MOLM-13 triple KO cells on the other flank. Mice were treated with vehicle, His-MP0533 at 0.5mg/kg or CD33-CD3 BiTE at 0.5mg/kg eight days after tumor cell injection when tumors were established. Data are presented in average + SEM.

Figure 5. His-MP0533 induced T cell activation only in MOLM-13 tumors. Mice were inoculated and treated as in Figure 4. Three days after the first injection, mice were sacrified and tumors were harvested and dissociated. Tumor cell suspension were analyzed by FACS to check T cell activation.

Conclusions

- MP0533 allows MOLM-13 tumor control in hPBMC mice model.
- MP0533 induced T cell activation in MOLM-13 tumors. Level of T cells activation correlated with His-MP0533 efficacy *in vivo*.
- MP0533 induces cytokine and chemokine release in tumors but not in circulation.
- Level of cytokines/chemokines release correlated with MP0533 efficacy and T cell activation in tumors only.
- MP0533 efficacy is specific to TAA expressing cells. MP0533 did not show efficacy and T cell activation against MOLM-13 triple KO tumors.
- Our data confirm MP0533 avidity-driven MOA as a solution to tackle the DLT of TCEs in the clinic and support its development for the treatment of AML.