C-KIT x CD16a x CD47 Switch-DARPin With Conditional Blockade of CD47: A Next-Generation Targeted Conditioning For Hematopoietic Stem Cell Transplantation

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EHA2024
JUNE 13–16
MADRID
POSTER P1294

INTRODUCTION

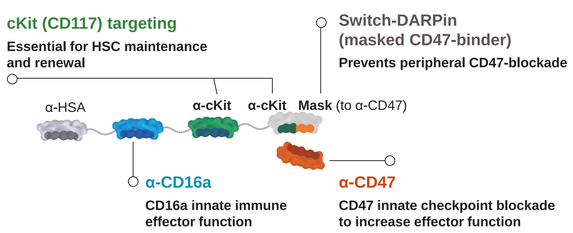
- HSCT offers a potential cure for patients with AML and other malignant and non-malignant diseases. However:
  - Many patients still relapse post-HSCT
  - Current MAC is associated with toxicity which limits patient eligibility while RIC further increases the risk of relapse
- More effective and safe conditioning regimens are needed to improve outcomes in AML and allow broader access to HSCT in non-malignant diseases
- cKit (CD117), a critical receptor for stem cell maintenance, is an attractive target to selectively eliminate HSCs as well as LSCs in AML
- Preclinical studies in mice suggest that cKit-targeting Abs alone do not sufficiently deplete HSCs for efficient HSCT and would benefit from concomitant blockade of the CD47 "do-not-eat-me" signal[1]
  - However, α-CD47 Abs are limited by toxicities linked to CD47 expression on a wide range of healthy cells
- Hence, simply combining cKit-targeting and systemic CD47-blocking Abs for conditioning is not a viable approach in patients

AIM

- To achieve a safe and effective targeted conditioning by combining inhibition of c-Kit signaling with an active Fc-effector function and conditional blockade of CD47 on target cells in a single molecule to induce potent but selective depletion of c-Kit+ cells

METHOD

- Generation of MP0621, a cKit x CD16a x CD47 Switch-DARPin
  - Containing a masked CD47-blocker that is released conditionally upon binding to cKit
  - Triggering immune cell-mediated killing of HSCs by engaging macrophages and natural killer cells via CD16a



- Functional activity was assessed using macrophage phagocytosis assays
- The ability to conditionally block CD47 on cKit+ target cells was tested using CD47 competition and CD47/SIRPα inhibition in *in vitro* assays
- Serum half-life was assessed using mouse PK studies
- Efficacy to deplete cKit+ cells in BM was assessed using a hCD34+ humanized NSG mouse model

RESULTS

MP0621 INDUCES cKIT-CONDITIONAL BLOCKADE OF CD47

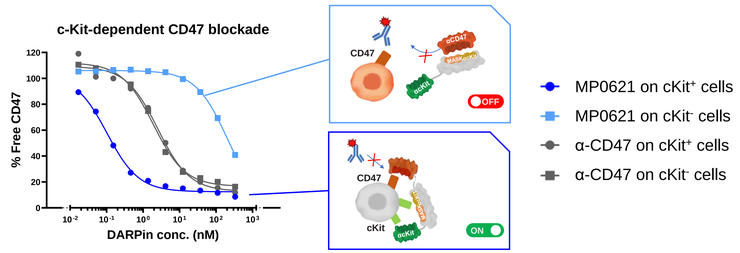


Figure 1. The Switch DARPin MP0621 allows conditional blockade of CD47 on cKit+ target cells, while preventing CD47-engagement on cKit- cells expressing similar amounts of CD47. *Titrated amounts of DARPins were added to the cKit+ AML cell line Kasumi-1 as well as a cKit- cell line engineered to express CD47 (CHO-hCD47). After incubation and washing, a biotinylated α-CD47 detection agent was added to the cells, followed by streptavidin-AF647. The signal obtained reflects the level of free CD47 available on the cell surface. Representative example of at least two independent experiments.*

CONDITIONAL CD47 BLOCKADE ENHANCES PHAGOCYTOSIS OF cKIT+ TARGET CELLS WHILE SPARING OTHER CD47+ CELLS

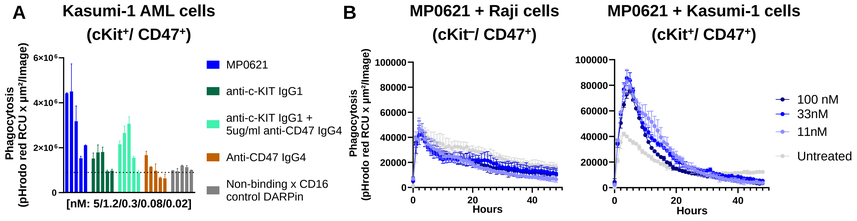


Figure 2. MP0621 induces MΦ-mediated phagocytosis comparable/superior to a combination of α-cKit and α-CD47 Abs of cKit+ target cells, while preventing phagocytosis of cKit- CD47+ cells. *(**A**) Switch-DARPin induced phagocytosis of pHrodo-labelled cKit-expressing AML cell line Kasumi-1 mediated by human monocyte-derived M0 MΦ at an E:T ratio of 1:4. The plots show the AUC obtained upon 48-h co-culture in presence of increasing concentrations of MP0621, α-cKit Ab (Fc-active version (IgG1) of JSP-191, reproduced in house) or a combination of α-cKIT and α-CD47 Abs. Representative example of at least three independent experiments. (**B**) MP0621-induced phagocytosis of pHrodo-labelled cKit- Raji cell line and cKit+ AML cell line Kasumi-1 at ascending concentrations of MP0621 mediated by human monocyte-derived M0 MΦ at an E:T ratio of 1:4. The plots show the signal obtained upon 48-h co-culture with the indicated amounts of DARPins. Representative example of at least three independent experiments.*

PHARMACOKINETIC PROFILE OF MP0621 IS COMPATIBLE WITH HSCT CONDITIONING APPLICATION

- PK studies in mice indicate approx. mono-exponential decline of serum concentrations following single i.v. injection
- Half-life values in the mono-exponential phase were calculated to range from 2.5-3 h in mice which are expected to scale to approx. 0.5 d in human based on scaling with the half-life of HSA[2]

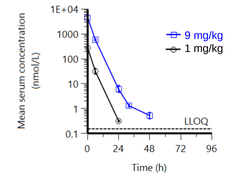
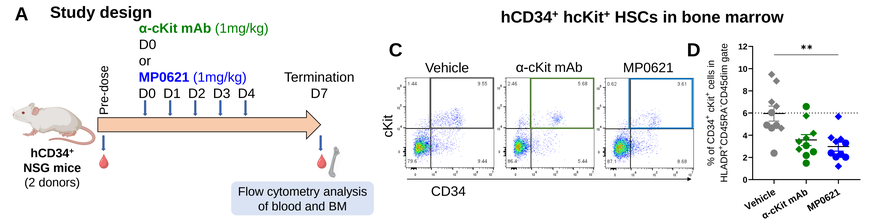


Figure 3. PK characteristics of MP0621 in female wild-type BALB/c mice following single i.v. bolus injection of 1 and 9 mg/kg of MP0621. *Results represent two groups with n=3 per time point (mean +/-max/min). Concentrations were measured by sandwich ELISA.*

MP0621 TARGETS cKIT+ CELLS IN BONE MARROW WITHOUT DEPLETING CIRCULATING hCD45+ CELLS IN hCD34+ NSG MICE



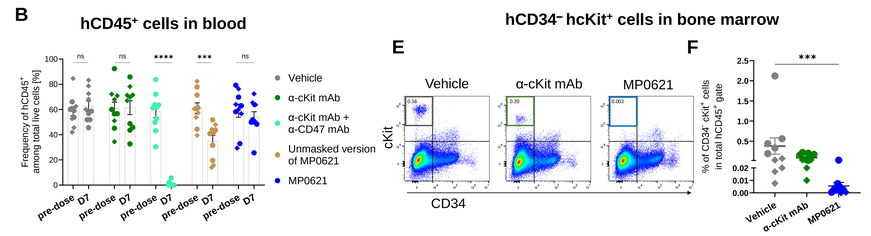


Figure 4. Treatment with MP0621 targets human cKit+ HSCs engrafted in CD34+ humanized NSG mice, while preventing elimination of peripheral hCD45+ cells observed with unconditional α-CD47 blockade. *(**A**) Study design (**B**) Flow cytometry analysis of hCD45+ cells in peripheral blood before and after treatment with MP0621, α-cKit mAb (IgG1), or a combination of α-cKit mAb (IgG1) and α-CD47 (IgG4) mAbs. Displayed data are calculated as in the following equation: %hCD45+ =(100*%hCD45+)/(%mCD45+ +%hCD45+); (Mann-Whitney test ****p<0.0001, ***p=0.007). (**C-F**) Flow cytometry assessment of human c-Kit+ cell populations in the bone marrow of hCD34-humanized NSG mice treated with MP0621 or α-cKit mAb vs vehicle (**C**) Representative flow cytometry dot plots and (**D**) frequency of human hCD34+ hcKit+ with HSC signature (hCD45dim CD45RA- HLADR+) (Kruskal-Wallis test ***p=0.004). (**E**) Representative flow cytometry plots and (**F**) frequency of BM hCD34- hcKit+ cell population of total hCD45+ cells. (Kruskal-Wallis test **p=0.002; ***p=0.0004). Round and square symbols depict mice humanized with hCD34+HSCs isolated from cord blood of two different donors.*

CONCLUSIONS

- Our preclinical results indicate that the c-KIT x CD16a x CD47 Switch-DARPin MP0621 can specifically target c-KIT+ HSCs with a suitable PK profile for a conditioning agent.
- The conditional CD47-binding enhances efficacy of cKit-targeting by blocking CD47 on target cells as well as safety by avoiding CD47-related off-target effects.
- Thus, MP0621 presents a novel targeted conditioning approach that has potential to improve the benefit/risk profile of HSCT strategies for patients with AML as well as non-malignant diseases.

ACKNOWLEDGEMENTS

We would like to thank the entire Molecular Partners team for their support and contribution.

CONTACT INFORMATION

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1. Chhabra et al., *Sci Transl Med* 2016;8(351); 2. Steiner et al., *Protein Eng Des Sel*. 2017 Sep 1;30(9):583-591; 3. Kwon et al., *Blood* 2019 May 9;133(19):2104-2108.

ABBREVIATIONS

mAb, (monoclonal) antibody; AML, acute myeloid leukemia; AUC, area under the curve; BM, bone marrow; ELISA, enzyme-linked immuno-sorbent assay E:T, effector to target; FACS, fluorescence-activated cell sorting; MAC, myeloablative conditioning; HSC(T), Hematopoietic stem cell (transplantation); HSA, human serum albumin; i.v., intravenous; LLOQ, lower limit of quantification; LSC, leukemic stem cell; MΦ, macrophage; PK, pharmacokinetic; RIC, reduced intensity conditioning; SIRPα, Signal regulatory protein α.