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Discovery and Characterization of DMR-001, an Fc-Null, Half-Life Optimized Monoclonal Antibody Selectively Targeting Mutant Calreticulin

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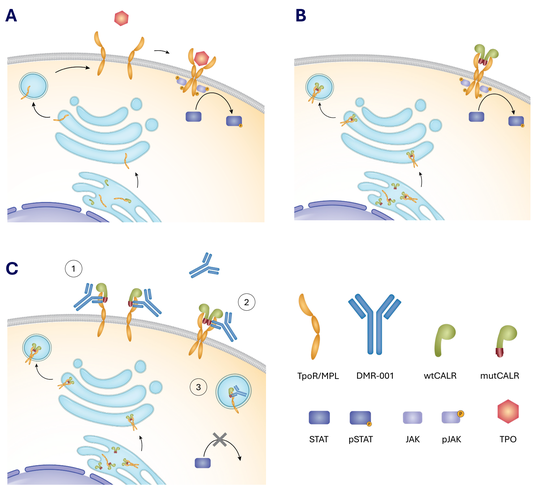
DAMORA THERAPEUTICS

Abstract PF873

Introduction

- Essential thrombocythemia (ET) and myelofibrosis (MF) are myeloproliferative neoplasms (MPNs) often driven by frameshift mutations in calreticulin (CALR).
- CALR mutations are found in ~25% of ET cases and ~35% of MF cases.
- CALR mutations produce a neomorphic mutant C-terminus that permits binding to and constitutive activation of the thrombopoietin receptor (TpoR), which drive a range of disease symptoms from increased risk of thrombosis to severe, life-threatening bone marrow fibrosis. The most common mutations in CALR are Type 1 (del52) and Type 2 (ins5).
- Disease modifying treatments selectively targeting mutant CALR (mutCALR) are needed to improve patient outcomes.
- Here we describe the discovery and preclinical activity of DMR-001, a novel, selective, anti-mutCALR mAb with high affinity and anti-proliferative potency against both Type 1 and Type 2 mutations and optimized PK properties.



(A) Normal function of wild-type calreticulin (wtCALR), with TPO-dependent activation of TpoR. **(B)** Ligand-independent signaling in the context of mutCALR binding to TpoR. **(C)** Inhibition of mutCALR signaling by DMR-001 via three possible mechanisms: 1) disruption of TpoR dimer, 2) inhibition of surface tetramer signaling, or 3) internalization of complex

Aims

To engineer and characterize an Fc-null, half-life extended mutCALR-targeted mAb with activity against both Type 1 and Type 2 mutCALR variants and drug-like properties enabling convenient clinical dosing.

Methods

Antibody discovery campaigns utilized engineered phage and yeast display libraries to identify clones specific to the mutCALR epitope of the C-terminus. Target specificity was increased through affinity maturation and iterative rounds of negative selection against wtCALR and positive selection against Type 1 and Type 2 mutCALR antigens. Antibodies were evaluated for inhibition of proliferation in Type 1 and Type 2 Ba/F3 cell lines to further refine potency.

Results

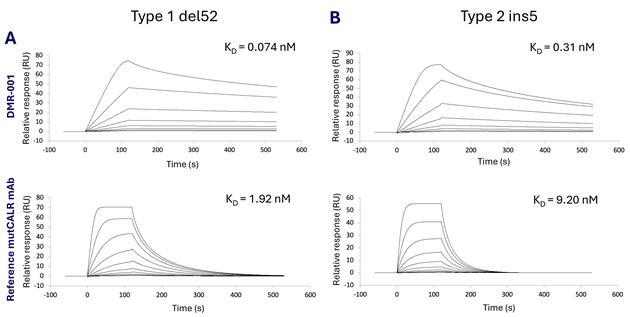
DMR-001 shows significantly improved Type 1 and Type 2 mutCALR affinity and PK properties predicted to enable less frequent SC dosing

	DMR-001	mutCALR reference mAb
Specificity	CALR mutant specific	CALR mutant specific
Affinity to Type 1 del52 mutCALR (K_D)	0.074nM	1.92nM
Affinity to Type 2 ins5 mutCALR (K_D)	0.31nM	9.20nM
Fc status	Effector null	Effector null
Non-Human Primate PK half life (days)	15	3.1
Expected Ph1 Dosing	SC Q4W	IV Q2W

Desired profile | Insufficient

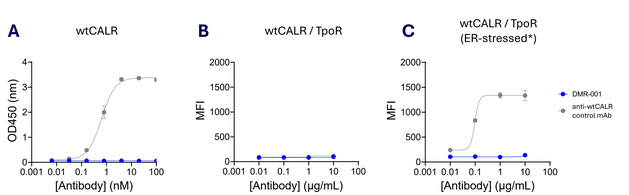
Reference mutCALR mAb produced recombinantly based on U.S. patent application US20230272055A1. All comparative studies of DMR-001 and mutCALR reference mAb were conducted in parallel under the same conditions.

Figure 1: DMR-001 shows higher affinity and slower off rate to Type 1 and Type 2 mutCALR compared to reference mutCALR mAb



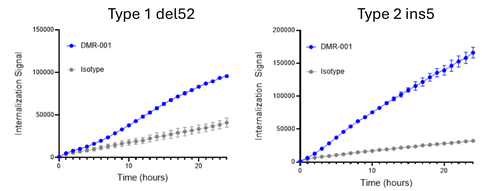
Binding affinity (K_D) of antibodies to mutCALR Type 1 del52 **(A)** & Type 2 ins5 **(B)** mutCALR proteins was determined by surface plasmon resonance (SPR) using a Biacore 8K SPR system (Cytiva). A 1:1 kinetic binding model was utilized to determine the dissociation rate constant (K_D). Exposure ranges tested in Type 1 del52 were DMR-001 0.02–2.5 nM and mutCALR reference mAb 0.02–10 nM (A), and in Type 2 ins5 were DMR-001 0.04–5 nM and mutCALR reference mAb 0.04–20 nM (B). Data shown are fits of the raw sensorgrams.

Figure 2: DMR-001 shows no off-target binding to wtCALR



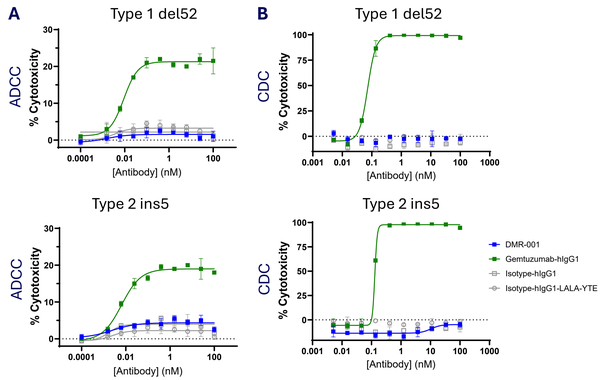
A) An ELISA assay demonstrates no binding of DMR-001 to wtCALR. **B, C)** DMR-001 binding was assessed against wtCALR expressed in Ba/F3-TpoR cells. Thapsigargin treatment induces ER stress (C), which translocates wtCALR to the cell surface. DMR-001 did not bind to cell-associated wtCALR. Control wtCALR antibody was included in (A-C).

Figure 3: DMR-001 induces internalization of surface Type 1 and Type 2 mutCALR



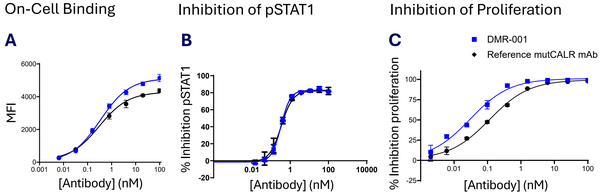
Ba/F3-TpoR cells expressing either Type 1 del52 mutCALR or Type 2 ins5 mutCALR were used to assess antibody internalization. Test antibodies were mixed with a pH-sensitive dye that is highly fluorescent in the low pH environment of lysosomes, which is a readout of internalization. Images were acquired every hour for 24 hours in an Incucyte. Internalization signal was measured as the integrated fluorescent intensity per well normalized to cell confluence.

Figure 4: DMR-001 does not cause ADCC or CDC



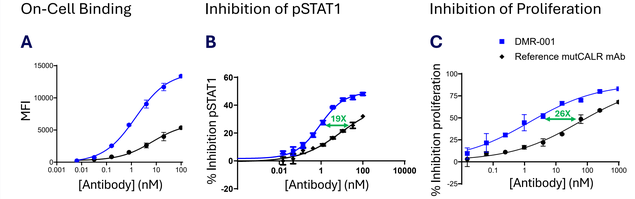
A) DMR-001 did not elicit antibody-dependent cellular cytotoxicity (ADCC) in a primary human NK cell assay to assess Fc-mediated effector function. ADCC activity was evaluated against ELF153-TpoR target cells expressing either Type 1 del52 mutCALR or Type 2 ins5 mutCALR using NK cells at an effector to target ratio of 5:1. **B)** DMR-001 did not elicit complement-dependent cytotoxicity (CDC) against ELF153-TpoR target cells expressing either Type 1 del52 mutCALR or Type 2 ins5 mutCALR. ELF153 cells express CD33, therefore gemtuzumab (anti-CD33) was used as a positive control for ADCC and CDC activity.

Figure 5: DMR-001 shows strong on-cell binding, inhibition of pSTAT1 signaling, and inhibition of proliferation in Type 1 mutCALR



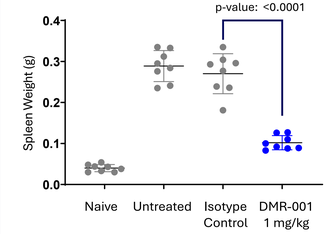
Ba/F3-TpoR cells expressing Type 1 del52 mutCALR were used for (A), (B) and (C). **A)** DMR-001 on-cell binding was assessed by flow cytometry. **B)** Cells were incubated with antibodies for 2 hours. A FACS assay was used to measure phos-STAT1 signal. The plot shown is a representative run from 4 independent experiments. **C)** Cells were incubated with antibodies for 3 days. A CellTiter-Glo assay was used to assess cellular proliferation. The graph shown is a representative run from at minimum 12 independent experiments.

Figure 6: DMR-001 shows greatly improved on-cell binding, inhibition of pSTAT1 signaling, and inhibition of proliferation in Type 2 mutCALR versus reference mutCALR mAb



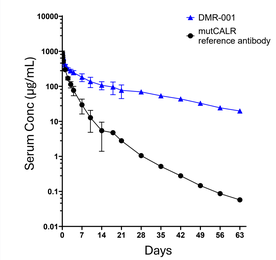
Ba/F3-TpoR cells expressing Type 2 ins5 mutCALR were used for (A), (B) and (C). **A)** DMR-001 on-cell binding was assessed by flow cytometry. **B)** Cells were incubated with antibodies for 2 hours. A FACS assay was used to measure phos-STAT1 signal. The plot shown is a representative run, and the fold decrease in IC50 relative to mutCALR reference antibody is an average of 4 independent experiments. **C)** Cells were incubated with antibodies for 3 days. A CellTiter-Glo assay was used to assay cellular proliferation. The graph shown is a representative run, and the fold decrease in IC50 relative to mutCALR reference antibody is an average of at minimum 12 independent experiments.

Figure 7: A single dose of DMR-001 results in near normalization of spleen weight in an *in vivo* model of Type 1 del52 mutCALR



On day 0, Ba/F3-TpoR cells expressing Type 1 del52 mutCALR were inoculated intravenously into the tail vein of female NOG mice. On day 9, mice were randomized into groups according to systemic Ba/F3 cell burden as measured by flow cytometry. Animals were dosed on day 10 as indicated. On day 13, spleen weight was recorded. P-value calculated using a two tailed t-test.

Figure 8: DMR-001 shows ~5x longer half-life in Non-Human Primates versus reference mutCALR mAb



	Half-life (days)
DMR-001	15
mutCALR reference mAb	3.1

Cynomolgus monkeys were dosed with 30mg/mg test article and PK followed out to 63 days. The concentration of test article in peripheral blood was measured at multiple timepoints and is plotted.

The estimated half-life of DMR-001 in NHP is 15 days, compared to the reference antibody which has a half-life of 3.1 days.

Conclusions

- DMR-001 has best-in-class potential based on superior preclinical anti-mutCALR activity and extended half-life, which is expected to enable both improved efficacy and dosing convenience.
  - DMR-001 is a highly potent and selective inhibitor of both Type 1 and Type 2 mutCALR.
  - DMR-001 demonstrates extended half-life predicted to enable prolonged target engagement and at least Q4W SC dosing.
- A Phase 1/1b study of DMR-001 in mutCALR-driven MPN (ET and MF) patients is planned to initiate in the third quarter of 2026.

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