

Molecular Characterization of Patients With Myeloproliferative Neoplasms Treated With INCA033989 Demonstrates Selective Targeting of *CALR* Mutant Hematopoietic Cells

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Myeloproliferative Neoplasms Are Clonal Disorders and *CALR* Mutations Are Frequent in ET and MF

- **25-35% of patients with essential thrombocythemia (ET) and myelofibrosis (MF) have a calreticulin (*CALR*) mutation (mut*CALR*)¹**
 - mut*CALR* type 1 is the most common mutation (50-70%)^{1,2}
- **All mutations result in aberrant expression of mut*CALR** on the cell surface in complex with thrombopoietin receptors (TPO-Rs)³⁻⁷**
 - **Activates the JAK/STAT pathway**
- **INCA033989 is a first-in-class antagonist antibody inhibiting mut*CALR***
 - INCA033989 is a novel, fully human, high-affinity, Fc-silenced, immunoglobulin monoclonal antibody that selectively targets mut*CALR* in complex with TPO-R to inhibit oncogenic signaling and proliferation of cells⁸
- INCA033989-101[†] and -102[‡] are phase 1, first-in-human, multicenter, open-label studies evaluating INCA033989 as monotherapy or in combination with ruxolitinib in patients with previously treated ET or MF harboring a *CALR* mutation

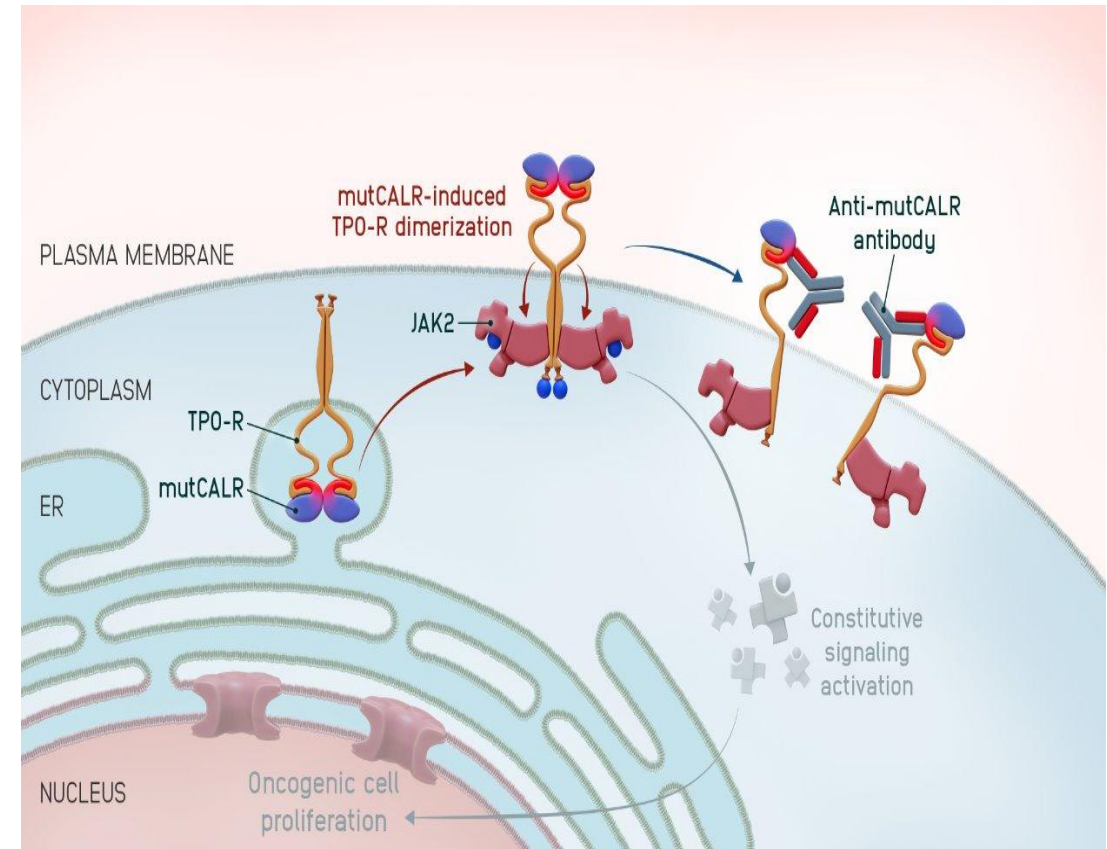


Figure reprinted from Reis E, et al. *Blood*. 2024;144:2336-2348 [visual abstract] with permission from Elsevier Inc. Copyright © 2024 American Society of Hematology.

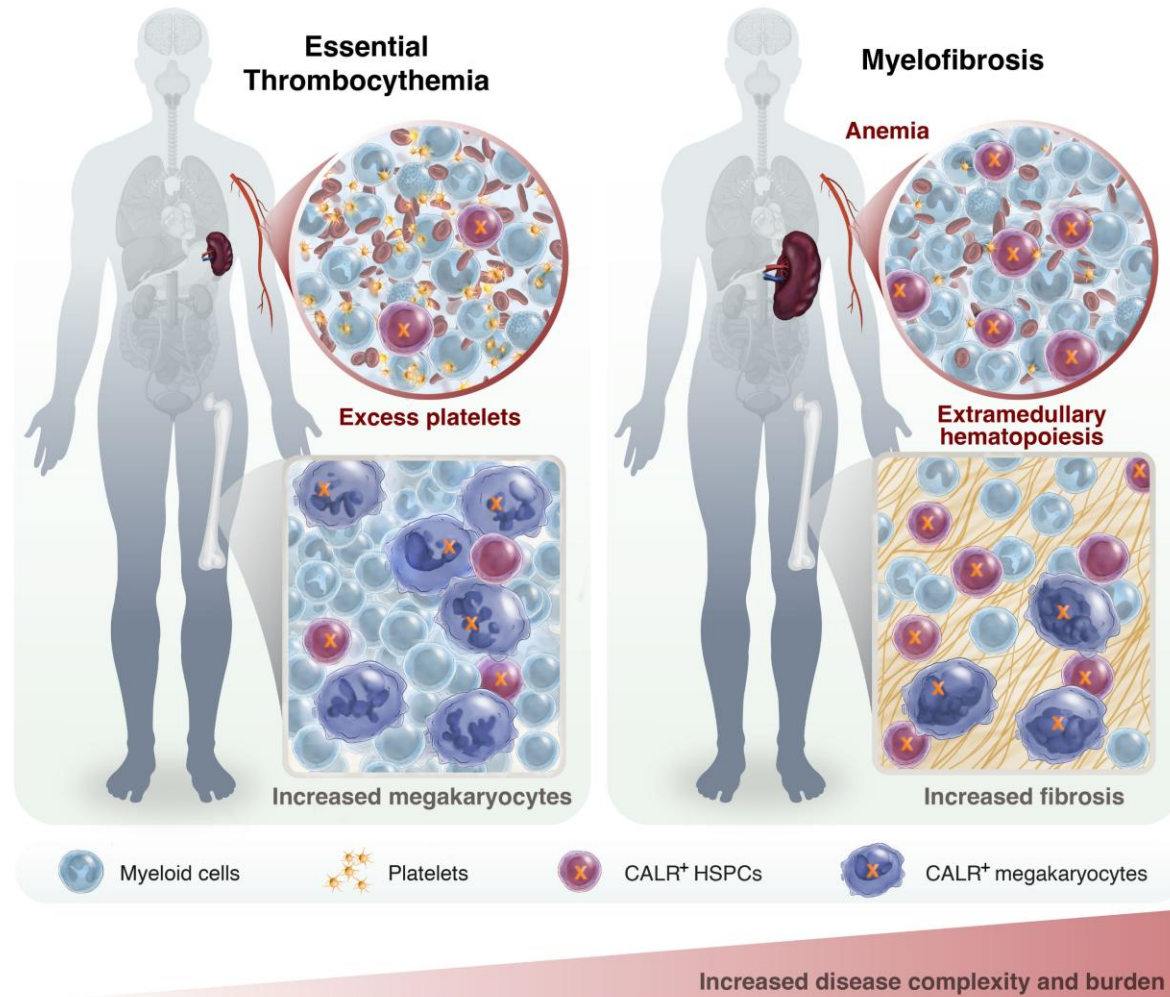
*mut*CALR*: *CALR* exon 9 frameshift mutations. [†]NCT05936359 (outside the US). [‡]NCT06034002 (US only).

1. Klampfl T, et al. *N Engl J Med*. 2013;369:2379-2390. 2. Cabagnols X, et al. *Blood*. 2014;124:1823. 3. Chachoua I, et al. *Blood*. 2016;127:1325-1335. 4. Elf S, et al. *Cancer Discov*. 2016;6:367-381. 5. Elf S, et al. *Blood*. 2018;131:782-786.

6. Papadopoulos N, et al. *Nat Commun*. 2023;14:1881. 7. Pecquet C, et al. *Blood*. 2019;133:2669-2681. 7. Reis ES, et al. *Blood*. 2024;22:2336-2348.

ER, endoplasmic reticulum; JAK2, Janus kinase 2; STAT, Signal transducer and activator of transcription.

ET and MF Share Common Oncogenic Drivers but Differ in Pathophysiology, Genomic Complexity, and Disease Burden



INCA033989-101/102 Studies
Higher CALR VAF in MF vs ET
at enrollment*

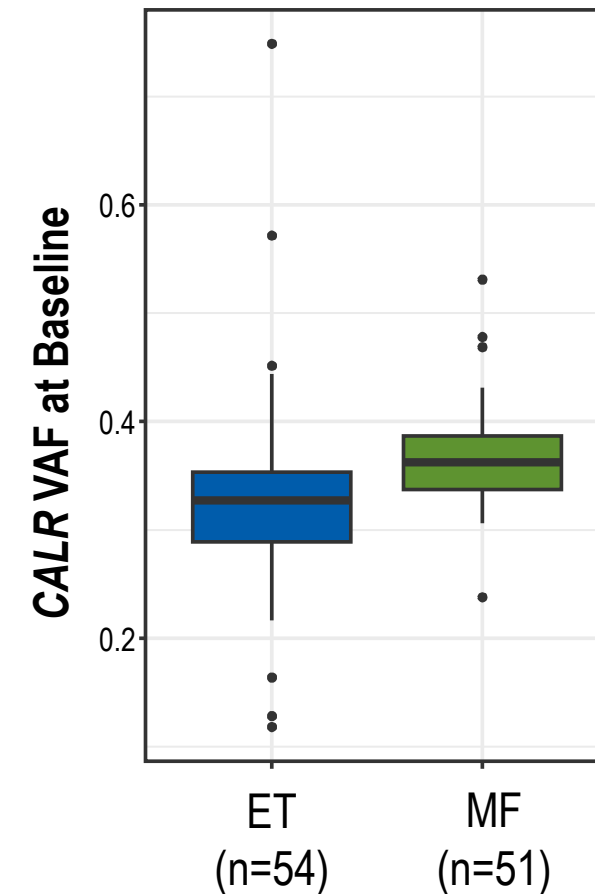


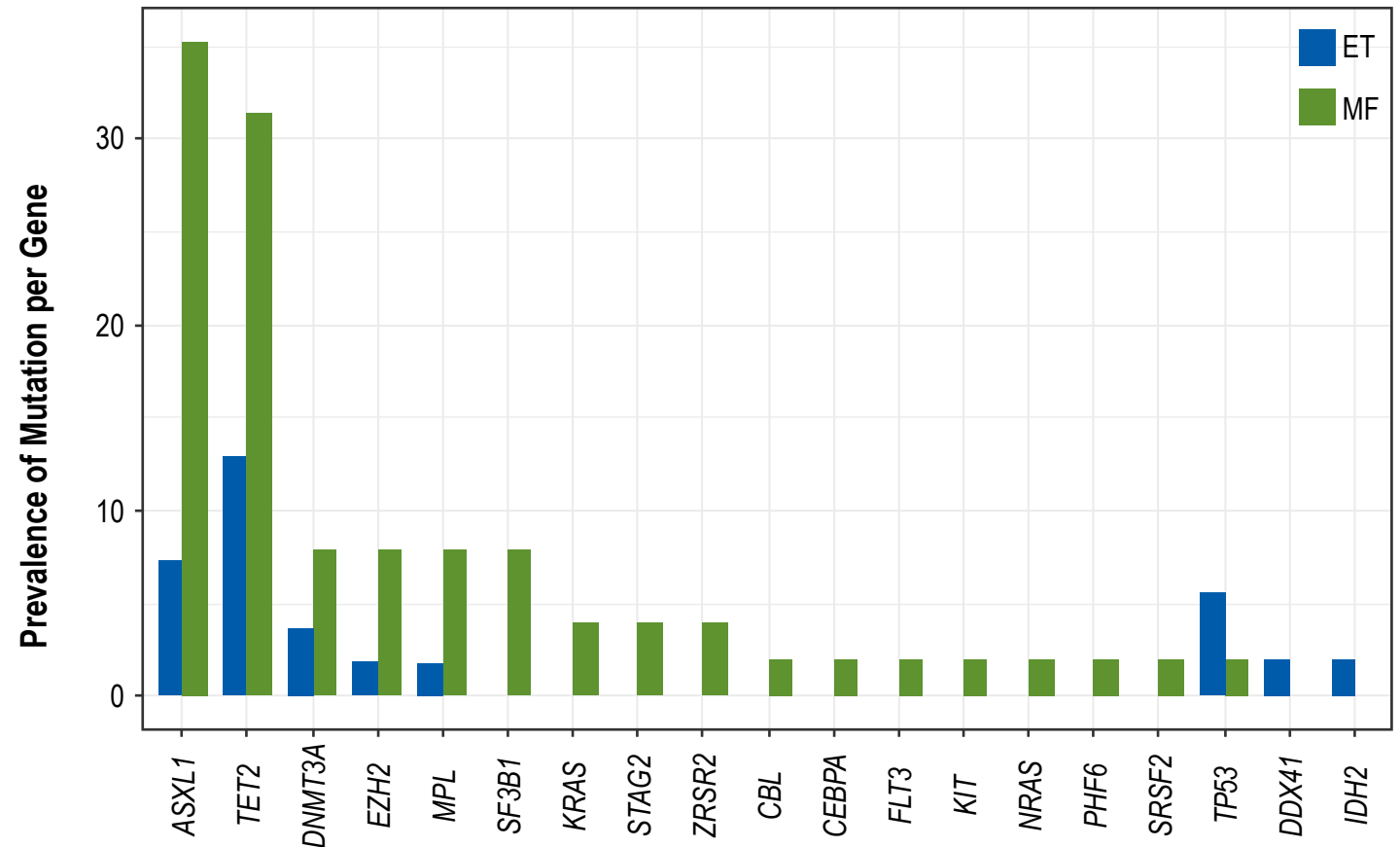
Illustration by DrawImpacts.

*Genomic data presented are from all ET and MF monotherapy patients with peripheral blood samples at screening measured by next-generation sequencing. CALR, calreticulin; ET, essential thrombocythemia; HSPC, hematopoietic stem/progenitor cell; MF, myelofibrosis; VAF, variant allele frequency.

Patients With MF Have Greater Clonal Complexity at Baseline Compared With ET

- Co-occurring mutations correlate with disease severity and progression in patients with ET and MF^{1,2}
- Most patients with MF had a co-occurring mutation (76.5%), compared with 32% of patients with ET (37 gene panel*)

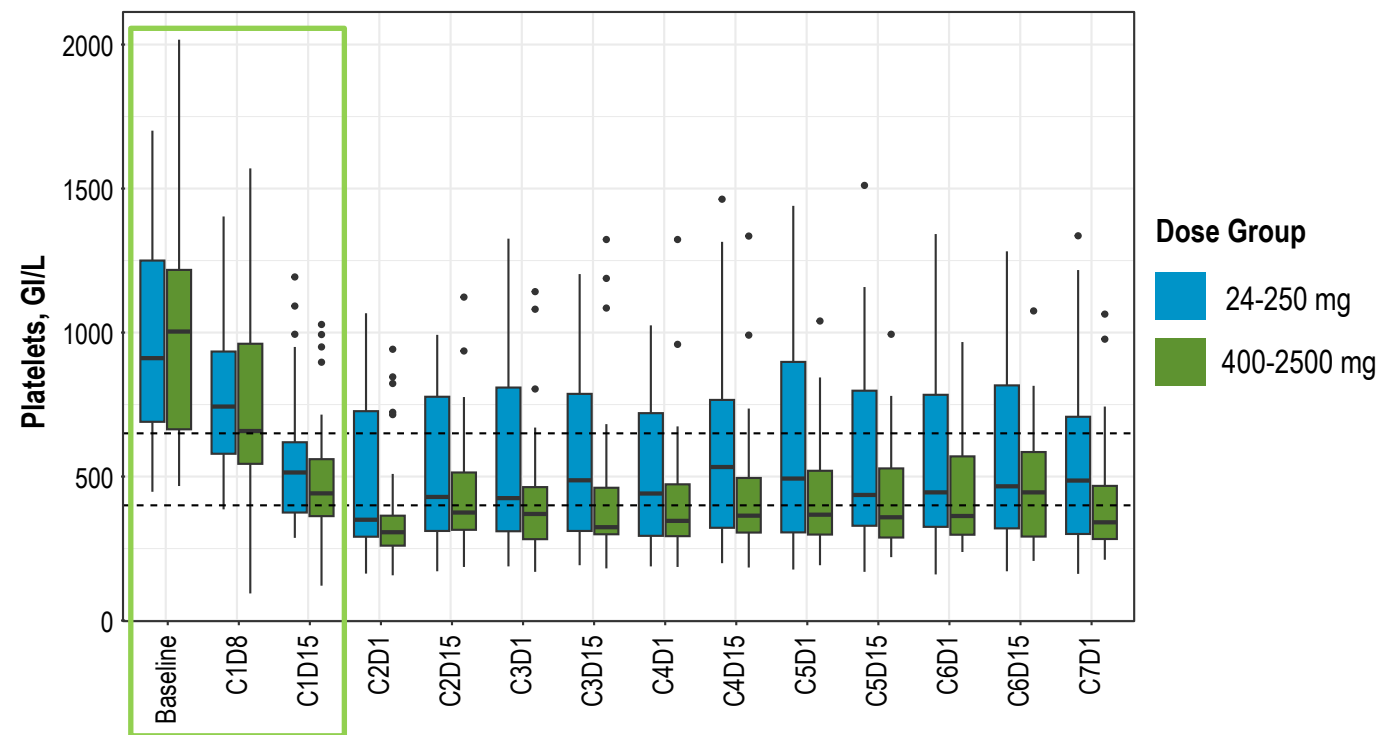
Prevalence of Co-occurring Mutations in Patients With ET and MF



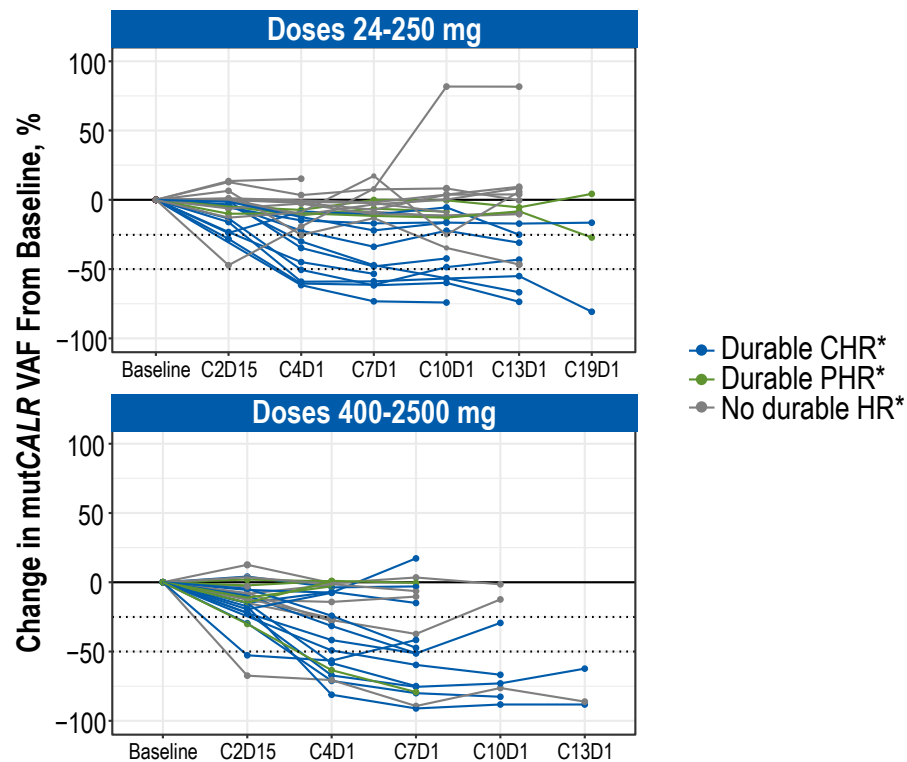
1. Mawalkar R, et al. *Blood*. 2024;144(Suppl 1):3173. 2. Tefferi A, et al. *Blood Adv*. 2016;1:21-30.
*ArcherDx™ library preparation followed by sequencing on the Illumina™ platform.
ET, essential thrombocythemia; MF, myelofibrosis.

Patients With ET Treated With INCA033989 Achieve Rapid and Durable Platelet Normalization With Correlated VAF Reductions

Durable Platelet Reduction (n=55)



mutCALR VAF Reduction (n=52[†])

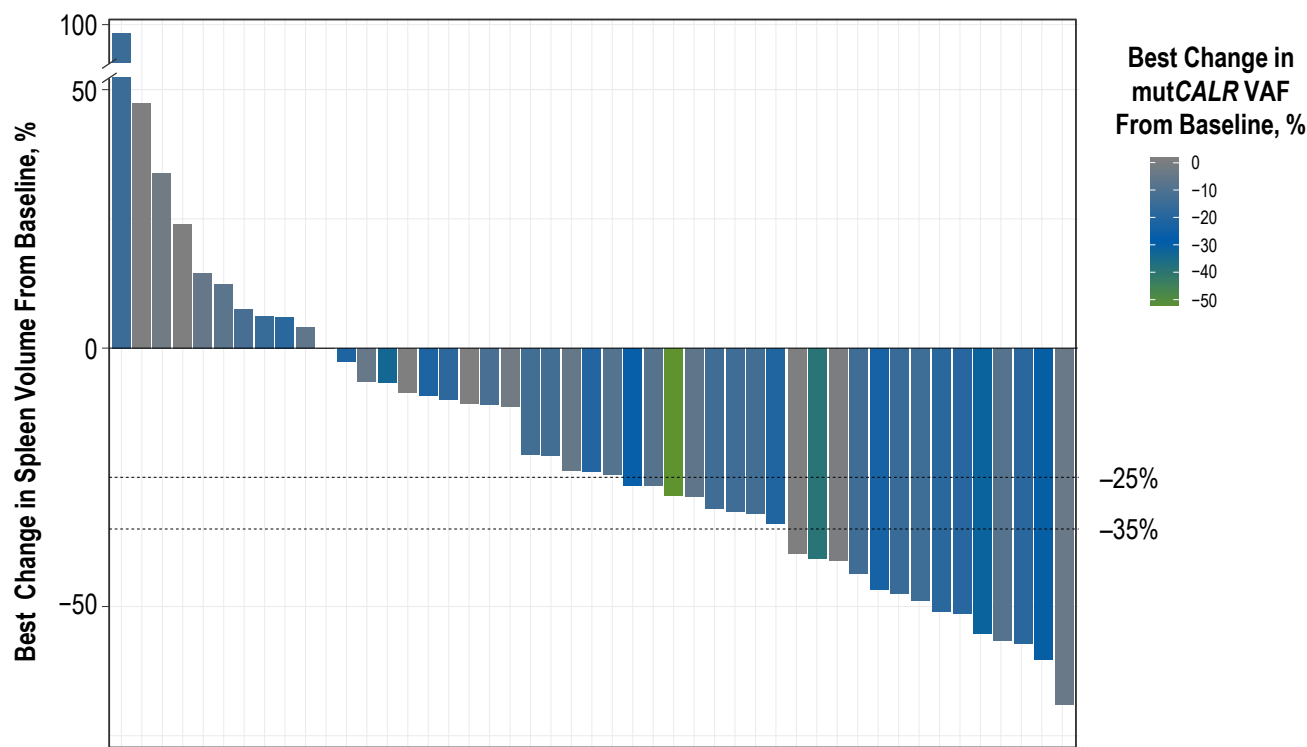


- Deeper and more consistent responses are observed with higher doses of INCA033989

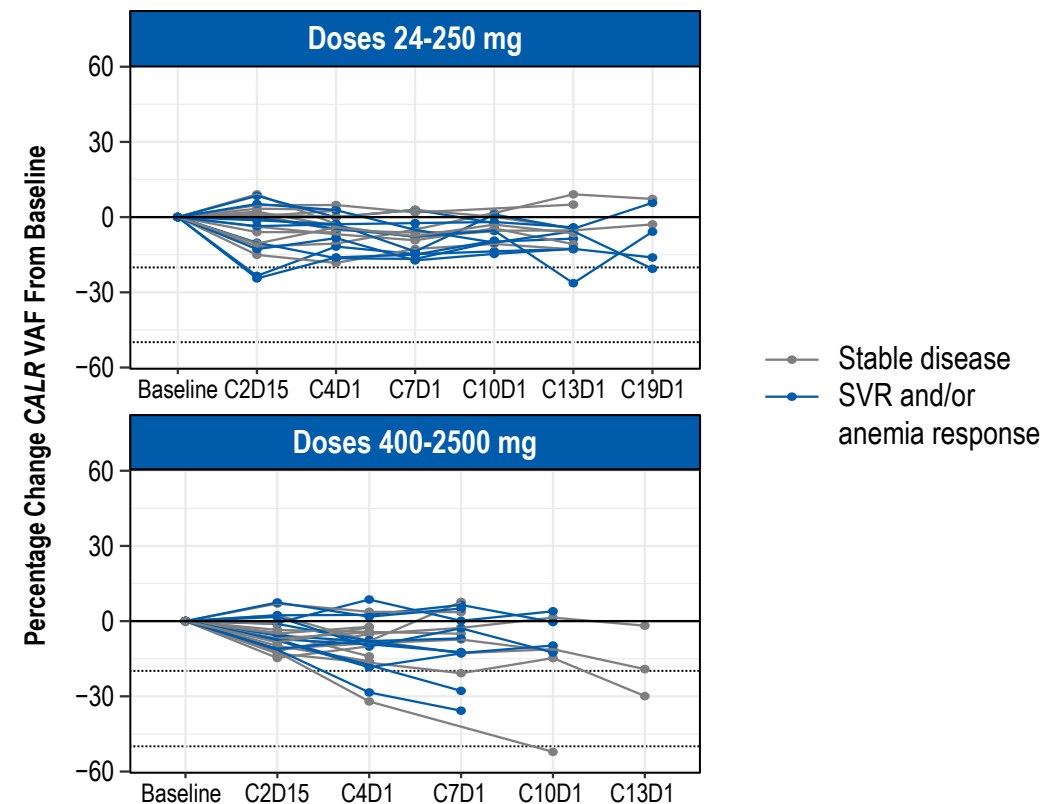
*CHR defined as platelet count $\leq 400 \times 10^9/L$ and leukocytes $< 10 \times 10^9/L$; PHR defined as platelet count $\leq 600 \times 10^9/L$ and leukocytes $< 10 \times 10^9/L$ (baseline platelet count $> 600 \times 10^9/L$). Durable response defined as maintaining for ≥ 12 weeks. [†]3 patients were excluded due to lack of postbaseline VAF assessment.
C, cycle; CHR, complete hematologic response; D, day; ET, essential thrombocythemia; HR, hematologic response; mutCALR, mutations of calreticulin; PHR, partial hematologic response; VAF, variant allele frequency.

Patients With MF Treated With INCA033989 Achieve Rapid Spleen Reductions With Deeper VAF Reductions at Higher Doses

Spleen Volume Reduction vs Molecular Response*



Percentage Change in mutCALR VAF From Baseline*

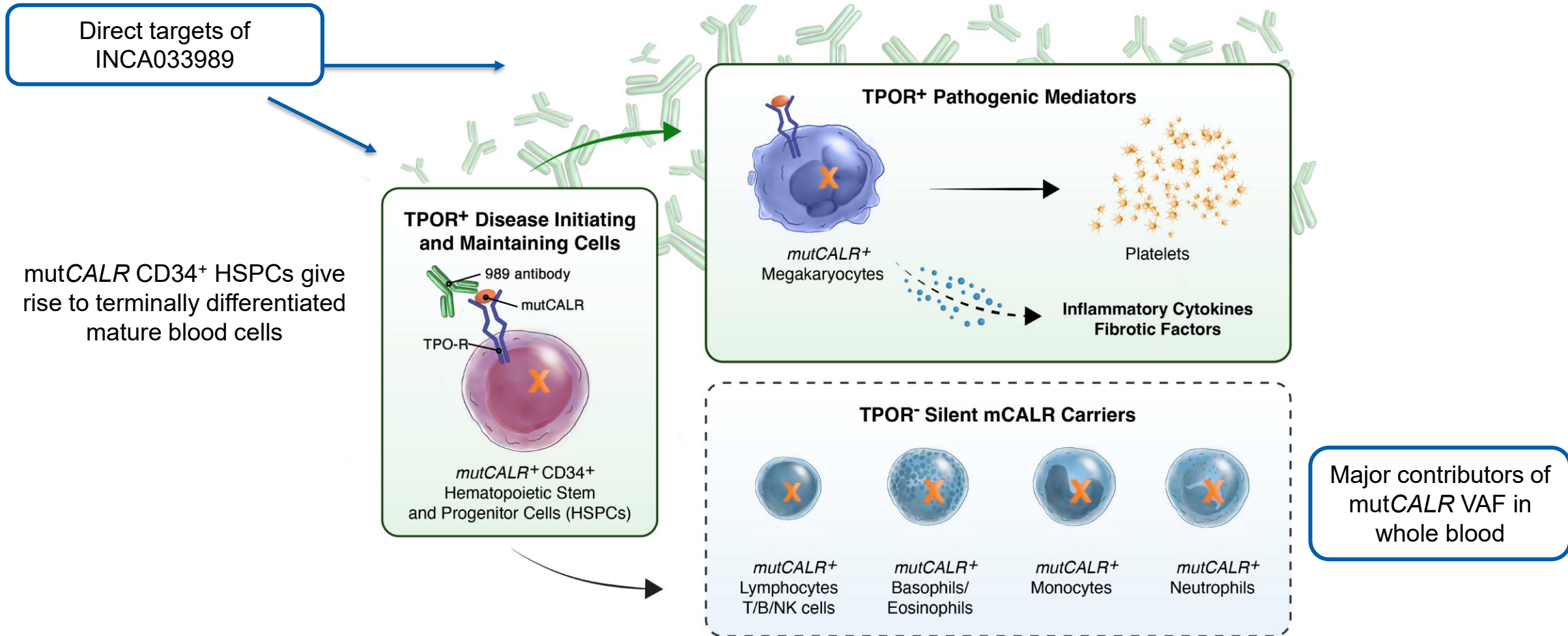


- For all patients with MF with SV measurement (n=48), 23 (47.9%) patients had SVR25, and 15 (31.3%) patients had SVR35
- For patients with ≥ 1 postbaseline VAF measurement (n=47), 42/47 (89.4%) had a reduction in mutCALR VAF
- Deeper reductions in VAF were observed with higher doses and SVR responses

*Data include only monotherapy MF patients with both SV and post-treatment VAF measurements (n=47).

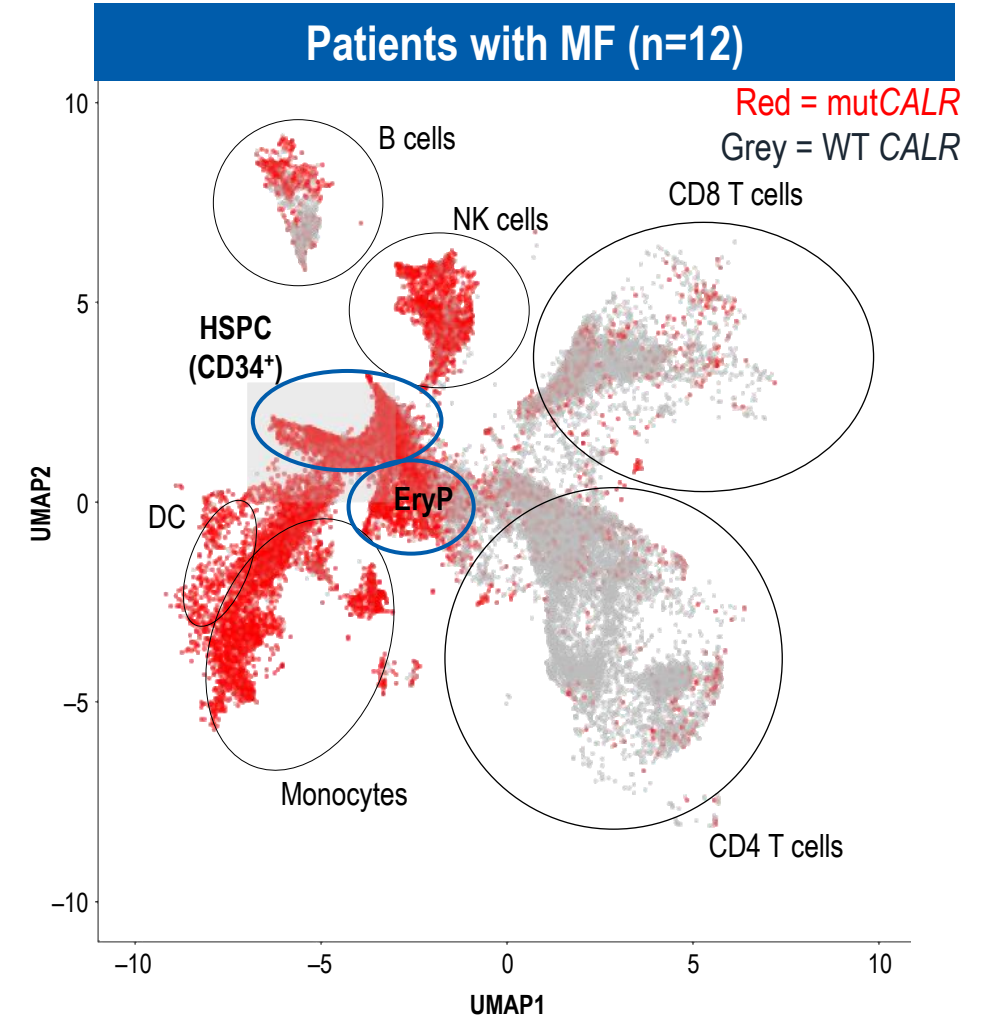
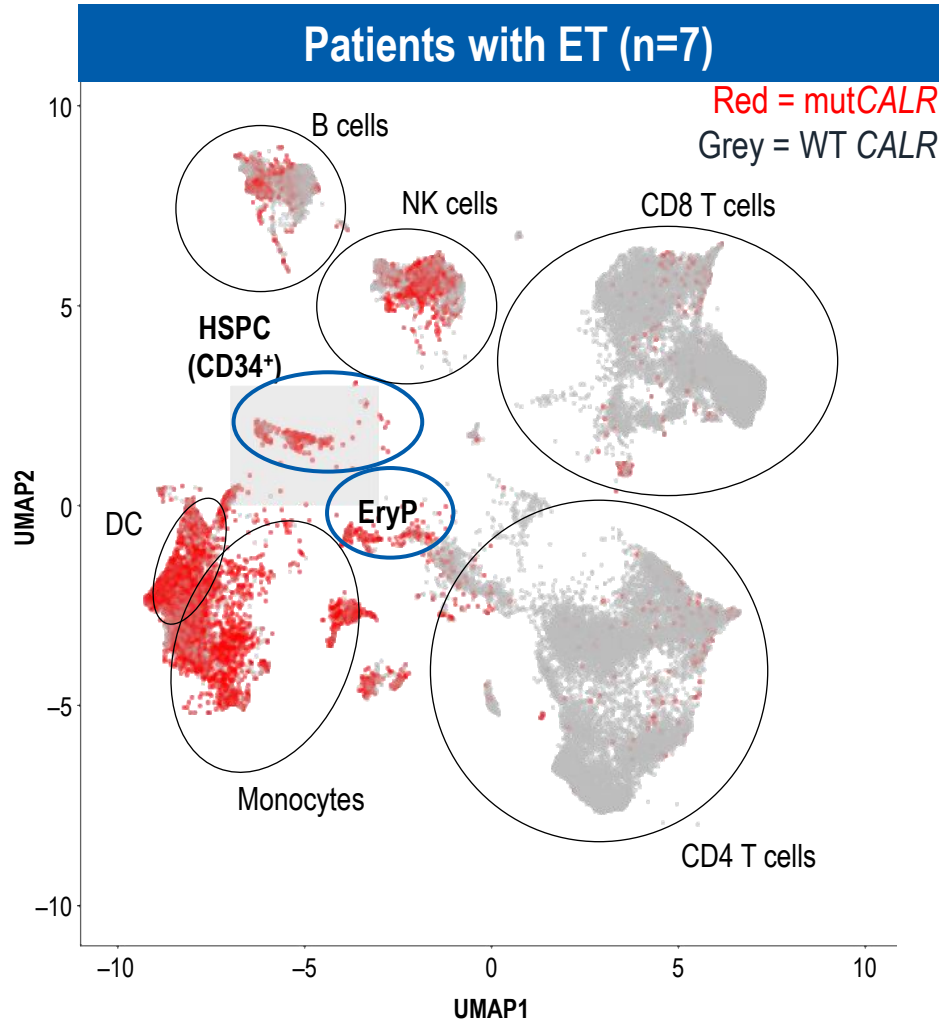
C, cycle; D, day; MF, myelofibrosis; mutCALR, mutations of calreticulin; SVR25, spleen volume reduction $\geq 25\%$; SVR35, spleen volume reduction $\geq 35\%$; VAF, variant allele frequency.

INCA033989 Targets Disease-Initiating and -Maintaining Cells, Including Hematopoietic Stem/Progenitor Cells (HSPCs) and Megakaryocytes



Single-Cell Immunophenotyping and Genotyping Demonstrates Differential Complexity of *mutCALR*⁺ Cells in PBMCs From Patients with ET and MF

- *mutCALR* peripheral blood mononuclear cells (PBMCs) ranged from 9% to 45% at baseline in patients with ET
- *mutCALR* PBMCs ranged from 28% to 62% at baseline in patients with MF
- Circulating HSPCs (CD34⁺) and progenitor cells were prominently detected in patients with MF

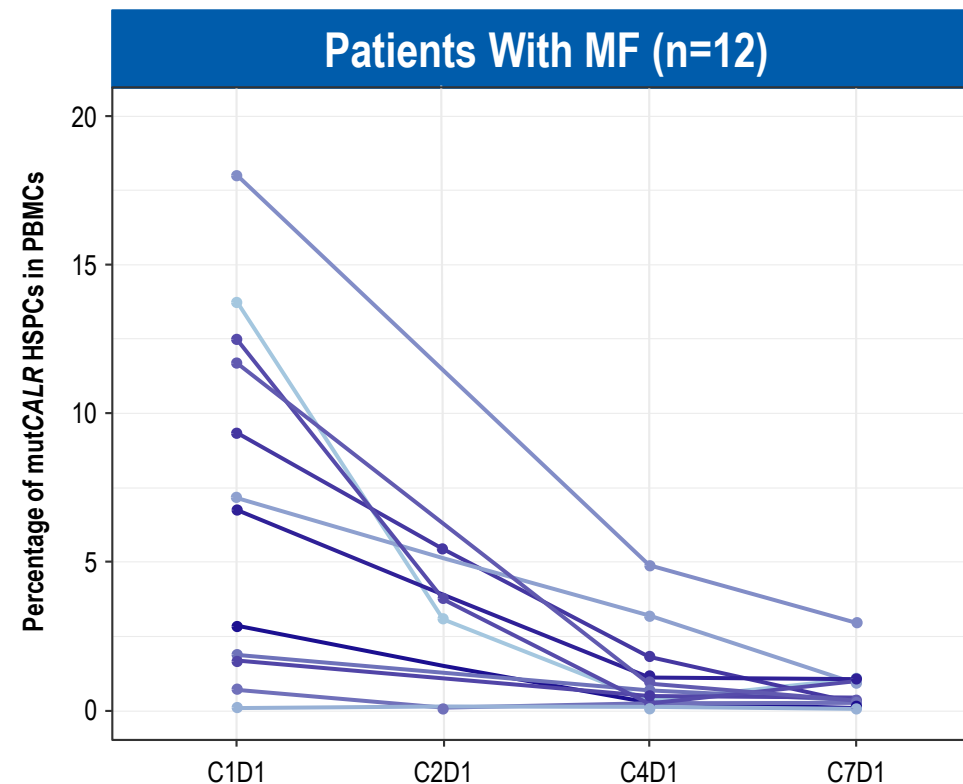
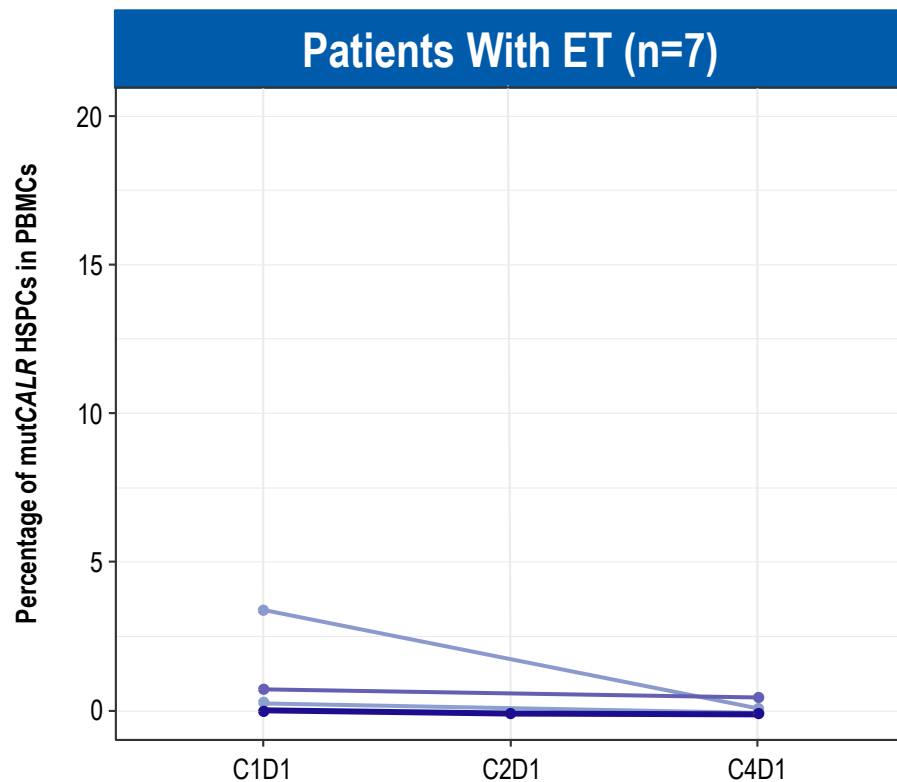


Presented single-cell DNA sequencing data (MissionBio™) are from available dose-escalation patient samples: ET (n=7, 70-750 mg), MF (n=12, 50-1500 mg). Cells were clustered and visualized using a UMAP based on cell surface expression of 46 proteins.

CALR, calreticulin; DC, dendritic cell; EryP, erythroid progenitor cell (CD71); ET, essential thrombocythemia; HSPC, hematopoietic stem/progenitor cell (CD34-high); MF, myelofibrosis; *mutCALR*, mutations in calreticulin; NK, natural killer; UMAP, uniform manifold approximation and projection; WT, wild-type.

INCA033989 Treatment Significantly Eliminates Disease-Initiating and -Maintaining CD34⁺ HSPCs in PBMCs From Patients With ET or MF

Single-Cell Data: mutCALR HSPCs (CD34⁺)

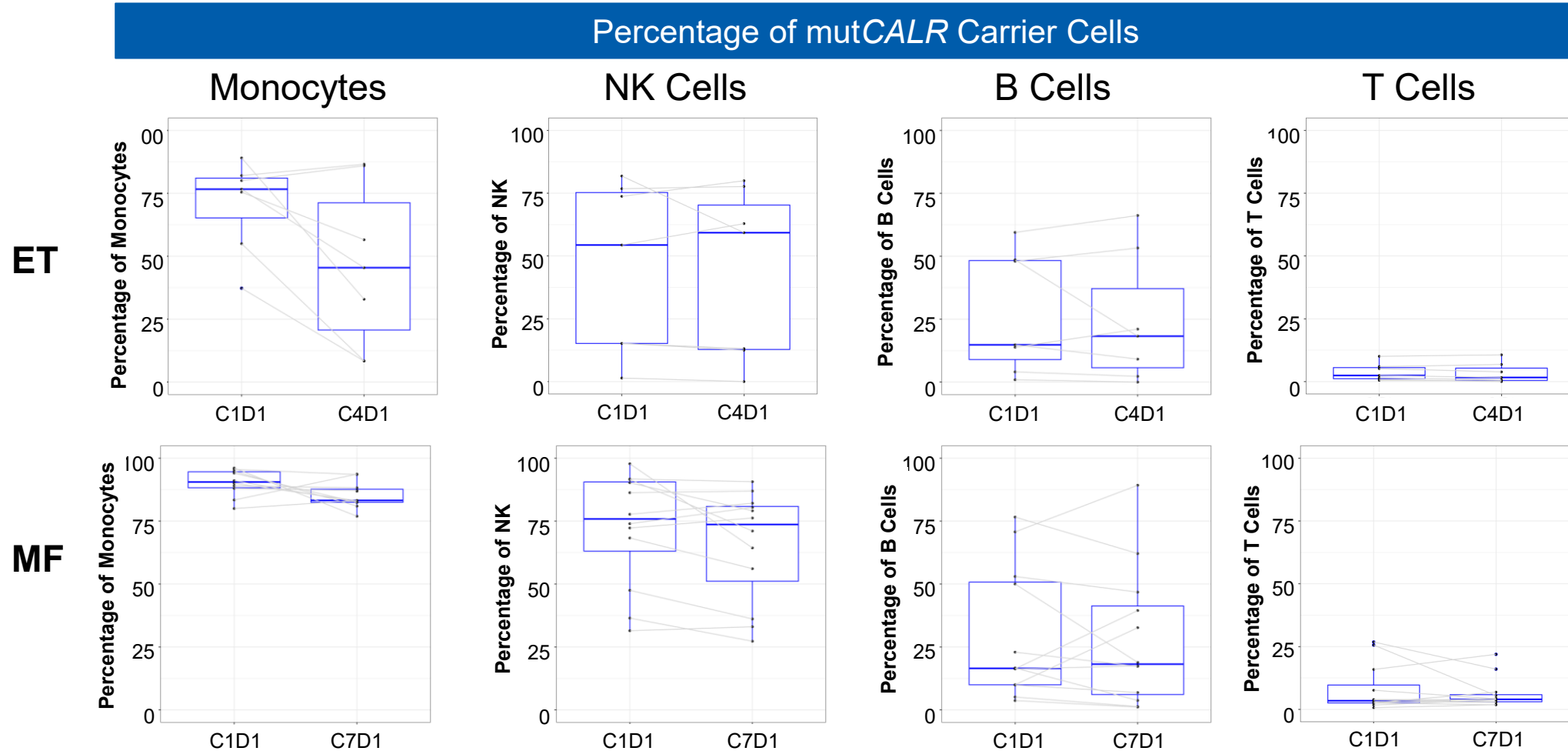


- High levels of mutCALR HSPCs (CD34⁺) are decreased with INCA033989 treatment in patients with MF
 - Lower levels of mutCALR HSPCs in PBMCs from patients with ET are also decreased

Presented single-cell DNA sequencing data is from available dose-escalation patient samples: ET (n=7, 70-750 mg), MF (n=12, 50-1500 mg).

C, cycle; D, day; ET, essential thrombocythemia; HSPC, hematopoietic stem/progenitor cell; MF, myelofibrosis; mutCALR, mutations in calreticulin; PBMC, peripheral blood mononuclear cell.

Single-Cell Analyses of MF and ET Samples Indicate Minimal Reduction in mutCALR^+ TPO-R $^-$ Cells at Early Time Points



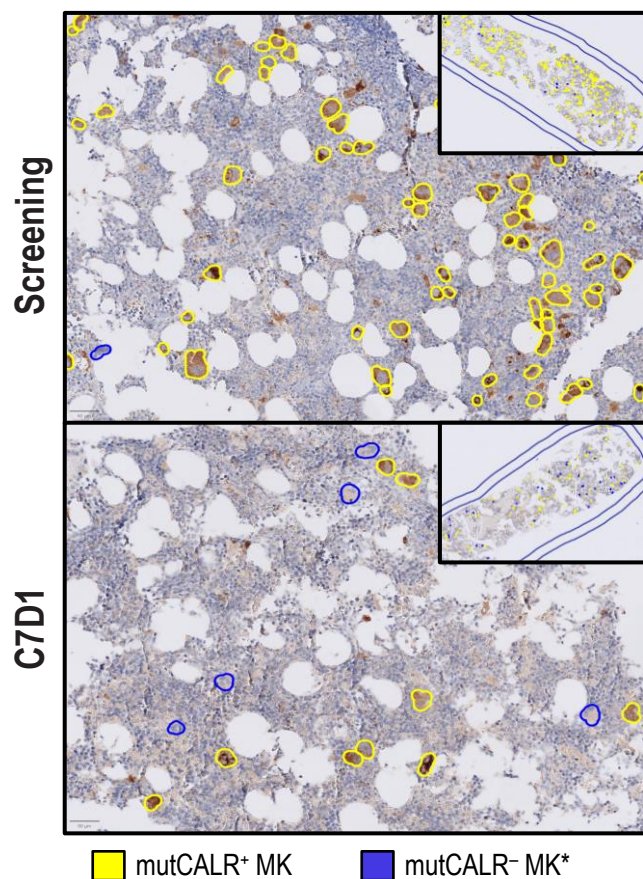
- The percentage of mutCALR^+ lymphocytes are relatively unchanged
- Among mutCALR carrier cells, the percentage of mutCALR^+ monocytes are consistently but modestly decreased

Presented single-cell DNA sequencing data is from available dose-escalation patient samples: ET (n=7, 70-750 mg), MF (n=12, 50-1500 mg).

C, cycle; D, day; ET, essential thrombocythemia; MF, myelofibrosis; mutCALR , mutations in calreticulin; NK, natural killer; TPO-R, thrombopoietin receptor.

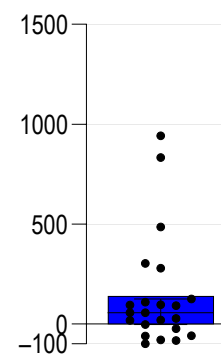
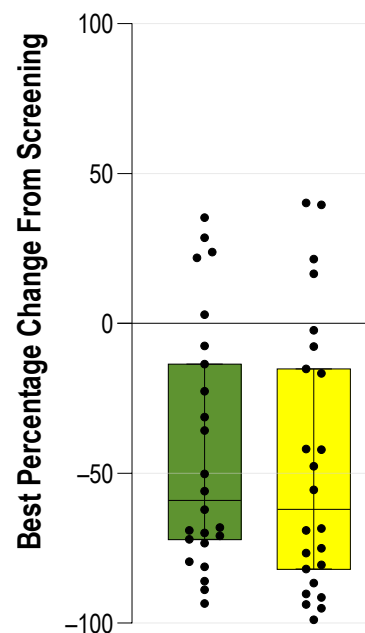
Treatment With INCA033989 Rapidly Decreases mutCALR⁺ Megakaryocytes in Bone Marrow Samples From Patients With ET and MF

MK Staining by mutCALR IHC (ET)

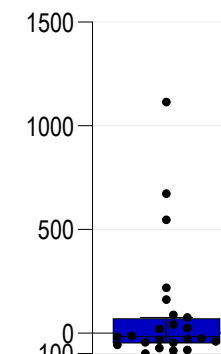
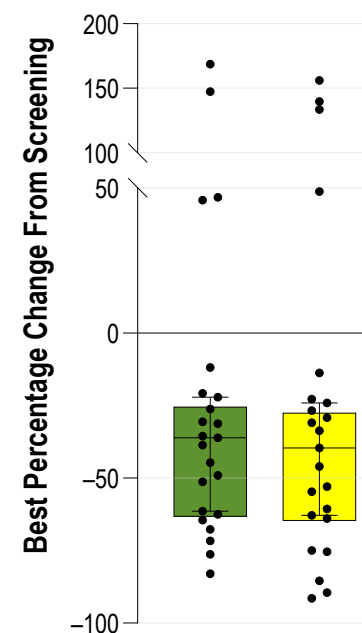


Best Percentage Change From Screening in Total, mutCALR⁺, and mutCALR⁻ MKs

Patients With ET (n=24)



Patients With MF (n=23)*



■ Total MKs ■ mutCALR⁺ MKs ■ mutCALR⁻ MKs

- Reductions in total and mutCALR⁺ megakaryocytes is accompanied by an increase in wild-type (mutCALR⁻) MKs

*1 patient with 0 mutCALR⁻ megakaryocytes at screening is not shown.

Bone marrow mutCALR immunohistochemistry quantitative assessments of mutCALR⁺ and mutCALR⁻ megakaryocytes were conducted by a pathologist at screening and at time points on-treatment (primarily 3 or 6 cycles).

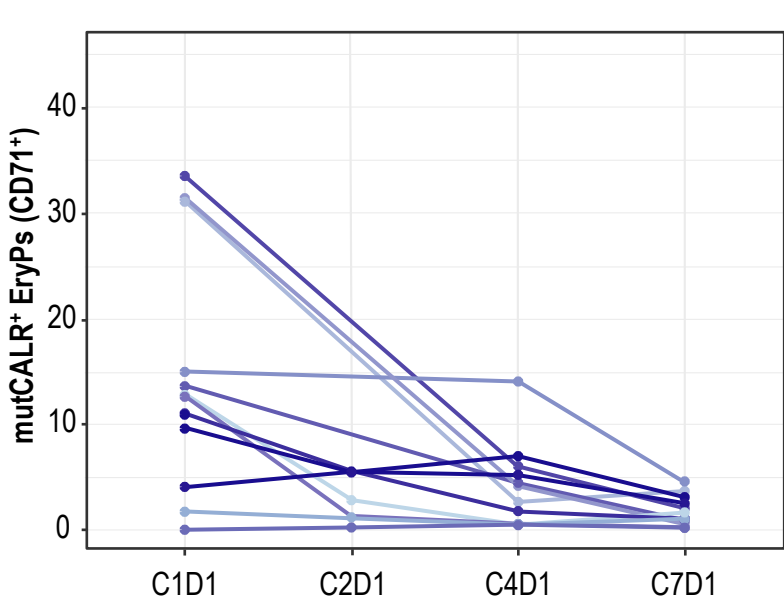
C, cycle; D, day; ET, essential thrombocythemia; IHC, immunohistochemistry; MF, myelofibrosis; MK, megakaryocyte.

INCA033989 Normalizes Erythropoiesis in Patients With MF and Is Associated With Anemia Response

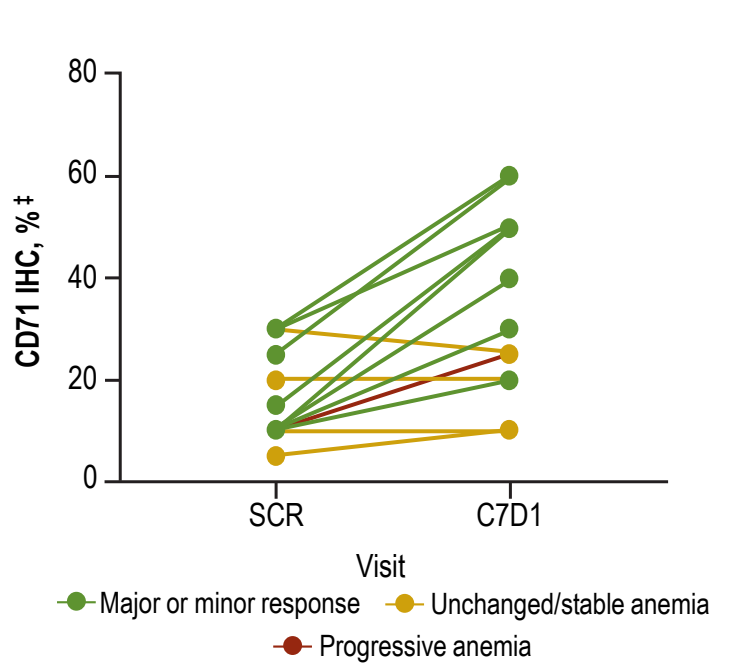
Anemia Response* in Evaluable Patients (MF)

Variable	TDA (n=5)	Non-TDA (n=20)	Total (n=25)
Best anemia response, n (%)			
Major response	1 (20)	9 (45)	10 (40)
Minor response	2 (40)	2 (10)	4 (16)
Stable anemia	1 (20)	7 (35)	8 (32)
Progressive anemia	1 (20)	1 (5)	2 (8)
Missing†	0	1 (5)	1 (4)

Atypical Circulating mutCALR⁺ EryPs (MF)



EryPs in BM of Anemic Patients With MF

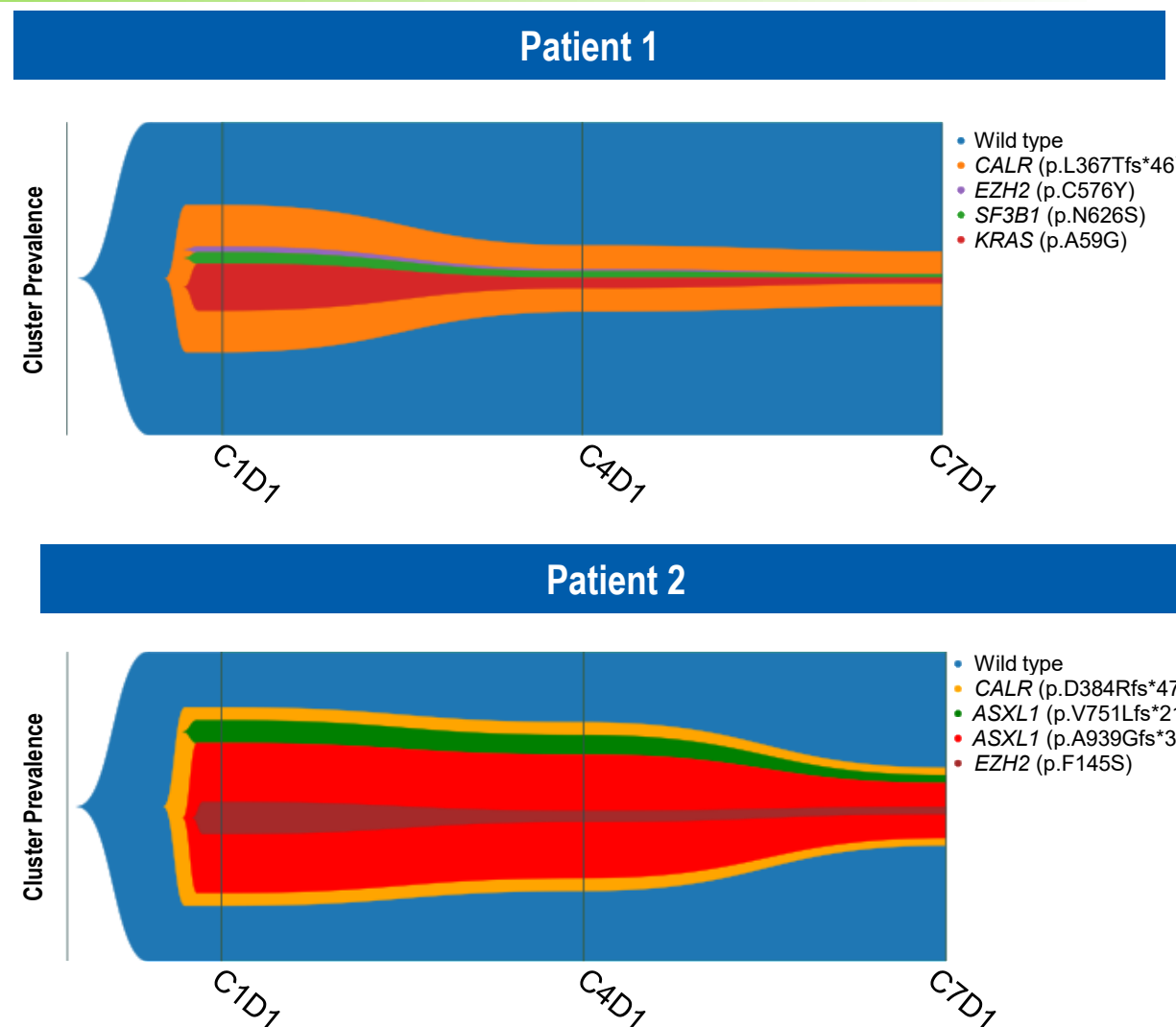


- Elevated levels of atypical circulating erythroid progenitor cells (EryPs) (CD71⁺) associated with extramedullary hematopoiesis were decreased in patients with MF with INCA033989 treatment
- EryPs (CD71 immunohistochemistry [IHC]) in bone marrow increased in anemic patients with MF, correlating with hemoglobin increase and clinical anemia response

Presented single-cell DNA sequencing data are from available dose-escalation patient samples: MF (n=12, 50-1500 mg).
*Criteria for baseline anemia and response based on Tefferi A. *Blood*. 2024;114:1813. Major and minor anemia responses were according to IWG-ELN response criteria. †Patient terminated treatment before 12 weeks. ‡CD71 IHC was centrally assessed by a single pathologist; data include all patients with baseline anemia and available CD71 IHC for screening and C7D1 (n=14).
BM, bone marrow; C, cycle; D, day; IWG-ELN, International Working Group-Myeloproliferative Neoplasms-European LeukemiaNet; MF, myelofibrosis; mutCALR, mutations in calreticulin; SCR, screening; TDA, transfusion-dependent anemia.

Reductions in mutCALR Clones in PBMCs are Evident, Regardless of the Presence of Co-occurring Mutations in Patients With MF

- 76.5% (39/51) of patients with MF had a co-occurring mutation (mean [range], 2.6 [1, 4])
- 40.5% (15/37*) of patients with a co-occurring mutation had SVR35 and/or anemia response
- 2 patients with MF (analyzed with single-cell sequencing) with high clonal complexity are displayed on the right and demonstrate reductions in all clones with mutCALR, independent of co-occurring mutations



*2 patients with co-occurring mutations at baseline did not have response data.

C, cycle; CALR, calreticulin; D, day; MF, myelofibrosis; mutCALR, mutations in calreticulin; PBMC, peripheral blood mononuclear cell; SVR35, spleen volume reduction $\geq 35\%$.

Conclusions

- INCA033989 results in rapid normalization of platelet counts in ET and splenomegaly and anemia responses in MF
- Clinical responses are associated with rapid reductions in mut*CALR* clone burden
 - Demonstrating speed and depth of molecular response
 - Highlighting VAF as a relevant, measurable endpoint
- Improvements in bone marrow are demonstrated by decreases in mut*CALR* megakaryocytes and increases in erythroid progenitor cells and are associated with anemia response
- Clonal responses in MF are also observed in patients who have co-occurring high-risk mutations, including those associated with increased risk of progression to acute myeloid leukemia
- Clinical safety, tolerability, and efficacy in the ET and MF cohorts will be presented in upcoming ASH sessions:
 - INCA033989 in MF (#484)—**Dr. John Mascarenhas, Dec 7, at 10:15 AM**
 - INCA033989 in ET (#1024)—**Dr. Vikas Gupta, Dec 8, at 5:15 PM**

Acknowledgments

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