MPN-553

Preclinical Evaluation of INCA035784, a Novel T-Cell–Redirecting Antibody for the Treatment of mutCALR-Driven Myeloproliferative Neoplasms (MPNs)

Presented at the
Society of Hematologic Oncology (SOHO) 2026 Annual Meeting
Houston, TX, USA • September 9-12, 2026
Originally presented at EHA 2026

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Background

- Mutations in exon 9 of the calreticulin gene (mut*CALR*) are the second most common drivers of myeloproliferative neoplasms (MPNs), occurring in ~25% and ~35% of essential thrombocythemia (ET) and myelofibrosis (MF), respectively[1]
- mutCALR proteins exhibit a novel, positively charged C-terminus that forms an abnormal complex with the thrombopoietin receptor (TPOR), resulting in cytokine-independent JAK/STAT signaling and malignant clonal growth[1,2]
- Type 1 (52-bp deletion) and Type 2 (5-bp insertion) mutations are the 2 major mut*CALR* classes, generating a shared mutant C-terminal sequence; however, more than 100 mut*CALR* variants have been described in MPNs, including atypical mutations that may significantly alter the C-terminal structure[3,4]
- Cell surface-expressed mutCALR in complex with TPOR represents an attractive cancer cell-specific therapeutic target, and antibodies directed against the mutant C-terminus are currently in phase 1 clinical trials[5–7]
- INCA035784 is a novel T-cell–redirecting antibody that binds within the conserved N-domain of mutCALR when it is in complex with TPOR, and is hypothesized to be equipotent across all C-terminal variants[8]
- This study evaluated the ability of INCA035784 to selectively deplete MPN-initiating stem cells and fibrosis-driving megakaryocytes (MKs) from patients with a range of *CALR* mutations

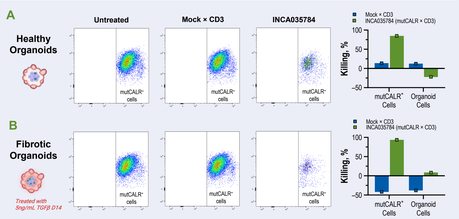
Summary of Patients With mutCALR-Driven MPNs

Disease Characteristics and Treatment Status of Patients With Samples Tested in This Study

Patient ID	Diagnosis	*CALR* Driver Mutation	VAF (%)	Secondary Mutations	Treatment at Time of Sampling
Pt A	PMF	Type 2	50	*EZH2, ASXL1*	ruxolitinib (25 mg bid)
Pt B	PMF	Type 2	40	*SF3B1*	ruxolitinib (15 mg bid)
Pt C	PMF	Type 2	39	*ASXL1, EZH2, TET2*	momelotinib (100 mg qd)
Pt D	ET	Type 1	NA	None	None
Pt E	PMF	Type 1	44	None	None
Pt F	Post-ET MF	Type 1	46	*ASXL1*	ruxolitinib (15 mg bid)
Pt G	PMF	Type 2-like*	39	*NFE2*	ruxolitinib (20 mg bid)
Pt H	PMF	Type 2	40	None	None
Pt I	PMF	Type 1	47	None	None
Pt J	Post-ET MF	Type 1	50	*CSF3R, PPM1D*	hydroxycarbamide
Pt K	PMF	Type 2	45	*ASXL1, TET2, EZH2, KRAS*	None
Pt L	Post-ET MF	Type 2	45	*TET2*	None
Pt M	PMF	Type 2	38	*DNMT3A*	None
Pt N	PMF	Type 1	50	*TET2, ASXL1*	None
Pt O	Post-ET MF	Type 2-like†	NA	NA	NA
Pt P	PMF	Type 1	48	*ASXL1*	None

*Atypical mut*CALR*: c.[1141del; 1221del] p.(Glu381ArgfsTer60).
†Atypical mut*CALR*: c.[1151_1154delinsCTTGTC;1214del].
bid, twice daily; CALR, calreticulin; ET, essential thrombocythemia; mutCALR, mutations of calreticulin; NA, data not currently available; PMF, primary myelofibrosis; post-ET MF, post-essential thrombocythemia myelofibrosis; Pt, patient; qd, once daily; VAF, variant allele frequency.

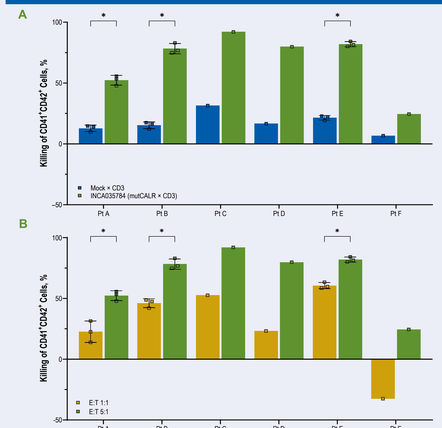
INCA035784 Selectively Reduces Target Cells in Both Healthy and Fibrotic Bone Marrow Organoids With Minimal Niche Toxicity



Representative flow plots and quantified killing of fluorescently labeled mutCALR⁺ cells (BFP⁺TPOR⁺ MARIMO) in healthy (A) and fibrotic (B) bone marrow organoids treated with INCA035784 or mock × CD3 (each 1 µg/mL). Each data point represents 6 organoids pooled together. Percent killing was normalized to untreated controls. Organoid fibrosis was induced by treatment with TGFβ.[9,10]
mutCALR, mutations of calreticulin.

- Healthy and fibrotic bone marrow organoids were engrafted with TPOR-expressing MARIMO cells (Type 1-like mut*CALR*) and healthy donor T cells (1:1 effector:target [E:T] ratio)
- Antibodies (1 µg/mL) were added to the organoids, and target cells were quantified after 72 hours by flow cytometry
- INCA035784 induced selective killing of mutCALR-expressing MARIMO cells in both healthy and fibrotic bone marrow organoids (>80% in both conditions), suggesting cytotoxic activity in both healthy (**A**) and fibrotic (**B**) bone marrow environments
- Minimal off-target toxicity to organoid niche cells was observed

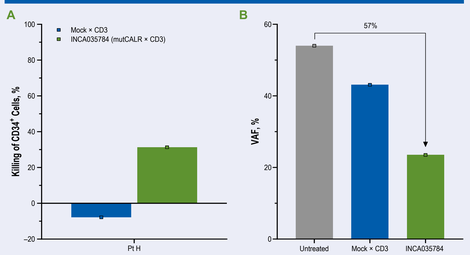
INCA035784 Demonstrates Robust Killing of CD34-Derived MK Progenitors (MKPs) From Patients Co-Cultured With Healthy Donor T Cells



*P<0.05 (unpaired t test).
Percent killing of patient-derived megakaryocyte (CD41⁺CD42⁺) cells with INCA035784 or mock × CD3 control and healthy donor T cells at 5:1 E:T (A), comparing percentage killing with INCA035784 at different E:T ratios (B). Percent killing was normalized to untreated controls. Error bars indicate standard deviation.
E, effector; mutCALR, mutations of calreticulin; Pt, patient; T, target.

- CD34⁺ cells from bone marrow or blood were differentiated towards the MK lineage for 6 days (thrombopoietin/stem cell factor) and then co-cultured with healthy donor T cells ± antibody (INCA035784 or mock × CD3; each 1 µg/mL) at 1:1 or 5:1 E:T ratios for 72 hours
- Cytotoxicity (% killing) was assessed by flow cytometry as reduction in CD41⁺CD42⁺ MKs normalized to untreated controls
- INCA035784 demonstrated killing of CD41⁺CD42⁺ MKs across all patient samples at 5:1 E:T, ranging from 25-92% (**A**)
- At a 1:1 E:T ratio, 5/6 patient samples showed target cell depletion when treated with INCA035784 (**B**)

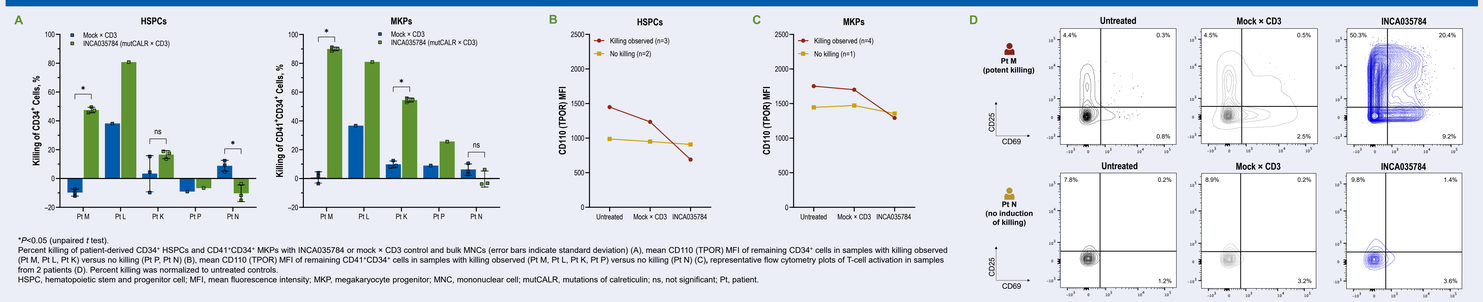
INCA035784-Induced Killing of Patient-Derived CD34⁺ Cells With Autologous T Cells Is Associated With a Decrease in Variant Allele Frequency (VAF)



Percent killing of CD34⁺ HSPCs derived from Pt H with INCA035784 or mock × CD3 control and autologous T cells at 1:1 E:T (A), with the change in VAF determined by droplet digital PCR (B). Percent killing was normalized to untreated controls.
E, effector; HSPC, hematopoietic stem and progenitor cells; mutCALR, mutations of calreticulin; Pt, patient; T, target; VAF, variant allele frequency.

- CD34⁺ hematopoietic stem and progenitor cells (HSPCs) and autologous CD3⁺ T cells were isolated from patient peripheral blood and co-cultured (1:1 ratio) ± antibody (INCA035784 or mock × CD3; each 1 µg/mL) for 72 hours
- Reduction in CD34⁺ cells was assessed by flow cytometry and droplet digital PCR (ddPCR) was performed on sorted CD34⁺ cells to determine VAF
- Samples derived from Pt H demonstrated target cell depletion with autologous T cells (**A**), with an associated 57% reduction in VAF, confirming selective depletion of the malignant clone (**B**)

INCA035784 Induces Killing of HSPCs and MKPs When Added to Total Mononuclear Cells (MNCs), With Activity Correlating With TPOR Expression and T-Cell Activation



*P<0.05 (unpaired t test).
Percent killing of patient-derived CD34⁺ HSPCs and CD41⁺CD34⁺ MKPs with INCA035784 or mock × CD3 control and bulk MNCs (error bars indicate standard deviation) (A), mean CD110 (TPOR) MFI of remaining CD34⁺ cells in samples with killing observed (Pt M, Pt L, Pt K) versus no killing (Pt P, Pt N) (B), mean CD110 (TPOR) MFI of remaining CD41⁺CD34⁺ cells in samples with killing observed (Pt M, Pt L, Pt K, Pt P) versus no killing (Pt N) (C), representative flow cytometry plots of T-cell activation in samples from 2 patients (D). Percent killing was normalized to untreated controls.
HSPC, hematopoietic stem and progenitor cell; MFI, mean fluorescence intensity; MKP, megakaryocyte progenitor; MNC, mononuclear cell; mutCALR, mutations of calreticulin; ns, not significant; Pt, patient.

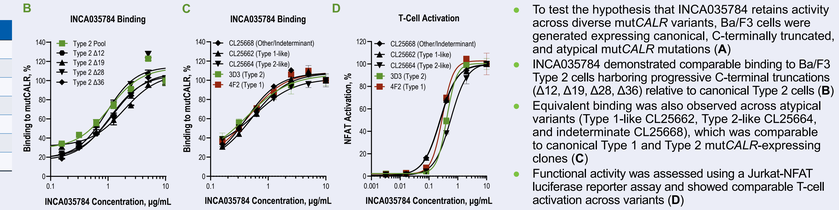
- To assess INCA035784 in a more physiological context, the antibody was added directly to total MNCs without manipulation of E:T ratios
- Reduction in HSPCs (CD34⁺ cells) and MKPs (CD41⁺CD34⁺) was assessed at 72 hours by flow cytometry
- INCA035784 induced depletion of HSPCs in 3/5 and MKPs in 4/5 patient samples (**A**)
- Where killing was observed, mean TPOR expression (used here as a surrogate marker for mutCALR) of the remaining cells was reduced following INCA035784 addition, consistent with on-target, specific killing of high TPOR-expressing cells (**B, C**)
- In these samples, INCA035784 also induced strong T-cell activation (CD25⁺CD69⁺) compared to untreated and mock × CD3 controls, whereas samples lacking cytotoxic activity showed minimal change in T-cell activation markers (**D**)

INCA035784 Demonstrates Binding and Activity in Ba/F3 Cells Expressing C-Terminally Truncated and Atypical mutCALR

A

Entity	C-Terminal Sequence
Wild-Type CALR	DEEQRLKEEEEDKKRKEEEEAEDKEDDEDKDEDEEDEEDKEEDEEEDVPGQAKDEL
mutCALR Type 1 (4F2)	DEEQR----------RRRRQRTRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA
mutCALR Type 2 (3D3)	DEEQRLKEEEEDKKRKEEEEAEDNCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTSCREACLQGWTEA
mutCALR Type 2 Ins. 5 Δ12	DEEQRLKEEEEDKKRKEEEEAEDNCRRMMRTKMRMRRMRRTRRKMRRKMSPARPRTS
mutCALR Type 2 Ins. 5 Δ19	DEEQRLKEEEEDKKRKEEEEAEDNCRRMMRTKMRMRRMRRTRRKMRRKMS
mutCALR Type 2 Ins. 5 Δ28	DEEQRLKEEEEDKKRKEEEEAEDNCRRMMRTKMRMRRMRRT
mutCALR Type 2 Ins. 5 Δ36	DEEQRLKEEEEDKKRKEEEEAEDNCRRMMRTKM

C-terminal sequences of engineered Ba/F3 mutCALR variants (A), INCA035784 binding to C-terminal truncation variants (B), INCA035784 binding (C) and mediated T-cell activation (D) across canonical and atypical mutCALR variants. INCA035784 binding was assessed by flow cytometry.
CALR, calreticulin; Ins, insertion; mutCALR, mutations of calreticulin; NFAT, nuclear factor of activated T cells.



- To test the hypothesis that INCA035784 retains activity across diverse mut*CALR* variants, Ba/F3 cells were generated expressing canonical, C-terminally truncated, and atypical mut*CALR* mutations (**A**)
- INCA035784 demonstrated comparable binding to Ba/F3 Type 2 cells harboring progressive C-terminal truncations (Δ12, Δ19, Δ28, Δ36) relative to canonical Type 2 cells (**B**)
- Equivalent binding was also observed across atypical variants (Type 1-like CL25662, Type 2-like CL25664, and indeterminate CL25668), which was comparable to canonical Type 1 and Type 2 mut*CALR*-expressing clones (**C**)
- Functional activity was assessed using a Jurkat-NFAT luciferase reporter assay and showed comparable T-cell activation across variants (**D**)

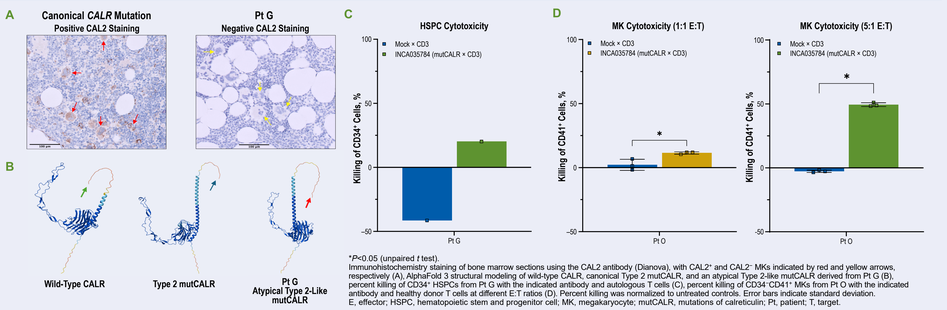
INCA035784 Selectively Depletes Target Cells in Samples From Patients With Atypical mutCALR Variants

Pt G

- 2 independent 1-bp deletions in cis, classified as a Type 2-like mut*CALR*:
  - Frameshift 1: E381Rfs*49
  - Frameshift 2: D408Mfs*22
- Bone marrow histology confirmed MF with characteristic MK hyperproliferation, but immunohistological staining using the CAL2 antibody, which is selective for the mutCALR C-terminus, was notably negative (**A**)
- Structural modeling of the protein using AlphaFold 3 predicted abnormal C-terminal folding compared to both wild-type CALR and canonical Type 2 mutCALR (**B**)
- Despite the aberrant C-terminal structure, INCA035784 induced ~20% killing of CD34⁺ HSPCs with autologous T cells at 1:1 E:T (**C**)

Pt O

- 2 independent mutations in cis, classified as a Type 2-like mut*CALR*:
  - Frameshift 1: D384Afs*47 (deletion-insertion)
  - Frameshift 2: E405Gfs*25 (1-bp deletion)
- No killing with a C-terminus–targeting monoclonal antibody (data on file)
- INCA035784 induced ~10% killing of MKs at 1:1 E:T and ~50% killing of MKs at 5:1 E:T with healthy donor T cells (**D**)



*P<0.05 (unpaired t test).
Immunohistochemistry staining of bone marrow sections using the CAL2 antibody (Dianova), with CAL2⁺ and CAL2⁻ MKs indicated by red and yellow arrows, respectively (A). AlphaFold 3 structural modeling of wild-type CALR, canonical Type 2 mutCALR, and an atypical Type 2-like mutCALR derived from Pt G (B), percent killing of CD34⁺ HSPCs from Pt G with the indicated antibody and autologous T cells (C), percent killing of CD34⁺CD41⁺ MKs from Pt O with the indicated antibody and healthy donor T cells at different E:T ratios (D). Percent killing was normalized to untreated controls. Error bars indicate standard deviation.
E, effector; HSPC, hematopoietic stem and progenitor cell; MK, megakaryocyte; mutCALR, mutations of calreticulin; Pt, patient; T, target.

Disclosures

Wang: Employment and stock ownership – *Incyte Corporation*.

Acknowledgments

The authors wish to thank Ruth Overton for help with obtaining patient samples. The authors gratefully acknowledge the collaboration with Merus B.V. for lead discovery and early-stage research activities. This study was sponsored by Incyte Corporation (Wilmington, DE, USA). Editorial and graphics support was provided by Envision Pharma Group (Fairfield, CT, USA) and funded by Incyte Corporation.

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Conclusions

- **INCA035784 induced T-cell–mediated cytotoxicity of mutCALR⁺ cells, including stem/progenitor cells and MK lineage cells**
- **Efficacy of INCA035784 was demonstrated against samples from patients with Type 1 and Type 2 mutations, including those with atypical *CALR* variants and additional high-risk co-mutations, in several assays:**
  - **Isolated CD34⁺ cells with autologous T cells**
  - **In vitro differentiated MKs with healthy donor T cells**
  - **Direct addition of antibody to total MNCs without manipulation of E:T ratios**
  - **Healthy and fibrotic human marrow organoids**
- **By targeting the conserved mutCALR N-domain, INCA035784 may have advantages over C-terminal–directed therapies, especially in patients harboring atypical mut*CALR* variants**