INCB052793, a JAK1 Selective Inhibitor Is Highly Efficacious in PDX and Xenograft Models of Acute Myeloid Leukemia Expressing Elevated Endogenous pSTAT3/pSTAT5

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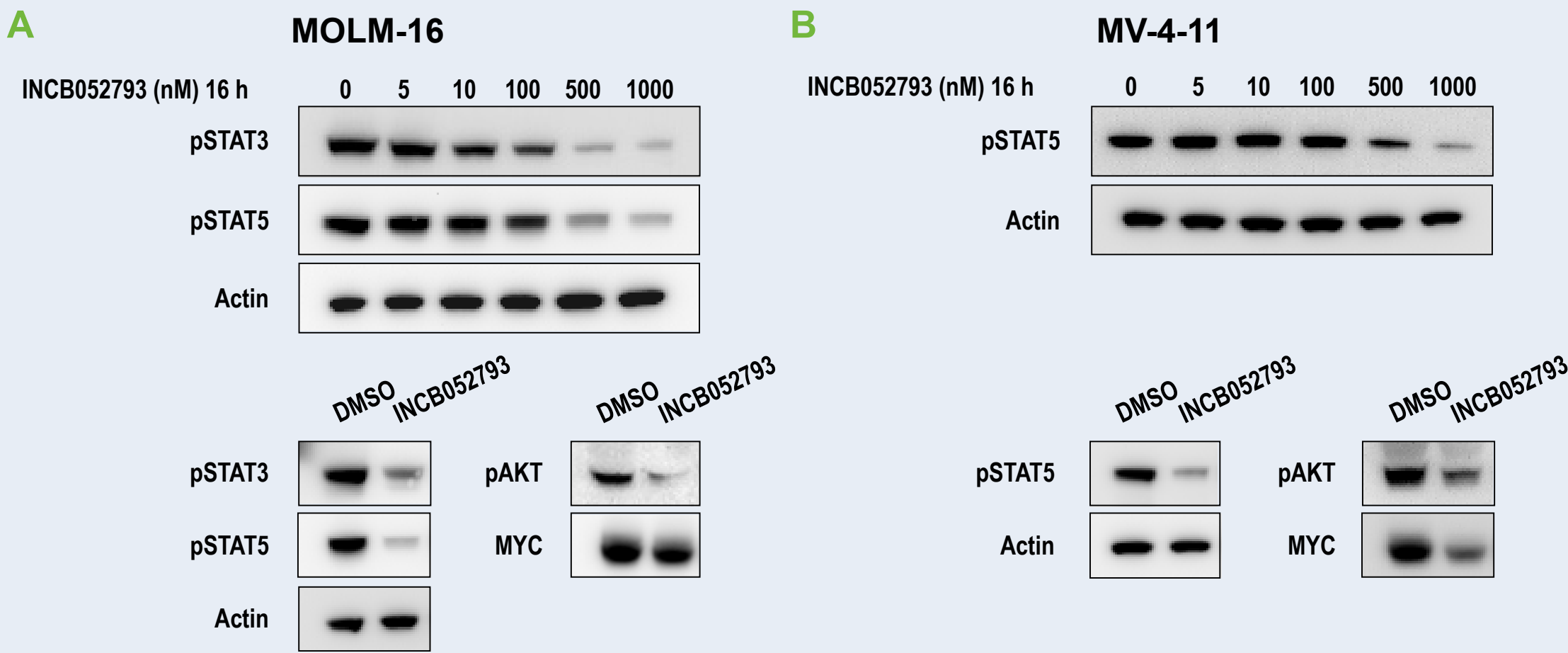
Abstract

Acute myeloid leukemia (AML) is characterized by infiltration of abnormally differentiated, clonal, and highly proliferative cells of the hematopoietic system that acquire successive genomic alterations. AML is the most common acute leukemia in adults. Current therapies of limited utility and involve a combination of cytarabine and anthracycline-based regimens with allogeneic stem cell transplantation for eligible candidates, but there is an urgent need to improve therapies for AML. JAK/STAT pathway dysregulation plays a role in the pathogenesis of AML, and the JAK2 V617F mutation is present in only a small percentage of these patients. Studies were conducted to evaluate the in vitro and in vivo activities of INCB052793, a highly JAK1-selective inhibitor having 100-fold selectivity for JAK1 over JAK2 in cell lines, xenograft, and PDX models of human AML having elevated endogenous pSTAT3 and/or pSTAT5 activation. In vitro, INCB052793 effectively inhibited pSTAT3 and/or pSTAT5 phosphorylation in MV-4-11, MOLM-16, and MOLM-13 cell lines and caused marked reductions in pAKT and MYC levels in MV-4-11 and MOLM-16 cells. Given these observations, oral administration of INCB052793 was evaluated at doses of 10 and 30 mg/kg twice daily in MOLM-16 xenografts and FLT3-ITD AML xenograft models, MV-4-11 and MOLM-13 in severe combined immunodeficiency (SCID) mice. INCB052793 administration significantly inhibited tumor growth in MOLM-16 xenografts in a dose-dependent manner and resulted in complete downregulation of pSTAT3 and pSTAT5 levels in MOLM-16 tumors. Similarly, INCB052793 administration was highly effective in inhibiting tumor growth in FLT3-ITD AML models, MV-4-11 and MOLM-13. Administration of INCB052793 in a systemic PDX model of AML with elevated endogenous levels of pSTAT3 and pSTAT5 resulted in amelioration of disease severity and demonstrated a significant effect on median survival in NOG mice. All dosing regimens of INCB052793 in both xenograft and PDX models were well tolerated. Since azacitidine and cytarabine are standards of care for the treatment of AML, the efficacy of INCB052793 was benchmarked against optimal dosing regimens of these agents. In the AML xenograft models evaluated, INCB052793 was comparably or more effective in reducing tumor burden than azacitidine and cytarabine. The combination of INCB052793 with cytarabine showed superior efficacy in comparison to single agents in the MOLM-16 xenograft model, and combinatorial studies are in progress in additional AML models. These findings suggest the therapeutic potential of INCB052793 as a single agent and in combination with standard-of-care chemotherapeutic regimens for the management of AML.

In Vitro Potency of INCB052793 in Cytokine and Growth Factor–Stimulated Cells

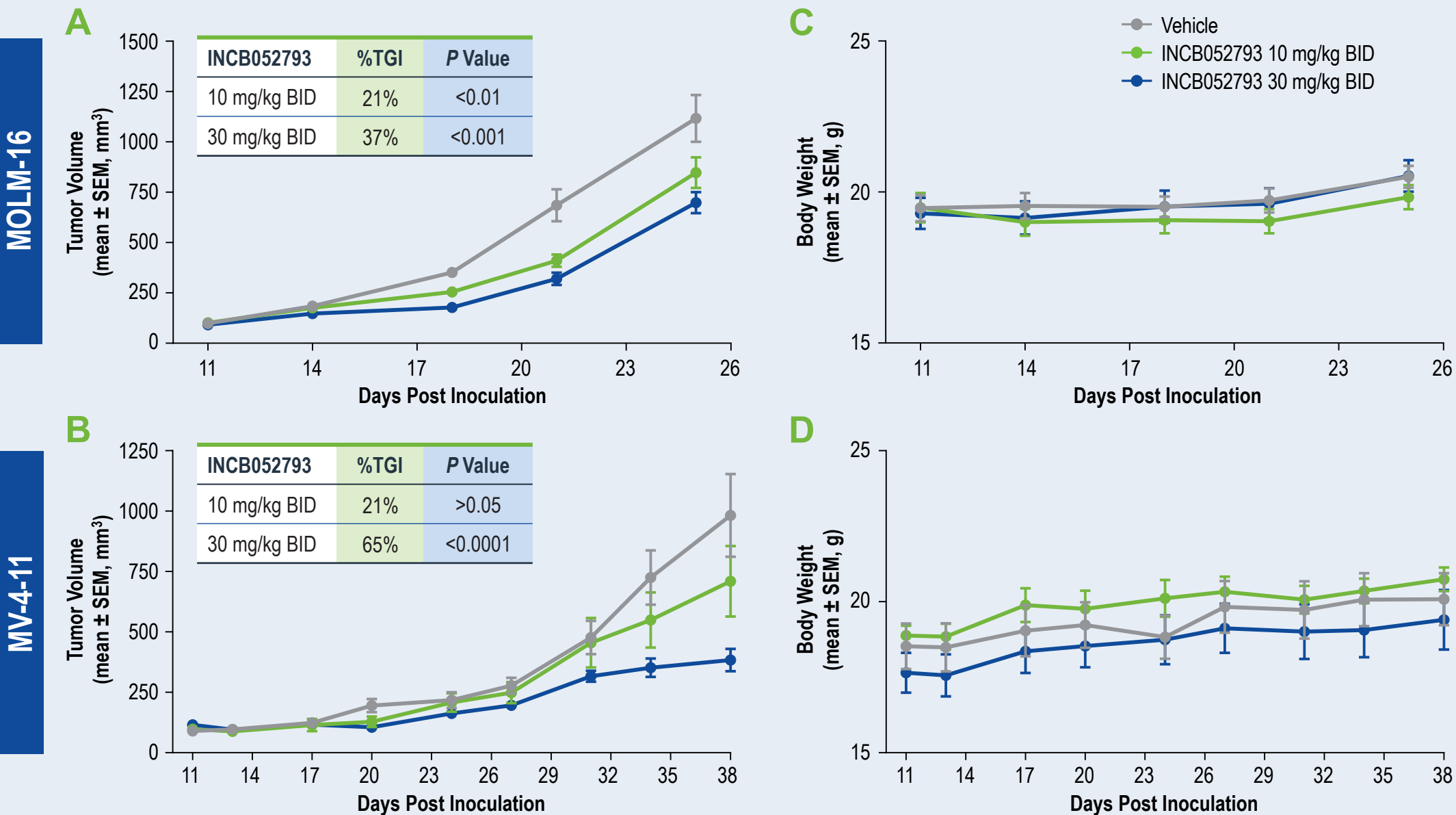| Assay Readout	Cell Type	Stimulus	JAK	Mean IC$_{50}$, nM*
Signaling assays				
pSTAT3	Human whole blood	IL-6	JAK1/JAK2	144 ± 8
pSTAT3	Human whole blood	TPO	JAK2	14,110 ± 349
pSTAT1	Human whole blood	IL-6	JAK1/JAK2	21
pSTAT5	Human whole blood	GM-CSF	JAK2	3774
pSTAT3/pSTAT5	Human PBMCs	IL-2	JAK1/JAK3	10–30
Functional assay				
IL-17 production	Human T cells	IL-23		10 ± 1
Proliferation	Human T cells	IL-2		12.4 ± 1.2
Proliferation	INA-6	IL-6		238 ± 29
MCP-1 production	Human PBMCs	IL-6		103 ± 21

*Values are presented as mean ± standard deviation.
GM-CSF, granulocyte macrophage colony-stimulating factor; JAK, Janus kinase; IC$_{50}$, half maximal inhibitory concentration; MCP, monocyte chemoattractant protein; PBMC, peripheral blood mononuclear cell; pSTAT, phosphorylated signal transducers and activators of transcription; TPO, thrombopoietin.

Effects of INCB052793 In Vitro in MOLM-16 and MV-4-11 Cells of AML

MOLM-16 cells were treated with increasing concentrations of INCB052793 and immunoblotted for pSTAT3 (Tyr705), pSTAT5 (Tyr694), and Actin. Effects of INCB052793 on signaling were determined after 16 hours of treatment (A). Similarly, MV-4-11 cells were treated as indicated and immunoblotted for pSTAT5 and Actin (B). Effects of INCB052793 (1000 nM) on signaling were evaluated by immunoblotting for pAKT (Ser473) and MYC in both MOLM-16 and MV-4-11 cells. Actin served as a loading control. INCB052793 substantially downregulated the expression of pSTAT3, pSTAT5, pAKT, and MYC in AML cells; pSTAT3 was not detected in the FLT3-ITD cell line MV-4-11 that harbors a t(4;11) translocation leading to an MLL-AF4 fusion.
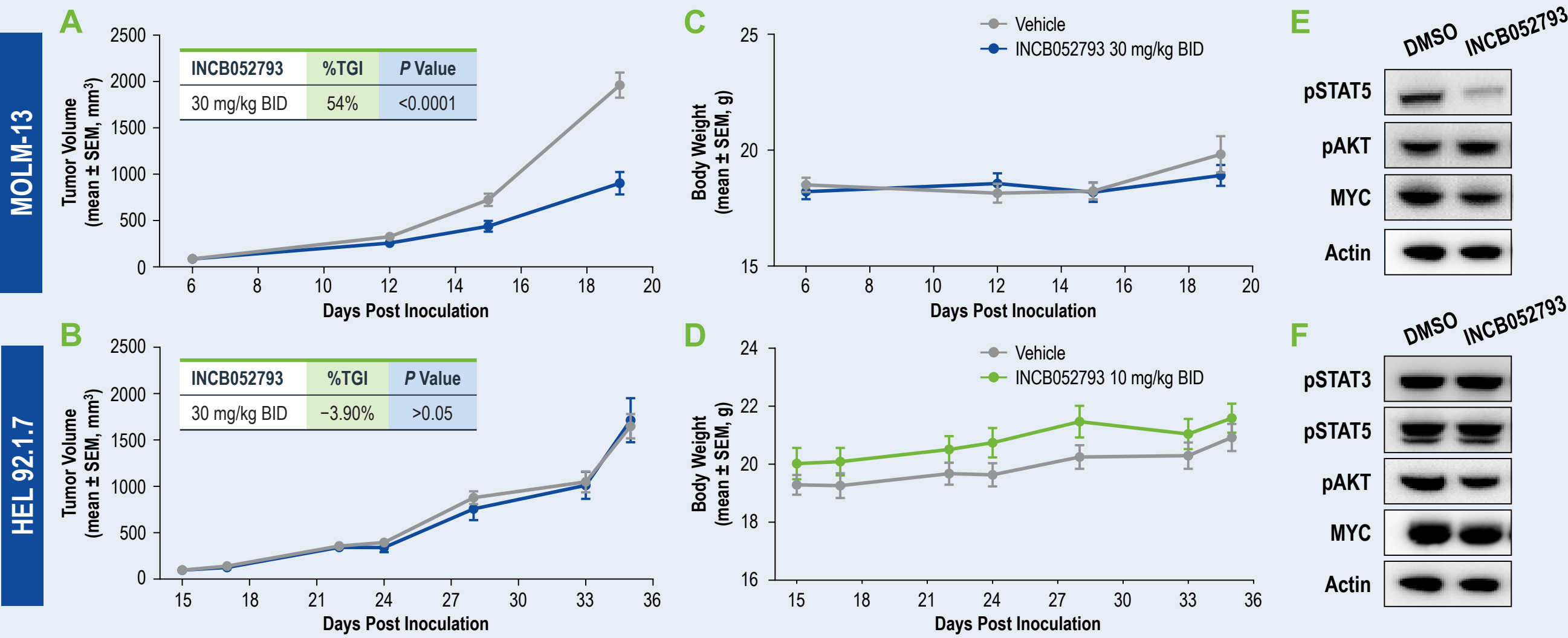
Efficacy of INCB052793 in the MOLM-16 and MV-4-11 Xenograft Model
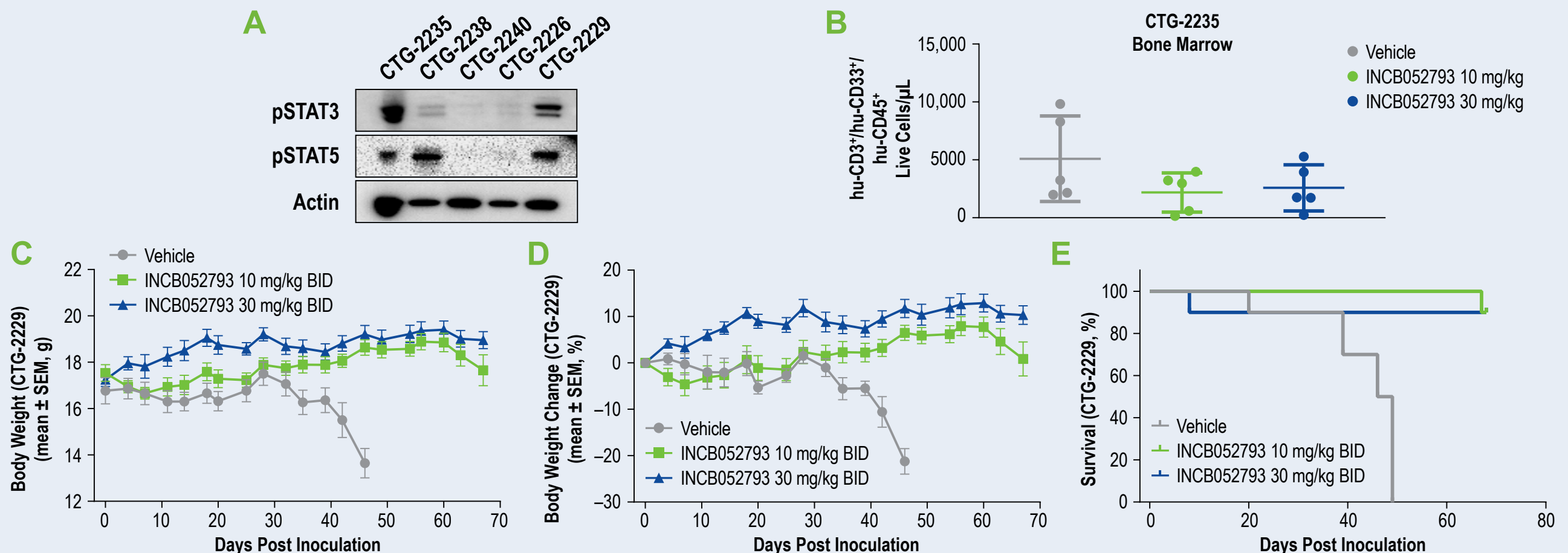

SEM, standard error of the mean.

Female SCID mice were subcutaneously inoculated with (A) 1×10^{7} MOLM-16 or with (B) MV-4-11 cells in Matrigel® (Corning Life Sciences, Tewksbury, MA) (n = 10 per group) and dosed daily with vehicle or either 10 mg/kg or 30 mg/kg twice daily (BID) INCB052793. Efficacy of INCB052793 was evaluated by assessing percentage of tumor growth inhibition (%TGI) over time. In the MOLM-16 xenograft model compared with the control, %TGI with single-agent INCB052793 at both 10 mg/kg BID (P < 0.01) and 30 mg/kg BID (P < 0.001) was significant (A). In the MV-4-11 xenograft model compared with the control, %TGI with single-agent INCB052793 at 30 mg/kg BID (P < 0.0001) was significant, with nonsignificant %TGI at 10 mg/kg BID (B). All dosing regimens were well tolerated (C, D). %TGI and statistical analyses were assessed by 2-way analysis of variance (ANOVA) with Šidák's multiple comparisons test.

Efficacy and In Vitro Effects of INCB052793 in MOLM-13 and HEL 92.1.7 (JAK2 V617F) AML Models
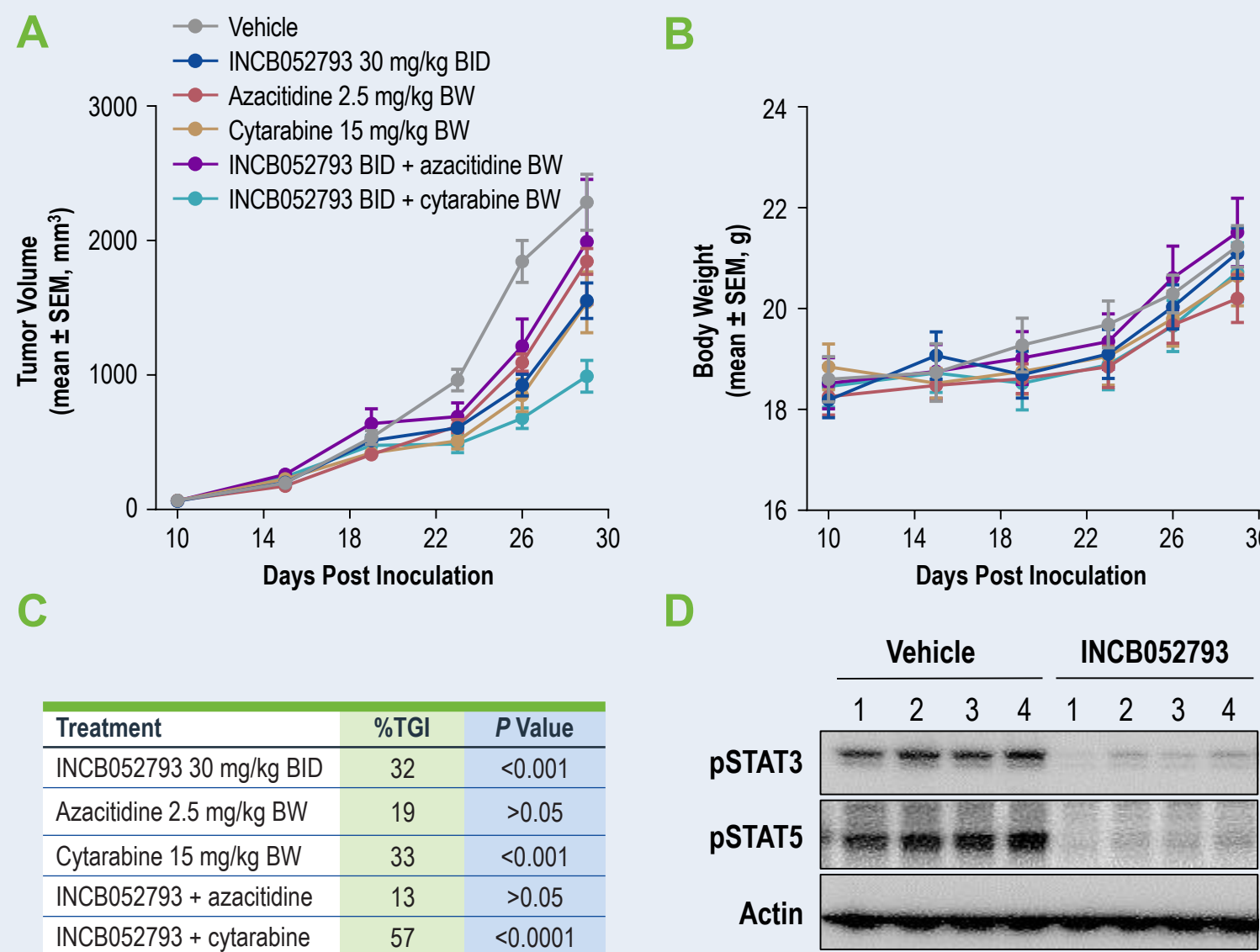

Female SCID mice were subcutaneously inoculated with (A) 1×10^{7} MOLM-13 or (B) HEL (JAK2 V617F) cells in Matrigel (n = 10 per group) and dosed daily with vehicle or INCB052793 30 mg/kg BID. Efficacy of INCB052793 was evaluated by assessing %TGI over time. Compared with the control, %TGI with single-agent INCB052793 at 30 mg/kg BID was significant in the MOLM-13 xenograft model (P < 0.0001) but not significant in the HEL (JAK2 V617F) xenograft model (P > 0.05) (A, B). %TGI was not significant in either model for INCB052793 10 mg/kg BID (data not shown). All dosing regimens were well tolerated (C, D). Effects of INCB052793 were evaluated on target inhibition and signaling in MOLM-13 and HEL (JAK2 V617F) cells treated with 1000 nM drug for 16 hours and immunoblotted for pSTAT3, pSTAT5, pAKT, MYC, and Actin. INCB052793 slightly downregulated the expression of pSTAT5 in the MOLM-13 xenograft model (E). As expected, INCB052793 was not effective in reducing tumor burden in the HEL (JAK2 V617F) xenograft model, which served as a negative control (F). pSTAT3 was not detected in the FLT3-ITD cell line MOLM-13 that harbors MLL-AF9 fusion. %TGI and statistical analyses were assessed by unpaired 2-tailed t test.

Efficacy Studies of INCB052793 in CTG-2229 and CTG-2235, Disseminated Models of Human AML Expressing High Endogenous Levels of pSTAT3/pSTAT5

Sub-lethally irradiated female NOG (NOD/SCID/IL-2Rγnull) mice were inoculated with human AML cells. Mice were monitored every 2 weeks starting at week 4 post AML inoculation to assess human AML engraftment using huCD4$/$huCD45$/$huCD33$/$huCD3 antibodies and BD TruCount™ beads (BD Biosciences, San Jose, CA). When individual animals had between 10 and 2000 CD33+ blasts/μL peripheral blood, they were randomized to treatment cohorts. Immunoblotting to determine pSTAT3 and pSTAT5 expression in patient-derived xenograft (PDX) mouse models (NOG) of AML were acquired from Champions Oncology, Inc. (Rockville, MD) (A). CTG-2229 and CTG-2235 PDX models were selected for further studies based on the pSTAT3/pSTAT5 phosphorylation levels. In the CTG-2235 PDX models, human CD45, CD33, and CD3 counts (day 60) in bone marrow samples by flow cytometry analysis were suppressed after treatment with INCB052793 at 10 and 30 mg/kg BID compared with vehicle (B). Mean body weight (C) and mean percentage body weight changes (D) in the CTG-2229 PDX model over time are shown. Treatment with INCB052793 at 10 and 30 mg/kg BID significantly increased survival to 67.9 ± 0.3 days (1 death) and 62.0 ± 19.0 days (1 death), respectively, compared with vehicle control (43.5 ± 9.2 days) (E).

Efficacy of INCB052793 in Comparison With Standard-of-Care Agents in MOLM-16 Xenograft Model

Treatment	%TGI	P Value
INCB052793 30 mg/kg BID	32	<0.001
Azacitidine 2.5 mg/kg BW	19	>0.05
Cytarabine 15 mg/kg BW	33	<0.001
INCB052793 + azacitidine	13	>0.05
INCB052793 + cytarabine	57	<0.001

Female SCID mice were subcutaneously inoculated with 1×10^{7} MOLM-16 Matrigel (n = 9 per group) and dosed daily with INCB052793 30 mg/kg BID, azacitidine 2.5 mg/kg twice weekly (BW), cytarabine 15 mg/kg BW, or combinations as indicated (A). All dosing regimens as single agents as well as combinations were well tolerated (B). Increased benefit was observed in the group treated with INCB052793 plus cytarabine with respect to %TGI. %TGI and statistical analyses were assessed by 2-way ANOVA with Šidák's multiple comparisons test (C). Tumors were harvested and immunoblotted for pSTAT3, pSTAT5, and Actin 2 hours post dose on day 19. INCB052793 substantially downregulated pSTAT3 and pSTAT5 expression in the MOLM-16 tumors (n = 4) (D).

Conclusions

- INCB052793 is a highly potent JAK1 inhibitor with selectivity for JAK1 (IC$_{50}$ = 144 nM) versus JAK2 (IC$_{50}$ = 14,410 nM) as observed in cell-based assays
- In vitro pSTAT3 and pSTAT5 expression was inhibited by INCB052793, as a measure of target inhibition across AML cell lines
- INCB052793 as a single agent effectively inhibited tumor growth rate in MOLM-16, MV-4-11, and MOLM-13 xenograft models
- As expected, INCB052793 was ineffective in inducing TGI in the HEL 92.1.7 xenograft model that harbors the JAK2 V617F mutation
- In PDX mouse models of AML expressing high endogenous levels of pSTAT3 and pSTAT5, INCB052793 substantially inhibited tumor burden and improved survival
- INCB052793 was more effective than the standard-of-care agent, azacitidine, and equally efficacious as cytarabine in the MOLM-16 xenograft model
- Combination of INCB052793 and cytarabine conferred added benefit in comparison with single agents
- INCB052793 effectively maintained target inhibition in vivo, as seen by a reduction in pSTAT3 and pSTAT5 levels in MOLM-16 tumors

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