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MYELOID NEOPLASIA

Synergistic targeting of *FLT3* mutations in AML via combined menin-MLL and FLT3 inhibition

Margarita M. Dzama,[1] Marlene Steiner,[1,2] Johanna Rausch,[1,2] Daniel Sasca,[1-3] Jonas Schönfeld,[1] Kerstin Kunz,[1] Martha C. Taubert,[1] Gerard M. McGeehan,[4] Chun-Wei Chen,[5] Annalisa Mupo,[6,7] Patricia Hähnel,[1,3] Matthias Theobald,[1-3] Thomas Kindler,[1-3] Richard P. Koche,[8] George S. Vassiliou,[6,7] Scott A. Armstrong,[9,10] and Michael W. M. Kühn[1-3]

[1]Department of Hematology, Medical Oncology, and Pulmonary Medicine, University Medical Center, Johannes Gutenberg-University, Mainz, Germany; [2]German Consortia for Translational Cancer Research, Mainz, Germany; [3]University Cancer Center Mainz, Mainz, Germany; [4]Syndax Pharmaceuticals, Inc, Waltham, MA; [5]Department of Systems Biology, Beckman Research Institute, City of Hope, Duarte, CA; [6]Welcome-Medical Research Center (MRC) Cambridge Stem Cell Institute, Department of Haematology, University of Cambridge, Cambridge, United Kingdom; [7]Wellcome Sanger Institute, Cambridge, United Kingdom; [8]Center for Epigenetics Research, Memorial Sloan-Kettering Cancer Center, New York, NY; [9]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA; and [10]Division of Hematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston, MA

KEY POINTS

- Inhibition of the menin-MLL interaction targets *FLT3* mutations transcriptionally via *MEIS1* in *NPM1*-mutant and *MLL*-rearranged leukemias.
- Combined menin-MLL and FLT3 inhibition is a synergistic therapeutic opportunity in these leukemia subtypes with concurrent *FLT3* mutation.

The interaction of menin (*MEN1*) and MLL (*MLL1, KMT2A*) is a dependency and provides a potential opportunity for treatment of *NPM1*-mutant (*NPM1*^{mut}) and *MLL*-rearranged (*MLL*-r) leukemias. Concomitant activating driver mutations in the gene encoding the tyrosine kinase FLT3 occur in both leukemias and are particularly common in the *NPM1*^{mut} subtype. In this study, transcriptional profiling after pharmacological inhibition of the menin-MLL complex revealed specific changes in gene expression, with downregulation of the MEIS1 transcription factor and its transcriptional target gene *FLT3* being the most pronounced. Combining menin-MLL inhibition with specific small-molecule kinase inhibitors of FLT3 phosphorylation resulted in a significantly superior reduction of phosphorylated FLT3 and transcriptional suppression of genes downstream of FLT3 signaling. The drug combination induced synergistic inhibition of proliferation, as well as enhanced apoptosis, compared with single-drug treatment in models of human and murine *NPM1*^{mut} and *MLL*-r leukemias harboring an *FLT3* mutation. Primary acute myeloid leukemia (AML) cells harvested from patients with *NPM1*^{mut} *FLT3*^{mut} AML showed significantly better responses to combined menin and FLT3 inhibition than to single-drug or vehicle control treatment, whereas AML cells with wild-type *NPM1*, *MLL*, and *FLT3* were not affected by either of the 2 drugs. In vivo treatment of leukemic animals with *MLL*-r *FLT3*^{mut} leukemia reduced leukemia burden significantly and prolonged survival compared with results in the single-drug and vehicle control groups. Our data suggest that combined menin-MLL and FLT3 inhibition represents a novel and promising therapeutic strategy for patients with *NPM1*^{mut} or *MLL*-r leukemia and concurrent *FLT3* mutation. (*Blood*. 2020;136(21):2442-2456)

Introduction

Acute myeloid leukemia (AML) is a neoplastic disease of hematopoietic progenitor cells with acquired genetic abnormalities characterized by impaired differentiation and clonal expansion.[1,2] Comprehensive sequencing studies of AML samples have provided a broad list of recurrent cytogenetic and mutational abnormalities that are considered potential driver events of AML pathogenesis. These studies confirmed nucleophosmin 1 (*NPM1*) and the fms-related tyrosine kinase 3 (*FLT3*) as the most commonly mutated genes in AML and discovered frequent genetic abnormalities in genes encoding epigenetic regulators of transcription, with about two-thirds of AML cases being affected.[3-5] Genetic abnormalities represent the basis for the current World Health Organization classification and for prediction of the outcome of AML.[1,6]

Despite our growing understanding of its pathogenesis, AML remains a therapeutic challenge. Curative treatment efforts still rely on intensive chemotherapy as a backbone.[6,7] Currently, only 35% to 40% of the younger and 5% to 15% of the elderly patients (>60 years) can be cured, and the outcome of patients who are not eligible for intensive treatment is even more dismal.[2] Hope arises from the introduction of mechanism-based agents that target AML-specific genetic abnormalities or epigenetic vulnerabilities.[7,8] Since 2017, eight novel drugs have been approved by the U.S. Food and Drug Administration for the treatment of AML, and numerous novel agents are currently under

clinical investigation, many of them targeting epigenetic mechanisms.[9,10]

One promising therapeutic opportunity for tackling an epigenetic vulnerability in AML is to inhibit the interaction between the histone methyltransferase MLL and the chromatin-associated oncogenic cofactor menin.[11] This approach was initially proposed for the treatment of *MLL*-rearranged (*MLL*-r) leukemias, in which a chromosomal translocation leads to the formation of an oncogenic MLL-fusion protein.[12,13] These fusion proteins require the interaction with menin for chromatin binding to activate leukemogenic gene expression that includes the *MEIS1*, *PBX3*, and *MEF2C* transcription factors and to drive the leukemic transformation of hematopoietic stem or progenitor cells.[14-19] Both can be reversed by pharmacological inhibition of the menin-MLL interaction,[11,20-25] and 2 small-molecule inhibitors are currently entering clinical trials (NCT04067336 and NCT04065399).

We have recently demonstrated that the menin-MLL interaction is also a dependency in the *NPM1*-mutant (*NPM1*^{mut}) leukemias, which represent the most common AML subtype in adults. These leukemias lack an MLL-fusion protein but express a leukemogenic gene program, including *MEIS1*, *HOX*, *PBX3*, and *FLT3* that is very similar to the program expressed in *MLL*-r leukemias.[26] As in *MLL*-r leukemias, small-molecule inhibition of this interaction reversed expression of these genes and showed dramatic antileukemic activity against human and murine models of *NPM1*^{mut} leukemias in vitro and in vivo.[26] These findings were recently confirmed by other groups that used medicinal chemistry to develop menin-MLL inhibitors with better drug-like properties.[22,27,28]

Of note, we and others found *FLT3* a putative transcriptional MEIS1 target to be among the genes most dramatically downregulated by menin-MLL inhibition in *NPM1*^{mut} AML.[26] This finding attracted our interest, because activated mutations within *FLT3* are important leukemic drivers. *FLT3* mutations occur across all AML subgroups, including *MLL*-r leukemias (10%), and are particularly common in *NPM1*^{mut} AML (60%).[29-32] An internal tandem duplication (ITD), the most common type of *FLT3* mutation, is associated with adverse treatment outcome and, if present at a high allelic ratio, also converts the relatively favorable *NPM1*^{mut} AML subtype into an intermediate prognostic category.[6,33-36] Also, *FLT3* mutations represent an important therapeutic target and midostaurin, a first-generation multitargeted inhibitor of FLT3 phosphorylation has been shown to improve survival rates of patients with AML who have de novo *FLT3* mutations, when given in combination with intensive chemotherapy.[37] Next-generation FLT3 inhibitors with higher potency and selectivity, such as quizartinib, gilteritinib, and crenolanib, induce high remission rates in relapsed/refractory patients with *FLT3*-ITD^{+} AML as single agents.[38-40] Gilteritinib was recently approved for this indication in the United States and Europe, but none of the currently used FLT3 inhibitors has induced long-term remissions as single agents.[38,39] The development of a curative treatment is therefore currently focused on the exploration of these compounds in combination treatment regimens.[41]

FLT3-transcription is consistently downregulated upon menin-MLL inhibition in *NPM1*^{mut} AML, and we therefore hypothesized that combining these agents with FLT3 inhibitors would enhance their therapeutic efficacy. In this study, we demonstrated that combined menin-MLL and FLT3 inhibition has enhanced on-target activity against activating *FLT3* mutations in *MLL*-r or *NPM1*^{mut} AML cells and shows remarkably superior activity compared with single-drug treatment in vivo.

Materials and methods

Cell culture

All human AML cell lines used in this study were authenticated by Multiplex Cell Authentication by Multiplexion (Heidelberg, Germany), as described previously,[42] and maintained in standard conditions, as described.[26,43] The murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ leukemia model, as well as murine retrovirally transformed *Mll-Af9*-cells and their culture, have been described.[26,44,45]

Primary AML blast cells and coculture assay

Primary human *NPM1*^{mut}*FLT3*^{ITD} AML samples were obtained from patients treated at the University Medical Center, Mainz, under Institutional Review Board–approved protocols and in accordance with the Declaration of Helsinki. The coculture treatment assays were performed as reported previously[26,43] and are also described in the supplemental Methods, available on the *Blood* Web site.

In vitro studies

In vitro drug treatment, cell viability assays, annexin-V staining, RNA isolation, cDNA synthesis, quantitative real-time-PCR (qRT-PCR), chromatin-immunoprecipitation (ChIP), western blot analysis, flow cytometry, viral transduction, and colony-forming unit assays were performed according to standard procedures.[26,43] Drug synergism was calculated using the Chou-Talalay Method.[46] A detailed description is provided in the supplemental Methods.

RNA sequencing and analysis

For gene expression analysis, OCI-AML3, MOLM13, and MV411 cells were treated in 3 independent experiments. Normalization on synthetic RNA spike-in controls, as proposed by Lovén and colleagues,[47] was performed as described.[26,47] See the supplemental Methods for RNA preparation, normalization details, and sequencing analysis.

AML xenograft model

Six- to 10-week-old NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/ SzJ (NSG) mice were purchased from the Translational Animal Research Center at the University Medical Center, Mainz. For in vivo experiments, they were injected via the tail vein with 5×10^6 MV411 cells. Animals were randomized into the following treatment groups: vehicle (25% DMSO, 25% PEG400, and 50% phosphate buffered saline), MI-503 (50 mg/kg; twice daily intraperitoneally), quizartinib (10 mg/kg; PO, once daily), or a combination of both drugs. For assessment of leukemia burden, the mice were euthanized after drug treatment. Harvested bone marrow cells were analyzed for human CD45 expression by flow cytometry. For survival analysis, treatment was initiated on day 12 after transplantation and continued until day 45, with a 2-day treatment break to allow partial recovery from local irritation at the injection sites. Moribund animals were euthanized when they displayed signs of terminal leukemic disease. All mouse experiments were approved by the National Investigation Office Rheinland-Pfalz.

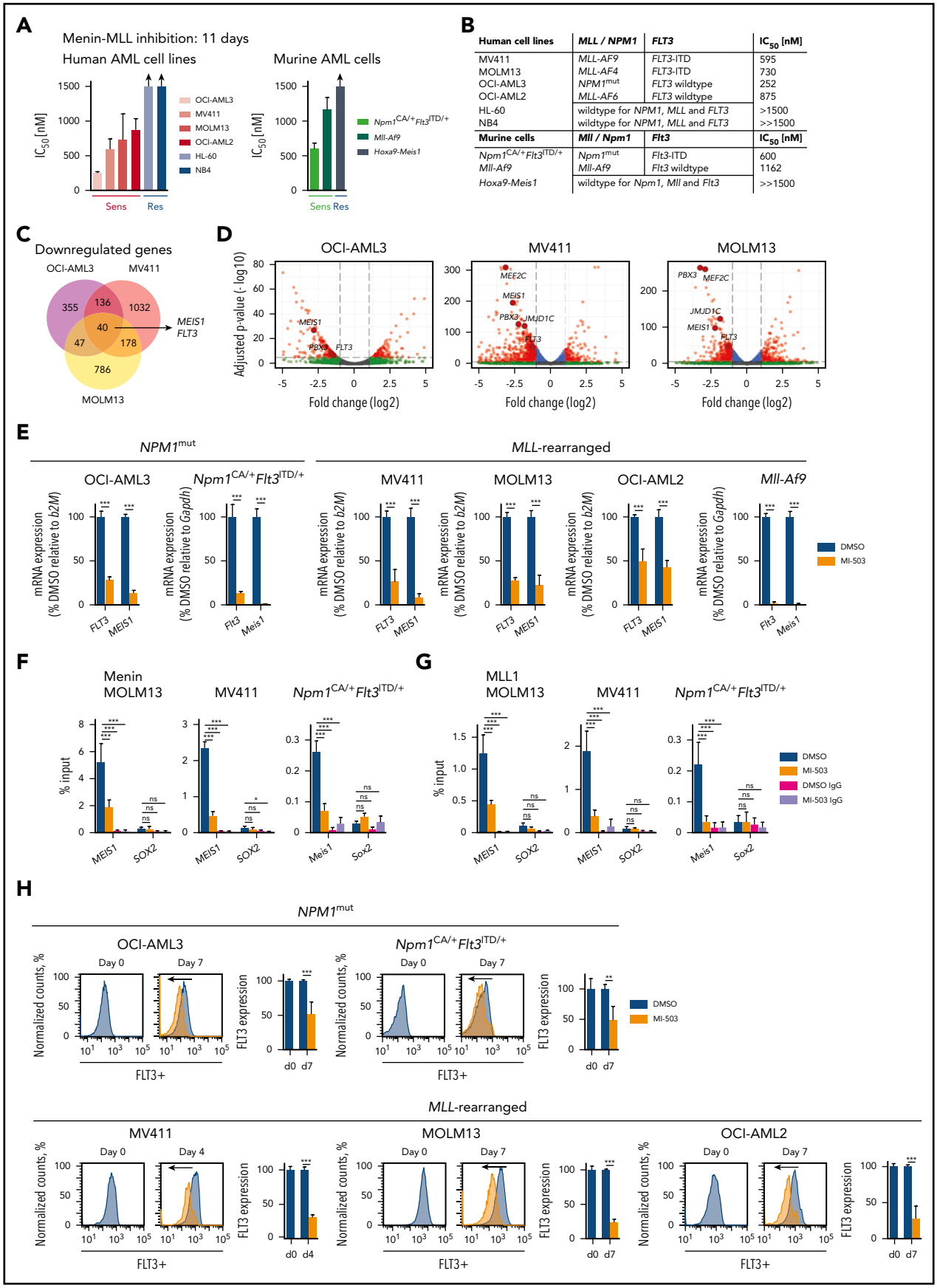


Figure 1.

Results

MEIS1 and *FLT3* transcription is uniformly suppressed by menin-MLL inhibition in *MLL*-rearranged and *NPM1*^{mut} leukemia

Because we found MEIS1 and its putative transcriptional target FLT3 to be dramatically downregulated upon menin-MLL inhibition in our previous study, we wanted to explore whether *MEIS1* and *FLT3* are uniformly suppressed after pharmacological disruption of the menin-MLL interaction in *MLL*-r and *NPM1*^{mut} leukemias.

We first confirmed the selective inhibitory effects of menin-MLL inhibition on cell proliferation in a selection of *MLL*-r and *NPM1*^{mut} human and murine leukemia cells, with and without activating *FLT3* mutations, using the small-molecule inhibitor MI-503 (*MLL*-r: MOLM13 [*FLT3*-ITD], MV411 [*FLT3*-ITD], OCI-AML2 [wild-type (wt) *FLT3*] and murine *Mll-Af9* [wt*Flt3*]; and *NPM1*^{mut}: OCI-AML3 [wt*FLT3*], murine *Npm1*$^{cA/+}$*Flt3*$^{ITD/+}$). In accordance with previous reports[20,26] the inhibitory effects of MI-503 on proliferation were assessed after 7 and 11 days, because this compound affects cell growth with a latency of several days, most likely as a consequence of differentiation induction (supplemental Figure 1). As expected, we observed a profound dose-dependent reduction in proliferation of all *MLL*-r and *NPM1*^{mut} cells, whereas leukemia cells lacking *MLL-r* or *NPM1*^{mut} showed only minor responses to very high concentrations of the drug (human APL cell lines: NB4; HL-60; murine *Hoxa9-Meis1*-transformed cells), with 50% inhibitory concentrations (IC_{50}) of >1500 nM (Figure 1A-B; supplemental Figure 1A-C).

To assess global transcriptional changes after menin-MLL inhibition in *NPM1*^{mut} and *MLL*-r leukemia cells, we performed RNA-seq analysis after MI-503 treatment in OCI-AML3, MOLM13, and MV411 cells. After 4 days of treatment, gene expression was assessed in OCI-AML3 and MOLM13 cells and after 3 days in MV411 as these exhibited an earlier antiproliferative response to the treatment. In the *MLL-r FLT3*-ITD^{+} leukemia cells, we found that 1051 (MOLM13) and 1386 (MV411) genes were downregulated by at least twofold (adjusted $P < .05$; Figure 1C; supplemental Table 1). Many of the most profoundly downregulated genes were known *MLL*-target genes, including *MEIS1*, *PBX3*, *JMJD1C*, and *MEF2C*. As hypothesized, we also found *FLT3* expression to be dramatically suppressed (Figure 1D). In the *NPM1*^{mut} OCI-AML3 cells, MI-503 led to downregulation of 578 genes, including *MEIS1*, *PBX3*, and *FLT3*, when using the same thresholds (Figure 1C-D; supplemental Table 1). It is interesting to note that, in contrast with the previously published menin-MLL inhibitor MI-2-2, *HOXA/B* genes were not substantially repressed in these cells (supplemental Figure 2A). Forty genes were significantly downregulated in both *NPM1*^{mut} and *MLL*-r leukemia cells and included *MEIS1* and *FLT3* (Figure 1C). Using qPCR, we confirmed that *MEIS1* and *FLT3* were uniformly downregulated by menin-MLL inhibition in all other *NPM1*^{mut} and *MLL*-r leukemia cells that we assessed in this study (Figure 1E). Dramatic suppression of *FLT3* in MV411 cells known to harbor a hemizygous *FLT3*-ITD mutation (with no remaining *FLT3* wild-type [WT] copy) indicated that menin-MLL inhibition also suppresses the mutated *FLT3*-ITD transcript. qPCR, with individually designed ITD-specific primers, confirmed this finding in MOLM13 and MV411 cells (supplemental Figure 2B).

ChIP, followed by qPCR, revealed that transcriptional downregulation of *MEIS1* by MI-503 treatment was accompanied by abrogation of menin and MLL protein binding to the *MEIS1* gene locus (Figure 1F-G; supplemental Figure 2C-D). These data support the view that binding of the menin-MLL complex is necessary for *MEIS1* gene expression in these AML-subtypes.

Next, to assess FLT3 protein expression after 7 days of MI-503 treatment (except MV411, 4-day treatment), we performed flow cytometry with antibodies that recognize an extracellular FLT3 epitope. In fact, FLT3 protein expression was also significantly reduced in all *NPM1*^{mut} and *MLL*-r leukemias (Figure 1H).

These data showed that pharmacological menin-MLL inhibition causes uniform downregulation of *MEIS1* and of WT and mutant *FLT3* in *NPM1*^{mut} and *MLL*-r leukemias.

Combined menin-MLL and FLT3 inhibition exerts a synergistic effect against *MLL*-r or *NPM1*^{mut} leukemias that harbor a concurrent *FLT3*-ITD

The data presented thus far indicate that mutant *FLT3* expression can be targeted via menin-MLL inhibition in *MLL*-r and *NPM1*^{mut} leukemias. We therefore sought to assess the effects of combining menin-MLL inhibition with direct FLT3 kinase inhibitors that have been shown to be highly active against *FLT3* mutant AML. First, we determined the IC_{50} concentrations of the specific and potent FLT3 inhibitors quizartinib, crenolanib, gilteritinib, and ponatinib in human *MLL*-r *FLT3*-ITD^{+} leukemia cell lines after 48 hours of treatment. Both cell lines were highly sensitive to the FLT3 inhibitors (Figure 2A; supplemental Figure 3A). Consistent with published data, quizartinib was the most potent inhibitor and was highly selective against *FLT3*-ITD^{+} leukemias, with IC_{50} values within the subnanomolar range, whereas AML cells without *FLT3* mutation were unaffected (Figure 2A-B; supplemental Figure 3B-C).

Figure 1. Gene and protein expression changes upon menin-MLL inhibition in *NPM1*^{mut} and *MLL*-r AML. (A) Human (left) and murine (right) AML cells were treated for 11 days with MI-503. Viable (4′,6-diamidino-2-phenylindole [DAPI]–negative) cells were assessed by flow cytometry, and IC_{50} values were calculated with GraphPad Prism software. (B) Summary of IC_{50} values (MI-503), *MLL*-rearrangement, and *NPM1* and *FLT3* mutation status in the AML cells assessed. (C) Venn diagram showing downregulated genes identified by RNA-seq (more than twofold decrease; adjusted $P < .05$), in *NPM1*^{mut} OCI-AML3, *MLL-r* MOLM13, and MV411 cells after MI-503 treatment (2.5 μM) compared with the DMSO control. (D) Volcano plots of RNA-seq data obtained from OCI-AML3, MOLM13, and MV411 cells treated with MI-503 (2.5 μM). FLT3 and selected MLL-fusion targets are labeled. (E) *FLT3* and *MEIS1* mRNA expression in human and murine leukemia cells after 4 days of MI-503 treatment (2.5 μM), as assessed by qRT-PCR. ChIP was performed with antibodies against menin (F) or MLL1 (G) and IgG as the negative control, followed by qPCR to detect a sequence within the *MEIS1* gene body or *SOX2* as negative control. Cells were treated with MI-503 (2.5 μM) or vehicle control for 4 days. (H) FLT3 protein (cell surface) expression assessed by flow cytometry in human and murine *NPM1*^{mut} and *MLL*-rearranged AML cells after MI-503 treatment (2.5 μM for 4 or 7 days, as indicated). Representative histograms of 3 independent experiments are shown. Bar graphs in panels A and E-G represent the mean of 3 independent experiments, each performed in technical triplicate. Bar graph in panel H showing *Npm1*$^{cA/+}$*Flt3*$^{ITD/+}$ cells represents 2 independent experiments performed in technical triplicate. Error bars represent standard deviation.

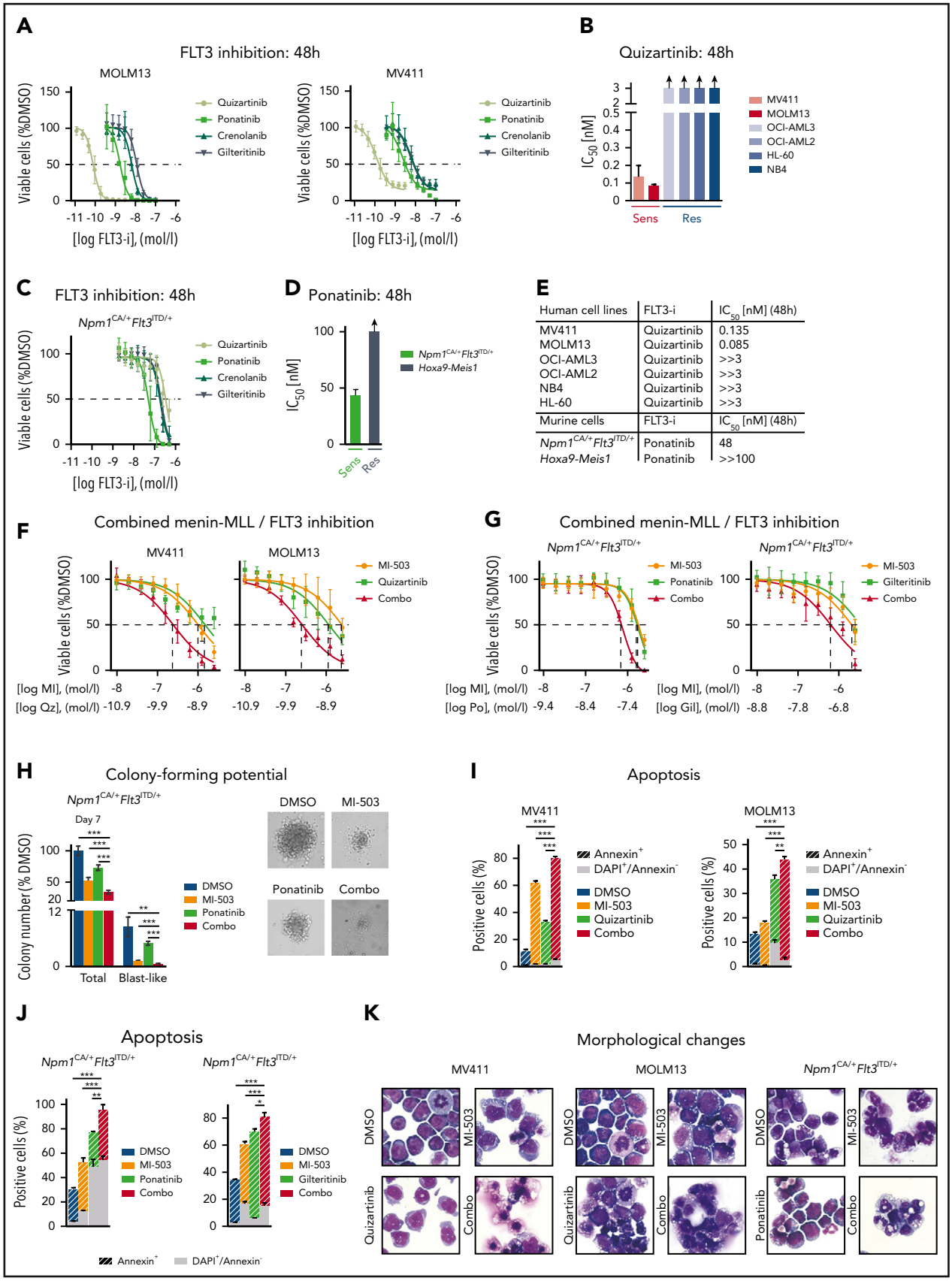


Figure 2. Synergistic effects of combined menin-MLL and FLT3 inhibition. (A) Dose-response curves from cell-viability assays after 48 hours of treatment with various FLT3 inhibitors in *FLT3*-ITD$^+$ MOLM13 and MV411 cells. (B) *FLT3*-ITD$^+$ and *FLT3* WT human leukemia cell lines were treated with quizartinib for 48 hours. IC_{50} values were graphically determined by GraphPad Prism. (C) Dose-response curves from cell-viability assays after 48 hours of treatment with various FLT3 inhibitors in murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells.

We next assessed FLT3 inhibition in the murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ leukemia cells known to harbor the F692L point ("gatekeeper") mutation that mediates resistance to most FLT3 inhibitors.[48] In accordance with published data, the cells showed hardly any response to quizartinib, and the IC_{50} values of ponatinib, crenolanib, and gilteritinib, were shifted to higher concentrations compared with the values determined from the MOLM13 and MV411 cells that lacked the F692L mutation. Murine *Hoxa9-Meis1*–transformed cells without an *FLT3* mutation were not affected by FLT3 inhibition (Figure 2C-E; supplemental Figure 3D-F). Because the multityrosine kinase inhibitor ponatinib showed the lowest IC_{50} (48 nM; 48 hours) among the different compounds, we used this inhibitor for the treatment of the murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ leukemias and validated the results with higher doses of the more specific drug gilteritinib. Quizartinib was chosen for experiments in the human *MLL*-r *FLT3*-ITD^{+} cells.

Next, we sought to assess combined menin-MLL and FLT3 inhibition in the *MLL*-r *FLT3*-ITD human and the murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ leukemia cells (Figure 2F-G). Because single-drug treatment with menin-MLL inhibitors inhibits proliferation with a latency of several days[20,26] and single-drug FLT3 inhibition induces a more rapid cytotoxic response in vitro, we pretreated the leukemia cells for 2 (MV411) or 3 days (MOLM13, murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells) with MI-503 and then added the FLT3 inhibitor for an additional 24 hours (combination treatment). Menin-MLL and FLT3 inhibition resulted in dramatically enhanced inhibition of proliferation compared with single-drug treatment in all 3 of the assessed leukemias (Figure 2F-G; supplemental Figure 3G). Notably, for all of those leukemias we found dramatic drug synergism when using the Chou-Talalay algorithm (supplemental Figure 3H-I). Human and murine AML cells without *MLL*-r, *NPM1*^{mut}, or *FLT3* mutation (HL-60, NB4, and *Hoxa9-Meis1*–transformed cells), which served as negative controls, were not affected by single-drug or combinatorial treatment (supplemental Figure 3J-K). Murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ leukemia cells treated with both drugs in methylcellulose for 7 days showed significantly better suppression of total and blast colony formation compared with single-drug treatment or vehicle control (Figure 2H; supplemental Figure 4A).

The enhanced killing of *MLL*-r *FLT3*-ITD^{+} and *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ leukemia cells was associated with a significantly enhanced induction of apoptosis compared with single-drug or vehicle treatment (Figure 2I-J; supplemental Figure 4B). Menin-MLL inhibitors are known to inhibit proliferation by apoptosis and induction of differentiation in *MLL*-r and *NPM1*^{mut} leukemias with a latency of several days, and we wondered whether early-onset induction of differentiation also contributes to the quick-killing effect of the combination treatment. Therefore, we assessed cell morphology in all 3 cell types at the time when efficient inhibition of proliferation was noted with combination treatment (3 and 4 days of menin-MLL inhibition and 24 hours of FLT3 inhibition). The mild-to-moderate myelomonocytic differentiation observed at this early time point (Figure 2K) indicates that apoptosis induction may be the main mechanism of the antileukemic activity of the combination treatment.

These data indicate that combined menin-MLL and FLT3 inhibition enhances apoptosis induction compared with single-drug treatment and synergistically inhibits proliferation in *NPM1*^{mut} and *MLL*-r leukemias with *FLT3*-ITD.

Menin-MLL inhibition enhances FLT3-inhibitor–mediated abrogation of phosphorylated FLT3

To characterize the effects of combined menin-MLL and FLT3 inhibition in more detail, we first assessed *MEIS1* and *FLT3* gene expression after combinatorial drug treatment. As described, menin-MLL inhibition alone or in combination with an FLT3 inhibitor suppressed *MEIS1* and *FLT3* transcription in *MLL*-r *FLT3*-ITD^{+} and *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ leukemias. As expected, we did not find significant downregulation of *MEIS1* and *FLT3* gene expression with single inhibition of FLT3-phosphorylation. In fact, *FLT3* transcription was even upregulated in MV411 and *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells with single FLT3 inhibition, which is consistent with previous reports[49-51] (Figure 3A).

These transcriptional changes translated into similar changes on the protein level, as assessed by immunoblot analysis. Although total FLT3 protein expression was reduced with menin-MLL or combination treatment, single-drug FLT3 inhibition caused no change (MOLM13) or upregulation (MV411) of total FLT3 protein levels (Figure 3B; supplemental Figure 5A).

Assessment of FLT3-receptor phosphorylation (pFLT3; activated FLT3) in these cell lines showed a strong reduction in pFLT3 after its direct inhibition by quizartinib and was also mildly reduced after menin-MLL inhibition, the latter most likely reflecting the decreased total FLT3 protein level. Combined-drug treatment caused even more pronounced reduction of pFLT3 compared with single-drug treatment, as assessed by immunoblot analysis (Figure 3B; supplemental Figure 5A). The observed reduction of pFLT3 was also associated with reduced phosphorylation of the downstream signaling proteins STAT5 (pSTAT5) and ERK (pERK). Similar to pFLT3, we observed reduced phosphorylation of both proteins with single-drug treatment that was even more pronounced with combinatorial treatment for pERK in MV411 and MOLM13 cells and for pSTAT5 in MV411 cells, whereas the

Figure 2 (continued) (D) Ponatinib IC_{50} concentrations in murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ and *Hoxa9-Meis1*–transformed cells after 48 hours of treatment. (E) Summary of FLT3 inhibitor IC_{50} concentrations in the human and murine leukemia cell lines assessed in this study. (F) Dose-response curves from cell-viability assays of MV411 and MOLM13 cells, comparing MI-503 (MI; 3 days for MV411 cells and 4 days for MOLM13 cells), quizartinib (Qz; 24 hours), and combinatorial MI-503 (3 or 4 days) and quizartinib (24 hours) treatment. Dashed lines indicate IC_{50} values. (G) Dose-response curves from cell-viability assays of *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells comparing MI-503 (MI, 4 days), ponatinib (Po, 24 hours, left), and gilteritinib (Gil, 24 hours; right), or their combination (4 days, MI-503; 24 hours, ponatinib and gilteritinib). Dashed lines indicate IC_{50} values. (H) Effect of MI-503 (2.5 μM), ponatinib (100 nM), and combinatorial treatment (2.5 μM and 100 nM) on the number of total and blast-like colonies in murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells, normalized to DMSO. Micrographs were taken at ×20 amplification. (I-J) Percentage of apoptotic (annexin V) and dead (4′,6-diamidino-2-phenylindole [DAPI]-stained) cells after single and combinatorial treatment with MI-503 (2.5 μM) and quizartinib (3 nM) in human cell lines (I) or MI-503 (2.5 μM) and ponatinib (100 nM) or gilteritinib (400 nM) in murine cells (J). (K) Giemsa-stained cytospins showing human MV411 and MOLM13 cells and murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells after single and combinatorial treatment with MI-503 (2.5 μM; 4 days and 3 days for MV411) and FLT3 inhibitor (quizartinib, 3 nM, 24 hours; ponatinib, 100 nM, 24 hours) or their combination (day 4/24 hours and day 3/24 hours for MV411, respectively). Micrographs were taken at ×100 amplification. Error bars represent SD of 3 independent experiments, each performed in 3 technical replicates.

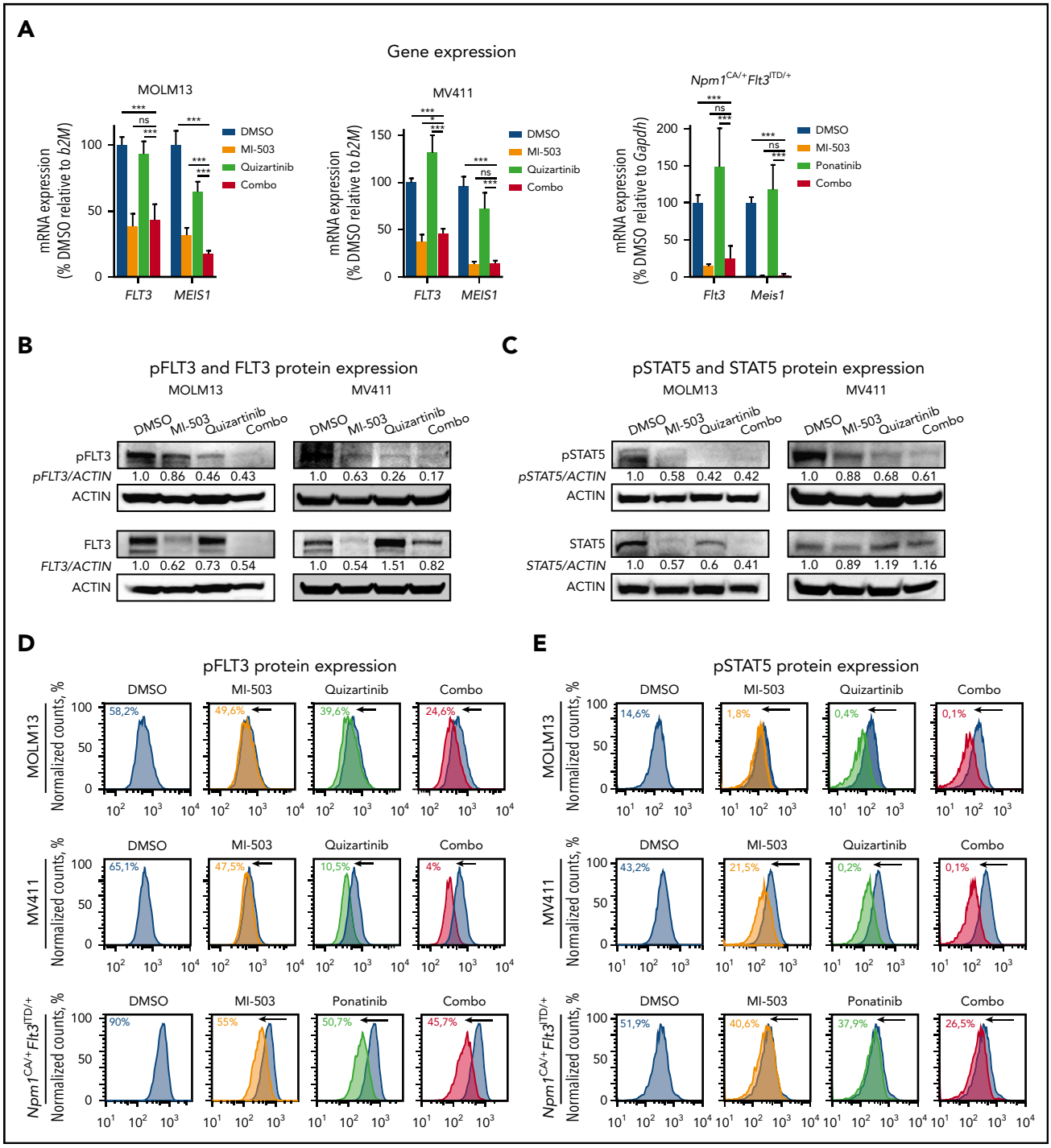


Figure 3. Effects of single and combined menin-MLL and FLT3 inhibition on FLT3 and phosphorylated FLT3 protein levels. (A) *FLT3* and *MEIS1* mRNA expression in human MOLM13 (left) and MV411 (middle) cells and murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ (right) leukemia cells after single or combinatorial treatment with MI-503 (2.5 μM; 4 days for MOLM13 and *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells and 3 days for MV411 cells) and FLT3 inhibitors (quizartinib, 3 nM; ponatinib, 100 nM, 24 hours) as assessed by qRT-PCR. Bar graphs represent the mean with SD of 3 independent experiments, each performed in technical triplicate. (B) Immunoblot analysis of FLT3 and phosphorylated (p)FLT3 in MOLM13 cells (left) and MV411 cells (right) upon treatment with 2.5 μM MI-503 (for 3 and 4 days in MV411 and MOLM13 cells), quizartinib (3 nM, 24 hours), or the 2 combined. One representative blot of 3 independent experiments is shown. Numbers indicate the DMSO-normalized quantification of western blot signals, relative to the loading control, performed by densitometry with ImageJ software. (C) Immunoblot analysis of STAT5 and phosphorylated (p)STAT5 in MOLM13 (left) and MV411 (right) cells after treatment as described in panel B. One representative blot of 3 independent experiments is shown. Numbers indicate the DMSO-normalized quantification of western blot signals, relative to the loading control, performed by densitometry with ImageJ software. pFLT3 (D) and pSTAT5 (E) protein expression in human MOLM13 and MV411 and murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells after treatment with MI-503 (2.5 μM; 4 days for MOLM13 and *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells; 3 days for MV411 cells), FLT3 inhibitor (quizartinib, 3 nM, and ponatinib, 100 nM; 24 hours) or their combination, as assessed by flow cytometry. One representative histogram of 3 independent experiments is shown. The colored numbers in the flow histograms indicate the percentage of pFLT3$^+$ and pSTAT5$^+$ cells, respectively.

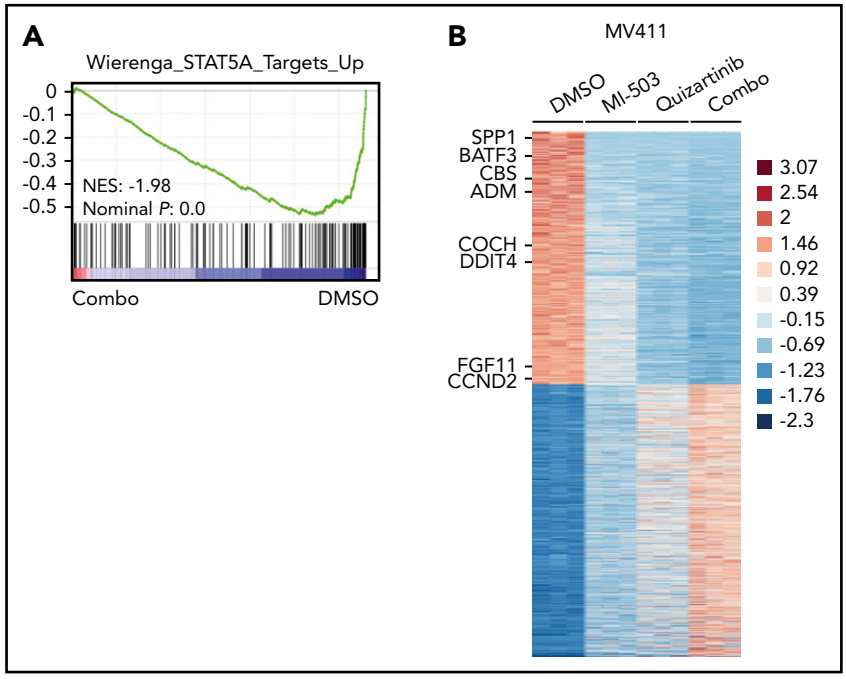


Figure 4. Downregulated genes after combined menin-MLL and FLT3 inhibition are enriched for STAT5A target genes. (A) Gene set enrichment analysis of gene expression changes in MV411 cells treated with combined MI-503 (2.5 μM; 3 days) and quizartinib (3 nM; 24 hours) compared with STAT5A targets. (B) Heatmap of differentially expressed genes (log2 change >1 and < −1, and adjusted $P < .05$) in MV411 cells after single and combined treatment with MI-503 (2.5 μM, 3 days) and quizartinib (3 nM, 24 hours). Target genes of STAT5A are indicated.

dramatic reduction of pSTAT5 observed with quizartinib in MOLM13 was not enhanced (Figure 3C; supplemental Figure 5B).

Similar results were obtained when we assessed pFLT3 by flow cytometry in the human *MLL*-r *FLT3*-ITD and the *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ leukemia cells, in which combined treatment abrogated pFLT3 levels significantly more than single-drug or vehicle treatment (Figure 3D; supplemental Figure 5C-D). The specificity of the antibody for pFLT3 is indicated by FLT3 ligand-stimulation experiments (supplemental Figure 5E). Again, the decrease in the FLT3 downstream signaling proteins pSTAT5 and pERK was more pronounced after combinatorial treatment than after single-drug treatment in MV411 cells and murine leukemia cells examined by flow cytometry, whereas quizartinib and combinatorial treatment showed a similar reduction of pSTAT5 and pERK in MOLM13 cells (Figure 3E; supplemental Figure 5F-J).

To explore the global transcriptional consequences of the enhanced reduction of pFLT3, we performed RNA-seq analysis in the MV411 cells and compared combinatorial vs single-drug or vehicle treatment. Gene set enrichment analysis revealed that the genes downregulated by combinatorial treatment were significantly enriched for target genes of the FLT3-activated transcription factor STAT5A (Figure 4A). When comparing the expression levels of STAT5A target genes between the different

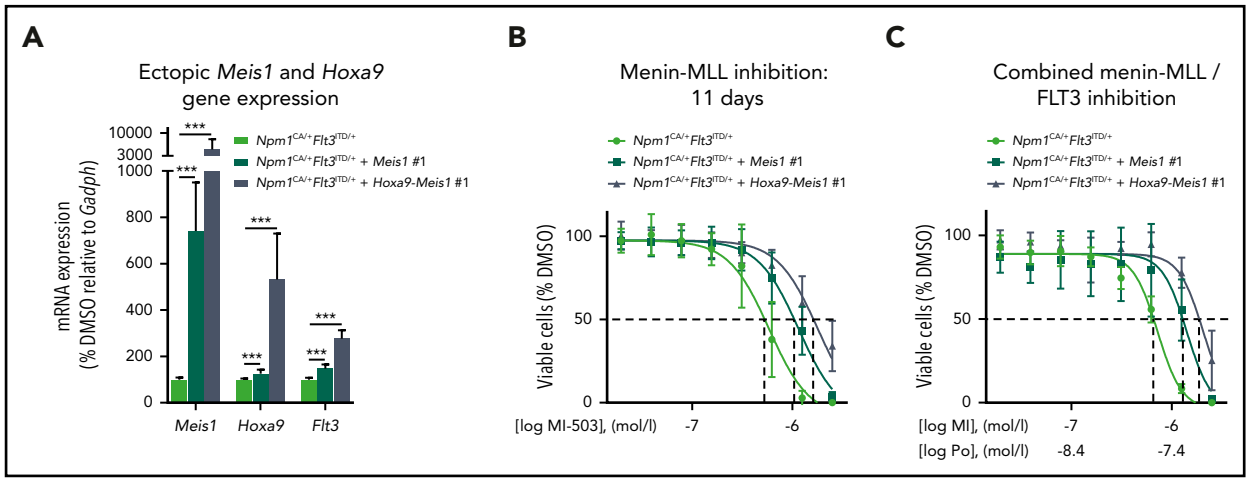


Figure 5. Effects of ectopic *Meis1* expression in murine *Npm1*^{mut} *Flt3*-ITD^{+} leukemias. (A) Relative mRNA expression of *Meis1*, *Hoxa9*, and *Flt3* in murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells ectopically expressing *Meis1* or both *Meis1* and *Hoxa9*, normalized to cells with just endogenous *Meis1* and *Hoxa9* expression. (B) Dose-response curves from cell viability assays after 11 days of MI-503 treatment comparing *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells vs *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells overexpressing *Meis1* or *Meis1-Hoxa9*. Dashed lines indicate the shift of IC_{50} values. (C) Dose-response curves from cell viability assays after combinatorial treatment with MI-503 (MI, 6 days) and ponatinib (Po; 72 hours) comparing *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells vs *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells overexpressing *Meis1* or *Meis1-Hoxa9*. Dashed lines indicate the shift of IC_{50} values. Cells with ectopic *Meis1* and *Hoxa9* expression in panels A-C were each obtained from 2 different clones after retroviral transduction. Shown are the average results of 3 different experiments with clone #1, all performed in triplicate. Error bars, standard deviation.

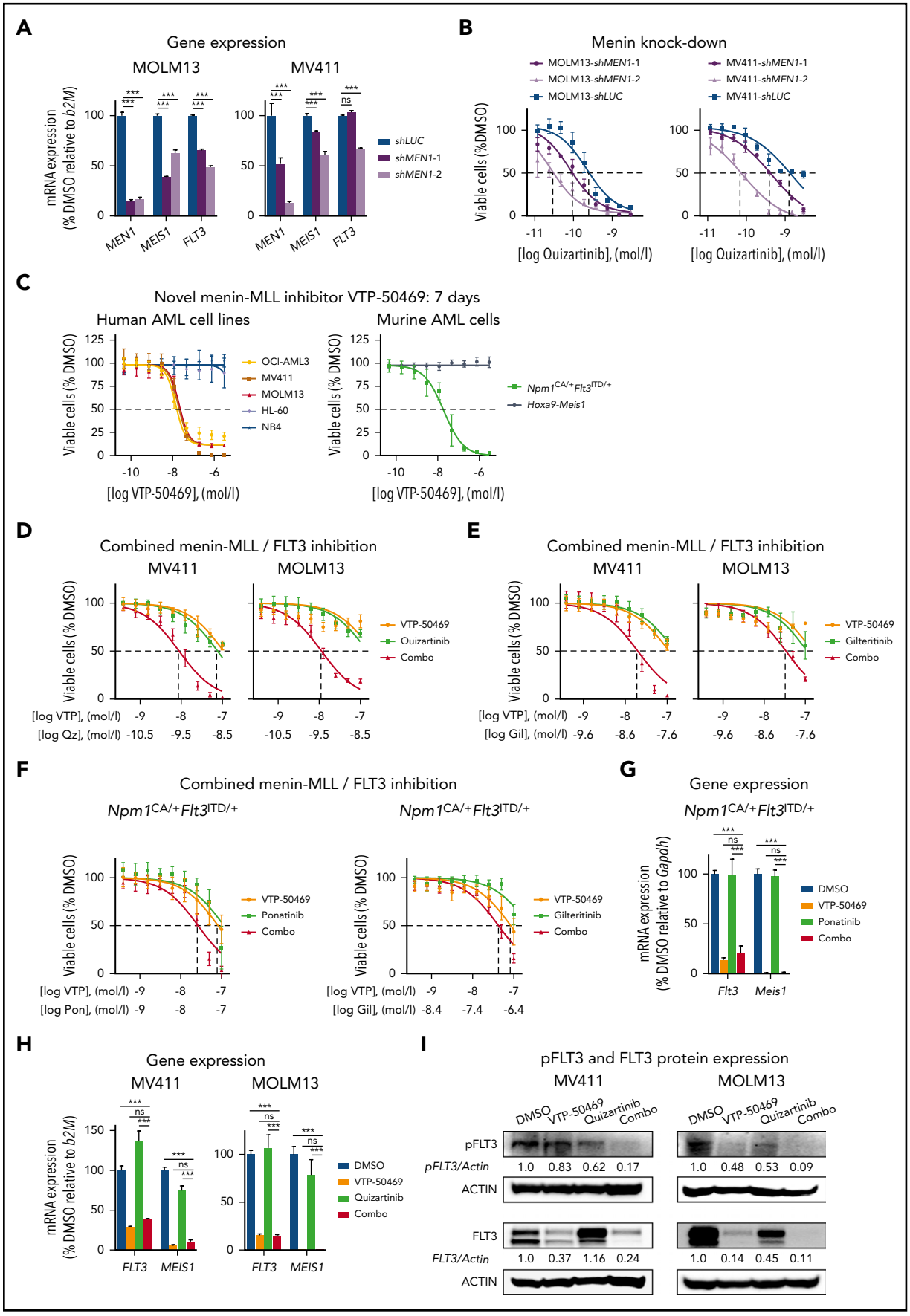


Figure 6.

treatment groups in more detail, we found most of these genes to be moderately suppressed by single-drug treatment with either menin-MLL or FLT3 inhibitors. The combinatorial treatment further reduced the expression levels of these genes more efficiently (Figure 4B; supplemental Figure 6A-B; supplemental Table 2).

As *FLT3* is a reported MEIS1 and HOXA9 transcriptional and potential binding target,[52-54] we next assessed the effect of retrovirally induced ectopic expression of *Meis1* and *Hoxa9-Meis1* on *Flt3* expression in *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ murine leukemia cells. Both scenarios increased the expression of endogenous *Flt3* by 1.5- and 2.8-fold, respectively (Figure 5A; supplemental Figure 7A). Of interest, ectopic *Meis1* and *Hoxa9-Meis1* expression partially rescued the *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells from the antiproliferative activity of the menin-MLL inhibitor and of the combinatorial treatment (Figure 5B-C; supplemental Figure 7B-C). Similar rescue effects on colony-forming potential were observed when the cells were treated in methylcellulose (supplemental Figure 8A-B).

These data support the concept that *FLT3* expression is driven by the MEIS1 transcription factor in these leukemias and that transcriptional suppression of FLT3 via menin-MLL inhibition contributes to a more efficient reduction of phosphorylated FLT3.

The novel menin-MLL inhibitor VTP-50469, in combination with FLT3 inhibitors, exerts a highly synergistic effect against *NPM1*^{mut} and *MLL*-r leukemias

To confirm the effects of pharmacological menin-MLL inhibition by MI-503, we used 2 validated small-hairpin RNAs to perform knockdown of *MEN1* in MOLM13 and MV411 cells. At 48 hours after lentiviral transduction, we observed downregulation of *MEIS1* and *FLT3* gene expression in a *MEN1* suppression–dependent manner. Also, we found that *MEN1* knockdown consistently sensitized the *MLL*-r AML cells to pharmacological FLT3 inhibition, thereby phenocopying the effects observed with MI-503 (Figure 6A-B).

During the work on the revision of this study, VTP-50469, a novel and more selective menin-MLL inhibitor with clinical utility was described and became available to us. We used VTP-50469 to independently validate the effects observed with MI-503. First, we confirmed selective growth inhibition of VTP-50469 on *MLL*-r and *NPM1*^{mut} leukemia cells (Figure 6C; supplemental Figure 9A-B). Next, we assessed its combination with the FLT3 inhibitors quizartinib and gilteritinib in the human *MLL*-r and with ponatinib and gilteritinib in the murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ AML cells. Similar to MI-503, all combinations resulted in synergistic inhibition of cell proliferation compared with single-drug treatment (Figure 6D-F; supplemental Figure 9C-G). VTP-50469 also suppressed *MEIS1* and *FLT3* expression dramatically and when combined with FLT3 inhibitors resulted in more pronounced abrogation of pFLT3 protein levels than each of the single-drug treatments alone (Figure 6G-I). These data independently confirm the synergistic antileukemic effects of combined menin-MLL and FLT3 targeting.

Combined menin-MLL and FLT3 inhibition suppresses primary *NPM1*^{mut}*FLT3*^{ITD} AML cells in vitro

To investigate combinatorial menin-MLL and FLT3 inhibition in samples from patients with primary AML, we used a previously described coculture assay that allowed us to maintain and treat these leukemia cells in serum-free medium with cytokines on a (HS27) stromal cell layer in vitro (Figure 7A). Five de novo N*PM1*^{mut} *FLT3*-ITD samples from patients with AML were treated for 7 days with DMSO, MI-503, quizartinib, or the combination of the drugs. All 5 samples showed significantly enhanced reduction in the number of cells with combinatorial treatment compared with single-drug treatment. In all samples, MI-503 and quizartinib also reduced the number of viable cells significantly compared with the cells exposed to the drug vehicle (Figure 7B-C). To control for potential nonspecific drug toxicity, we also treated 2 primary AML samples lacking *NPM1*^{mut}, *MLL*-r, and *FLT3*-ITD. In both samples the number of viable cells was not significantly affected by either single-drug or combinatorial treatment (Figure 7D). Four of the 5 primary *NPM1*^{mut} *FLT3*-ITD samples were also treated in methylcellulose. Similar to the proliferation assays, blast-colony formation was significantly reduced by the drug combination vs all other treatment groups (Figure 7E).

These data from primary *NPM1*^{mut} *FLT3*-ITD^{+} AML patient samples further support single and combinatorial menin-MLL and FLT3 inhibition as a potentially efficacious therapeutic concept for *FLT3*-mutated *NPM1*^{mut} leukemia.

Combined in vivo treatment significantly prolongs survival of *MLL*-r *FLT3*-ITD^{+} leukemic mice

We next sought to explore the therapeutic potential of combined menin-MLL and FLT3 inhibition in vivo. First, we assessed the effects of these drugs on leukemic burden in a disseminated human MV411 xenotransplantation model. MV411 cells were transplanted into NSG mice via tail-vein injection, and the animals were randomly divided into 4 groups receiving MI-503, quizartinib, a combination of the 2 drugs, or vehicle control treatment. The treatment was started 1 week after transplantation (day 7) and the animals were euthanized after 14 days of treatment. Leukemia

Figure 6. Synergistic inhibition of proliferation and FLT3 activation after combined treatment with next-generation menin-MLL and FLT3 inhibitors. (A) Dose-response curves of MOLM13 and MV411 cells treated with quizartinib for 24 hours, comparing cells transduced with short hairpin RNAs against *MEN1* with control-transduced cells (*shLUC*). (B) mRNA expression levels of *MEN1*, *MEIS1*, and *FLT3* in MOLM13 and MV411 cells with *MEN1* knock down or control-transduced cells, assessed 48 hours after transduction. (C) Dose-response curves from cell-viability assays after 7 days of treatment with VTP-50469 in human (left) and murine (right) leukemia cells. Viable (4′,6-diamidino-2-phenylindole [DAPI]-negative) cells were assessed by flow cytometry. (D-E) Dose-response curves from cell viability assays of MV411 and MOLM13 cells comparing VTP-50469 (VTP; 3 days for MV411 and 4 days for MOLM13 cells), quizartinib (Qz, 24 hours; D), gilteritinib (Gil, 24 hours; E), and combinatorial VTP-50469 (3 or 4 days) and FLT3 inhibition (24 hours) treatment. Dashed lines indicate IC_{50} values. (F) Dose-response curves from cell viability assays of *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells comparing VTP-50469 (VTP; 6 days), ponatinib (Po, 24 hours, [left]), or gilteritinib (Gil, 24 hours; [right]) with their combination (6 days VTP50469, 24 hours FLT3 inhibition). Dashed lines indicate IC_{50} values. (G-H) *FLT3* and *MEIS1* mRNA expression in murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ (G), human MV411 (H; left), and MOLM13 (H; right) leukemia cells after single or combined treatment with VTP-50469 (100 nM; 4 days for MOLM13 and *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ cells and 3 days for MV411 cells) and FLT3 inhibitors (quizartinib, 3 nM and ponatinib, 100 nM; 24 hours) as assessed by qRT-PCR. Bar graphs represent the mean with standard deviation of 3 independent experiments, each performed in technical triplicate. (I) Immunoblot analysis of FLT3 and phosphorylated (p)FLT3 in MV411 cells (left) and MOLM13 cells (right) after treatment with VTP-50469 (100 nM; 3 and 4 days in MV411 and MOLM-13, respectively) and quizartinib (3 nM, 24 hours), or their combination. Numbers indicate the DMSO-normalized quantification of western blot signals, relative to the loading control, performed by densitometry using the ImageJ software tool.

burden, as defined by the percentage of bone marrow cells expressing human CD45, was significantly reduced within the animal group treated with the drug combination vs all other groups (vehicle, 15%; MI-503, 8%; quizartinib, 3%; combination [combo], 1.5%; $P < .05$ for combo vs other groups; Figure 7F).

In a separate experiment, we assessed survival in the disseminated MV411 xenograft leukemia model. Drug treatment was initiated on day 12 after transplantation and continued until day 45. Combinatorial MI-503 and quizartinib treatment resulted in a significant survival advantage compared with single-drug– or vehicle-treated animals (hazard ratios for death, 0.15 and 0.35; 95% confidence intervals, 0.02845-0.8061 and 0.0285-0.8783; $P = .0269$ and $P = .0350$ for combo vs MI-503 and combo vs quizartinib, respectively; Figure 7G).

In summary, these data confirm that the combination of menin-MLL and FLT3 inhibition substantially improves survival of mice engrafted with *MLL*-r *FLT3*-ITD^{+} leukemia compared with single-drug– or vehicle-treated animals and supports this therapeutic concept as a synergistic approach to combat these leukemias.

Discussion

Intensive chemotherapy remains the backbone of curative treatment of AML.[6] This aggressive therapy can induce long-term remissions in only about half of the patients with *NPM1*^{mut} AML and one-third of the patients with *MLL*-r AML.[3] Explanations for these relatively unsatisfactory survival rates is older age at diagnosis and the presence of concurrent poor prognostic disease markers, such as the *FLT3*-ITD mutations.[33,34,55] These mutations are associated with treatment failure, relapse, and death[56] and also represent important therapeutic targets. FLT3 inhibitors have been shown to increase survival rates in de novo and in relapsed or refractory AML,[38,39,57,58] and potent, highly selective second-generation FLT3 inhibitors can induce high response rates as single agents. However, without further consolidating treatment, almost all patients relapse and ultimately succumb to their disease.[38,39] Emerging resistance mutations have been reported as one reason for resistance to FLT3 inhibition,[58,59] but there are other potential explanations. First, AML is commonly driven by multiple oncogenic mechanisms and not by a single *FLT3* mutation. Recent deep-sequencing efforts found a median number of 5 and at least 2 genetic driver events to be present per AML case.[5] Second, *FLT3* mutations are considered late events during leukemogenesis.[60-62] Therefore, FLT3 inhibitors target the dominant *FLT3*-mutant AML blast population, but potentially, not the AML founding clone from which relapse may originate. Because of its biologic heterogeneity, AML is generally not believed to be cured by single-drug treatment.[63]

In our study, we developed a synergistic treatment regimen that combines menin-MLL and FLT3 inhibitors to target leukemogenic gene expression in *NPM1*^{mut} or *MLL*-r leukemia and activating *FLT3* mutations. The development of this approach was based on the previous findings that inhibition of the menin-MLL interaction reverses leukemogenic gene expression in *NPM1*^{mut} AML, including the most pronounced *MEIS1* transcription factor and its putative transcriptional target gene *FLT3*.[26] In a detailed assessment, we also demonstrated that both WT and mutant *FLT3* transcript levels were consistently downregulated in response to MI-503 in *NPM1*^{mut} and *MLL*-r AML models.

As *FLT3* is a transcriptional target gene of MEIS1,[53,54] FLT3 downregulation after menin-MLL inhibition is therefore a likely result of the dramatically suppressed *MEIS1* expression. Reduced total FLT3 levels were also associated with the lower level of pFLT3 that we observed after menin-MLL inhibition. This may explain the superior reduction of pFLT3 that we observed with the combination treatment, compared with direct inhibition of pFLT3 or menin-MLL inhibition alone.

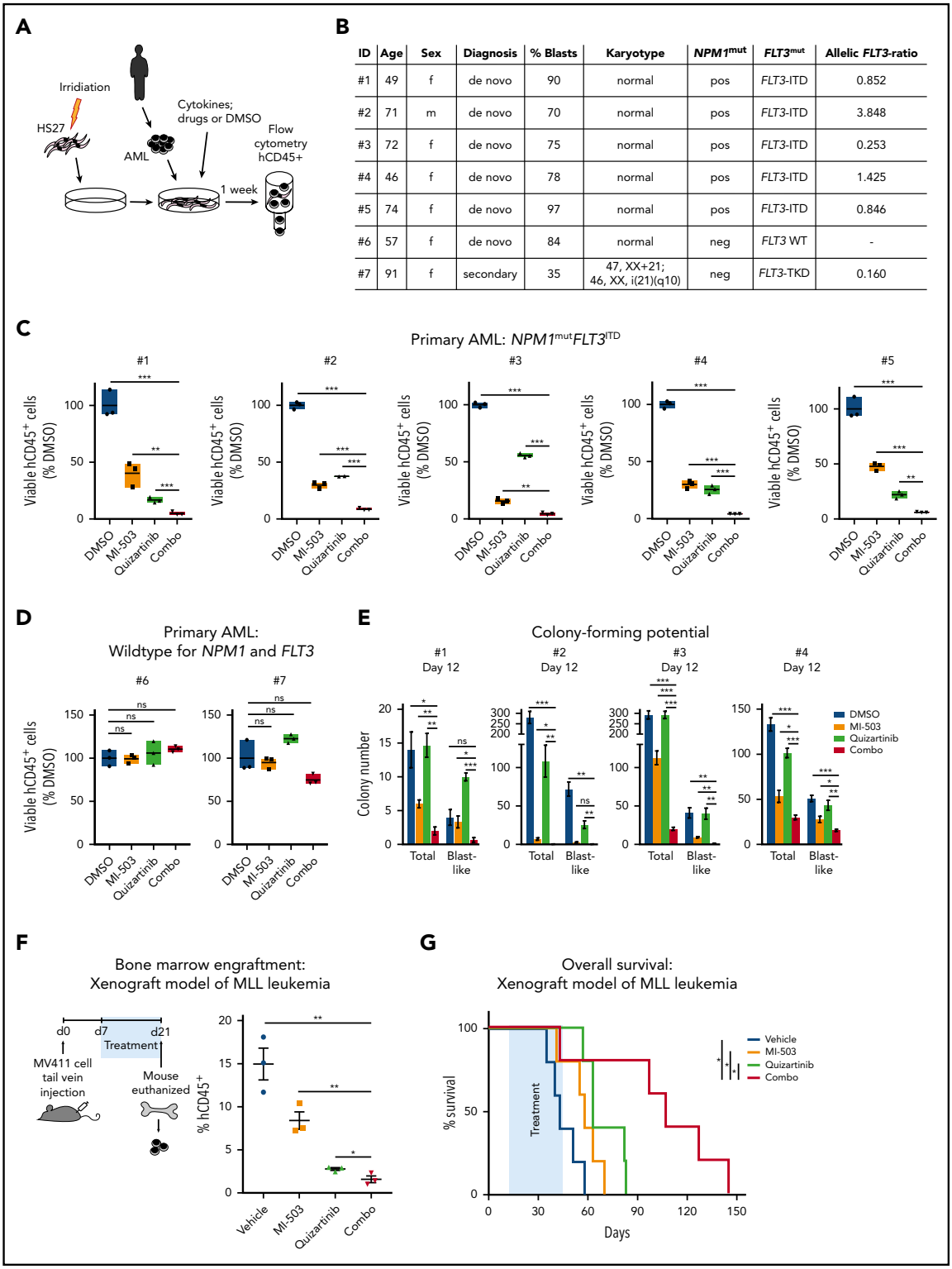
It is of interest to note that the pronounced reduction of pFLT3 after combinatorial menin-MLL and FLT3 inhibition also resulted in significantly enhanced suppression of STAT5A target genes in MV411 cells. Because STAT5A contributes to leukemia maintenance and is an important downstream mediator of activating *FLT3* mutations,[64-66] the synergistic antileukemic activity of combined menin-MLL and FLT3 inhibition is probably caused by its enhanced on-target activity in preventing FLT3 signaling.

We have also shown that upregulation of FLT3, which is commonly observed after FLT3 inhibition,[49-51] can be reversed with the addition of the menin-MLL inhibitor. Although this mechanism may contribute to enhanced activity of FLT3 inhibitors, future studies will determine whether this concept may help to prevent or overcome resistance to FLT3 inhibition.

Menin-MLL inhibitors are particularly attractive partners in a combined treatment, as they allow the effective targeting of a core leukemogenic gene expression program driven by *NPM1* mutations or MLL fusions including the transcription factors *MEIS1* and *PBX3*.[15,53,67,68] Our assessment of blast colony formation upon drug treatment is consistent with the view that menin-MLL inhibition targets immature leukemia-initiating cells, most likely via suppression of self-renewal–associated MEIS1- and PBX3-driven gene expression. Blast colony formation can be further inhibited by the addition of a FLT3 inhibitor, whereas FLT3 inhibition alone has only minor effects. The mechanisms behind this observation remain to be determined in detailed studies assessing single and combined drug effects on the leukemia-initiating fraction in these leukemia subtypes.

In contrast to previous menin-MLL inhibitors such as MI-2-2,[25] many other MLL target genes including the HOX transcription factors were only moderately suppressed (*MLL*-r and murine *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$) or unchanged (human *NPM1*^{mut} OCI-AML3) with MI-503. This is consistent with findings from recent studies assessing novel and more selective menin-MLL inhibitors, such as VTP-50469 and MI-3454, that also did not lead to broad downregulation of *HOX* genes and other MLL-fusion targets.[22,28] These findings are in line with the observation that MLL fusion occupancy is lost only on a subset of genes in response to menin-MLL inhibition.[22] Although we do not know the exact mechanism behind the different responses at specific loci, the 2 novel menin-MLL inhibitors also dramatically suppress a core transcriptional program that includes *PBX3*, *MEIS1*, and *FLT3*. In accordance with these observations, we present clear evidence that the novel menin-MLL inhibitor VTP-50469 has dramatic synergistic antileukemic activity in combination with various FLT3 inhibitors.

In summary, our data show that menin-MLL inhibition targets *FLT3* transcriptionally via suppression of the leukemogenic transcription factor MEIS1, which is driven by *NPM1* mutations or



ID	Age	Sex	Diagnosis	% Blasts	Karyotype	*NPM1*^{mut}	*FLT3*^{mut}	Allelic *FLT3*-ratio
#1	49	f	de novo	90	normal	pos	*FLT3*-ITD	0.852
#2	71	m	de novo	70	normal	pos	*FLT3*-ITD	3.848
#3	72	f	de novo	75	normal	pos	*FLT3*-ITD	0.253
#4	46	f	de novo	78	normal	pos	*FLT3*-ITD	1.425
#5	74	f	de novo	97	normal	pos	*FLT3*-ITD	0.846
#6	57	f	de novo	84	normal	neg	*FLT3* WT	-
#7	91	f	secondary	35	47, XX+21; 46, XX, i(21)(q10)	neg	*FLT3*-TKD	0.160

Figure 7. Effects of single and combined menin-MLL and FLT3 inhibition on primary *NPM1*^{mut} *FLT3*^{ITD} AML patient samples and on survival of in vivo–treated *MLL*-r *FLT3*-ITD leukemic xenograft mice. (A) The human stromal cell coculture assay, performed to maintain and treat patients' primary AML blasts. (B) Summary of characteristics of patients providing the samples used in panels C-E. (C-D) Number of viable cells in de novo AML samples treated in coculture for 7 days with DMSO, MI-503 (2 μM), quizartinib (6 nM), or combinatorial MI-503 and quizartinib treatment. (C) Five independent samples of de novo *NPM1*^{mut}*FLT3*^{ITD} AML. (D) Two independent samples of de novo AML, WT for *NPM1*, *FLT3*, and *MLL*. Depicted are 4′,6-diamidino-2-phenylindole [DAPI]$^{-}$, human CD45^{+} cell numbers as assessed by flow cytometry. (E) Effect of MI-503 (2.5 μM), quizartinib (3 nM), and combinatorial treatment (2.5 μM and 3 nM) on total and blast-like CFUs in primary patient sample cells. (F) Experimental setup for the treatment of MV411-derived leukemic xenograft mice (left); percentage of human CD45^{+} cells in the bone marrow of leukemic mice (right) after treatment with drug vehicles, MI-503 (50 mg/kg; twice daily IP), quizartinib (10 mg/kg; PO; once daily), or combined MI-503 and quizartinib. (G) Kaplan-Meier survival analysis of MV411-derived leukemic xenograft mice treated with drug vehicles, MI-503 (50 mg/kg; twice daily IP), quizartinib (10 mg/kg; PO; once daily), or combinatorial MI-503 and quizartinib (n = 5 mice/group). The treatment period is displayed in blue. The log-rank (Mantel-Cox) test was used to calculate the *P*-values.

MLL fusions in AML and is not amenable to direct pharmacological inhibition. We further demonstrate that the combination of menin-MLL and FLT3 inhibition results in enhanced on-target activity against pFLT3, resulting in synergistic antileukemic effects against *NPM1*^{mut} or *MLL*-r leukemias harboring the prognostically adverse *FLT3*-ITD mutation. This drug combination therefore represents a promising opportunity for the treatment of these leukemia subtypes that is already available for clinical testing.

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Authorship

Contribution: M.W.M.K. designed the research, analyzed and interpreted the data, wrote and revised the manuscript, and supervised the study; M.M.D. performed the experiments, analyzed and interpreted the data, wrote the original draft of the manuscript and edited the manuscript; M.S. performed experiments, analyzed and interpreted the data, and edited the manuscript; J.R. performed experiments and analyzed and interpreted the data; K.K., J.S., and M.C.T. performed experiments and analyzed the data; D.S. and R.P.K. analyzed and interpreted the RNA-seq data; A.M. provided *Npm1*$^{CA/+}$*Flt3*$^{ITD/+}$ murine leukemia cells and revised the manuscript; G.M.M. provided the menin-MLL inhibitor VTP-50469 and revised the manuscript; P.H. and T.K. provided administrative support and patient samples and revised the manuscript; and C.-W.C., M.T., G.S.V., and S.A.A. analyzed and interpreted the data and revised the manuscript.

Conflict-of-interest disclosure: M.W.M.K, is a consultant for Pfizer and Abbvie and receives travel support from Celgene and Daiichi Sankyo. S.A.A. is a consultant and/or shareholder for Epizyme Inc, Vitae/Allergan Pharmaceuticals, Imago Biosciences, Cyteir Therapeutics, C4 Therapeutics, Syros Pharmaceuticals, OxStem Oncology, Accent Therapeutics, and Mana Therapeutics and has received research support from Janssen, Novartis, and AstraZeneca. G.S.V. is a consultant for Kymab and Oxstem. G.M.M. is a shareholder of Syndax Pharmaceuticals. The remaining authors declare no competing financial interests.

ORCID profiles: C.-W.C., 0000-0002-8737-6830; A.M., 0000-0002-2771-0462; G.S.V., 0000-0003-4337-8022.

Correspondence: Michael W. M. Kühn, University Medical Center, Johannes Gutenberg-University Mainz, Langenbeckstr 1, 55131 Mainz, Germany; e-mail: michael.kuehn@unimedizin-mainz.de.

Footnotes

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The RNA-seq data reported in this article have been deposited Gene Expression Omnibus database (accession number GSE144759).

Original data are available by e-mail request to the corresponding author Michael W. M. Kühn (michael.kuehn@unimedizin-mainz.de).

The online version of this article contains a data supplement.

There is a *Blood* Commentary on this article in this issue.

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