# Co-clinical Modeling of the Activity of the BET Inhibitor Mivebresib (ABBV-075) in AML

DANIEL H. ALBERT[1], NEAL C. GOODWIN[2], ANGELA M. DAVIES[2], JENNY ROWE[3],
GEROLD FEUER[3], MICHAEL BOYIADZIS[4], KATHLEEN A. DORRITIE[4], MARIA MANCINI[2],
REGINA GANDOUR-EDWARDS[5], BRIAN A. JONAS[6], GAUTAM BORTHAKUR[7],
IBRAHIM ALDOSS[8], DAVID A. RIZZIERI[9], OLATOYOSI ODENIKE[10], THOMAS PREBET[11],
SANJANA SINGH[1], RELJA POPOVIC[1], YU SHEN[1], KEITH F. MCDANIEL[1], WARREN M. KATI[1],
DIMPLE A. MODI[1], MONICA MOTWANI[1], JOHANNES E. WOLFF[1] and DAVID J. FROST[1]

[1]AbbVie, Inc., North Chicago, IL, U.S.A.;
[2]Champions Oncology, Inc., Hackensack, NJ, U.S.A.;
[3]HuMurine Technologies, La Verne, CA, U.S.A.;
[4]University of Pittsburgh Medical Center, Hillman Cancer Center, Pittsburgh, PA, U.S.A.;
[5]Department of Pathology & Laboratory Medicine, UC Davis School of Medicine, Sacramento, CA, U.S.A.;
[6]University of California Davis School of Medicine, Sacramento, CA, U.S.A.;
[7]Department of Leukemia, The University of Texas MD Anderson, Houston, TX, U.S.A.;
[8]Department of Hematology and Hematopoietic Cell Transplantation, City of Hope, Duarte, CA, U.S.A.;
[9]Medical Oncology, Novant Health Cancer Institute, Charlotte, NC, U.S.A.;
[10]Section of Hematology/Oncology, The University of Chicago Medicine Comprehensive
Cancer Center, Chicago, IL, U.S.A.;
[11]Section of Hematology, Department of Internal Medicine, Yale School of Medicine and Yale
Cancer Center, New Haven, CT, U.S.A.

**Abstract.** *Background/Aim: The therapeutic potential of bromodomain and extra-terminal motif (BET) inhibitors in hematological cancers has been well established in preclinical and early-stage clinical trials, although as of yet, no BET-targeting agent has achieved approval. To add insight into potential response to mivebresib (ABBV-075), a broad-spectrum BET inhibitor, co-clinical modeling of individual patient biopsies was conducted in the context of a Phase I trial in acute myeloid leukemia (AML). Materials and Methods: Co-clinical modeling involves taking the patient's biopsy and implanting it in mice with limited passage so that it closely retains the original characteristics of the malignancy and allows comparisons of response between animal model and clinical data. Procedures were developed, initially with neonate NOD/Shi-scid-IL2rγnull (NOG) mice and then optimized with juvenile NOG-EXL as host mice, eventually resulting in a robust rate of engraftment (16 out of 26, 62%). Results: Results from the co-clinical AML patient-derived xenograft (PDX) modeling (6 with >60% inhibition of bone marrow blasts) were consistent with the equivalent clinical data from patients receiving mivebresib in monotherapy, and in combination with venetoclax. The modeling system also demonstrated the activity of a novel BD2-selective BET inhibitor (ABBV-744) in the preclinical AML setting. Both agents were also highly effective in inhibiting blast counts in the spleen (10/10 and 5/6 models, respectively). Conclusion: These findings confirm the validity of the model system in the co-clinical setting, establish highly relevant in vivo models for the discovery of cancer therapy, and indicate the therapeutic value of BET inhibitors for AML and, potentially, myelofibrosis treatment.*

*Correspondence to:* Daniel H. Albert, Dept. R4N2, Bldg. AP3, Rm. 316, 1 North Waukegan Road, North Chicago, IL 60064-6098, U.S.A. Tel: +1 8479373844, Fax: +1 8479383266, e-mail: daniel.h.albert@abbvie.com

Mivebresib is a broad-spectrum, highly potent bromodomain and extra-terminal motif (BET) inhibitor with a promising preclinical profile in solid and hematological cancers (1-6). Preclinically, mivebresib induces strong apoptosis in cell

Table I. *Extent of engraftment in neonate NOD/Shi-scid-IL2rγnull (NOG), NOG®, and NOG-EXL® mice following inoculation of peripheral blood mononuclear cells from acute myeloid leukemia (AML) patients that were independent of AbbVie's co-clinical trial (CTG-2357, University of Pittsburgh clinical trials).*

| Model | # Cells inoculated | N | Timepoint (weeks) | Bone marrow counts/μl (%) | Spleen counts/μl (%) | Peripheral blood counts/μl (%) | Take rate % (bone marrow) (%) |
|---|---|---|---|---|---|---|---|
| Neonate NOG | $0.2 \times 10^6$ | 13 | 20 | 52,243 (37%) | 11,250 (7.2%) | 3,735 (9.4%) | 92 |
| Juvenile NOG | $0.5 \times 10^6$ | 7 | 12 | 36,020 (14%) | 145 (0.2%) | 169 (0.5%) | 42 |
| Juvenile NOG | $1 \times 10^6$ | 7 | 12 | 85,276 (23%) | 2,316 (1.5%) | 707 (2.1%) | 100 |
| Juvenile NOG | $2 \times 10^6$ | 7 | 12 | 106,242 (45%) | 2,104 (2.1%) | 1,553 (2.3%) | 100 |
| Juvenile NOG-EXL | $0.5 \times 10^6$ | 8 | 12 | 127,527** (52%**) | 5,642*** (7.3%***) | 977*** (2.6%***) | 100 |
| Juvenile NOG-EXL | $1 \times 10^6$ | 8 | 12 | 271,958*** (78%***) | 29,842*** (18%***) | 5,374*** (13%**) | 100 |
| Juvenile NOG-EXL | $2 \times 106$ | 8 | 12 | 235,586*** (84%***) | 25,226*** (20%***) | 4,369* (9.3%***) | 100 |

*p vs*. juvenile NOG: *<0.05, **<0.01, ***<0.005. NOG: CIEA NOG mouse®; NOG-EXL: NOG-EXL mouse®.

lines originating from hematological malignancies, including acute myeloid leukemia (AML), by epigenetically regulating the intrinsic apoptotic pathway through, in part, down-regulation of MCL1 (1). The impact of mivebresib on apoptosis and the resulting synergy with the BCL-2 inhibition in preclinical models of AML provided the rationale for clinical evaluation of mivebresib as monotherapy and in combination with venetoclax (1).

As a class of targeted cancer therapy, BET inhibitors have exhibited activity in hematological cancer trials, but no BET-targeting agent has yet achieved approval (7). The clinical effectiveness of mivebresib is currently under investigation (8, 9). In this regard, as part of a clinical trial to define the safety and biological activity of mivebresib in AML, we optimized procedures for AML patient-derived xenograft (PDX) modeling in the co-clinical setting for use with biopsies collected from the Phase I trial. In the studies described herein, the *in vivo* anti-cancer activity of mivebresib was observed in a subset of AML PDX models that correlated with the biological activity observed clinically as monotherapy or in combination with the BCL-2-targeting agent venetoclax. In addition, a recently described BD2-selective BET inhibitor (ABBV-744), although not part of the Phase I study, was evaluated and found to be active in a subset of the co-clinical PDX models (10, 11). The results presented support a therapeutic potential for pan- and BD2-selective BET inhibitors in AML and their combination with venetoclax.

## Materials and Methods

*Patient samples*. Bone marrow aspirates were obtained from the University of Pittsburgh Biospecimen Core (PBC) for PDX model development. Patients consented to sample donation with the acknowledgement that they would receive no direct benefit, clinical or otherwise. For the PDX pharmacology studies, patient samples were obtained from subjects entering AbbVie's M14-546 Phase I

study for patients receiving either mivebresib as monotherapy or in combination with venetoclax (9). Patients aged ≥18 years with AML for whom no standard-of-care therapy exists or who were refractory after standard-of-care therapy were eligible. Inclusion and exclusion criteria and subject demographics (age and weight) are presented elsewhere (9). In one case, one of the samples evaluated was obtained from a patient (#129) that crossed over from mono- to combination therapy. Patients consented to provide tissue and clinical information with acknowledgement that resulting data may be used for pre-clinical tumor model development and that the results generated from PDX studies would not inform on-going treatment decisions.

PDX modeling was optimized using AML peripheral blood mononuclear cells (PBMCs) collected from low volume (14 ml) patient samples (CTG-2241 and 2357, Champions Oncology, Hackensack, NJ, USA). For development of PDX models from the Phase I study, patient bone marrow aspirates obtained at screening were Ficoll gradient purified using Accuspin System Histopaque kits (Sigma-Aldrich, St. Louis, MO, USA) following the suppliers' instructions and viably cryopreserved for use with pharmacology studies. In one case, a patient's bone marrow aspirate from the combination cohort that successfully engrafted was used for two efficacy studies (#43 com –1 and –2).

*Drug formulation*. Mivebresib, venetoclax, and ABBV-744 were synthesized at AbbVie. The dose of mivebresib was limited to ≤0.5 mg/kg to approximate exposure achievable with a non-toxic clinical dose. Doses of ABBV-744 were limited to ≤10 mg/kg (~¼ murine maximum tolerated dose) to approximate the efficacious dose in cell-derived xenograft models with efficacy roughly comparable to the pre-clinical efficacy achieved with mivebresib (1, 10). Mivebresib, venetoclax, and ABBV-744 were formulated and administered orally in 2% DMSO, 30% PEG-400, and 68% Phosal-50PG, respectively.

*Animal studies. Neonate*. To optimize co-clinical modeling, neonate NOD/Shi-scid-IL2rγnull (CIEA NOG mouse®, NOG, Taconic Bioscience, NY, USA) mice were produced by mating NOG mice. The progeny was then evaluated as PDX hosts after intrahepatic (i.h.) inoculation of AML isolates (12). U.C. Davis Animal Care and Use Committee approved all neonate experimental procedures.
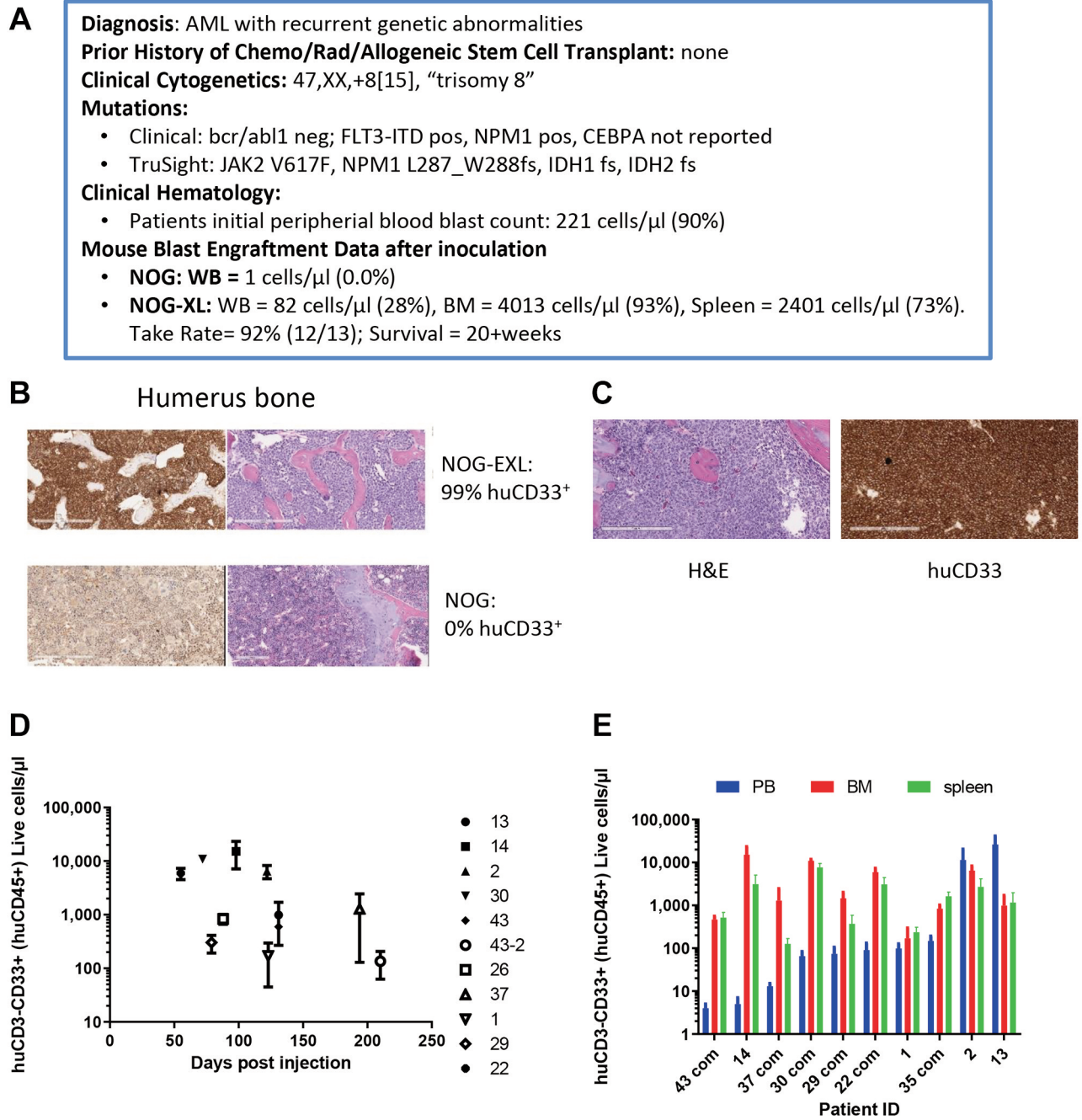
**A**

**Diagnosis**: AML with recurrent genetic abnormalities
**Prior History of Chemo/Rad/Allogeneic Stem Cell Transplant:** none
**Clinical Cytogenetics:** 47,XX,+8[15], "trisomy 8"
**Mutations:**
- Clinical: bcr/abl1 neg; FLT3-ITD pos, NPM1 pos, CEBPA not reported
- TruSight: JAK2 V617F, NPM1 L287_W288fs, IDH1 fs, IDH2 fs

**Clinical Hematology:**
- Patients initial peripherial blood blast count: 221 cells/μl (90%)

**Mouse Blast Engraftment Data after inoculation**
- **NOG: WB =** 1 cells/μl (0.0%)
- **NOG-XL:** WB = 82 cells/μl (28%), BM = 4013 cells/μl (93%), Spleen = 2401 cells/μl (73%). Take Rate= 92% (12/13); Survival = 20+weeks

**B** Humerus bone

NOG-EXL: 99% huCD33⁺

NOG: 0% huCD33⁺

**C**

H&E   huCD33

**D**

**E**

Figure 1. *Patient-derived xenograft (PDX) and acute myeloid leukemia (AML) co-clinical model development. A) Patient and PDX characteristics of CTG-2241, an AML sample that previously failed to engraft in juvenile NOD/Shi-scid-IL2γnull (NOG) mice. B) Humerus bone stained for huCD33+ and H&E. C) Immunohistochemical confirmation for co-clinical sample engraftment. D) Time to engraftment for pharmacology studies using bone marrow samples. Each symbol represents a different study. Values are expressed as mean±SE (N=10). E) Extent of engraftment during the expansion phase for pharmacology studies. Values are expressed as mean±SE (N=10).*

*Young adult.* Juvenile NOG mice and juvenile human transgenic IL-3/GMCSF NOD/Shi-*scid*-IL2rγ*null* mice (juvenile NOG-EXL®, Taconics Bioscience) were evaluated for engraftment efficiency following intravenous inoculation of AML isolates. Bone marrow

aspirates, splenocytes, and PBMCs from host mice were evaluated by fluorescence-activated cell sorting (FACS) at 12 weeks post AML inoculation for engraftment. Humerus bones from inoculated animals from selected models were also evaluated by human CD33

Table II. *Engraftment results of patient-derived xenograft (PDX) models.*

| PtnD | Days since implant | N | PB* | BM* | Spleen* | Eng | Eff |
|---|---|---|---|---|---|---|---|
| 13 | 131 | 4 | 26,261±14,941 (86%) | 986±717 (60%) | 1,162±803 (66%) | Y | 1 |
| 16 | 129 | 2 | 0.0 (1.7%) | Nd | Nd | N | |
| 4 | 129 | 5 | 0.6±0.3 (0.0%) | 140±139 (4%) | 717±699 (13%) | N | |
| 14 | 98 | 10 | 5±2 (0.7%) | 15,226±8,062 (63%) | 3,107±1,990 (34%) | Y | 2 |
| 8 | 82 | 4 | 68±44 (42%) | 979 (72%) pooled | 1,823 (14%) pooled | Y | |
| 2 | 122 | 4 | 11,429±9,066 (66%) | 6,492±1,796 (97%) | 2,706±1,465 (90%) | Y | 3 |
| 30 (+VEN) | 72 | 10 | 65±19 (18%) | 10,964±816 (87%) | 7,669±1,783 (78%) | Y | 4 |
| 20 (+VEN) | 49 | 6 | 0.1±07 (0.01%) | 5 (0.0%) pooled | 0 (0.0%) pooled | N | |
| 43 (+VEN) | 166 | 12 | 4±1 (2.1%) | 465±95 (20%) | 516±167 (5.5%) | Y | 5, 6 |
| 26 (+VEN) | 88 | 9 | 147±45 (21%) | 837±173 (63%) | 1,622±430 (61%) | Y | 7 |
| 35 (+VEN) | 199 | 9 | 0.02±0.01 (0.1%) | 0.1 (0.0%) pooled | 0.05 (0.1%) pooled | N | |
| 37 (+VEN) | 194 | 9 | 13±2 (6.9%) | 1,288±1,159 (39%) | 126±43 (14%) | Y | 8 |
| 25 (+VEN) | 228 | 7 | 0.02±0.01 (0.0%) | 0.0 (0.0%) pooled | 0.0 (0.0%) pooled | N | |
| 45 | 125 | 2 | 0.0±0.00 (0.0%) | Nd | Nd | N | |
| 17 | 137 | 5 | 0.27±0.17 (0.0%) | Nd | Nd | N | |
| 6 | 123 | 10 | 0.0±0.00 (0.0%) | 0.0 (0.0%) pooled | 0.03 (0.1%) pooled | N | |
| 15 | 137 | 9 | 201±31 (46%) | 5,013 (94%) pooled | 854 (47%) pooled | Y | |
| 12 | 100 | 18 | 62,661±26,858 (97%) | Nd | Nd | Y | |
| 1 | 123 | 10 | 99±28 (8.2%) | 171±126 (29%) | 236±74 (95%) | Y | 9 |
| 10 | 109 | 10 | 962±164 (74%) | 16,013 (99%) pooled | 46,047 (74%) pooled | Y | |
| 19 | 88 | 5 | 0.02±0.01 (0.0%) | 105 (2%) pooled | 1 (8.4%) pooled | N | |
| 9 | 123 | 10 | 0.0±0.00 (0.02%) | 0.0 (0.0%) pooled | 0.00 (0.0%) pooled | N | |
| 29 (+VEN) | 44 | 4 | 74±31 (30%) | 1,454±553 (46%) | 370±218 (9.9%) | Y | 10 |
| 33 (+VEN) | 95 | 8 | 193±45 (61%) | 6,407 (95%) pooled | 5,371 (81%) pooled | Y | |
| 34 (+VEN) | 117 | 7 | 87±39 (32%) | 5,072 (73%) pooled | 12,149 (88%) pooled | Y | |
| 22 (+VEN) | 55 | 4 | 90±41 (34%) | 4,658±1,106 (99%) | 3,085±1,345 (78%) | Y | 11 |

*Mean blast count±SE (%blasts). **blood counts >50 cells/μl or bone marrow counts >100 cells/μl. Eng: engraftment; Eff: efficacy study; Nd: not determined; VEN: venetoclax.

immunohistochemistry using clone PWS44 (Cell Marque, Rocklin, CA, USA) on an automated staining platform.

For co-clinical efficacy studies, purified and cryopreserved AML patient isolates were used to inoculate sub-lethally irradiated female NOG-EXL mice. Following inoculation (300,000-500,000 cells/mouse), engraftment was monitored in the blood and bone marrow for up to 32 weeks. After engraftment was confirmed in surrogate mice, the remaining cohorts were screened and randomized based on blast counts in blood into groups (n=11) and placed on trial. Investigators were blinded to the identity of the treatment. After two weeks of treatment, the presence of human blast cells in peripheral blood was assessed by flow cytometry. Treatment was continued for a total of 4 weeks. One week after cessation of treatment (day 35), terminal samples were collected to determine blast counts in peripheral blood, bone marrow, and spleen. In one study, the effect on "survival" (time to development of morbidity) was assessed and compared using Log-Rank statistical analysis (JMP 14, SAS, NC, USA). These studies were performed under protocols approved by AbbVie's and Champions' Institutional Animal Care and Use Committee and the adherence to the NIH Guidelines for the Care and Use of Laboratory Animals.

*FACS analysis and mutational profiling.* The number of human blasts in whole blood, bone marrow aspirates, and spleen samples was determined by flow cytometry using the criteria: huCD45+/muCD45−/huCD33+/huCD3− normalized with BD TruCount™ beads (13). For data analysis ($p<0.05$) comparison of means was performed using Dunnett's Method (% blast *vs.* vehicle control group) and paired Student's *t*-test (inhibition of blast count).

Mutation profiling on bone marrow aspirates was conducted using the TruSight Myeloid Sequencing Panel (Illumina), which covers 54 genes commonly mutated in myeloid malignancies. FLT3-ITD status was determined using capillary electrophoresis. One patient sample set (#13) was not available for mutation profiling.

## Results

*Model development and characterization.* The neonatal PDX platform exhibited engraftment characteristics (Neonate NOG, Table I). Even at lower PBMC inoculums ($2×10^5$), the neonates had a high extent of engraftment and efficient take rate in bone marrow. However, although the neonatal platform had efficient engraftment, the complexities of sample injections and timing of pregnancies led us to optimize and adopt a juvenile system that replaced NOG mice with humanized mice (NOG-EXL). Previous studies had demonstrated the potential for superior engraftment: a patient inoculum (CTG-2241, Champions Oncology,

Table III. *Efficacy summary of monotherapy bromodomain and extra-terminal motif (BET) inhibitors and in combination with venetoclax.*

| | | PDX model | | | Clinical response | | |
|---|---|---|---|---|---|---|---|
| | | Response (% inhibition *vs.* vehicle) | | | | | |
| Ptn ID cohort | Treatment (mg/kg) | PB d14/d35 | BM d35 | Spleen d35 | Initial mivebresib dose (mg) | Best % Change BM Blast | Response (>50% reduction in blast) |
| | Mivebresib 0.5 QD | 50/43 | 89*[1] | 98* | 1.5 | −70 | Biological |
| #14 | Mivebresib 2 3/wk | 25/−43 | 69 | 97* | | | activity |
| Monotherapy | ABBV-744 10 QD | 9/6 | 98*[1] | 99* | | | |
| | ABBV-744 25 3/wk | 51/33 | 94* | 98* | | | |
| | Mivebresib 0.25 QD | 24/36 | 79* | 86* | 2.5/400 | 42 | No biological |
| #29 | ABBV-744 2.5 QD | 34*/32 | 85* | 76* | | | activity |
| Monotherapy | ABBV-744 5 QD | 53*/57 | 93* | 95* | | | |
| | ABBV-744 10 QD | 50*/46 | 46 | 99* | | | |
| | Mivebresib 0.5 QD | 3/84 | 19[1] | 59 | 2 | | Not evaluable |
| #2 | Mivebresib 1.3 3/wk | 16/28 | 5[1] | 44 | | | |
| Monotherapy | ABBV-744 9.4 QD | −7/88 | 0[1] | 88 | | | |
| | Mivebresib 0.25 QD | −43/13 | −55 | 97* | 2.5 | | Not evaluable |
| #1 | Mivebresib 0.5 QD | −85/50* | −1,020 | 86* | | | |
| Monotherapy | ABBV-744 2.5 QD | −5/60* | −342 | 79* | | | |
| | ABBV-744 5 QD | 24/74* | −17 | 79* | | | |
| | ABBV-744 10 QD | −39/87* | 81 | 97* | | | |
| | Mivebresib 0.5 QD | 57*/82 | 67* (0% ↑survival) | 96* | 1.5 | −72 | Biological |
| #13 | Mivebresib 1.3 3/wk | 79*/-82 | −1 (49% ↑survival) | 71 | | | activity |
| Monotherapy | ABBV-744 9.4 QD | 91*/80 | −42 (104% ↑survival) | 33 | | | |
| | Mivebresib 0.5 QD | −9/24 | 43* | 89* | 0.5 | 27 | No biological |
| #30 | Venetoclax 50 | 42*/69* | 31* | 94* | | | activity |
| Combination | Mivebresib QD/ven | 7/43 | 53* | 84* | | | |
| | Mivebresib 0.5 QD | 3/94* | 83* | 99* | 1 | −58 | Biological |
| #43-1 | Venetoclax 50 | 9/29 | 44* | 95* | | | activity |
| Combination | Mivebresib QD/ven | 40/97* | 94* | 100* | | | |
| | Mivebresib 0.5 QD | 44*/39 | 2 | 97* | 1 | −85 | Biological |
| #37 | Venetoclax 50 | 86*/79* | 86 | 98* | | | activity |
| Combination | Mivebresib QD/ven | 92*/65* | 96 | 93* | | | |
| | Mivebresib 0.5 QD | 25/54 | 46[1] | 92*[1] | 1 | | Not evaluable |
| #26 | Venetoclax 50 | 52*/26 | 0[1] | 90*[1] | | | |
| Combination | Mivebresib QD/ven | 63*/term[2] | Term[2] | Term[2] | | | |
| | Mivebresib 0.5 QD | 46* | Term[2] | Term[2] | 2.5/100 | | Not evaluable |
| #22 | Venetoclax 50 | 74* | Term[2] | Term[2] | | | |
| Combination | Mivebresib QD/ven | 89* | Term[2] | Term[2] | | | |
| | Mivebresib 0.5 QD | −29/74* | 54 | 95* | 1 | −58 | Biological |
| #43-2 | ABBV-744 5 QD | 14/74* | 63 | 92* | | | activity |
| Combination | Venetoclax 50 | 33/20 | 45 | 77* | | | |
| | ABBV-744 QD/ven | 29/95* | 72[3] | 99* | | | |

[1]10% Weight loss, dosing holidays. [2]>15% weight loss, dosing holidays, mortality. [3]N=2 due to disease-related mortality. *Indicates mean blasts differs significantly from vehicle ($p<0.05$). ven: Venetoclax.

University of Pittsburgh clinical patient sample) that showed no engraftment in juvenile NOG mice exhibited a 92% take rate with 93% bone marrow (BM) tumor burden in NOG-EXL mice (Figure 1A and B). This work was confirmed by comparing engraftment obtained with another patient PBMC source (CTG-2357, University of Pittsburgh clinical patient sample) using juvenile NOG and juvenile NOG-EXL as host mice. Comparing the juvenile settings, efficiency in the number of juvenile mice engrafted (100% *vs.* 42%) and the extent of BM engraftment (52% *vs.* 14%) following a $5 \times 10^5$ PBMC inoculation was improved with the NOG-EXL mice (Table I). Based on these results, IL-3/GM-CSF NOG-EXL mice were used as hosts for subsequent co-clinical PDX modeling.
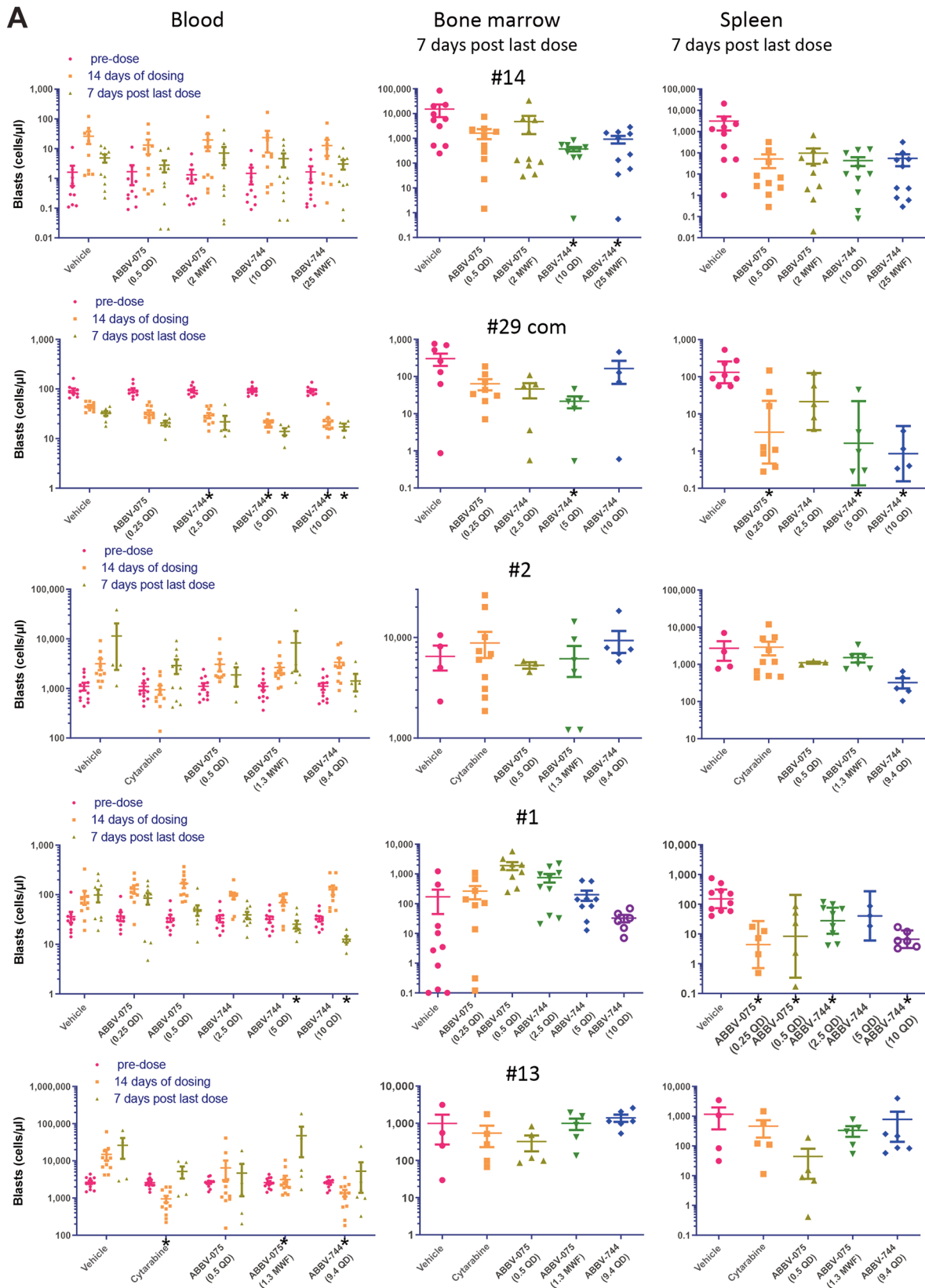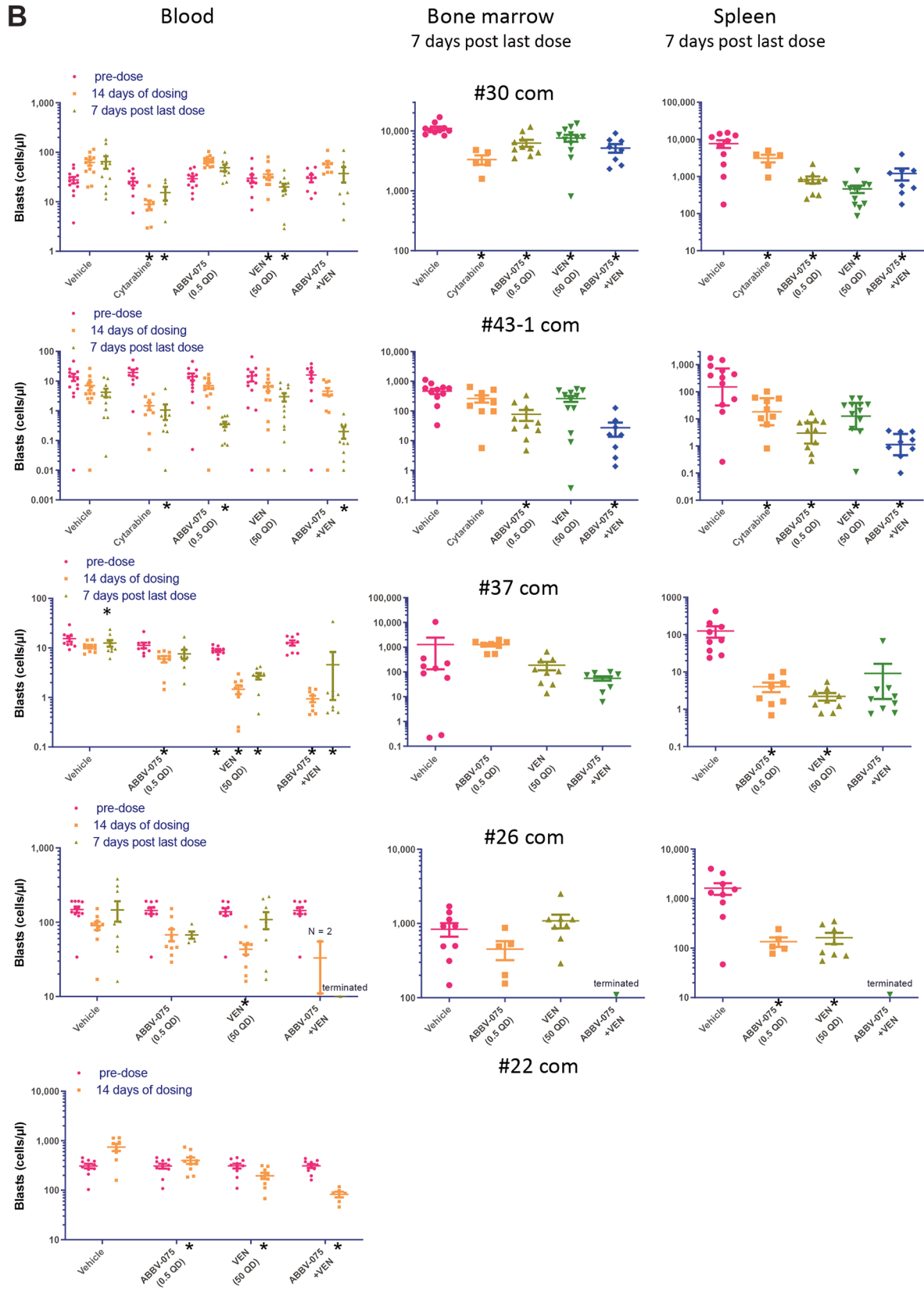
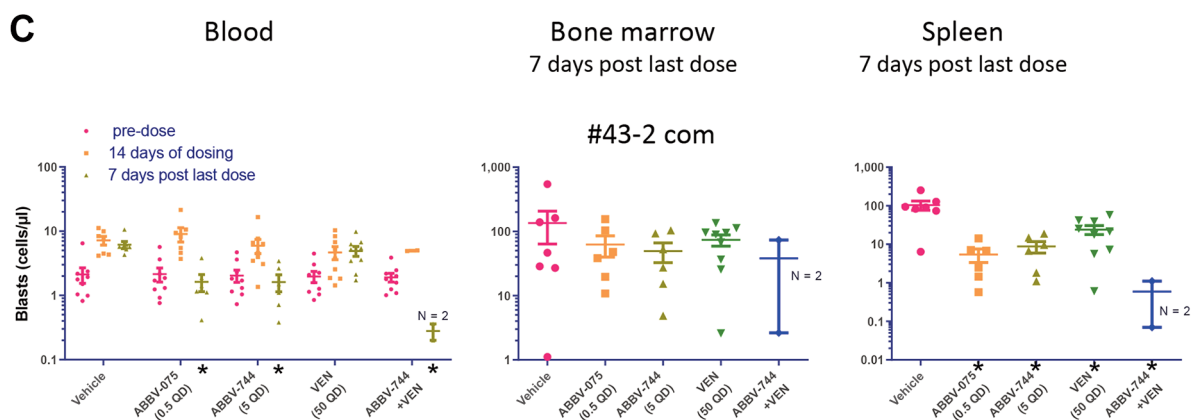Figure 2. *Continued*

Figure 2. *Continued*

Figure 2. *Patient-derived xenograft (PDX) results (blasts/μl) in blood, bone marrow, and spleen compartments: pharmacology models derived from subjects that received mivebresib (ABBV-075) and venetoclax (VEN) combination therapy. ABBV-744 is another BET inhibitor that was evaluated in vivo in comparison with mivebresib. Values are expressed as mean±SE (N=4-10). *Indicates mean differs significantly (p<0.05) from vehicle.*

Using the NOG-EXL mice, 16 of 26 patient inoculates from the mivebresib Phase I trial resulted in engraftment (defined as blood counts >50 cells/μl or bone marrow counts >100 cells/μl) (Table II). Pharmacological studies proceeded with 10 of the engrafted models (the remaining models required a scalable expansion phase for efficacy studies and were deprioritized and slated for expansion at a later date). In selected models, bone marrow engraftment was confirmed by immunohistochemistry (Figure 1C). The extent and timing of engraftment varied considerably, as illustrated by the plot of baseline bone marrow counts in Figure 1D. Furthermore, the distribution of the cells (PB, BM, spleen) also varied markedly, suggesting that in some cases the number of PB cells can underestimate the overall disease burden (Figure 1E), despite their reported fidelity in mimicking disease in terms of their mutational profile (14). In 7 of 16 successful engraftments, the bone marrow and spleen were the predominate sites of engraftment (Table II), which is typical of AML PDX modeling (15).

*Efficacy studies.* A subset of isolates that exhibited successful engraftment, which were representative of patients receiving mono- and combination therapy, were selected for pharmacology studies. For the purposes of assessing a quantitative response to therapy, we focused on inhibition of blast count, which is summarized in Table III. The profiles of treatment effects on blast count in the three tissue compartments from individual studies are provided in Figure 2. Although in some cases statistical significance compared to vehicle treated control was not achieved, trends were evident. As monotherapy, mivebresib was effective (TGI >50%) in reducing tumor burden in the bone marrow compartment 1 week after the last dose in 5/10 models (Figure 3A). ABBV-744 also exhibited activity in 4/6 models

(Figure 3A). Interestingly, mivebresib and ABBV-744 were generally more effective in inhibiting blast counts in the spleen (10/10 and 5/6 models, respectively), indicating that in the mouse this organ is highly susceptible to disease inhibition. Response in the blood compartment tended to be less pronounced and more variable compared to the bone marrow and spleen. For models that both agents were evaluated, the responses to both agents were generally similar across the three compartments. The notable exception was #13, which was broadly sensitive to mivebresib but relatively insensitive to ABBV-744 (Figure 3A).

The treatment groups from the pharmacology study with one model (#13) were subdivided to allow assessment of disease progression (Figure 3B). A survival benefit was demonstrated with the higher dose of mivebresib and with ABBV-744. The survival benefit tracked with blast inhibition (79% and 91%) was measured in the blood compartment during the dosing cycle (d14, Table III), suggesting that transient inhibition can have a beneficial effect on survival in these models. However, with another model (#22 com), all groups succumbed to disease prior to completion of the study, indicating that survival benefit is model-dependent.

PDX modeling of BET inhibitors and venetoclax combination was conducted with inoculates from patients receiving combination therapy (Table III, "combination"). There was a trend for better efficacy in the bone marrow and spleen compartments from the combination groups of 3/5 models receiving the mivebresib/venetoclax combination (+VEN, Figure 3A). Strong single agent activity may have obscured the potential benefit of combination therapy in the spleen. Limited data also support a potential benefit from co-administration of venetoclax with the BD2-selective BET inhibitor ABBV-744 (Figure 3A).
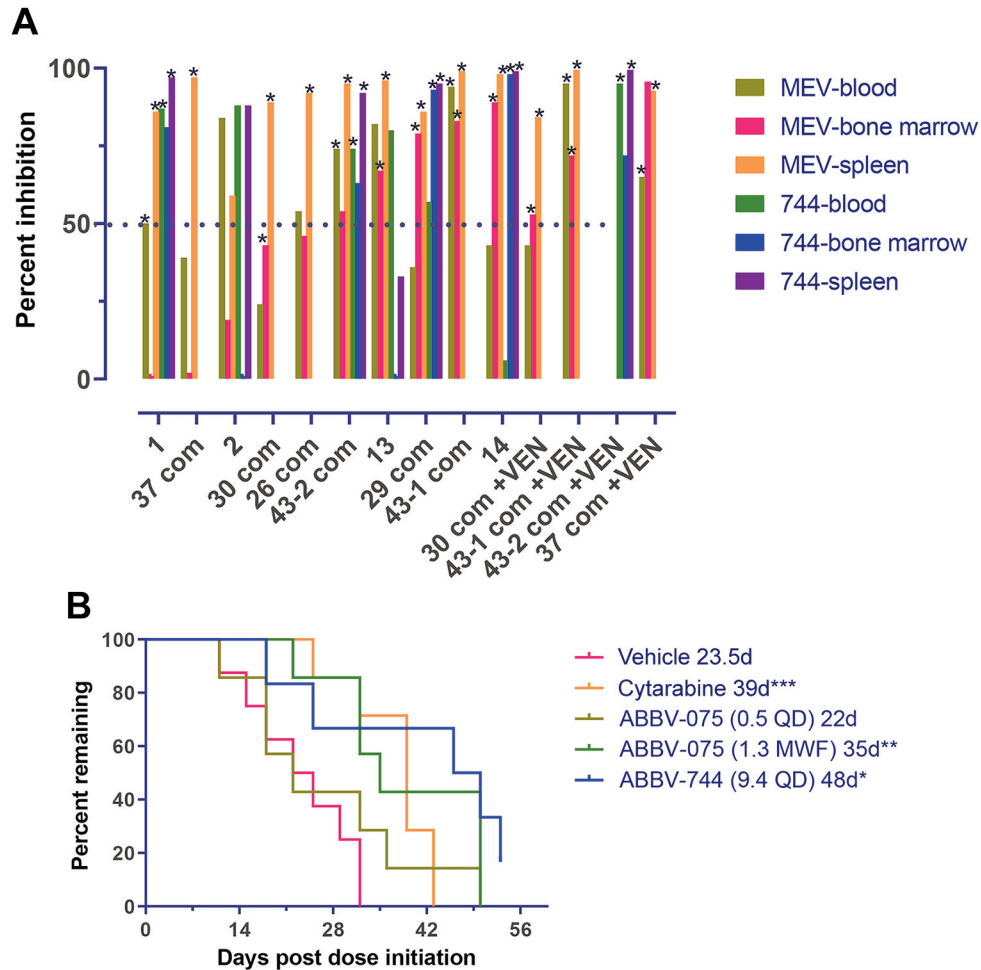
Figure 3. *Efficacy of bromodomain and extra-terminal motif (BET) inhibitors in co-clinical models. A) Inhibition of blast counts in blood, bone marrow and spleen from pharmacology models derived from subjects that received monotherapy or venetoclax combination therapy. Illustrated results include mivebresib and ABBV-744 monotherapy groups and groups in combination with venetoclax (indicated by the "+VEN" suffix). *Indicates that the mean of blasts differs significantly from vehicle (p<0.05). B) Effect of therapeutic agents on time to endpoint in a co-clinical patient-derived xenograft model derived from a patient receiving monotherapy (model #13, days 0-28). Treatment, median survival, and statistical analysis vs. vehicle are given in the legend (*p<0.05; **p<0.01; ***p<0.005).*

For comparison of the co-clinical model efficacy to clinical findings in the ABBV-075 trial, patient response was defined as biologically active if there was 50% reduction in blasts clinically. A waterfall plot comparing best clinical activity with co-clinical decrease in peripheral blood blast count revealed that a PDX response of >25% reduction in blasts was associated with the clinical biological activity of evaluable patients (Spearman rank correlation coefficient=0.98, *p*<0.05) (Figure 4A). An association with co-clinical response in the bone marrow with clinical response was also evident, although not as statistically robust (*p*=ns). In contrast, there was no apparent correlation between inhibition in the spleen (>50% decrease for each model) and clinical activity.

Results of mutational analysis of inoculated samples are summarized in Figure 4B (shown are genes that harbor

mutations). Genomic profiling identified a pattern of mutations in genes associated with spliceosome components, chromatin modulators, and MAPK signaling similar to the pattern of mutations detected with the initial clinical isolates (8). While sample numbers are small, making it difficult to confirm correlations, we did observe biological activity in *RAS* and *PTPN11* mutant models, which are considered more difficult to treat.

## Discussion

The primary goals of the studies reported here were: first, to establish and provide validation of a co-clinical AML PDX model system; and second, to define the *in vivo* activity of BET inhibitors in these clinically relevant models. For model
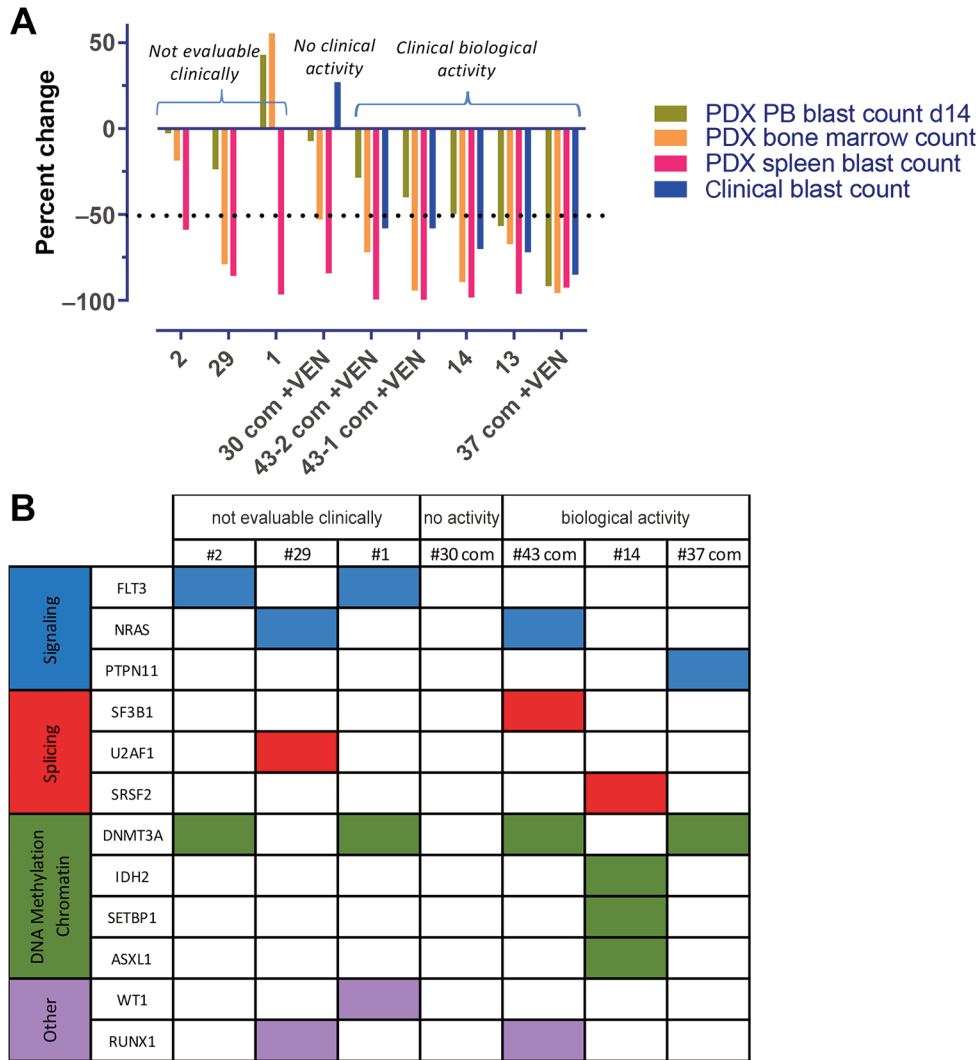
Figure 4. *Comparison of patient-derived xenograft model results to patient response. A) Clinical, biological activity, defined as ≥50% decrease in blast count. B) Mutational profile.*

development, we expanded procedures described in the literature with the goal to enhance take rate, while maintaining feasibility (15-17). We initially experimented with neonatal models as a potential platform for our co-clinical studies, but the intensive labor associated with this system shifted the use to juvenile mice. Additional co-clinical modeling demonstrated that *i.v.* inoculation of AML clinical samples in juvenile NOG–EXL mice was advantageous over NOG mice, presumably a consequence of human IL-3 and GMCSF expression. These results are in concert with previous studies reported by Wunderlich *et al.* demonstrating that "humanized" mice can be permissive hosts for AML engraftment (15).

The juvenile NOG–EXL approach led to the generation of informative models of clinical response and a rate of

engraftment (62%) in the co-clinical setting that compares with the previously 49% reported for the generation of AML engraftment PDX xenograft studies (16). Importantly, inhibition of disease tracked with clinical biological activity, thereby providing clinical validation of these PDX models. This was exemplified by the studies with patients #13 and #14 that revealed strong PDX monotherapy activity coupled with biological activity observed in the clinical trial.

It is interesting to note that, whereas the site of engraftment of these NOG–EXL PDX models generally included the three compartments evaluated, the spleen of BET inhibitor-treated mice typically exhibited higher rates of blast count inhibition. This result suggests that the spleen is a more sensitive compartment than the blood and bone marrow compartments

for BET inhibitor treatment. Whether this difference is due to differences in tissue physiology, variations in the engrafting tumor cell, or differences in drug distribution remains to be determined. Interestingly, co-clinical activity in the blood compartment provided the best correlation with clinical activity, although the bone marrow compartment would seem to be more relevant to AML. It is worth noting that strong antiproliferative activity in the spleen, a target organ in myeloproliferative diseases, suggests a potential utility for BET inhibitors in myelofibrosis that is currently being explored in the clinical setting (18, 19). In any event, the co-clinical activity generated in these studies that associated with clinical biological activity provides a comparison of AML co-clinical PDX activity to clinical biological activity and can serve as a template for future hematological disease co-clinical studies.

Establishment of the co-clinical AML PDX models provided the opportunity to define the activity of two BET inhibitors. Both mivebresib and ABBV-744, as single agents, proved to be broadly active. The effectiveness of mivebresib, which corresponded with the biological activity observed in the clinic, correlated with the well-established activity of other broad-spectrum BET inhibitors in preclinical AML models (7). PDX activity with the experimental BET inhibitor ABBV-744 provides evidence of the potential utility of domain-selective BET inhibitors for the treatment of AML. ABBV-744's novel BD2-selectivity may provide a favorable toxicity profile and is currently undergoing a phase I trial in AML (20).

Both agents were also effective in the PDX models when given in combination with venetoclax. The PDX response (high single agent activity) made it difficult to demonstrate either additive or synergistic activity. Nonetheless, evaluating combination activity deserves further exploration.

Taken together, these results demonstrate the potential scientific value of biologically viable samples obtained during on-going clinical studies from enrolled patients, to whom the scientific community owes a profound debt of gratitude. The co-clinical results generally are in line with cell line-derived xenograft studies (1) and provide confidence that these models will be useful in defining effective dosing regimens and alternative mono- and combination therapies. However, it should be recognized that the co-clinical approach to PDX model development may have only limited utility as a tool to prognostically predict therapeutic response of individual patients, given the typically long development time of most PDX models. Therefore, the primary value of these validated models is that they can be used as tools to discover and characterize novel therapeutic agents. For the current example, the PDX modeling results, taken together with the Phase I study, suggest that combining BET inhibition with BLC2 inhibition (venetoclax) deserves further consideration. Furthermore,

activity in these models, coupled with genomic signatures of sensitivity, confirm biological response to the investigational BET inhibitors mivebresib and the novel BD2-selective inhibitor ABBV-744.

## Funding

## Conflicts of Interest

DHA, SS, RP, YS, WMK, DAM, and DJF are employees of AbbVie. KFM, MM, and JEW were employees of AbbVie at the time of the study. NCG, AMD, and MM were employees of Champions Oncology (AbbVie provided funding for a co-clinical PDX collaboration study in conjunction with an Abbvie-sponsored clinical investigation). RGE and BAJ were employees at UC Davis at the time of the study (RGE was a collaborator with Champions Oncology). BAJ was a consultant/advisor for AbbVie, BMS, Celgene, Genentech/Roche, Gilead, GlycoMimetics, Jazz, Pfizer, Servier, Takeda, Tolero, and Treadwell; protocol steering committee member for GlycoMimetics; data monitoring committee member for Gilead; received travel reimbursement from AbbVie; and received research funding to his institution from 47, AbbVie, Accelerated Medical Diagnostics, Amgen, AROG, BMS, Celgene, Daiichi Sankyo, F. Hoffmann-La Roche, Forma, Genentech/Roche, Gilead, GlycoMimetics, Hanmi, Immune-Onc, Incyte, Jazz, Loxo Oncology, LP Therapeutics, Pfizer, Pharmacyclics, Sigma Tau, and Treadwell. JR and GF were employees of HuMurine Technologies, Inc. (were collaborators with Champions Oncology, no Abbvie funding to disclose). MB and KAD were employees of UPMC Hillman Cancer Center (were collaborators with Champions Oncology, no Abbvie funding to disclose). GB is an employee of the University of Texas MD Anderson Cancer Center (was investigator in Abbvie-sponsored study and received research support from AbbVie). DAR is an employee of Novant Health Cancer Institute (received Advisory board and speaker fees from AbbVie and was an investigator in Abbvie- sponsored study). OO: is an employee of University of Chicago Medicine Comprehensive Cancer Center (received funding from AbbVie for consulting/advisory role and was an investigator in Abbvie- sponsored study). IA is an employee of City of Hope, Duarte, California, USA (Advisory Board with Abbvie and was an investigator in Abbvie- sponsored study). TP was an employee of Yale (was an investigator in Abbvie- sponsored study).

## Authors' Contributions

DHA: designed pharmacology studies, analyzed and interpreted the data, wrote the original draft, and edited the manuscript. NCG: designed the concept, analyzed and interpreted data, provided administrative support, reviewed and edited the manuscript. AMD: designed the concept, analyzed and interpreted data, provided

## References

1  Bui MH, Lin X, Albert DH, Li L, Lam LT, Faivre EJ, Warder SE, Huang X, Wilcox D, Donawho CK, Sheppard GS, Wang L, Fidanze S, Pratt JK, Liu D, Hasvold L, Uziel T, Lu X, Kohlhapp F, Fang G, Elmore SW, Rosenberg SH, McDaniel KF, Kati WM and Shen Y: Preclinical characterization of BET family bromodomain inhibitor ABBV-075 suggests combination therapeutic strategies. Cancer Res *77(11)*: 2976-2989, 2017. PMID: 28416490. DOI: 10.1158/0008-5472.CAN-16-1793

2  Faivre EJ, Wilcox D, Lin X, Hessler P, Torrent M, He W, Uziel T, Albert DH, McDaniel K, Kati W and Shen Y: Exploitation of castration-resistant prostate cancer transcription factor dependencies by the novel BET inhibitor ABBV-075. Mol Cancer Res *15(1)*: 35-44, 2017. PMID: 27707886. DOI: 10.1158/1541-7786.MCR-16-0221

3  Fiskus W, Cai T, DiNardo CD, Kornblau SM, Borthakur G, Kadia TM, Pemmaraju N, Bose P, Masarova L, Rajapakshe K, Perera D, Coarfa C, Mill CP, Saenz DT, Saenz DN, Sun B, Khoury JD, Shen Y, Konopleva M and Bhalla KN: Superior efficacy of cotreatment with BET protein inhibitor and BCL2 or MCL1 inhibitor against AML blast progenitor cells. Blood Cancer J *9(2)*: 4, 2019. PMID: 30647404. DOI: 10.1038/s41408-018-0165-5

4  Lam LT, Lin X, Faivre EJ, Yang Z, Huang X, Wilcox DM, Bellin RJ, Jin S, Tahir SK, Mitten M, Magoc T, Bhathena A, Kati WM, Albert DH, Shen Y and Uziel T: Vulnerability of small-cell lung cancer to apoptosis induced by the combination of BET bromodomain proteins and BCL2 inhibitors. Mol Cancer Ther *16(8)*: 1511-1520, 2017. PMID: 28468776. DOI: 10.1158/1535-7163.MCT-16-0459

5  Lin X, Huang X, Uziel T, Hessler P, Albert DH, Roberts-Rapp LA, McDaniel KF, Kati WM and Shen Y: HEXIM1 as a robust pharmacodynamic marker for monitoring target engagement of BET family bromodomain inhibitors in tumors and surrogate tissues. Mol Cancer Ther *16(2)*: 388-396, 2017. PMID: 27903752. DOI: 10.1158/1535-7163.MCT-16-0475

6  McDaniel KF, Wang L, Soltwedel T, Fidanze SD, Hasvold LA, Liu D, Mantei RA, Pratt JK, Sheppard GS, Bui MH, Faivre EJ, Huang X, Li L, Lin X, Wang R, Warder SE, Wilcox D, Albert DH, Magoc TJ, Rajaraman G, Park CH, Hutchins CW, Shen JJ, Edalji RP, Sun CC, Martin R, Gao W, Wong S, Fang G, Elmore SW, Shen Y and Kati WM: Discovery of N-(4-(2,4-Difluorophenoxy)-3-(6-methyl-7-oxo-6,7-dihydro-1H-pyrrolo[2,3-c]pyridin-4-yl)phenyl) ethanesulfonamide (ABBV-075/Mivebresib), a potent and orally available bromodomain and extraterminal domain (BET) family bromodomain inhibitor. J Med Chem *60(20)*: 8369-8384, 2017. PMID: 28949521. DOI: 10.1021/acs.jmedchem.7b00746

7  Alqahtani A, Choucair K, Ashraf M, Hammouda DM, Alloghbi A, Khan T, Senzer N and Nemunaitis J: Bromodomain and extra-terminal motif inhibitors: a review of preclinical and clinical advances in cancer therapy. Future Sci OA *5(3)*: FSO372, 2019. PMID: 30906568. DOI: 10.4155/fsoa-2018-0115

8  Piha-Paul SA, Sachdev JC, Barve M, LoRusso P, Szmulewitz R, Patel SP, Lara PN Jr, Chen X, Hu B, Freise KJ, Modi D, Sood A, Hutti JE, Wolff J and O'Neil BH: First-in-human study of Mivebresib (ABBV-075), an oral pan-inhibitor of bromodomain and extra terminal proteins, in patients with relapsed/refractory solid tumors. Clin Cancer Res *25(21)*: 6309-6319, 2019. PMID: 31420359. DOI: 10.1158/1078-0432.CCR-19-0578

9  Borthakur G, Odenike O, Aldoss I, Rizzieri DA, Prebet T, Chen C, Popovic R, Modi DA, Joshi RH, Wolff JE and Jonas BA: A phase 1 study of the pan-bromodomain and extraterminal inhibitor mivebresib (ABBV-075) alone or in combination with venetoclax in patients with relapsed/refractory acute myeloid leukemia. Cancer *127(16)*: 2943-2953, 2021. PMID: 33934351. DOI: 10.1002/cncr.33590

10 Faivre EJ, McDaniel KF, Albert DH, Mantena SR, Plotnik JP, Wilcox D, Zhang L, Bui MH, Sheppard GS, Wang L, Sehgal V, Lin X, Huang X, Lu X, Uziel T, Hessler P, Lam LT, Bellin RJ, Mehta G, Fidanze S, Pratt JK, Liu D, Hasvold LA, Sun C, Panchal SC, Nicolette JJ, Fossey SL, Park CH, Longenecker K, Bigelow L, Torrent M, Rosenberg SH, Kati WM and Shen Y: Selective inhibition of the BD2 bromodomain of BET proteins in prostate cancer. Nature *578(7794)*: 306-310, 2020. PMID: 31969702. DOI: 10.1038/s41586-020-1930-8

11 Zhang L, Cai T, Lin X, Huang X, Bui MH, Plotnik JP, Bellin RJ, Faivre EJ, Kuruvilla VM, Lam LT, Lu X, Zha Z, Feng W, Hessler P, Uziel T, Zhang Q, Cavazos A, Han L, Ferguson DC, Mehta G, Shanmugavelandy SS, Magoc TJ, Rowe J, Goodwin NC, Dorritie KA, Boyiadzis M, Albert DH, McDaniel KF, Kati WM, Konopleva M and Shen Y: Selective inhibition of the second bromodomain of BET family proteins results in robust antitumor activity in preclinical models of acute myeloid leukemia. Mol Cancer Ther *20(10)*: 1809-1819, 2021. PMID: 34253595. DOI: 10.1158/1535-7163.MCT-21-0029

12 Ellegast JM, Rauch PJ, Kovtonyuk LV, Müller R, Wagner U, Saito Y, Wildner-Verhey van Wijk N, Fritz C, Rafiei A, Lysenko V, Dudkiewicz E, Theocharides AP, Soldini D, Goede JS, Flavell RA and Manz MG: inv(16) and NPM1mut AMLs engraft human cytokine knock-in mice. Blood *128(17)*: 2130-2134, 2016. PMID: 27581357. DOI: 10.1182/blood-2015-12-689356

13 Sanchez PV, Perry RL, Sarry JE, Perl AE, Murphy K, Swider CR, Bagg A, Choi JK, Biegel JA, Danet-Desnoyers G and Carroll M: A robust xenotransplantation model for acute myeloid leukemia. Leukemia *23(11)*: 2109-2117, 2009. PMID: 19626050. DOI: 10.1038/leu.2009.143

14 Tong WG, Sandhu VK, Wood BL, Hendrie PC, Becker PS, Pagel JM, Walter RB and Estey EH: Correlation between peripheral blood and bone marrow regarding FLT3-ITD and NPM1 mutational status in patients with acute myeloid leukemia. Haematologica *100(3)*: e97-e98, 2015. PMID: 25527567. DOI: 10.3324/haematol.2014.118422

15 Wunderlich M, Chou FS, Link KA, Mizukawa B, Perry RL, Carroll M and Mulloy JC: AML xenograft efficiency is significantly improved in NOD/SCID-IL2RG mice constitutively expressing human SCF, GM-CSF and IL-3. Leukemia *24(10)*: 1785-1788, 2010. PMID: 20686503. DOI: 10.1038/leu.2010.158

16 Pearce DJ, Taussig D, Zibara K, Smith LL, Ridler CM, Preudhomme C, Young BD, Rohatiner AZ, Lister TA and Bonnet D: AML engraftment in the NOD/SCID assay reflects the outcome of AML: implications for our understanding of the heterogeneity of AML. Blood *107(3)*: 1166-1173, 2006. PMID: 16234360. DOI: 10.1182/blood-2005-06-2325

17 Wunderlich M, Mizukawa B, Chou FS, Sexton C, Shrestha M, Saunthararajah Y and Mulloy JC: AML cells are differentially sensitive to chemotherapy treatment in a human xenograft model. Blood *121(12)*: e90-e97, 2013. PMID: 23349390. DOI: 10.1182/blood-2012-10-464677

18 Mascarenhas J, Gerds A and Verstovsek S: Paradigm shift: combination BET and JAK inhibition in myelofibrosis. Leukemia *35(12)*: 3361-3363, 2021. PMID: 34480105. DOI: 10.1038/s41375-021-01405-z

19 AbbVie, Safety and Tolerability Study of Oral ABBV-744 tablet alone or in combination with oral ruxolitinib tablet or oral navitoclax tablet in adult participants with myelofibrosis, 2020. Available at: https://ClinicalTrials.gov/show/NCT04454658 [Last accessed on May 10, 2022]

20 AbbVie, A study evaluating the safety and pharmacokinetics of ABBV-744 in participants with relapsed/refractory acute myeloid leukemia (AML) cancer, 2018. Available at: https://ClinicalTrials.gov/show/NCT03360006 [Last accessed on May 10, 2022]