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American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

Azacitidine and Durvalumab in First-line Treatment of Elderly Patients With Acute Myeloid Leukemia

Tracking no: ADV-2021-006138R1

Amer Zeidan (Yale University, United States) Isaac Boss (Bristol Myers Squibb, United States) CL Beach (Bristol Myers Squibb, United States) Wilbert Copeland (Bristol Myers Squibb, United States) Ethan Thompson (Bristol Myers Squibb, United States) Brian Fox (Bristol Myers Squibb, United States) Vanessa Hasle (Bristol Myers Squibb, United States) Andrzej Hellmann (Medical University of Gdansk, Poland) David Taussig (The Royal Marsden Hospital, United Kingdom) Mar Tormo (Hospital Clinico Universitary. INCLIVA Research Institute, Spain) Maria Teresa Voso (Universita' di Roma Tor Vergata, Italy) Jamie Cavenagh (St. Bartholomew's Hospital, United Kingdom) Tim O'Connor (Bristol Myers Squibb, United States) Aessandro Previtali (Bristol Myers Squibb, Switzerland) Shelonitda Rose (Bristol Myers Squibb, United States) Lewis Silverman (Mount Sinai School of Medicine, United States)

Abstract:

Evidence suggests that combining immunotherapy with hypomethylating agents may enhance antitumor activity. This phase 2 study investigated the activity and safety of durvalumab, a programmed death ligand 1 (PD-L1) inhibitor, combined with azacitidine for patients aged {greater than or equal to}65 years with acute myeloid leukemia (AML), including analyses to identify biomarkers of treatment response. Patients were randomized to first-line therapy with azacitidine 75 mg/m^2 on days 1-7 with (Arm A, n= 64) or without (Arm B, n=65) durvalumab 1500 mg on day 1 every 4 weeks. Overall response rate (complete response [CR] + CR with incomplete blood recovery [CRi]) was similar in both arms (Arm A, 31.3%; Arm B, 35.4%), as were overall survival (A, 13.0 months; B, 14.4 months) and duration of response (A, 24.6 weeks; B, 51.7 weeks; P=0.0765). No new safety signals emerged with combination treatment. The most frequently reported treatment-emergent adverse events were constipation (Arm A, 57.8%; Arm B, 53.2%) and thrombocytopenia (A, 42.2%; B, 45.2%). DNA methylation, mutational status, and PD-L1 expression were not associated with response to treatment. In this study, first-line combination therapy with durvalumab and azacitidine in older patients with AML was feasible, but did not improve clinical efficacy compared with azacitidine alone. ClinicalTrials.gov: NCT02775903

Conflict of interest: COI declared - see note

COI notes: Amer M. Zeidan - A.M.Z. is a Leukemia and Lymphoma Society Scholar in Clinical Research and was also supported by a NCI's Cancer Clinical Investigator Team Leadership Award (CCITLA). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. A.M.Z. received research funding (institutional) from Celgene/BMS, Abbvie, Astex, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Trovagene/Cardiff oncology, Incyte, Takeda, Novartis, Amgen, Aprea, and ADC Therapeutics. A.M.Z participated in advisory boards, and/or had a consultancy with and received honoraria from AbbVie, Otsuka, Pfizer, Celgene/BMS, Jazz, Incyte, Agios, Boehringer-Ingelheim, Novartis, Acceleron, Astellas, Daiichi Sankyo, Cardinal Health, Taiho, Seattle Genetics, BeyondSpring, Trovagene/Cardiff Oncology, Takeda, Ionis, Amgen, Janssen, Epizyme, Syndax, Gilead, Kura, Aprea, Janssen, and Tyme. A.M.Z served on clinical trial committees for Novartis, Abbvie, Geron, Gilead, Kura, and Celgene/BMS. A.M.Z received travel support for meetings from Pfizer, Novartis, and Cardiff Oncology. Isaac Boss, CL Beach, Wilbert B. Copeland, Ethan Thompson, Brian A. Fox, Vanessa E. Hasle, Tim O'Connor, Alessandro Previtali, Shelonitda Rose - are employees of Celgene, a Bristol Myers Squibb company, and may be shareholders. David C. Taussig - Supported by the National Institute for Health Research Royal Marsden/Institute of Cancer Research Biomedical Research Centre. Research funding from Celgene. James Cavenagh - No potential conflicts of interest. Lewis R. Silverman - Research funding from Celgene, Onconova Therapeutics, Forty-seven, Medimmune, Takeda Maria Teresa Voso - Research funding from Celgene; speakers bureau for Celgene, Astellas, Jazz, Abbvie Andrzej Hellmann - No potential conflicts of interest. Mar Tormo Consultancy for Celgene, Astellas, Novartis; sponsored conferences and meetings with Celgene, Astellas, Amgen, Novartis, Pfizer, Servier

Preprint server: No;

Author contributions and disclosures: Study design: Isaac Boss contributed the translational plan to the study design; Alessandro Previtali Study investigator: Amer M. Zeidan, David C. Taussig Enrolled patients: Amer M. Zeidan, David C. Taussig, Andrzej Hellmann, Mar Tormo, Maria Teresa Voso, James Cavenagh, Lewis R. Silverman Collection and assembly of data: Amer M. Zeidan, Isaac Boss, Wilbert B.

Copeland, Brian A. Fox Data analysis: Isaac Boss, CL Beach, Wilbert B. Copeland, Vanessa E. Hasle, Ethan Thompson, Brian A. Fox, Alessandro Previtali, Tim O'Connor Data interpretation: All authors Manuscript preparation: Brian Fox, Amer M. Zeidan Manuscript review and revisions: All authors Final approval of manuscript: All authors

Non-author contributions and disclosures: Yes; The authors would like to thank the patients enrolled in the study as well as associated clinical trial teams. The authors received editorial support in the preparation of this manuscript from Lauren Gallagher, RPh, PhD, of Peloton Advantage, LLC, an OPEN Health company, Parsippany, NJ, USA, sponsored by Bristol Myers Squibb, Summit, NJ, USA. The authors, however, directed and are fully responsible for all content and editorial decisions for this manuscript.

Agreement to Share Publication-Related Data and Data Sharing Statement: BMS policy on data sharing may be found at https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html. Data were analyzed by Isaac Boss, Wilbert Copeland, Vanessa Hasle, Ethan Thompson, Brian Fox, Ken Ogasawara, and Alessandro Previtali. All authors had access to primary data of the trial.

Clinical trial registration information (if any): https://clinicaltrials.gov/ct2/show/NCT02775903

Original Research

Azacitidine and Durvalumab in First-line Treatment of Elderly Patients With Acute Myeloid Leukemia

Amer M. Zeidan[1], Isaac Boss[2], CL Beach[2], Wilbert B. Copeland[2], Ethan Thompson[2], Brian A. Fox[2], Vanessa E. Hasle[2], Andrzej Hellmann[3], David C. Taussig[4], Mar Tormo[5], Maria Teresa Voso[6], James Cavenagh[7], Tim O'Connor[2], Alessandro Previtali[2], Shelonitda Rose[2], Lewis R. Silverman[8]

[1]Yale University and Yale Cancer Center, New Haven, CT, USA; [2]Bristol Myers Squibb, Princeton, NJ, USA; [3]University Clinical Center of Medical University of Gdańsk, Gdańsk, Poland; [4]The Royal Marsden NHS Foundation Trust, Sutton, United Kingdom; [5]Hospital Clinico Universitario de Valencia and INCLIVA Biomedical Research Institute, Valencia, Spain; [6]AF Oncoematologia – Universita' di Roma Tor Vergata, Rome, Italy; [7]Barts Health NHS Trust, St. Bartholomew's Hospital, West Smithfield, London, United Kingdom; [8]Icahn School of Medicine at Mount Sinai, Tisch Cancer Institute, New York, NY, USA

Corresponding Author:

Amer M. Zeidan, MD

Associate Professor of Internal Medicine (Hematology)

Hematology Early Therapeutics Research, Director

Section of Hematology, Department of Internal Medicine

Yale University and Yale Cancer Center

37 College Street. First floor

New Haven, CT 06510, USA

Phone: 203-737-7103

Fax: 203-737-3401

amer.zeidan@yale.edu

Keywords: AML, durvalumab, azacitidine, PD-L1, immune checkpoint inhibitors

Text word count: 4352/4000

Abstract: 199/250

Figures/tables: 5/2 (7/7 total)

References: 21

Running title: Azacitidine and Durvalumab in Elderly Patients With AML

ABSTRACT

Evidence suggests that combining immunotherapy with hypomethylating agents may enhance antitumor activity. This phase 2 study investigated the activity and safety of durvalumab, a programmed death ligand 1 (PD-L1) inhibitor, combined with azacitidine for patients aged ≥65 years with acute myeloid leukemia (AML), including analyses to identify biomarkers of treatment response. Patients were randomized to first-line therapy with azacitidine 75 mg/m^2 on days 1–7 with (Arm A, n= 64) or without (Arm B, n=65) durvalumab 1500 mg on day 1 every 4 weeks. Overall response rate (complete response [CR] + CR with incomplete blood recovery [CRi]) was similar in both arms (Arm A, 31.3%; Arm B, 35.4%), as were overall survival (A, 13.0 months; B, 14.4 months) and duration of response (A, 24.6 weeks; B, 51.7 weeks; *P*=0.0765). No new safety signals emerged with combination treatment. The most frequently reported treatment-emergent adverse events were constipation (Arm A, 57.8%; Arm B, 53.2%) and thrombocytopenia (A, 42.2%; B, 45.2%). DNA methylation, mutational status, and PD-L1 expression were not associated with response to treatment. In this study, first-line combination therapy with durvalumab and azacitidine in older patients with AML was feasible, but did not improve clinical efficacy compared with azacitidine alone.

ClinicalTrials.gov: NCT02775903

KEY POINTS

- This is the first reported randomized trial of immune checkpoint inhibitor therapy in elderly patients with acute myeloid leukemia.
- Azacitidine combined with the PD-L1 inhibitor durvalumab was feasible but did not improve outcomes over azacitidine alone.

INTRODUCTION

Increased expression of inhibitory checkpoint molecules (programmed cell death protein 1 [PD-1], OX40, T cell immunoglobulin and mucin domain-containing protein 3 [TIM-3], and lymphocyte-activation gene 3 [LAG3]) by effector T-cell subsets in the bone marrow (BM) has been reported in patients with acute myeloid leukemia (AML) versus healthy donors and is further enhanced in patients with multiple AML relapses.[1] Programmed death ligand 1 (PD-L1; CD274) expression on tumor blasts has also been reported to be elevated in patients with tumor protein p53 [*TP53*]-mutated AML.[1] A recent analysis of RNA-sequencing and mutation data from the Cancer Genome Atlas database suggested that high co-expression of certain checkpoint molecules, such as PD-1/cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and PD-L2/CTLA-4, may be predictive of poor overall survival (OS) in AML.[2]

Epigenetic therapies, including azacitidine, may shift the tumor environment from immune evasion to immune recognition through multiple mechanisms such as reversal of epigenetic silencing/hypermethylation to elicit expression of tumor antigens,[3-5] upregulation of antigen processing and presentation,[6] increase in T-cell infiltration and chemokines,[6-8] and induction of interferon response.[6,9] Epigenetic therapies, however, also upregulate the expression of inhibitory checkpoint molecules such as PD-L1, which may lead to treatment resistance.[10-12] Immune checkpoint (ICP) blockade, both as monotherapy and in combination with hypomethylating agents (HMAs), has shown clinical benefit in patients with relapsed or refractory AML, including following relapse after allogeneic stem cell transplantation.[13-15]

Durvalumab is a PD-L1–blocking antibody that is approved for the treatment of select patients with advanced lung cancers and is under investigation in numerous cancer types.[16] Blockade of PD-L1 with durvalumab in combination with azacitidine may enhance T-cell–mediated antitumor activity and improve clinical outcomes in patients with AML.

We report the final results from FUSION-AML-001, the first randomized study to compare the efficacy and safety of first-line combination therapy with durvalumab and azacitidine versus azacitidine monotherapy in elderly patients with AML. Additionally, extensive exploratory biomarker analyses including global DNA methylation, peripheral blood (PB) and BM immunophenotyping, mutation profiling, and RNA sequencing of genes of interest, were performed to identify biomarkers of response to combination therapy and infer the impact of treatment in the tumor microenvironment (TME).

METHODS

Patients

Eligible patients were aged ≥65 years with centrally confirmed *de novo* AML (BM blasts ≥20%), or AML secondary to prior MDS or exposure to potentially leukemogenic agents with the primary malignancy in remission for at least 2 years. Patients were required to have centrally confirmed intermediate- or poor-risk status based on cytogenetics and Eastern Cooperative Oncology Group performance status 0–2. Patients with prior hematopoietic stem cell transplantation (HSCT) or who were eligible for HSCT were ineligible, as were those who had previously received any formulation of azacitidine or decitabine. Complete exclusion criterion are provided in the Supplementary Methods.

Study Design and Treatments

FUSION-AML-001 (NCT02775903) was an open-label, international, randomized, phase 2 study (**Supplementary Figure S1**). Patients were randomized 1:1 to combination therapy with azacitidine 75 mg/m^2 subcutaneously days 1–7 and durvalumab 1500 mg intravenously day 1 every 4 weeks (Q4W) (Arm A) or monotherapy with azacitidine 75 mg/m^2 subcutaneously days 1–7 Q4W (Arm B) and stratified according to cytogenetic risk (intermediate versus poor). Treatment continued through 6 cycles, and if an overall response or other clinical benefit was obtained, continued until disease progression or unacceptable toxicity.

Dose Modifications

The azacitidine dose could be modified as follows: nonhematologic toxicity: dose interruption, delay, or discontinuation, depending on toxicity grade; hematologic toxicity: dose delay followed by dose reduction if platelet count and absolute neutrophil count (ANC) did not recover within 14 days; unexplained reductions in serum bicarbonate (<20 mmol/L)—dose reduction by 50% on the next cycle; and unexplained elevations in serum creatinine or blood urea nitrogen ≥2-fold above baseline values and above the ULN, with next cycle delayed until values returned to normal or baseline and dose reduction by 50% on the next treatment cycle.

With durvalumab, modifications to address immune-mediated AEs were based on toxicity grade per National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. For grade 2 or 3 events, the dose was interrupted until toxicity resolved to grade ≤1. Durvalumab was discontinued permanently (1) for recurrence of a prior grade 3 treatment-related AE after resuming treatment, (2) for a grade 4 event or a grade 2 or 3 event with high likelihood of morbidity/mortality, and (3) if the corticosteroid dose could not be reduced to ≤10 mg prednisone per day, or equivalent, within 12 weeks after the final dose. In the event that a participant assigned to the combination treatment arm discontinued treatment with durvalumab or subcutaneous azacitidine because of drug-related toxicity, treatment with single-agent durvalumab or subcutaneous azacitidine may have continued until any discontinuation criterion was met.

Disease status was centrally evaluated at the end of cycles 3 and 6 (before day 1 procedures of cycles 4 and 7) and at the end of every third treatment cycle thereafter. All patients were followed every 3 months until death, loss to follow-up, or withdrawal of consent to further follow-up, for survival and subsequent AML-related therapies.

The study protocol was approved by each study site’s institutional review board/independent ethics committee before commencement (**Supplementary Table**). The sponsor, its authorized representative, and investigators abided by Good Clinical Practice, as described in the International Council for Harmonisation guideline E6 and in accordance with the general ethical principles outlined in the Declaration of Helsinki.

The study was conducted in accordance with applicable national, state, and local laws of the relevant regulatory authorities. All patients or their guardians provided informed consent.

Endpoints and Assessments

Efficacy

The primary endpoint was the overall response rate (ORR), defined as CR or CRi based on modified International Working Group (IWG) 2003 response criteria for AML.[17] Key secondary endpoints were (1) time to response, per IWG 2003 criteria, defined as the time from randomization to first documented response, (2) duration of response, defined as time from response/improvement until relapse or disease progression (IWG 2003 criteria), (3) hematologic improvement (HI) rate, defined as HI-N (HI – neutrophil response) +HI-P (HI – platelet response) +HI-E (HI – erythroid response) (IWG 2003 criteria), (4) complete cytogenetic response, defined as the proportion of patients who achieved complete cytogenetic response (IWG 2003 criteria), (5) relapse-free survival, defined as time from CR, partial remission (PR), or marrow complete remission until first relapse, death from any cause, or loss to follow-up, (6) 1-year survival, and (7) OS, defined as time from randomization to any-cause death.

Safety

Safety was assessed using AEs, laboratory assessments, electrocardiogram, vital signs, and physical examination. These assessments were made at screening, at regular predetermined time points during treatment, at treatment discontinuation, and at days 28 (azacitidine) and 90 (durvalumab) of follow-up. Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 criteria.

Pharmacokinetics

Blood samples for pharmacokinetic (PK) assessment were collected at the end of infusion on day 1 of cycles 1 and 4 (considered peak concentrations) and preinfusion (90 to 5 minutes before dosing) on day 1 of cycles 2, 4, and 6 (considered as trough concentrations in the previous cycles). Serum durvalumab concentrations were measured using a validated electrochemiluminescence method.[18] Pharmacokinetic concentration data and summary statistics were tabulated.

Exploratory Biomarker Analysis

DNA Methylation Analyses

DNA was extracted from PB samples that were collected at cycle 1 day 1 (C1D1; pretreatment) and C2D1 (on-treatment). DNA was quantified with the PicoGreen DNA kit (LabCorp, Burlington, NC). The Infinium MethylationEPIC Array (Illumina, San Diego, CA) was used to determine the global DNA methylation score (GDMS) and focal DNA demethylation in PD-L1 and PD-L2 regulatory regions. Beta-values were calculated by taking the ratio of methyl-probe intensities to all probe intensities. GDMS was calculated by tabulating the percentage of highly methylated loci [beta-value >0.7], with change in GDMS calculated by subtracting GDMS at C2D1 from GDMS at C1D1.

Immunophenotyping and Immune Checkpoint Molecule Expression

PB collected at C1D1 (pretreatment), C1D8, C1D15, C2D1, and C2D15 was analyzed by Q^2 Solutions (Morrisville, NC and Edinburgh, UK). Three separate panels of antibodies were used to measure the abundance of T-cell subsets. Changes in abundance were determined by measuring percent change at each time point to the mean at C1D1.

BM aspirates were collected at screening, C3D22, and C6D22. Processing and flow cytometry was performed at the Munich Leukemia Laboratory (https://www.mll.com/en.html). Flow cytometry antibodies were used to detect granulocytes, lymphocytes, monocytes, T cells, and tumor blasts that were gated on the basis of CD34 and CD117 variant expression. BM cells were also assessed for expression of surface PD-L1 (detected by using clone 29E.2A3), PD-1, and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3). QuantiBRITE beads were used to measure protein expression, which was reported as molecules of equivalent soluble fluorochrome (MESF).

Mutation Profiling

The Munich Leukemia Laboratory used a next-generation targeted sequence assay to assess and characterize gene mutations of samples collected at screening. The 38 genes assessed included those frequently mutated in AML. The mean sequencing coverage across the panel and samples was approximately 3000x. Genetic alterations not matching the reference sequence were classified as mutated, wild-type (or common single nucleotide polymorphism [SNP]), or as a nondetrimental variant. Mutational load and coverage were assessed at each non-reference location.

RNA sequencing

EA Genomics (Q^2 Solutions, Morrisville, NC) used the Qiagen Micro RNeasy kit (Hilden, Germany) to sequence RNA of BM aspirates collected at screening and at C3D22. RNA sequencing was performed on an Illumina HiSeq 2500, with 2 x 50 bp read lengths using TruSeq SBS v4 chemistry (Illumina, San Diego, CA). Strand-specific libraries were prepared by using polyA enrichment and included barcodes.

Alignment was performed using a two-pass mode with STAR (v2.5.2b) on the full hg38 human genome, and gene level counts were obtained using the quantmode GeneCounts option. Gene expression was then normalized with the function “voom” in the R package “limma.” Where gene expression is presented in box plots, boxes represent the mean and SEM, which were calculated by using ggplot after the data were normalized with voom and $\log_2$ transformed.

Statistical Analyses

Assuming a treatment effect of 100% relative improvement of CR and CRi (from 25% to 50% absolute CR and CRi rates), a sample size of 110 patients was needed to provide 80% power to detect such an effect at the 5% level of statistical significance. The primary analysis was conducted after all patients completed 6 cycles and had assessment of their disease.

Study populations including the following: The intent-to-treat (ITT) population was defined as all randomized patients. The efficacy evaluable (EE) population included patients in the ITT population who completed 6 treatment cycles, unless they established an earlier response or discontinued the study due to death or disease progression. The safety population was defined as all patients who received at least 1 dose of any study treatment. The PK population was defined as all patients who received at least 1 dose of study treatment and had at least 1 measurable durvalumab concentration.

Efficacy analyses were performed on the ITT population and supported by analyses conducted in the EE population. Continuous variables were summarized by descriptive statistics (n, mean, SD, Q1 and Q3, median, minimum [min], and maximum [max]). Early safety monitoring meetings were held after 12 patients completed 2 treatment cycles. Additional analyses were conducted approximately 12 months after the last patient was enrolled.

ORR was summarized together with a two-sided 95% confidence interval (CI). Patients who discontinued before treatment cycle 6 without achieving an objective response were counted as nonresponders. The OS curve and OS at 6 and 12 months were estimated with the Kaplan-Meier (KM) method. Patients who were alive at the time of the clinical data cut-off date were censored at the earlier of last assessment at which the patient was known to be alive or the cut-off date. All patients lost to follow-up before the clinical data cut-off date were also censored at the time of last contact. Statistical analyses for the primary and secondary endpoints were conducted with SAS version 9.3 or higher.

RESULTS

Patients and Treatment

The study was conducted from June 3, 2016, to October 31, 2018, at clinical sites in Europe and the United States. A total of 129 patients were randomized, with 64 in Arm A and 65 in Arm B. As of the clinical data cut-off date, 18 patients (8 in Arm A, 10 in Arm B) continued to receive treatment and 111 patients (56 in Arm A, 55 in Arm B) had discontinued. Baseline demographics and disease characteristics were generally balanced across arms. Approximately one-quarter had poor cytogenetic risk (Arm A: 25.0%; Arm B: 24.6%). Tumor mutations in *TP53* were found in 33.0% of patients in Arm A and 26.0% in Arm B (**Table 1**). The median duration of follow-up was 15.7 months. Treatment exposure was similar in both arms. The median number of treatment cycles was 6.5 and 6.7 in Arms A and B, respectively, and more than half of patients completed ≥4 treatment cycles. (**Supplementary Figure S2**).

Efficacy

No statistically significant difference in ORR was observed between treatment arms in the intent-to-treat (ITT) population (Arm A, 31%; Arm B, 35%; *P*=0.6180; **Table 2**). Rates of morphologic complete response (CR) and CR with incomplete blood recovery (CRi) were similar in both arms (CR: Arm A, 17%; Arm B, 22%; CRi: Arm A, 14%; Arm

B, 14%); PR was observed in 6 patients (4 [6%] in Arm A and 2 [3%] in Arm B). The single recipient of a subsequent allogeneic stem cell transplant (Arm B) achieved a CR. Among 43 responders, response was ongoing in 16 (37.2%) patients (**Supplementary Figure S3**). Median duration of response was 24.6 weeks in Arm A and 51.7 weeks in Arm B (*P*=0.0765). The median times to first response and best overall response were similar in both arms. Median OS was also similar, at 13.0 months in Arm A and 14.4 months in Arm B.

In terms of cytogenetic response, both treatments resulted in a similar cytogenetic response. Among evaluable patients, a complete cytogenetic response was identified in 6 of 53 patients in Arm A (11.3%; 95% CI: 2.79, 19.85) and 8 of 50 patients in Arm B (16.0%; 95% CI: 5.84, 26.16). Blast reduction was reported in 34/43 (79%) patients in Arm A and 34/44 (77%) patients in Arm B. Maximal blast reduction for responders is shown in **Figure 1.**

Safety

The overall incidence of AEs was similar in both arms of the study. All patients in Arm A and >98% in Arm B experienced at least 1 treatment-emergent AE (TEAE). In Arm A, AEs were treatment related in 50 (78.1%) patients receiving durvalumab and 56 (87.5%) of those receiving azacitidine; in Arm B, AEs were treatment related in 50 (80.6%) patients. The most frequently reported TEAEs were constipation (Arm A, 57.8%; Arm B, 53.2% of patients) and thrombocytopenia (Arm A, 42.2%; Arm B, 45.2%) (**Supplementary Figure S4**). Anemia occurred in 30% and 31% of patients in Arms A and B, respectively, and neutropenia in 36% and 34%. An AE ≥grade 3 was experienced by 96.9% of patients in Arm A and 88.7% in Arm B, with hematologic toxicities being the most frequently reported grade 3 or 4 TEAEs. Serious AEs (SAEs) were numerically more frequent in Arm A (87.5%) than in Arm B (71.0%); the most frequent SAEs occurring in ≥15% of patients were febrile neutropenia (Arm A, 35.9%; Arm B, 22.6%) and pneumonia (Arm A, 23.4%; Arm B, 12.9%).

Permanent treatment discontinuation due to AEs was reported in 11 (20%) patients in Arm A and 2 (4%) patients in Arm B, and 12/126 (9.5%) patients required azacitidine dose reduction due to AEs (Arm A, 10 patients; Arm B, 2 patients). The most common AEs related to discontinuation were pneumonitis and febrile neutropenia. A total of 18 immune-mediated AEs (imAEs) due to durvalumab were reported, including 5 cases of pneumonitis (1 grade 1; 3 grade 3, 1 grade 4), 2 cases of dermatitis (1 grade 2; 1 grade 3), and 1 case each of neuropathy, synovitis, TH1 and above peripheral sensory neuropathy (all grade 2), enteritis, arthritis, myocarditis, hepatitis, thyroiditis, bullous pemphigoid (all grade 3), colitis, and progressive multifocal leukoencephalopathy (both grade 4). imAEs occurred throughout treatment. Patients who experienced grade 3 and 4 imAEs discontinued durvalumab. One case of progressive multifocal leukoencephalopathy in Arm A was a potentially immune-mediated SAE that had not resolved at the time of treatment discontinuation; the patient subsequently died due to AML progression 6 months later.

There were 35 on-treatment deaths (24 [37.5%] in Arm A and 11 [17.7%] in Arm B), of which 20 were due to AEs (Arm A, 11 [17.2%]; Arm B, 9 [14.5%]). Deaths due to AML, pneumonia, and sepsis occurred more often in Arm A than in Arm B. In Arm A, deaths (all-cause) were due to AML (n=9, 14.1%), pneumonia (n=3, 4.7%), sepsis (n=2, 3.1%), and cardiopulmonary failure, general physical health deterioration, respiratory tract infection, death, septic shock, lactic acidosis, cerebrovascular accident, ischemic stroke, dyspnea, and respiratory failure (n=1 patient each, 1.6%). In Arm B, deaths (all-cause) were due to AML (n=3, 4.8%), and cardiac failure chronic, pneumonia, sepsis, septic shock, cerebral hemorrhage, cerebral infarction, acute kidney injury, and respiratory failure (n=1 patient each, 1.6%).

Pharmacokinetic Analyses

Geometric means (geometric coefficient of variation [CV] %) of peak durvalumab concentrations in cycles 1 and 4 in Arm A were 302 µg/mL (210%, n=59) and 348 µg/mL (71%, n=35), respectively. Geometric means (geometric CV%) of trough durvalumab concentrations in cycles 1, 3, and 5 were 46.0 µg/mL (75%, n=51), 50.8 µg/mL (370%, n=38), and 72.0 µg/mL (422%, n=30), respectively.

Biomarker Analyses

Global DNA methylation was assessed in PB samples following 1 cycle of treatment with or without durvalumab. Results showed a decrease of the GDMS in 50/55 (90.9%) patients with a negligible difference between Arms A and B (**Figure 2**). Mean GDMS in

AML patients was reduced from a mean of 482,375 pretreatment to 463,645 after 1 cycle of azacitidine monotherapy.

Changes in surface PD-L1 expression on BM cells during treatment was evaluated by flow cytometry and presented in **Figure 3**. At baseline, mean PD-L1 surface expression in BM immune cells was highest in monocytes (Arm A: 1397 MESF; Arm B: 1514 MESF), followed in Arm A by tumor blasts (1003 MESF) and granulocytes (645 MESF) and in Arm B by granulocytes (776 MESF) and tumor blasts (585 MESF) (**Figure 3A**). In Arm A, PD-L1 expression on monocytes was noticeably increased at the end of treatment cycle 3 for matched patients, while changes in expression on granulocytes and tumor blasts were minimal (**Figure 3B**). In Arm B, PD-L1 expression was unchanged in monocytes, granulocytes, and myeloid blasts (**Figure 3A, B)**. Changes in surface PD-L2 expression are shown in **Supplementary Figure S5**. A trend toward lower PD-L2 surface expression on myeloid blasts was not observed after treatment for unmatched patients (**Supplementary Figure S5A**) but was observed for matched patients in Arm B only (**Supplementary Figure S5B**).

To evaluate for treatment-mediated changes in BM immune cells, flow cytometry analysis was carried out, and **Supplementary Figure S6** summarizes changes in abundance of lymphocytes, CD3-positive T-cells, and tumor blasts. A reduction in tumor blasts was noted in both groups of unmatched (**Supplementary Figure S6A**) and matched (**Supplementary Figure S6B**) patients after treatment cycle 3, indicating a reduction in tumor burden. Because there was no difference between treatment arms, reduction in tumor cell burden was likely a response to azacitidine alone. There were no clinically meaningful differences between arms in lymphocytes and T-cells (**Supplementary Figure S6A,B**). Analysis of PB CD4-positive (**Supplementary Figure S7A)** and CD8-positive (**Supplementary Figure S7B)** T-cells in Arms A and B showed no differences in abundance from baseline through cycle 2.

RNA sequencing analysis of BM mononuclear cells was used to identify changes in gene expression of immune-related genes. Results showed that expression of T-cell genes, PD-L1, and the interferon-gamma (γ) signature (the mean of *CD274*, *LAG3*, *IFNG*, and *CXCL9*) was increased on treatment compared with baseline (**Figure 4A**), with similar increases in both arms. For example, in patients who provided both a screening and cycle 3 sample, CD3D increased by a mean of 2x in both Arms. Many additional T-cell genes shared this pattern (**Supplementary Figure S8A**). Other genes of interest are tumor marker CD34, which was consistently reduced on treatment, and PNMA Family Member 5 (PNMA5), a cancer testis antigen that was highly upregulated in both arms (**Figure 4A**). Additionally, within both arms, the interferon-γ signature increased on treatment in paired samples as shown by a 90% CI >0 (**Figure 4A**). When patients were stratified by response, the increase was observed only in responders (**Figure 4B,** left and right panels), and the additional T-cell genes had similar patterns (**Supplementary Figure S8B**).

In a mutational analysis of 38 genes using targeted deep sequencing, the probability of overall response in patients with a mutation in a gene was compared to the probability of response in patients with wild-type (or non-detrimental) variants of that gene. This revealed not only that mutations in *TP53* were present in similar proportions of patients in Arms A and B (**Figure 5A**), but also that patients with *TP53* mutations had an overall response rate (35% ORR [80% CI: 25%, 47%]) that was similar to those with wild-type *TP53* (34% ORR [80% CI: 27%,41%]) when patients of Arms A and B were grouped into 2 cohorts based on mutation status (**Figure 5B**). None of the other genes had a significant influence on ORR (**Figure 5B**). When patients with a mutated gene in Arm A were compared with those with mutations of that gene in Arm B, one gene—RUNX family transcription factor 1 (*RUNX1*)— was associated with a significantly decreased probability of ORR, while the probability of ORR with neurofibromin 1 (*NF1)* mutations trended towards significance (**Supplementary Figure S9**).

DISCUSSION

Elderly patients with AML have very poor clinical outcomes with standard treatments. While azacitidine provides a benefit when compared with other chemotherapies, low survival rates and resistance to therapy remain ongoing challenges. We sought to determine if durvalumab-mediated blockade of PD-L1 could synergize with azacitidine to enhance antitumor activity and improve outcomes.

FUSION-AML-001 is the first randomized study to compare the efficacy and safety of the combination of durvalumab with azacitidine versus azacitidine alone as first-line treatment of patients with AML who were 65 years or older, and to explore biomarkers of response to treatment. The results indicate that combination therapy was feasible and the safety profile of azacitidine plus durvalumab compared favorably to that of venetoclax plus azacitidine, decitabine, or low-dose cytarabine, which are combination therapies recently approved for older adults with newly diagnosed AML.[19] The large number of patients with AML studied in this trial enabled the profile of imAEs with azacitidine and durvalumab to be determined. The imAEs observed in the combination treatment arm were expected when considering the safety profile of durvalumab, and all imAEs resolved after treatment.

In terms of efficacy, both overall antitumor response and survival were similar among patients who received durvalumab and azacitidine and those who received azacitidine alone. The addition of durvalumab did not result in a clinically meaningful improvement in ORR, HI, time to first or best response, duration of response, cytogenetic response, or any metrics of survival versus azacitidine monotherapy. Our results can be considered together with those of studies evaluating other combination treatments. In a single-arm trial of combination therapy with azacitidine and nivolumab in 70 patients with relapsed/refractory AML, the ORR was 33%, with 15 (22%) CR/CRis, 1 PR, and 1 HI maintained for >6 months.[15] Median OS was 6.3 months. Pretreatment percentages of BM and PB CD3 and CD8 cells were significant predictors of response.[15]

Our extensive exploratory biomarker analysis has provided insight as to why no difference in efficacy was observed between treatment arms. First, combination treatment in elderly patients with AML promoted global hypomethylation and led to increased PD-L1 surface expression that was restricted to monocytes. These data suggest that differentiated cells in the TME might exert immune suppressive effects. Second, patients in both arms also had an increase in the percentage of lymphocytes, possibly due to improve BM function. Finally, the addition of durvalumab had no apparent effect on reversing T-cell exhaustion, based on a lack of increased PB and BM T-cell abundance. The RNA-sequencing analysis, which showed that T-cell genes, PD-L1, and the interferon-γ signature increased similarly in patients of both arms, was consistent with the observed increase in PD-L1 surface expression and BM immune changes during treatment. A phase 1b/2 study of azacitidine and avelumab in relapsed/refractory AML found that the combination was well tolerated and clinical activity was limited. BM blasts analyzed for immune-related markers by mass cytometry demonstrated significantly higher PD-L2 expression compared with PD-L1 both before and during therapy, with PD-L2 expression increasing on therapy, suggesting that high PD-L2 expression on BM blasts may be a mechanism of resistance to anti–PD-L1 therapy in AML. These results, together with the results from our study, indicate that anti–PD-L1 therapies are unlikely to be clinically beneficial in AML.[20] A randomized phase 2 study to evaluate the efficacy of combining HMAs plus venetoclax with PD-1 blockade in the first-line setting for elderly patients with AML has been initiated (NCT04284787) and should provide additional information about the interaction of epigenetic therapies and the TME.[21]

In conclusion, first-line combination therapy with durvalumab and azacitidine was feasible in older patients with AML but did not provide response or survival advantages over azacitidine monotherapy. The absence of improved response was consistent with the lack of immune changes observed in the combination arm. Our findings are an important contribution to the field as they are derived from a controlled study that included in-depth analyses of tumor mutations, biomarkers, and immune cell populations. Thus, they provide high-level evidence that can be used to inform treatment decisions and maximize the benefits of ICP therapy for older patients with AML who were not eligible for HSCT. While anti–PD-L1 therapy may be of limited value in AML, other ICP blocking agents such as anti–PD-1, CTLA-4, CD47, TIM-3, and other novel agents may have a place in AML therapy based on promising early clinical data. Ongoing clinical trials with these and similar agents should clarify whether there is a role for immune checkpoint blockade in the management of AML.

DATA SHARING STATEMENT

BMS policy on data sharing may be found at https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html.

Data were analyzed by Isaac Boss, Wilbert Copeland, Vanessa Hasle, Ethan Thompson, Brian Fox, Ken Ogasawara, and Alessandro Previtali. All authors had access to primary data of the trial.

ACKNOWLEDGMENTS

The authors would like to thank the patients enrolled in the study and their families as well as associated clinical trial teams.

The authors received editorial support in the preparation of this manuscript from Lauren Gallagher, RPh, PhD, of Peloton Advantage, LLC, an OPEN Health company, Parsippany, NJ, USA, sponsored by Bristol Myers Squibb, Summit, NJ, USA. The authors, however, directed and are fully responsible for all content and editorial decisions for this manuscript.

FUNDING DISCLOSURE

This trial (NCT02775903) was sponsored by Celgene, a Bristol Myers Squibb company, and supported by AstraZeneca/MedImmune.

AUTHORSHIP

Contribution: Isaac Boss contributed the translational plan to the study design; Alessandro Previtali contributed to the study design; Amer M. Zeidan and David C. Taussig were study investigators; Amer M. Zeidan, David C. Taussig, Andrzej Hellmann, Mar Tormo, Maria Teresa Voso, James Cavenagh, and Lewis R. Silverman enrolled patients; Amer M. Zeidan, Isaac Boss, Wilbert B. Copeland, and Brian A. Fox collected and assembled data; Isaac Boss, CL Beach, Wilbert B. Copeland, Vanessa E. Hasle, Ethan Thompson, Brian A. Fox, Alessandro Previtali, and Tim O’Connor analyzed data; all authors interpreted data; Brian Fox and Amer M. Zeidan prepared the manuscript; all authors reviewed and revised the manuscript; all authors approved the final manuscript.

CONFLICT-OF-INTEREST DISCLOSURES

Amer M. Zeidan – is a Leukemia and Lymphoma Society Scholar in Clinical Research and was also supported by a NCI's Cancer Clinical Investigator Team Leadership Award (CCITLA). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. A.M.Z. received research funding (institutional) from Celgene/BMS, AbbVie, Astex, Pfizer, Medimmune/AstraZeneca, Boehringer-Ingelheim, Trovagene/Cardiff oncology, Incyte, Takeda, Novartis, Amgen, Aprea, and ADC Therapeutics. A.M.Z participated in advisory boards, and/or had a consultancy with and received honoraria from AbbVie, Otsuka, Pfizer, Celgene/BMS, Jazz, Incyte, Agios, Boehringer-Ingelheim, Novartis, Acceleron, Astellas, Daiichi Sankyo, Cardinal Health, Taiho, Seattle Genetics, BeyondSpring, Trovagene/Cardiff Oncology, Takeda, Ionis, Amgen, Janssen, Epizyme, Syndax, Gilead, Kura, Aprea, Janssen, and Tyme. A.M.Z served on clinical trial committees for Novartis, AbbVie, Geron, Gilead, Kura, and Celgene/BMS. A.M.Z received travel support for meetings from Pfizer, Novartis, and Cardiff Oncology.

Isaac Boss, CL Beach, **Wilbert B. Copeland, Ethan Thompson, Brian A. Fox, Vanessa E. Hasle, Tim O'Connor, Alessandro Previtali, Shelonitda Rose** – are employees of Celgene, a Bristol Myers Squibb company, and may be shareholders.

David C. Taussig – is supported by the National Institute for Health Research Royal Marsden/Institute of Cancer Research Biomedical Research Centre. Research funding from Celgene.

James Cavenagh – has no potential conflicts of interest.

Lewis R. Silverman – has received research funding from Celgene, Onconova Therapeutics, Forty-seven, Medimmune, Takeda

Maria Teresa Voso – has received research funding from Celgene; speakers bureau for Celgene, Astellas, Jazz, AbbVie

Andrzej Hellmann – has no potential conflicts of interest.

Mar Tormo – reports consultancy for Celgene, Astellas, Novartis; sponsored conferences and meetings with Celgene, Astellas, Amgen, Novartis, Pfizer, and Servier

Correspondence: Amer M. Zeidan, MD, Associate Professor of Internal Medicine (Hematology), Hematology Early Therapeutics Research, Director Section of Hematology, Department of Internal Medicine, Yale University and Yale Cancer Center, 37 College Street, First floor, New Haven, CT 06510, USA; Phone: 203-737-7103; Fax: 203-737-3401; Email: amer.zeidan@yale.edu

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TABLES

Table 1. Baseline Demographics and Disease Characteristics

Parameter	**Arm A** **Azacitidine + Durvalumab** **n=64**	**Arm B** **Azacitidine** **n=65**
Median age (range), y	76.0 (65, 89)	75.0 (65, 87)
Sex, M/F, n	40 / 24	31 / 34
ECOG status 0/1/2, %	29.7 / 62.5 / 7.8	40.0 / 49.2 / 10.8
Median hemoglobin, g/L	88.5	93.0
Median transfusion burden, units/28 d	2.00	2.00
Median platelet count, x 10^9/L	55.5	42.0
Low platelets (<100,000), n (%)	0	0
Median ANC, x 10^9/L	0.595	0.430
Low ANC (<100), n (%)		
Band form	0	0
Segmented	58 (91)	55 (92)
Median time since diagnosis, months	1.00	0.80
Secondary AML, %		
Yes	42.2	41.5
No	56.3	56.9
Cytogenetic risk category per NCCN		

guidelines, %		
Intermediate	39.1	40.0
Poor	25.0	24.6
Missing	35.9	35.4
Median bone marrow blasts, %	34.00	32.00
Bone marrow blast % category, %[a]		
<20	3.1	6.2
20 to <30	37.5	35.4
≥30	57.8	58.5
Main WHO classes, %		
AML with MDS-related changes	39.1	58.5
AML not otherwise specified	39.1	27.7
TP53 mutation status, %[b]		
Wild-type	67	74
Mutated	33	26

[a] Missing category not displayed.

[b] n=105.

AML, acute myeloid leukemia; ANC, absolute neutrophil count; ECOG, Eastern Cooperative Oncology Group; F, female; IPSS-R, Revised International Prognostic Scoring System; M, male; MDS, myelodysplastic syndrome; WHO, World Health Organization.

Table 2. Treatment Response, ITT Population[a]

Response, n (%) [95% CI]	**Arm A Azacitidine + Durvalumab n=64**	**Arm B Azacitidine n=65**
ORR (CR+CRi)	20 (31.3) [19.89, 42.61]	23 (35.4) [23.76, 47.01]
CR	11 (17.2) [7.94, 26.43]	14 (21.5) [11.54, 31.53]
CRi	9 (14.1) [5.55, 22.58]	9 (13.8) [5.45, 22.24]
PR	4 (6.3) [0.32, 12.18]	2 (3.1) [0.0, 7.28]
HI	27 (42.2) [30.09, 54.29]	25 (38.5) [26.63, 50.29]
SD	23 (35.9)	21 (32.3)

[a] Defined as all randomized patients.

CR, complete remission; CRi, CR with incomplete blood recovery; HI, hematologic improvement; ITT, intent to treat; ORR, overall response rate; PR, partial remission

FIGURE LEGENDS

Figure 1. Maximal blast reduction from baseline for responders. The waterfall plot shows the percentage of blast reduction for all responders. Each responder’s blast percentage is illustrated as a bar along the x-axis. The y-axis shows the change from baseline in the percentage of blasts.

Figure 2. Analysis of methylation patterns during treatment. The figure shows changes in GDMS in PB of patients with AML upon treatment with azacitidine or azacitidine + durvalumab. AML, acute myeloid leukemia; GDMS, global DNA methylation score; PB, peripheral blood.

Figure 3. Changes in surface PD-L1 expression on bone marrow cells during treatment. (A) This plot represents the mean (circles) and 90% CIs (error bars) for surface PD-L1 (CD274) abundance (MESF) at screening and C3D22 on three different cell types: granulocytes, monocytes, and tumor blasts. PD-L1 is notably higher at C3D22 compared with screening on monocytes for Arm A patients because the CIs are not overlapping. (B) This plot represents the mean (circles) and 90% CIs (error bars) of the C3D22 minus screening values for patients who had both time points measured. In this plot, we also observed that PD-L1 has an increased density on monocytes for Arm A patients because the CIs do not span the dashed line at zero. However, PD-L1 on tumor cells is not increased beyond the CI in either plot. AML, acute myeloid leukemia; C3D22, cycle 3 day 22; CI, confidence interval; Combo, combination therapy; MESF, molecules of equivalent soluble fluorochrome; Mono, monotherapy; PD-L1, programmed death ligand 1.

Figure 4. RNA-sequencing analyses. (A) Increase or decrease of gene expression from screening to C3D22, split by treatment arm. *CD3D* (T-cell gene), *PDCD1* (PD-1), *CD274* (PD-L1), interferon γ signature (mean of 4 genes [*CD274*, *LAG3*, *IFNG*, *CXCL9*]), *CD34* (gene expressed on tumor cells), and *PNMA5* (cancer testis antigen). The x-axis is the $\log_2$ of the fold change between C3D22 and screening for patients with samples at both time points. (B) Interferon-γ signature at screening and at C3D22 in responders versus nonresponders to treatment with azacitidine and durvalumab (Arm A) or azacitidine monotherapy (Arm B). The plot on the left shows the screening samples and C3D22 samples separately, with lines connecting those which are from the

same patient. The y-axis is the expression score. The right plot shows the fold change for those patients with both time points. The y-axis is the $\log_2$ of the fold change between C3D22 and screening. For all plots in A and B, the line in the middle of the box is the mean, and the edges of the boxes are the 90% CIs. C3D22, cycle 3 day 22; CI, confidence interval.

Figure 5. **Effect of mutation status (wild type versus mutated) on overall response.** (A) The bar graph summarizes the mutation frequency for genes analyzed in this study. The x-axis presents the number of patients with a mutation and the y-axis presents a bar for the genes of interest. Black bars, Arm A; Grey bars; Arm B. (B) The graph summarizes probabilities of overall response among patients stratified by mutation status. The x-axis presents the odds ratio (circles) with 80% confidence intervals (error bars) for overall response and the y-axis presents the genes of interest. Black circles and error bars, wild type gene status; Red circles and error bars, mutated gene status.

Figure 1

Fig 1

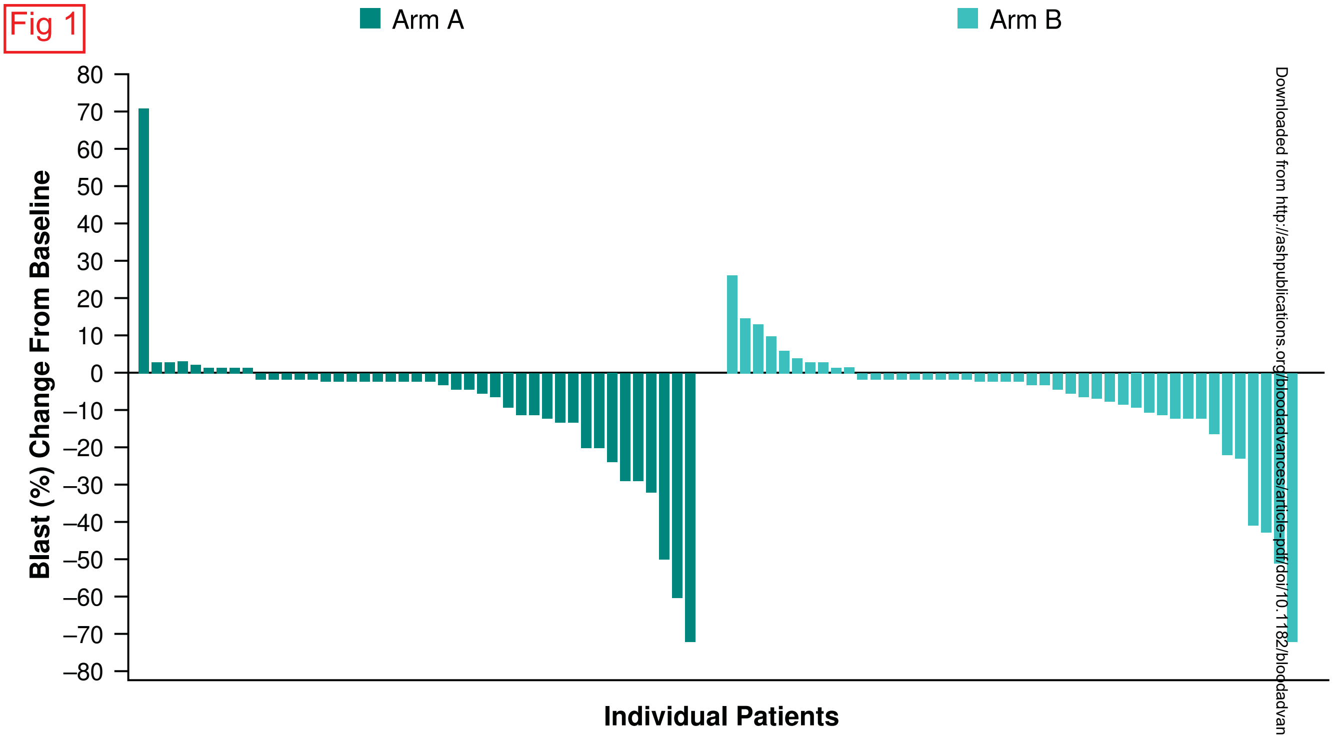

Figure 2

Fig 2

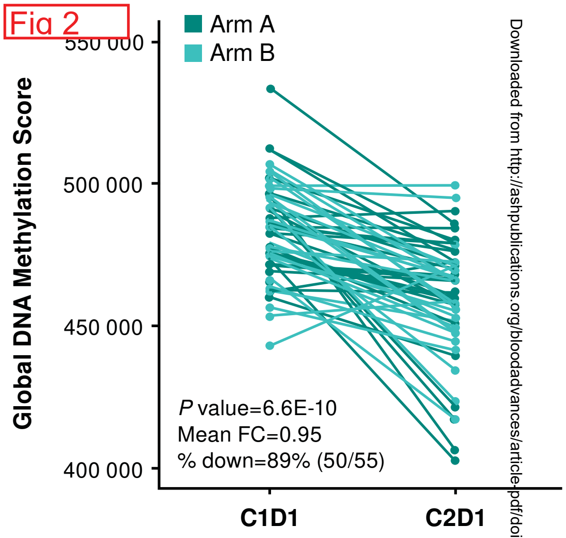

Figure 3

Fig 3

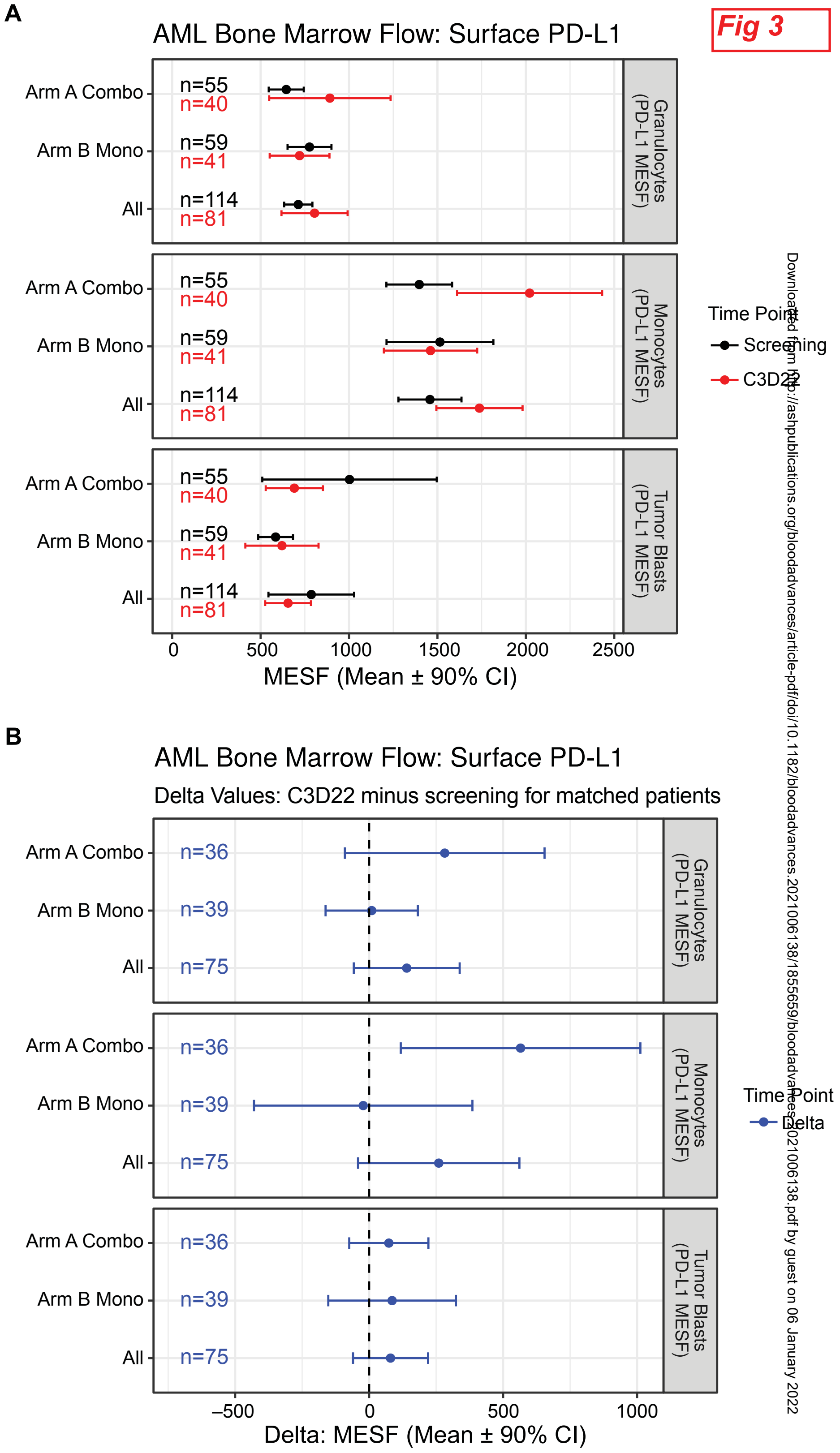

Figure 4

Fig 4

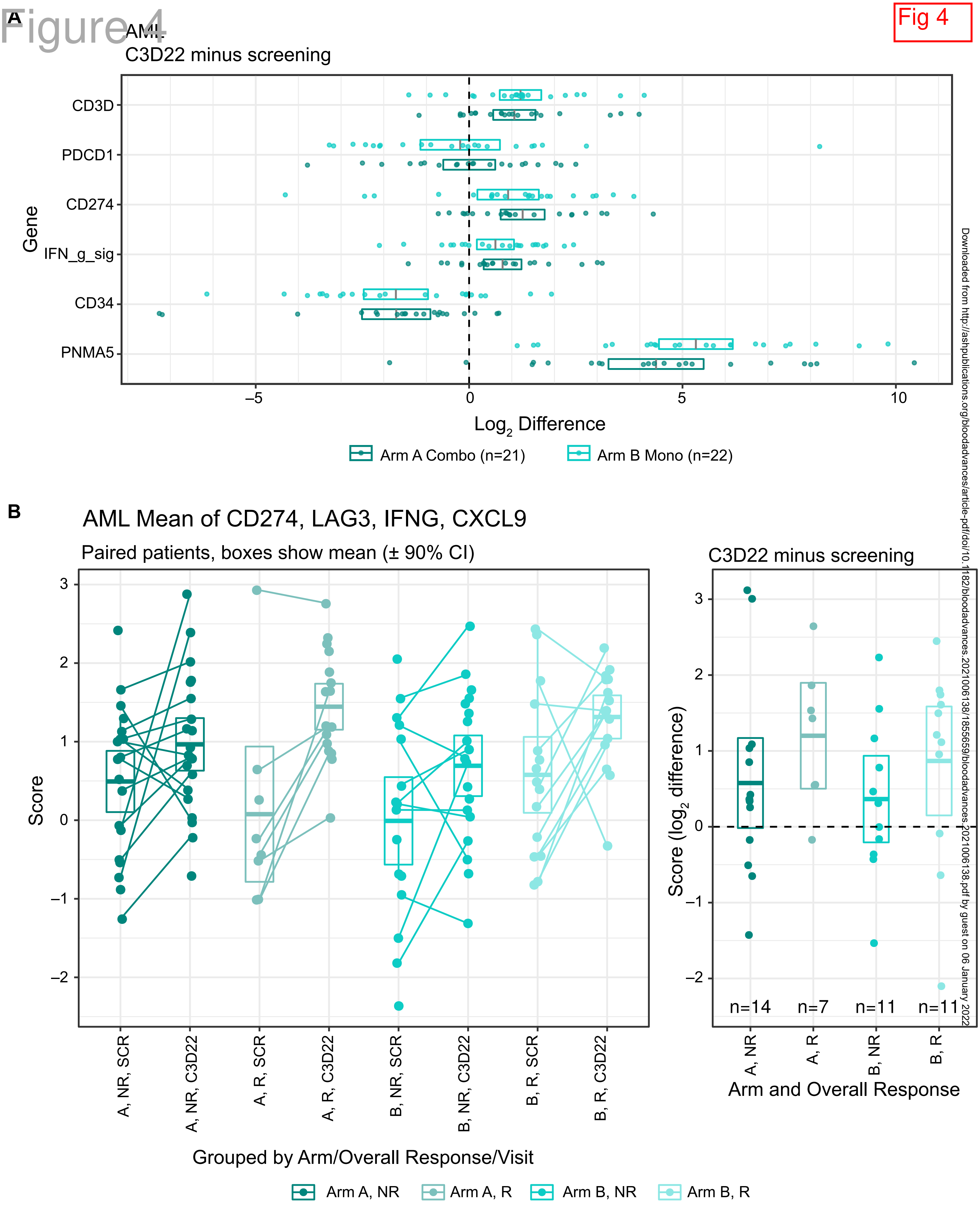

Figure 5

Fig 5