A precision medicine classification for treatment of acute myeloid leukemia in older patients

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Abstract

Background: Older patients (≥ 60 years) with acute myeloid leukemia (AML) often have multiple, sequentially acquired, somatic mutations that drive leukemogenesis and are associated with poor outcome. Beat AML is a Leukemia and Lymphoma Society-sponsored, multicenter umbrella study that algorithmically segregates AML patients based upon cytogenetic and dominant molecular abnormalities (variant allele frequencies (VAF) ≥ 0.2) into different cohorts to select for targeted therapies. During the conception of the Beat AML design, a historical dataset was needed to help in the design of the genomic algorithm for patient assignment and serve as the basis for the statistical design of individual genomic treatment substudies for the Beat AML study.

Methods: We classified 563 newly diagnosed older AML patients treated with standard intensive chemotherapy on trials conducted by Cancer and Leukemia Group B based on the same genomic algorithm and assessed clinical outcomes.

Results: Our classification identified core-binding factor and NPM1-mutated/FLT3-ITD-negative groups as having the best outcomes, with 30-day early death (ED) rates of 0 and 20%, respectively, and median overall survival (OS) of > 1 year and 3-year OS rates of ≥ 20%. All other genomic groups had ED rates of 17–42%, median OS ≤ 1 year and 3-year OS rates of ≤ 15%.

Conclusions: By classifying patients through this genomic algorithm, outcomes were poor and not unexpected from a non-algorithmic, non-dominant VAF approach. The exception is 30-day ED rate typically is not available for intensive induction for individual genomic groups and therefore difficult to compare outcomes with targeted therapeutics. This Alliance data supported the use of this algorithm for patient assignment at the initiation of the Beat AML study. This outcome data was also used for statistical design for Beat AML substudies for individual genomic groups to determine goals for improvement from intensive induction and hopefully lead to more rapid approval of new therapies.

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Background

Acute myeloid leukemia (AML) is not a single entity but a multitude of diseases that differ with regard to pre-treatment genetic features including cytogenetics and gene mutations [1–3]. Despite this disease heterogeneity, initial AML treatment approaches have been essentially the same for the past forty years, with patients either receiving intensive induction approaches (i.e., \(7 + 3\)) or palliative treatment including hypomethylating agents (HMA), subcutaneous cytarabine, supportive care, or hospice care. Over the past few years, numerous new agents have been added to the treatment arsenal of AML, including venetoclax combined with HMA or subcutaneous cytarabine, \(IDH1\), \(IDH2\), and \(FLT3\) inhibitors, liposomal daunorubicin/cytarabine, gemtuzumab ozogamicin, and glasdegib combined with subcutaneous cytarabine [4–13]. In the upfront setting, venetoclax, glasdegib, ivosidenib, and liposomal daunorubicin/cytarabine are approved by the Food and Drug Administration for certain older patient populations or for patients with comorbidities that prevent them from tolerating intensive induction therapy. Although these treatments lead to improved outcomes, including increased complete remission (CR) rates, disease-free survival (DFS), and overall survival (OS) for some AML patient populations, currently none of these therapies are considered curative unless the patients are able to undergo allogeneic stem cell transplantation in initial CR.

It is well known that older AML patients (aged \(\geq 60\) years) have worse outcomes than younger patients, but the reasons for this are not entirely clear. Some contributing factors include higher incidence of high-risk cytogenetic and molecular genetic features, secondary or therapy-related AML, and comorbidities that limit more intensive treatment approaches including allogeneic stem cell transplantation [14]. However, even among patients with favorable-risk features such as core-binding factor (CBF) or NPM1+/FLT3-ITD-mutated AML who are able to tolerate and undergo intensive chemotherapy, older patients have worse outcomes compared with younger patients with these same genetic characteristics [15–17]. Vasu et al. showed that a 10-year DFS rate of older AML patients treated with intensive induction who were not able to receive allogeneic transplantation in first CR was 2.4% [18].

In the era of high throughput sequencing (HTS) and the availability of targeted therapies, the question remains whether an individualized treatment approach based on the results of genetic tests performed at the time of diagnosis could improve the currently poor outcomes of older AML patients. The Leukemia and Lymphoma Society (LLS) has sought to answer this question through the Beat AML Master Study. Gene mutation analysis using HTS, cytogenetic analysis, and polymerase chain reaction (PCR)-based analysis for internal tandem duplication of the \(FLT3\) gene (\(FLT3\)-ITD) are performed at the time of diagnosis in older patients with AML in a comprehensive and timely manner. Patients are then assigned to more individualized therapy based on the presence of cytogenetic and/or mutational drivers detected in the patients’ leukemic clones by inferred variant allele frequency (VAF) [19]. However, to determine whether this approach constitutes improvement upon existing standard of care, it was necessary to have a historical perspective on particular genetic groups of older AML patients and their actual outcomes. This information allowed for study planning relative to a null hypothesis for outcome expectation in specific molecular/cytogenetic groups and provide a reference for regulatory agencies when evaluating new therapies relevant to these groups.

We analyzed data from 563 older newly diagnosed de novo AML patients treated on the Cancer and Leukemia Group B (CALGB, now part of the Alliance for Clinical Trials in Oncology) trials and retrospectively assigned them to several genetic groups based on an algorithm that incorporates treatable cytogenetic abnormalities and mutational drivers with high VAF. We aimed to determine 1) whether this algorithmic approach would lead to a genetic group assignment in the majority of patients and 2) the outcomes of patients assigned to each of the genetic groups to serve as a benchmark and allow comparisons with the results of treatment with new therapeutic agents.

Methods

Patients, treatment, and cytogenetic studies

We analyzed 563 adults \(\geq 60\) years of age with newly diagnosed de novo AML (excluding acute promyelocytic leukemia) whose pretreatment bone marrow (BM) or blood samples underwent HTS analysis [20]. Patients who underwent allogeneic transplantation in first
complete remission (CR) were excluded as per required for the eligibility of the CALGB/Alliance protocols. HTS analysis was not performed in all patients with CBF AML because this subtype of AML represents an already recognized, curable entity and is at the top of the LLS prioritization schema. The patients were treated on CALGB trials which included a range of time from 1984 to 2013 with all receiving standard intensive treatment (Table 1 and further details in the Additional file 1) [21–30]. As patients on the RATIFY study (CALBG 10603) were eligible only from ages 18 to 59, there are not any patients included in our analysis who received midostaurin as part of their chemotherapy regimen. All patients were considered for outcome analyses including those who suffered early death (ED), defined as death within 30 days after starting therapy irrespective of cause. Cytogenetic analyses of pretreatment BM and/or blood samples were performed by institutional CALGB/Alliance-approved laboratories. The results were confirmed by central karyotype review [31]. Patients provided written informed consent to participate in companion protocols CALGB 8461 (cytogenetic studies), CALGB 9665 (leukemia tissue bank), and CALGB 20,202 (molecular studies), which involved collection of pretreatment BM and blood samples. Treatment protocols were in accordance with the Declaration of Helsinki and approved by the institutional review boards at each center, and all patients provided written informed consent.

Molecular analyses
Mononuclear cells were enriched through Ficoll-Hypaque gradient centrifugation and cryopreserved until use. Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). The mutational status of 81 protein-coding genes was determined centrally at The Ohio State University by targeted amplicon sequencing using two different gene panels on the MiSeq platform (Illumina, San Diego, CA; see Additional file 1 for details). MuCor was used for integrative data analysis [32]. Details about the variant calling are outlined in the Additional file 1. In addition to the 81 genes assessed by HTS, testing for CEBPA mutations was performed as previously described, thus resulting in mutational status of 82 genes being assessed in our study [33]. Only patients with biallelic CEBPA mutations were considered as mutated [2]. The presence or absence of FLT3-ITD, as well as quantification of the FLT3-ITD to FLT3 wild-type allelic ratio, was determined as previously described [34].

Genetic algorithm/assignment
This precision medicine-based stratification of AML patients was initially designed in 2015 and took into consideration “assignment to curative therapy with 7 and 3” for known responsive groups [i.e., CBF AML and NPM1-mutated/FLT3-ITD-negative (NPM1m/FLT3-ITD–) patients] followed by genetic groups where high rationale therapeutic options were or soon would be available (KMT2A-rearranged, IDH2m, IDH1m). These groups were first formed by high-risk genetic/cytogenetic groups which could confound prognostic impact of other gene mutations and typically lack other common co-mutations (TP53m and complex karyotype with wild-type TP53), next followed by FLT3-mutated [including both FLT3-ITD and mutations in the tyrosine kinase domain of the FLT3 gene (FLT3-TKD)], then followed by the hypermethylation group [encompassing patients with TET2m [35, 36] or WT1m [37]], and then the marker-negative group. The following priority schema (in order from highest to lowest) was used for the treatment assignment algorithm: CBF AML (CBF); NPM1m/FLT3-ITD–; 11q23/KMT2A-rearranged (KMT2A); IDH2 mutated (IDH2m); IDH1 mutated (IDH1m); TP53 mutated (TP53m); complex karyotype/TP53 wild-type (complex karyotype/TP53wt); FLT3-ITD (both high and low allelic ratios included) or FLT3-TKD (FLT3m); WT1 mutated or TET2 mutated (WT1m or TET2m); and marker-negative group (i.e., all other karyotypes and mutations that did not occur as co-mutations and were not included in the aforementioned grouping) (Fig. 1). The presence of a clonal cytogenetic aberration, FLT3-ITD allelic ratio of ≥0.05 and VAF ≥0.3 was assessed initially for treatment assignment. Patients were assigned in dominant clone fashion if clones harboring mutations with VAF ≥0.3 determined by HTS, FLT3-ITD allelic ratio of ≥0.05 or particular cytogenetic abnormalities were identified. For patients not assigned to any genetic group during the initial stratification, a second run-through of the algorithm was performed searching for a clone with mutations with VAF ≥0.2 excluding FLT3-ITD. As this algorithm is designed to assign therapy and assess outcome on an intent-to-treat basis, patients who suffered ED were included.

Statistical analyses
We made comparisons among groups regarding baseline characteristics, co-occurring mutations, and outcomes using Fisher’s exact test for categorical variables, the Kruskal–Wallis test for continuous variables, and the Kaplan–Meier method and log rank test for survival endpoints [38]. Data collection and statistical analyses were performed by the Alliance Statistics and Data Center using SAS 9.4 and TIBCO Spotfire S+8.2 with a dataset locked on September 12, 2019, and median follow-up of 8.6 years. Clinical endpoints are defined in the Additional file 1.
| Protocol | Induction | Maintenance |
|----------|-----------|-------------|
| 8321 \(n=3\) | Randomized to: | n/a |
|  | *DNR Days 1–3 (45 mg/m²/day < 60 years or 30 mg/m²/day ≥ 60 years) | |
|  | Ara-C Days 1–7 (100 mg/m²/day) | |
|  | **VERSUS** | |
|  | *DNR Days 1–3 (45 mg/m²/day < 60 years or 30 mg/m²/day ≥ 60 years) | |
|  | Ara-C Days 1–7 (200 mg/m²/day CIV) | |
|  | 6-TG Days 1–10 (100 mg/m²/day q12 hour x 10 doses) | |
|  | DNR Day 57 (45 mg/m²/day < 60 years or 30 mg/m²/day ≥ 60 years) | |
|  | Ara-C Days 1–10, 29–38, 57–66, 84–92 (per Induction assignment BID q12 hours x 10 doses) | |
|  | VCR Days 29 and 84 (2 mg/m² (max 2 mg)) | |
|  | Prednisone Days 29–33, 84–88 (40 mg/m²/day x 5 days) | |
| 8525 \(n=41\) | Randomized to 4 Cycles: | |
|  | *DNR Days 1–3 (45 mg/m²/day < 60 years or 30 mg/m²/day ≥ 60 years) | |
|  | Ara-C Days 1–5 (100 mg/m²/day CIV) | |
|  | **VERSUS** | |
|  | Days 1–5 (400 mg/m²/day CIV) | |
|  | **VERSUS** | |
|  | Days 1,3,5 (3gm/m² every 12 h for 6 total doses) | |
| 8721 \(n=1\) | 2 Cycles: | n/a |
|  | Ara-C Days 1,2,8,9 (3gm/m² every 12 h – 8 doses total) | |
|  | L-asparaginase Days 2, 9 (6000 IU/m²) | |
|  | Allowed to repeat on Days 15,16 for both agents if no response | |
| 8821 \(n=1\) | Randomized to Course 1 followed by Course 2 VERSUS Course 2 followed by Course 1: | n/a |
|  | *DNR Days 1–3 (45 mg/m²/day) | |
|  | Ara-C Days 1–7 (200 mg/m²/day CIV) | |
|  | Course 1: (1 cycle) | |
|  | Mitoxantrone Days 1–3 (12 mg/m²/day) | |
|  | Diaziquone Days 1–5 (28 mg/m²/day CIV) | |
|  | Course 2: (1 cycle) | |
|  | Etoposide Day 1 (2 mg/m² (max 2 mg)) | |
|  | Cytoxan Days 3–6 (50 mg/kg/day) | |
| 8923 \(n=38\) | Randomized to: | |
|  | *DNR Days 1–3 (45 mg/m²/day) | |
|  | Ara-C Days 1–7 (200 mg/m²/day CIV) | |
|  | G-CSF Starting Day 8 | |
|  | **VERSUS** | |
|  | *DNR Days 1–3 (45 mg/m²/day) | |
|  | Ara-C Days 1–7 (200 mg/m²/day CIV) | |
|  | Placebo Starting Day 8 | |
|  | Course 1: (up to 4 cycles) | |
|  | Ara-C Days 1–5 (100 mg/m²/day CIV) | |
|  | Course 2: (up to 2 cycles) | |
|  | Ara-C Days 1–3 (500 mg/m² q 12 h x 6 doses) | |
|  | Mitoxantrone Days 1–3 (5 mg/m² x 6 doses) | |
| 9420 \(n=18\) | Randomized to: | |
|  | *DNR Days 1–3 (dose-escalated to MTD 40 mg/m²/day) | |
|  | Etoposide Days 1–3 (dose-escalated to MTD 60 mg/m²/day) | |
|  | Ara-C Days 1–7 (100 mg/m²/day CIV) | |
|  | PSC-833 1.5gm/kg IV Days 1–3 for 2 h, followed by 10 mg/kg/day CIV for 72 h | |
|  | **VERSUS** | |
|  | *DNR Days 1–3 (dose-escalated to MTD 40 mg/m²/day) | |
|  | Etoposide Days 1–3 (dose-escalated to MTD 60 mg/m²/day) | |
|  | Ara-C Days 1–7 (100 mg/m²/day CIV) | |
|  | No PSC-833 | |
|  | Randomized to 1 Cycle: | |
|  | DNR Days 1–2 (30 mg/m²/day) | |
|  | Etoposide Days 1–2 (60 mg/m³/day) | |
|  | Ara-C Days 1–5 (100 mg/m²/day CIV) | |
|  | PSC-833 1.5gm/kg IV Days 1–3 for 2 h, followed by 10 mg/kg/day CIV for 72 h | |
|  | **VERSUS** | |
|  | DNR Days 1–2 (30 mg/m²/day) | |
|  | Etoposide Days 1–2 (60 mg/m³/day) | |
|  | Ara-C Days 1–5 (100 mg/m²/day CIV) | |
|  | No PSC-833 (based on initial induction assignment) | |
|  | Randomized to: | |
|  | R-IL2 (0.9 × 10⁶ SQ Days 1–14, 19–28, 33–42, 47–56, 61–70, 75–90 and 12 × 10⁶ Day 15–17,29–31, 43–45, 57–59, 71–73) | |
|  | No maintenance | |
Table 1 (continued)

| Protocol | Induction | Maintenance |
|----------|-----------|-------------|
| 9720 (<n = 233) | Randomized to: | Randomized to 1 Cycle: | Randomized to: |
| | *DNR Days 1–3 (40 mg/m²/day) | DNR Days 1–2 (30 mg/m²/day) | R-IL2 (0.9 × 10⁶ SQ Days 1–14, 19–28, 33–42, 47–56, 61–70, 75–90 and 12 × 10⁶ Day 15–17, 29–31, 43–45, 57–59, 71–73) |
| | Ara-C Days 1–7 (100 mg/m²/day CV) | Etoposide Days 1–2 (60 mg/m²/day) | VERSUS |
| | PSC-833 1.5 mg/kg IV Days 1–3 for 2 h, followed by 10 mg/kg/day CV for 72 h | PSC-833 1–3 (1.5 mg/kg IV for 2 h, followed by 10 mg/kg/day CV for 72 h) | VERSUS |
| | | VERSUS |
| 10201 (<n = 168) | Randomized to: | Randomized to 2 Cycles: | Randomized to: |
| | *DNR Days 4–6 (60 mg/m²/day) | Ara-C Days 4–8 (2000 mg/m²/day) | n/a |
| | Ara-C Days 4–10 (100 mg/m²/day) | n/a |
| | Oblimersen Days 1–10 (7 mg/kg/day CV) | No Oblimersen |
| 10502 (<n = 35) | Randomized to: | 2 Cycles: | n/a |
| | *DNR Days 1–3 (60 mg/m²/day) | Ara-C Days 1–5 (2 gm/m²/day) | Ara-C Days 4–8 (2000 mg/m²/day) |
| | Ara-C Days 1–7 (100 mg/m²/day) | Bortezomib per dose escalation Days 1,4,8,11 | n/a |
| | | VERSUS |
| 10801 (<n = 13) | Randomized to: | 4 cycles: | Dasatinib 100 mg PO daily up to 12 months |
| | *DNR Days 1–3 (60 mg/m²/day) | Ara-C Days 1–7 (2 gm/m²/day) | Ara-C Days 1–5 (2 gm/m²/day) |
| | Ara-C Days 1–7 (200 mg/m²/day) | Ara-C Days 1,3,5 (3 gm/m² q12 hours ≤ 60 years and 1 gm/m² q12 hours ≥ 60 years) | Ara-C Days 4–8 (2000 mg/m²/day) |
| | Dasatinib Days 8–21 (100 mg PO Daily) | Dasatinib Days 1–26 (100 mg PO daily) | Dasatinib Days 4–8 (2000 mg/m²/day) |
| 11001 (<n = 11) | Randomized to: | 2 Cycles: | Sorafenib 400 mg PO BI Days 1–28 up to 12 cycles |
| | *DNR Days 1–3 (60 mg/m²/day) | Ara-C Days 1–7 (100 mg/m²/day) | Ara-C Days 1–5 (2 gm/m²/day) |
| | Ara-C Days 1–7 (100 mg/m²/day) | Ara-C Days 4–8 (2000 mg/m²/day) | Sorafenib Days 1–28 (400 mg PO BID) |

Ara-C, cytarabine; BID, twice daily; CV, continuous intravenous infusion; DNR, daunorubicin; gm, gram; h, hour; IU, international units; kg, kilogram; m, meter; mg, milligram; MTD, maximum tolerated dose; n, number; n/a, not applicable; PO, orally; q12, every 12; SQ, subcutaneous; VCR, vincristine; 6-TG, 6-Thioguanine

1Reduction therapy allowed

Results

Patient genetic group assignment

We sought to establish the outcomes of patients treated on CALGB trials who were retrospectively assigned to specific genetic groups to serve as a historical control for comparison with future results of the Beat AML trial. Using the algorithm, 498 (88%) patients were assigned to a genetic group based upon cytogenetic findings or the presence of a dominant mutational clone with VAF ≥ 0.3. This number increased to 508 (90%) when an additional 10 (2%) patients were reclassified following detection of a clone with a mutation with VAF ≥ 0.2. There were 75 (13%) patients assigned to the CBF group, 107 (19%) to the NPM1/m/FLT3-ITD group, 13 (2%) to the KMT2A group, 59 (10%) to the IDH2m group, 35 (6%) to the IDH1m group, 50 (9%) to the TP53m group, 28 (5%) to the complex karyotype/TP53wt group, 99 (18%) to the FLT3m group, and 42 (7%) to the TET2m or WT1m group. The remaining 56 (10%) patients were assigned to the marker-negative group (Table 2).

Clinical, cytogenetic and molecular genetic characteristics of patients classified into genetic groups

Baseline clinical characteristics among groups were similar with the following exceptions: (1) CBF and NPM1/m/FLT3-ITD—patients had an almost equal male-to-female ratio whereas other groups had predominance of male patients; (2) platelet counts were highest in the IDH1m group; (3) the white blood cell counts were highest in the FLT3m, KMT2A and NPM1/m/FLT3-ITD—groups; and (4) percentage of BM blasts were highest in the KMT2A, IDH1m, and the FLT3m groups (Table 3).

We next analyzed occurrence of mutations belonging to the previously reported functional groups [39] within each of the genetic groups identified in the current study (Additional file 1: Table S1). We found that NPM1/m/FLT3-ITD-patients had most often gene mutations in the methylation-related (87%), RAS pathway (47%), and spliceosome (28%) functional groups. In the KMT2A group, gene mutations were infrequent as previously reported [40] and the mutations occurring most frequently were those in genes belonging to
Genetic group assignment algorithm. Patients were assigned to a genetic group with initial run-through of the algorithm based on cytogenetic features or molecular mutational clones with VAF $\geq 0.3$. For patients not assigned to any genetic group during the initial stratification, a second run-through of the algorithm was performed with assignment after assessing for a mutational clone with VAF $\geq 0.2$.

CBF, Core-Binding Factor; Complex, Complex karyotype

The methylation-related (23%) functional group. Both IDH2m and IDH1m frequently had mutations in genes from spliceosome (57 and 34%, respectively), chromatin remodeling (29 and 31%), kinases (25 and 46%), and transcription factors (29 and 21%) functional groups. The most common co-mutations in TP53m patients were in genes from the methylation-related functional group (24%). In the complex karyotype/TP53wt genetic group, most often mutated were genes from spliceosome (36%) and methylation-related (32%) functional groups. The FLT3m genetic group had high frequency of methylation-related (59%), NPM1 (55%), transcription factors (29%) and spliceosome (26%) mutations. Patients in the TET2m or WT1m and the marker-negative groups had high frequency of mutations in genes belonging to spliceosome (61 and 45%), transcription factors (36 and 64%), chromatin remodeling (50 and 46%), and RAS pathway (24 and 36%) functional groups (Additional file 1: Table S1).

Frequencies of individual gene mutations in patients assigned to the genetic groups are provided in Additional file 1: Table S2. For better visualization of the mutational spectrum of each of the genetic patient groups, we created an oncoprint depicting gene mutations found in each genetic group (Fig. 2). Of note, the NPM1m/FLT3-ITD– genetic group had concurrently occurring mutations in the TET2 (33%), IDH2 (21%), and IDH1 (21%), TP53 (1%) genes, and FLT3-TKD (13%). Patients in the IDH2m group did not harbor a concurrent IDH1 mutation nor did patients in the IDH1m group harbor simultaneously an IDH2 mutation. The complex karyotype/TP53wt genetic group did include a low frequency of TP53 mutations ($n=2$), and in both patients the VAF of these mutations was $<0.2$. The FLT3m genetic group had high frequency of NPM1 (55%), DNMT3A (40%) and TET2 (25%) mutations. The marker-negative genetic group included a relatively high frequency of RUNX1 (43%), ASXL1 (25%), NRAS (21%), and U2AF1 (20%) mutations.

Treatment outcome based on patient genetic groups
ED occurred in 20% of all patients, most commonly in the TP53m (42%), KMT2A (23%), IDH1m (23%), and FLT3m (23%) groups. All other groups had ED rates between 17 and 20%, except for the CBF group, which had no ED
The CR rates were above 50% in the two favorable risk groups: CBF (73%), and NPM1/m/FLT3-ITD− (68%) and 62% in the intermediate risk group KMT2A. However, in the other groups the CR rates ranged between 32 and 47%, except for TP53m group, in which CR rate was much lower at 16%. These CR rates were not affected by selection bias for patients surviving early AML treatment complications or progression and thus provide a historical control for new therapies in specific molecular/cytogenetic groups defined herein.

Concerning long-term outcomes, the median DFS was less than a year for all patients except for those in the NPM1/m/FLT3-ITD− group, for whom median DFS was exactly 1 year. The 3-year DFS rates were less than 15% for all genetic groups but the two favorable groups of CBF (30%) and NPM1/m/FLT3-ITD− (27%) that included some patients with long-term benefit from standard treatment (Table 4 and Fig. 3). Median OS was less than 1 year in all groups other than the two favorable risk ones: CBF with median OS of 1.5 years and NPM1/m/FLT3-ITD− with median OS of 1.3 years. The 3-year OS rates were less than 10% in all groups except for CBF (33%), NPM1/m/FLT3-ITD− (27%), and IDH2m (15%).

Discussion
Our analysis demonstrates the outcomes of classifying older AML patients based on a precision-based medicine assignment of the LLS Beat AML Master Study using both targetable cytogenetic abnormalities and gene mutations found in dominant mutational clones, defined as those having VAF ≥ 0.3, or VAF ≥ 0.2 (in cases with no selected mutations with VAF ≥ 0.3), or FLT3-ITD allelic ratio of ≥ 0.05. The majority of patients (90%) were assigned to a genetic group as a result of the run-through of the algorithm based initially on either cytogenetic findings, FLT3-ITD allelic ratio of ≥ 0.05 or detection of gene mutations with a VAF ≥ 0.3, followed by a second run-through of VAF ≥ 0.2. Only 10% of patients were assigned to the marker-negative group that included patients with mutations in the spliceosome (mostly SRSF2 and U2AF1), RUNX1, ASXL1, and NRAS genes (though the other treatment assignment genomic groups could include these mutations as well). These mutations are currently not targetable with any available therapeutic. However, as we have gained more knowledge since the original design of this algorithm, including potential mutations that lead to resistance in certain targeted therapeutics and new available therapeutics as aforementioned, other mutational genomic subgroups such as RAS mutated patients are being added to the algorithm and the reordering of the algorithm genomic subgroups (such as FLT3 mutations being higher up in the stratification) are occurring. Despite this, application of this dataset did not reveal any concerns in regard to our algorithm with inappropriate genetic assignments that would preclude patients from receiving curative therapy. The outcomes of this approach prompted inclusion of this assignment

Table 2  Retrospective assignment of 589 patients receiving standard therapy on CALGB/Alliance trials to Beat AML genetic treatment groups

| Assignment                      | Performed concurrently | Final assignment |
|---------------------------------|------------------------|------------------|
|                                 | Initial assignment     | Initial assignment | Reassignment | Total number of patients n (%) |
| Step 1                          | Step 2                 | Step 3           |             |                                   |
| Cytogenetics                    | VAF ≥ 0.3              | VAF ≥ 0.2         |             | n (%)                              |
| Core-binding factor             | 74                     | –                | –            | 74 (13)                             |
| NPM1/m/FLT3-ITD−                | –                      | 106              | 1            | 107 (19)                            |
| KMT2A                           | 13                     | –                | –            | 13 (2)                              |
| IDH2m                           | –                      | 56               | 3            | 59 (10)                             |
| IDH1m                           | –                      | 33               | 2            | 35 (6)                              |
| TP53m                           | –                      | 50               | –            | 50 (9)                              |
| Complex karyotype/TP53wt        | 28                     | –                | –            | 28 (5)                              |
| FLT3m                           | –                      | 96               | 3            | 99 (18)                             |
| TET2m or WT1m                   | –                      | 41               | 1            | 42 (7)                              |
| Marker-negative                  | –                      | 66               | – 10         | 56 (10)                             |
| Total number of assigned patients per column | 115 | 448 | 10 | 563 |

m, mutated; n, number; VAF, variant allele frequency; wt, wild-type
Table 3  Comparison of pretreatment clinical characteristics of older patients with acute myeloid leukemia assigned to the genetic groups

| Characteristic                          | CBF   | NPM1m/FLT3-ITD | KMT2A | IDH2m | IDH1m | TP53m | Complex karyotype/TP53wt | FLT3m | TET2m or WT1m | Marker-negative |
|----------------------------------------|-------|----------------|-------|-------|-------|-------|--------------------------|-------|---------------|-----------------|
|                                        | n=74  | n=107          | n=13  | n=59  | n=35  | n=50  | n=28                     | n=99  | n=42          | n=56            |
| Age, years                             |       |                |       |       |       |       |                          |       |               |                 |
| Median                                 | 66    | 68             | 69    | 71    | 71    | 70    | 69                       | 70    | 71            | 69              |
| Range                                  | (60, 78) | (60, 84)        | (60, 77) | (60, 86) | (60, 85) | (60, 84) | (61, 78)                   | (60, 89) | (62, 85) | (60, 86)         |
| Sex, n (%)                             |       |                |       |       |       |       |                          |       |               |                 |
| Male                                   | 37 (50) | 54 (50)         | 7 (54) | 36 (61) | 19 (54) | 29 (58) | 20 (71)                   | 55 (56) | 26 (62) | 35 (63)         |
| Female                                 | 37 (50) | 53 (50)         | 6 (46) | 23 (39) | 16 (46) | 21 (42) | 8 (29)                    | 44 (44) | 16 (38) | 21 (38)         |
| Race, n (%)                            |       |                |       |       |       |       |                          |       |               |                 |
| White                                  | 61 (86) | 98 (82)         | 11 (85) | 55 (95) | 31 (91) | 42 (86) | 23 (85)                   | 90 (94) | 38 (90) | 51 (93)         |
| Non-white                              | 10 (14) | 8 (8)           | 2 (15) | 3 (5)  | 3 (9)  | 7 (14) | 4 (15)                    | 6 (6)  | 4 (10) | 4 (7)           |
| Hemoglobin, g/dL                       |       |                |       |       |       |       |                          |       |               |                 |
| Median                                 | 8.8   | 9.4            | 8.5   | 9.3   | 9.4   | 9.2   | 9.4                      | 9.5   | 9.1           | 9.1             |
| Range                                  | (4.8, 12.4) | (5.3, 14.1)       | (5.7, 11.5)  | (5.2, 13.9) | (6.5, 13.8) | (6.8, 11.5) | (6.0, 14.7)                | (4.3, 15.0) | (3.0, 11.9) | (6.2, 12.7) |
| Platelet count, × 10^9/L               |       |                |       |       |       |       |                          |       |               |                 |
| Median                                 | 42    | 75             | 45    | 68    | 103   | 48    | 53                       | 41    | 56            |                 |
| Range                                  | (7, 237) | (6, 507)        | (8, 242)  | (5.673) | (5, 850) | (9, 224) | (4.426)                   | (9, 387) | (7, 510) | (7, 281)        |
| WBC count, × 10^9/L                    |       |                |       |       |       |       |                          |       |               |                 |
| Median                                 | 15.2  | 29.3           | 41.6  | 13.4  | 11.8  | 7.8   | 18.4                     | 52.4  | 23.5          | 145             |
| Range                                  | (14.2, 25.2) | (0.6, 30.85)      | (22.2, 172.9) | (0.6, 4341) | (0.7, 248.0) | (0.4, 1180) | (0.6, 1161)                | (0.8, 450.0) | (2.2, 2295) | (1.1, 1100)     |
| % Blood Blasts                         |       |                |       |       |       |       |                          |       |               |                 |
| Median                                 | 40    | 40             | 71    | 46    | 55    | 27    | 34                       | 69    | 57            | 33              |
| Range                                  | (0.96) | (0.97)         | (1.99) | (0.97) | (0.99) | (0.88) | (0.94)                   | (0.97) | (0.99) | (0.86)         |
| % Bone marrow blasts                   |       |                |       |       |       |       |                          |       |               |                 |
| Median                                 | 56    | 70             | 85    | 70    | 81    | 47    | 50                       | 80    | 62            | 60              |
| Range                                  | (10, 90) | (0.95)         | (46, 97) | (21, 99) | (34, 93) | (31, 90) | (16, 88)                   | (6.99) | (26, 96) | (17, 94)        |
| Extramedullary involvement, n (%)      | 14 (23) | 28 (28)        | 2 (15) | 15 (28) | 4 (12) | 7 (16) | 6 (24)                   | 28 (30) | 9 (22) | 7 (13)         |
| ECOG performance status, n (%)         |       |                |       |       |       |       |                          |       |               |                 |
| Grade 0                                | 17 (30) | 25 (26)        | 2 (17) | 13 (23) | 12 (40) | 9 (20) | 6 (26)                   | 18 (20) | 13 (33) | 18 (38)        |
| Grade 1                                | 25 (44) | 44 (45)        | 5 (42) | 27 (48) | 11 (37) | 21 (47) | 14 (61)                   | 40 (45) | 17 (43) | 21 (44)        |
| Grade 2                                | 11 (19) | 26 (27)        | 4 (33) | 11 (20) | 6 (20) | 9 (20) | 3 (13)                   | 25 (28) | 7 (18)  | 8 (17)         |
| Grade 3                                | 4 (7)  | 3 (3)          | 1 (8)  | 2 (4)  | 1 (3)  | 5 (11) | 6 (7)                     | 3 (8)  | 0 (0)     |                 |
| Grade 4                                | 0 (0)  | 0 (0)          | 0 (0)  | 3 (5)  | 0 (0)  | 1 (2)  | 0 (0)                     | 0 (0)  | 0 (0)     | 1 (2)           |

CBF, core-binding factor; dL, deciliter; ELN, European LeukemiaNet; g, gram; L, liter; m, mutated; n, number; WBC, white blood cell; wt, wild-type
With regard to outcomes of our patients treated with standard therapies who were assigned to genetic groups, the findings were similar to what has been reported in older AML patients. Patients in the more favorable genetic groups of CBF and NPM1/FLT3-ITD fared best, but overall the patients had poor long-term outcomes with standard treatment approaches [41, 42]. Although we are making progress with the addition of such agents as midostaurin [9], venetoclax [4], glasdegib [13, 43], and other newly approved targeted therapies to achieve short-term goals of improved CR rates, DFS and OS, these therapies remain non-curative and need to be built and improved upon further.

It is also of importance, with new treatment approaches becoming available, to understand how best to decide among multiple potential therapeutic options, both from a clinician and patient perspective. Data presented herein have served as the historical control for individual Beat AML genomic substudies statistical design at the initiation of this study and have served as benchmarks for clinical outcome improvement for particular AML genomic patient groups. This has allowed determination of clinical progress made in older AML patients and in specific genetic groups to advance novel therapies and aid in better selection of treatment. The inclusion of ED patients, who have typically been excluded from other prognostic studies, is important because this helps in assessing true outcomes of patients assigned to specific genetic groups. This information is also of value in determining if newer therapeutic options are superior to standard treatment and associated toxicities. Rates of ED observed in specific genetic groups could aid in making treatment decisions for patients that potentially influence quality of life, especially when deciding between therapies with non-curative intent. In this regard, it is notable that a very high-risk TP53m group had a 30-day ED rate of 41% that corresponds to the low induction success rate of 17%. These data clearly identify a distinct genetic group for which standard of care induction with 7+3 chemotherapy lacks therapeutic benefit. Notably, all other groups outside of CBF AML had an ED rate of 17% or more indicating that in the historical setting, 7+3 chemotherapy treatment and potential increased risk of infections and other complications arising from other comorbid illnesses brings early risk to elderly patients. Adaptation of functional assessment models [44, 45] or other pretreatment models [46, 47] to identify patients at risk for ED in choosing chemotherapy approaches is needed. Clinical trials in elderly AML have focused predominantly on OS as the primary endpoint for analysis,
Table 4  Outcomes of older patients with acute myeloid leukemia assigned to the genetic groups

| Outcome               | CBF n = 74 | NPM1m/FLT3-ITD- n = 107 | KMT2A n = 13 | IDH2m n = 59 | IDH1m n = 35 | TP53m n = 50 | Complex karyotype/ TP53 wt n = 28 | FLT3m n = 99 | TET2m or WT1m n = 42 | Marker-negative n = 56 |
|-----------------------|------------|--------------------------|--------------|--------------|--------------|--------------|-----------------------------------|--------------|-----------------------|------------------------|
| CR                    | 55 (74)    | 73 (68)                  | 8 (62)       | 24 (41)      | 14 (40)      | 8 (16)       | 9 (32)                            | 47 (47)      | 16 (38)               | 22 (39)                |
| Early death, n (%)    | 0 (0)      | 21 (20)                  | 3 (23)       | 11 (19)      | 8 (23)       | 21 (42)      | 5 (18)                            | 23 (23)      | 7 (17)                | 11 (20)                |
| Death in CR, n (%)    | 6 (11)     | 4 (5)                    | 3 (38)       | 1 (4)        | 1 (7)        | 0 (0)        | 0 (0)                             | 9 (19)       | 0 (0)                 | 1 (5)                  |
| Relapse rate, n (%)   | 34 (62)    | 59 (81)                  | 5 (63)       | 23 (96)      | 13 (93)      | 8 (100)      | 9 (100)                           | 37 (79)      | 16 (100)              | 18 (82)                |
| Number expired, n (%) | 56 (76)    | 95 (89)                  | 13 (100)     | 59 (100)     | 35 (100)     | 50 (100)     | 28 (100)                          | 97 (98)      | 42 (100)              | 52 (93)                |
| Disease-free survival (DFS) |           |                          |              |              |              |              |                                   |              |                       |                        |
| Median (years) (95% CI) | 0.9 (0.7–1.9) | 1.0 (0.8–1.2) | 0.5 (0.1–0.7) | 0.9 (0.4–1.8) | 0.6 (0.3–1.2) | 0.4 (0.2–0.4) | 0.4 (0.1–0.5) | 0.5 (0.3–0.6) | 0.7 (0.5–0.9) | 0.7 (0.4–1.0) |
| % Disease-free at 1 y (95% CI) | 48 (35–61) | 53 (41–64) | 13 (1–42) | 46 (26–64) | 29 (9–52) | 0 | 11 (1–39) | 15 (7–27) | 13 (2–33) | 41 (21–60) |
| % Disease-free at 3 y (95% CI) | 30 (18–42) | 27 (18–38) | 0 | 8 (1–23) | 0 | 0 | 0 | 7 (2–16) | 0 | 14 (3–31) |
| % Disease-free at 5 y (95% CI) | 30 (18–42) | 22 (13–32) | 0 | 4 (0–18) | 0 | 0 | 0 | 7 (2–16) | 0 | 14 (3–31) |
| Overall survival (OS) |           |                          |              |              |              |              |                                   |              |                       |                        |
| Median (years) (95% CI) | 1.5 (1.0–2.0) | 1.3 (1.0–1.5) | 0.5 (0–0.8) | 0.6 (0.4–0.9) | 0.6 (0.2–0.8) | 0.2 (0.1–0.3) | 0.2 (0.1–0.5) | 0.5 (0.4–0.6) | 0.6 (0.4–0.9) | 0.7 (0.3–1.1) |
| % Alive at 1 y (95% CI) | 61 (48–71) | 59 (49–68) | 8 (0–29) | 36 (24–48) | 23 (11–38) | 0 | 21 (9–38) | 18 (11–26) | 31 (18–45) | 39 (27–52) |
| % Alive at 3 y (95% CI) | 33 (23–44) | 27 (19–36) | 0 | 15 (8–26) | 6 (1–17) | 0 | 7 (1–20) | 7 (3–13) | 0 | 9 (3–18) |
| % Alive at 5 y (95% CI) | 30 (20–41) | 21 (14–29) | 0 | 8 (3–17) | 3 (0–13) | 0 | 0 | 5 (2–11) | 0 | 7 (2–16) |

CBF, core-binding factor; CR, complete remission; m, mutated; n, number; y, year; wt, wild-type
particularly when considering regulatory actions [11, 48]. Although this endpoint is of utmost importance, the potential impact of ED on the patient’s family well-being may serve to justify ED rate as another surrogate outcome for new therapies with less morbidity than chemotherapy in this disease. Examination of all aspects of ED is a major focus of the precision medicine LLS Beat AML Master Study.

The oncoprint depiction of the genetic groups defined in our prioritization is based on observations made by others [3, 49, 50]. Specifically, the KMT2A and TP53m genetic groups have very little overlap with other common AML mutations as reported by others, suggesting that both 11q23/KMT2A rearrangements [40] and TP53 mutations [3] are strong drivers of the disease. Notably, no (KMT2A) or little (TP53m) overlap occurred with prognostically favorable mutations such as NPM1 mutations or with mutations in the IDH2 and IDH1 genes, for which there are definitive targeted therapies with proven benefit [6–8]. As more directed FLT3 inhibitors are now available, the FLT3m group is moving higher up in the treatment algorithm. Also discerned from this oncoprint, one can observe that NRAS, KRAS and/or PTPN11 mutations overlap with the IDH1m, IDH2m, and FLT3m groups but are relatively infrequent. As targeted therapies directed at IDH2, IDH1, and FLT3 are available, it is notable that NRAS or PTPN11 mutations can represent pretreatment or acquired alterations that lead to primary or secondary resistance [51, 52]. As more data comes forth from studies with targeted therapy, it has been necessary

Fig. 3 Kaplan–Meier curves depicting the a disease-free survival and b overall survival of older patients with acute myeloid leukemia classified into genetic groups. Each genetic group is identified by color as outlined in the figure.
to re-examine prioritization of patients with NRAS and PTPN11 mutations and potentially those with other mutations, such as CBL or NFI, which activate RAS/MAPK signaling, to include these patients as a separate genomic subgroup. Finally, examination of the marker-negative group demonstrates enrichment of patients with RUNX1, ASXL1, and spliceosome mutations that might be amendable to specific targeted therapies in the future. Future decisions to change the algorithm for the Beat AML study will also be based upon the ability to identify relevant and best directed therapeutic options.

Limitations of this study include its retrospective nature, changes in practice patterns for AML therapy over time, and lack of measurable residual disease data (MRD) to correlate with outcomes. However, despite being treated on different Alliance protocols, outcomes in regard to CR, DFS, and OS are similar between all treatment arms of the various protocols (Fig. 4). Patients received intensive regimens with different doses of anthracycline for induction and varied consolidation therapies. There was also inclusions of patients on CALGB 9720 and 9420 which were closed early due to early mortality on the investigational arms but only 32 patients in our analysis were included in treatment arms with PSC-833 with 11 of these patients being included for early death. Another major limitation is the broad range of time for the study enrollment for patients analyzed in this dataset. Supportive care for AML has improved over time with inclusion of better anti-emetics, proton pump inhibitor drugs, antifungal prophylaxis, and treatment of infectious complications and other complications, which has led to improvement in outcomes for patients and may have improvement on ED rates than what is included in our dataset. However, data from the Swedish AML Registry which began collecting patient in 2005 included newly diagnosed AML patient 60–74 with AML with NPM1m/FLT3ITD-treated with intensive chemotherapy had only slightly improved survival data compared to our findings. These patients had a median OS of 1.49 years to our findings of 1.33 years and 3 year OS of 35.5% in comparison to our finding of 27% [53]. Patients included in this analysis were also enrolled onto clinical trials at multiple centers, which may not be reflective of treatment given outside of clinical trials. Also our patients were not transplanted in first CR, which likely contributes to poor long-term outcomes; however, this is likely more akin to real-world data as the majority of older AML patients are, unfortunately, still not being considered for transplantation and many still remain untreated [54–56]. Albeit, this may hopefully improve with additional new less intensive treatment options that be more feasible to give in the community setting.

Also, our analysis included only patients whose AML had both cytogenetic and mutational studies performed from diagnostic samples, a potential selection bias. Finally, our analysis lacks any MRD assessments and correlation to patient clinical outcomes although efforts to add this to Alliance/CALGB patient dataset analyses are currently underway. The use of MRD assessments in AML is evolving in regard to methodology and standardization, as well as timing and threshold of meaningful MRD positivity [57]. Although assessment of AML MRD remains complicated, current efforts are underway to implement multimodality MRD testing in clinical trials including the Beat AML study. It is hopeful that MRD testing can become standardized and a routine part of AML patient clinical care in order to continue to improve treatment outcomes. Despite these limitations to our dataset, our study represents one of the largest series of older AML patients with inclusion of all newly diagnosed patients regardless of early death in order to most accurately define outcomes of specific genetic groups relevant to the ongoing Beat AML study.

Conclusions
As more treatments in AML are explored in the upfront setting, this historical outcome data from patients treated with standard treatment on CALGB/Alliance protocols can aid in determining appropriate milestone achievements for potential trial design. This dataset has been used for Beat AML genomic Phase 1/2 substudy statistical designs to determine primary endpoints. If dramatic improvements with new therapeutics are seen from these baseline expectations, the goal is to lead to rapid drug approval in this older AML population. However, as outcome data matures for newer approved treatment modalities in older AML patients, new benchmarks will be set for therapeutic clinical trials to further improve upon the
recent progress that has been made in this patient population. This is particularly relevant for small genetic groups where randomized trials may not be possible. To extend the results reported herein, we will be validating our findings through analysis of a multi-institutional cohort and, ideally, in a series that includes other standards of care for elderly AML currently used in practice such as HMA [58] or venetoclax combined with HMA [4]. We also plan to assess the potential impact of co-mutations on treatment outcomes and compare data from our retrospective patient cohort in the prospective Beat AML patient data. Our hope is that genetics-based approaches will result in continued improved outcomes in both older and younger AML patient populations and lead to curative therapies, not just short-term improvements.

Abbreviations
AML: Acute myeloid leukemia; BM: Bone marrow; CALGB: Cancer and leukemia group B; CBF: Core-binding factor; CR: Complete remission; DFS: Disease-free survival; ED: Early death; FLT3/Flt3: FLT3-ITD (both high and low allelic ratios included); FK506: Tumor necrosis factor receptor superfamily, member 6 A; FTS: High throughput sequencing; IDH1/ITD: IDH1 Mutated; IDH2/ITD: IDH2 Mutated; KMT2A: 11q23/kMT2A-rearranged; LL: Leukemia Lymphoma Society; OS: Overall survival; PCR: Polymerase chain reaction; TET2/DM: TET2 Mutated; TP53: TP53 Mutated; TP53wt: TP53 Wild-type; VAF: Variant allele frequency; WT1/MT: WT1 Mutated.

Supplementary Information
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Authors’ contributions
ASM, JK, AB, RLL, UB, ES, BJD, and JCB contributed to the study design; ASM, JK, KM, and JCB contributed to the data interpretation; ASM, JK, KM, and JCB wrote the manuscript; ASM, A-KE, JSB, and SC performed laboratory-based research; JSB performed the data processing; JK performed statistical analysis; UB, ES, RMS, JKE, ESW, and JCB were involved directly or indirectly in the care of patients and/or sample procurement. All authors read and agreed on the final version of the manuscript. The authors dedicate this paper to CDB, who died unexpectedly as this manuscript was being completed. Her mentorship and support fostered all of us to pursue novel approaches to improve outcome for AML patients. All authors read and approved the final manuscript.

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Availability of data and materials
For original data, please contact Alice.Mims@osumc.edu.

Declarations
Ethics approval and consent to participate
Patients provided written informed consent to participate in companion protocols CALGB 8461 (cytogenetic studies), CALGB 9665 (leukemia tissue bank), and CALGB 20202 (molecular studies), which involved collection of pretreatment BM and blood samples. Treatment protocols were in accordance with the Declaration of Helsinki and approved by the institutional review boards at each center participating in the CALGB/Alliance treatment protocols, and all patients provided written informed consent.

Consent for publication
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Competing interests
The authors declare no conflicts of or competing interest.

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