CD74 is expressed in a subset of pediatric acute myeloid leukemia patients and is a promising target for therapy: a report from the Children's Oncology Group

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Supplementary Methods:

Patients and Treatment

AAML1031[1] was an open-label multicenter randomized trial for patients with previously untreated *de novo* acute myeloid leukemia (AML) aged 0 to 29.5 years. Patients with acute promyelocytic leukemia, juvenile myelomonocytic leukemia, bone marrow failure syndromes, or secondary AML were not eligible. Patients were randomly assigned at enrollment to either standard AML treatment of standard treatment with bortezomib. Patients with high allelic ratio (AR) FLT3-ITD were offered enrollment on a phase I sorafenib treatment arm,[2] patients who declined enrollment in this arm or who enrolled while the arm was suspended received treatment according to initial randomization. The primary end point of the study was event free survival (EFS) from study entry, defined as time from study entry until death, refractory disease, or relapse of any type, whichever occurred first.

Patients identified as 'low-risk' based on study protocol received four cycles of chemotherapy (with or without bortezomib) while 'high-risk' patients received three cycles (with or without bortezomib) followed by allogeneic donor hematopoietic cell transplantation (HCT). Risk group stratification for AAML1031 was performed using cytogenetic and molecular abnormalities at diagnosis and end-of-induction 1 (EOI1) MRD assessed by MDF as previously described.[1] The high-risk group included patients with a high allelic ratio (>0.4) *FLT3*-ITD and/or high-risk cytogenetics as well as patients with non-informative cytogenetic and molecular abnormalities who were MRD positive at EOI1. Recent refinement of risk stratification ahead of the successor COG AAML1831 trial (NCT04293562) includes the addition of several high-risk markers identified based on unfavorable outcomes.[3] A complete list of genetic markers used in risk stratification can be found in **Supplementary Table 1**.

CD74 Assessment

CD74 immunophenotyping was performed using titered FITC or PE conjugated anti-CD74 monoclonal antibody (ThermoFisher, clone: 5-329 with either fluorophore). Normal expression of CD74 came from bone marrows that were submitted for either: 1) MRD analysis with no detectable residual disease, 2) staging marrows from lymphoma patients, or 3) to rule out myeloid neoplasms. The conversion of CD74 MFI to molecules per cell (MPC) was performed using CD4 MFI on T cells as a reference, as previously described.[4]

CFU Assays

After the 250 cells were treated with STRO-001 or the control, they were mixed with 1 mL Methocult H4034 Optimum media (StemCell Technologies) supplemented with IL-6 (Shenandoah Biotechnology).

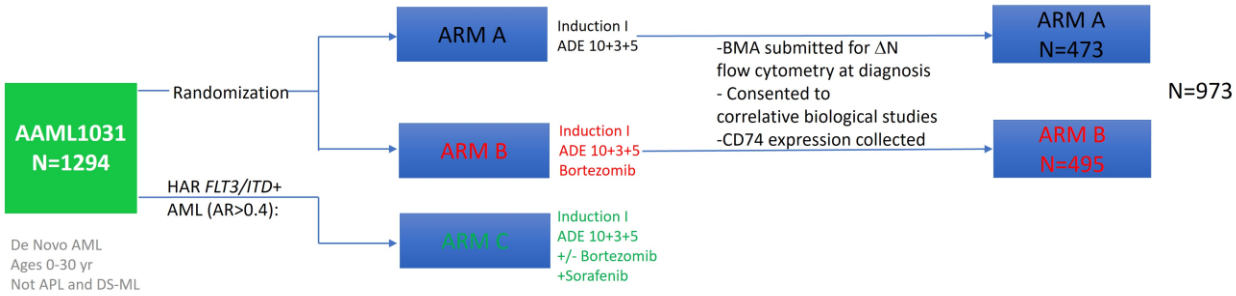
***In Vitro* Cytotoxicity Assays**

Primary cells were used immediately after thawing or post 1 day in culture. Cells were cultured in 200 µL per well of StemSpan SFEM II (StemCell Technologies) + 1% PenStrep (Gibco), and 50 ng/mL SCF (StemCell), 50 ng/mL FLT3 Ligand (StemCell), 50 ng/mL TPO (StemCell), 20ng/mL IL6 (Shenandoah), and 10ng/mL IL3 (Shenandoah). Prior to STRO-001 treatment, blocking antibody (SP7219) was added (1 µM).

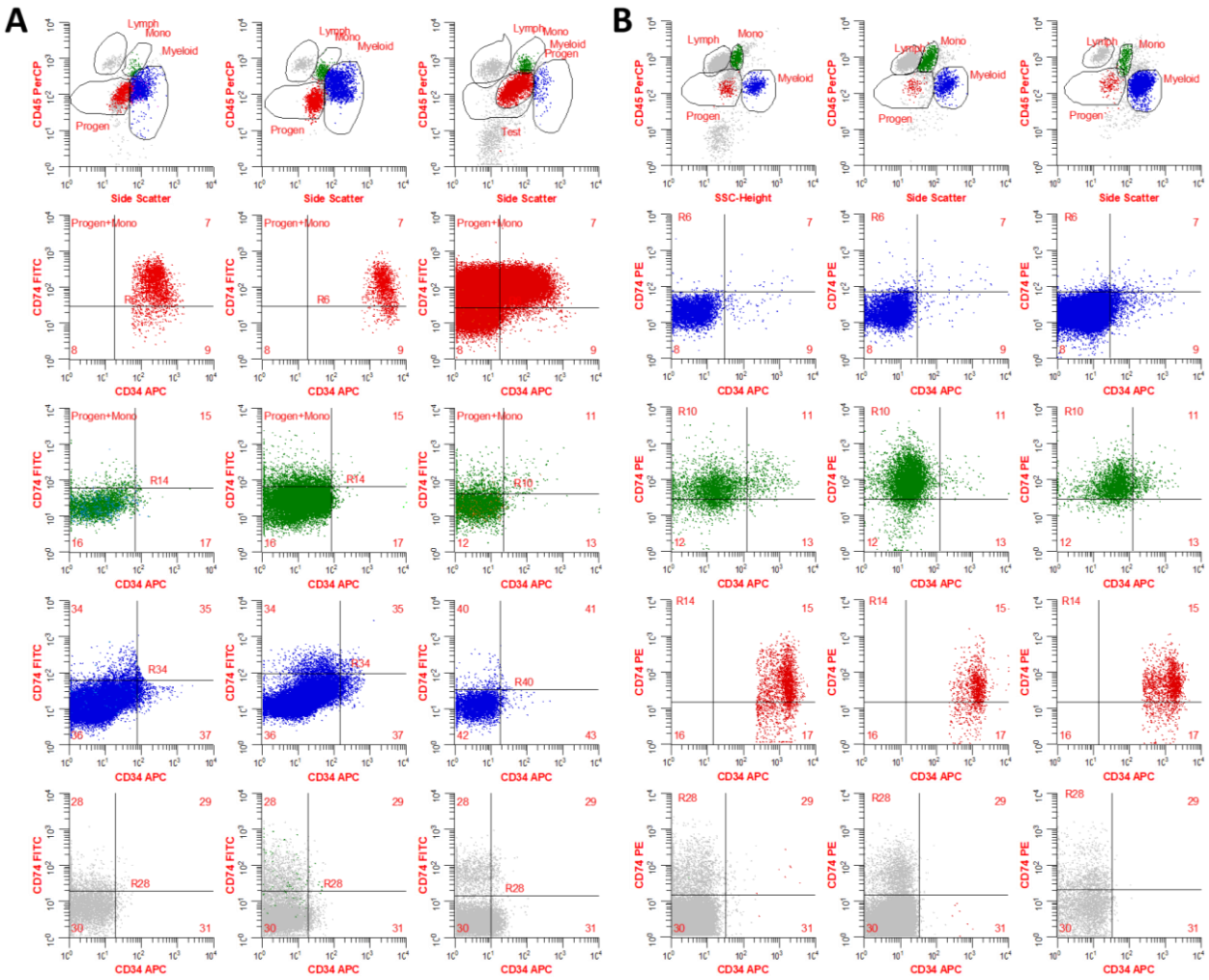
Statistical Analysis

OS was defined as the time from study entry to death from any cause or date of last follow-up in surviving patients. EFS was defined as the time from study entry until induction failure, relapse, or death. Complete remission (CR) was defined as a bone marrow aspirate containing <5% blasts by morphologic analysis and without evidence of extramedullary disease. The

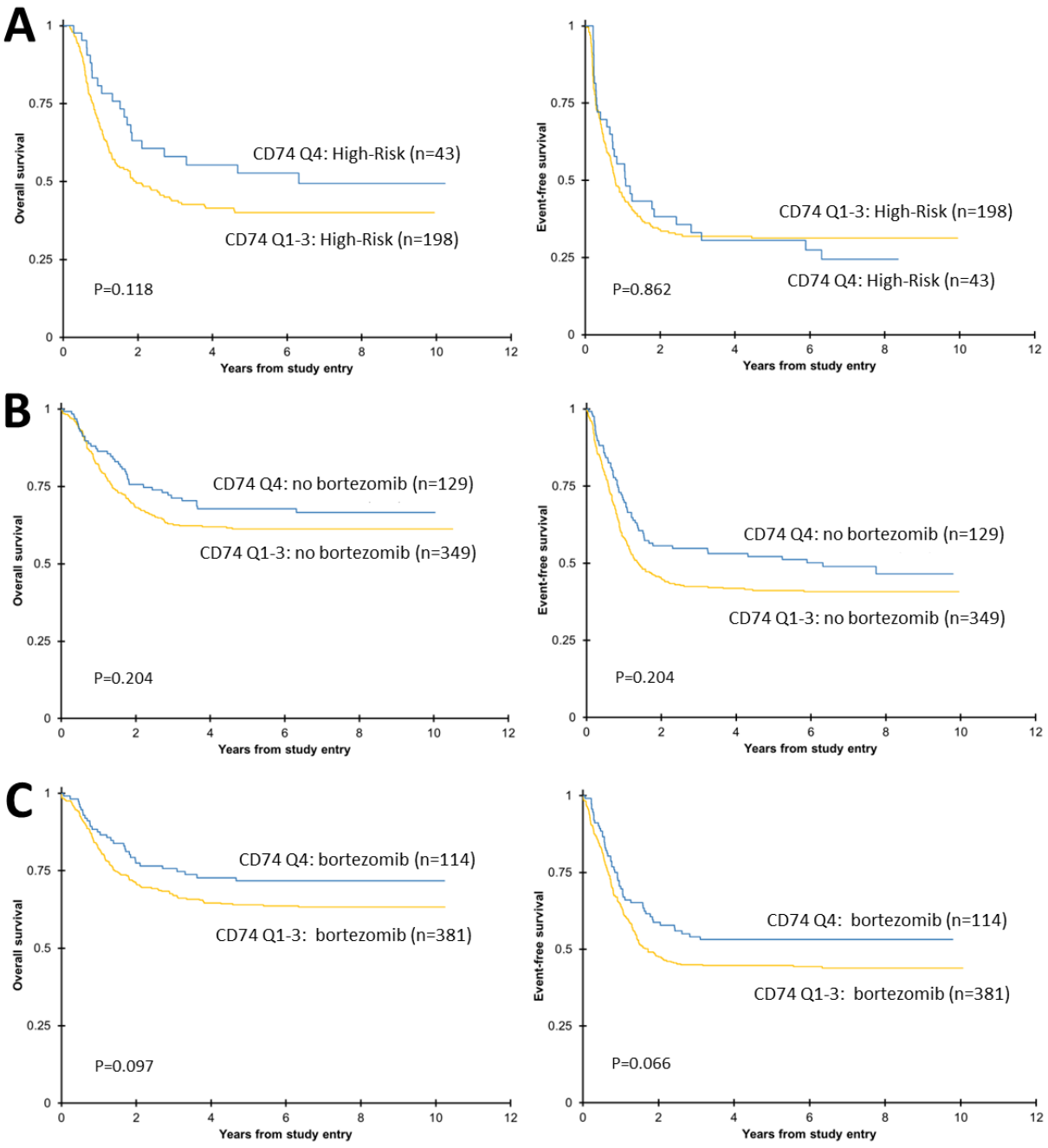
significance of predictor variables was tested with the log-rank test for OS and EFS. The Cox proportional hazards model was used to estimate hazard ratios for survival outcomes and univariable/ multivariable analysis. The Kruskal-Wallis test was used for comparison of continuous variables, and the chi-squared test was used to test for significance of observed differences in proportions.



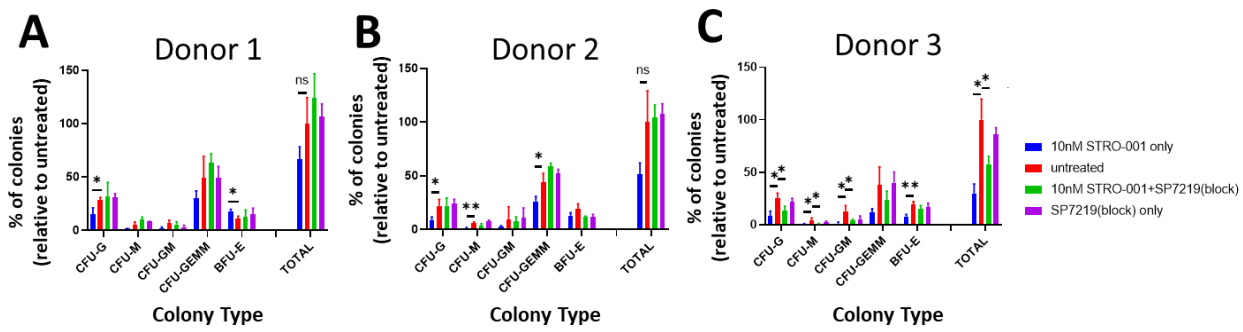
Supplementary Figure 1. Study Design for the AAML1031 Clincial Trial.



Supplmentary Figure 2 A) Representative data from three cases demonstrating expression of CD74 in normal regenerating bone marrow, stained with PE. The quadrants on the graphs represent the autofluorescence of the cell population shown. **B)** Three representative pediatric AML patients from Q4 (high CD74 expression) comparing expression of CD74, stained with FITC, on AML cells compared to normal cells from the same patient. The quadrants on the graphs represent the autofluorescence of the cell population shown.



Supplementary Figure 3. Additional Outcome Data. A) Overall and event free survival of high-risk patients stratified based on CD74 expression (Q4 vs. Q1-3). **B)** Outcomes for patients not treated with bortezomib (Arm A) stratified based on CD74 expression. **C)** Outcomes for patients treated with bortezomib (Arm B) in either CD74 Q4 or CD74 Q1-3.



Supplementary Figure 4. Breakdown of individual colony types from colony forming unit assay. Each type of colony normalized to the total number of colonies in the untreated group (red) for that patient.

Supplementary Table 1 (see excel file). Unfavorable and favorable prognostic markers for risk stratification on the AAML1031 and AAML1831 clinical trials.

Supplementary Table 2 (see excel file). Disease characteristics of CD74 Q4 and Q1-3. Analysis was done for the cohort as a whole as well as a subanalysis of Q4 and Q1-3 by treatment arm.

Supplementary Table 3 (see excel file). Cox univariable and multivariable regression analysis of CD74 expression (Q4 vs. Q1-3) and other prognostic factors.

Supplementary References

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