Bortezomib with standard chemotherapy for children with acute myeloid leukemia does not improve treatment outcomes: a report from the Children’s Oncology Group

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### Supplemental Table 1. Patient Enrollment and WHO classification

| Characteristic                                                                 | Overall | Arm A | Arm B | P-value |
|--------------------------------------------------------------------------------|---------|-------|-------|---------|
| N                                                                              | N       | %     | N     | %       | N     | %     | P-value |
| Total enrolled                                                                 |         |       |       |         |       |       |         |
| Ineligible                                                                     | 32      | 19    | 13    |         |       |       | 0.538   |
| Eligible, ITD high AR (enrolled on Arm C)                                     | 60      | 36    | 24    |         |       |       | 0.156   |
| Eligible, ITD high AR                                                          | 42      | 19    | 23    |         |       |       | 0.398   |
| Eligible, without ITD high AR                                                  | 1097    | 542   | 555   |         |       |       | 0.624   |
| WHO classification                                                             |         |       |       |         |       |       |         |
| AML, not otherwise categorized: Acute erythroid leukemia (Erythroleukemia, erythroid/myeloid) | 12      | 1%    | 7     | 1%      | 5     | 1%    | 0.538   |
| AML, not otherwise categorized: Acute erythroid leukemia (Pure erythroid leukemia) | 3       | 0%    | 3     | 1%      | 0     | 0%    | 0.121   |
| AML, not otherwise categorized: AML without maturation                         | 68      | 6%    | 28    | 5%      | 40    | 7%    | 0.156   |
| AML, not otherwise categorized: AML with maturation                            | 66      | 6%    | 36    | 7%      | 30    | 5%    | 0.398   |
| AML, not otherwise categorized: AML, with minimal differentiation              | 31      | 3%    | 14    | 3%      | 17    | 3%    | 0.624   |
| AML, not otherwise categorized: Acute myelomonocytic leukemia                  | 71      | 6%    | 32    | 6%      | 39    | 7%    | 0.440   |
| AML, not otherwise categorized: Acute monoblastic/acute monocytic leukemia     | 172     | 16%   | 89    | 16%     | 83    | 15%   | 0.521   |
| AML, not otherwise categorized: AML megakaryoblastic leukemia                  | 57      | 5%    | 25    | 5%      | 32    | 6%    | 0.382   |
| AML with t(8;21)(q22;q22); RUNX1-RUNX1T1                                     | 154     | 14%   | 78    | 14%     | 76    | 14%   | 0.758   |
| AML with inv(16)(p13q22) or t(16;16)(p13;q22); CBFB-MYH11                   | 101     | 9%    | 53    | 10%     | 48    | 9%    | 0.530   |
| AML with t(9;11)(p22;q23); MLLT3-MLL                                          | 94      | 9%    | 46    | 8%      | 48    | 9%    | 0.909   |
| AML with t(6;9)(p23;q34); DEK-NUP214                                         | 12      | 1%    | 8     | 1%      | 4     | 1%    | 0.232   |
| AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1                  | 1       | 0%    | 0     | 0%      | 1     | 0%    | 1.000   |
| AML (megakaryoblastic) with t(1;22)(p13;q13); RMB15-MKL                    | 13      | 1%    | 6     | 1%      | 7     | 1%    | 0.808   |
| AML with myelodysplasia-related changes                                        | 88      | 8%    | 41    | 8%      | 47    | 8%    | 0.570   |
| Provisional entity: AML with mutated CEBPA                                   | 37      | 3%    | 19    | 4%      | 18    | 3%    | 0.819   |
| Provisional entity: AML with mutated NPM1                                     | 32      | 3%    | 16    | 3%      | 16    | 3%    | 0.954   |
| AML, not otherwise categorized                                                | 69      | 6%    | 35    | 6%      | 34    | 6%    | 0.833   |
| Myeloid sarcoma                                                               | 14      | 1%    | 6     | 1%      | 8     | 1%    | 0.617   |
| Unknown                                                                        | 2       | 0%    | 2     |         |       |       |         |

Abbreviations: AML, acute myeloid leukemia; WHO, World Health Organization.
### Supplemental Table 2: Outcome by Risk Group:

| Risk Group | Overall | Arm A   | Arm B   | p-value |
|------------|---------|---------|---------|---------|
|            | N       | % ± 2 SE% | N       | % ± 2 SE% | N       | % ± 2 SE% |
| Low Risk   |         |         |         |         |         |
| 3 year DFS from end of Induction I | 805 | 52.9 ± 3.7 | 403 | 50.7 ± 5.2 | 402 | 55.1 ± 5.2 | 0.129 |
| 3 year OS from end of Induction I  | 805 | 74.1 ± 3.4 | 403 | 71.9 ± 4.9 | 402 | 76.3 ± 4.6 | 0.264 |
| 3 year CI of relapse from end of Induction I | 805 | 44.1 ± 3.7 | 403 | 45.7 ± 5.2 | 402 | 42.4 ± 5.2 | 0.214 |
| High Risk  |         |         |         |         |         |
| 3 year DFS from end of Induction I | 210 | 27.8 ± 6.6 | 103 | 31.7 ± 9.5 | 107 | 24.6 ± 8.9 | 0.279* |
| 3 year OS from end of Induction I  | 210 | 36.9 ± 7.6 | 103 | 38.0 ± 10.9 | 107 | 36.2 ± 10.5 | 0.924 |
| 3 year CI of relapse from end of Induction I | 210 | 58.8 ± 7.2 | 103 | 55.0 ± 10.2 | 107 | 62.1 ± 10.0 | 0.311* |

*Landmark analyses as proportional hazards assumption violated
Supplemental Table 3: Outcome by Cytogenetic Risk Group:

|                      | NPM+ patients only |                      | CEBPα+ patients only |                      |
|----------------------|--------------------|----------------------|----------------------|----------------------|
|                      | Overall            | Arm A                | Arm B                | p-value              |
|                      | N                  | % ± 2 SE%            | N                    | % ± 2 SE%            | N                  | % ± 2 SE%            |                      |
| 3 year EFS from study entry | 80                 | 72.2 ± 10.4          | 37                   | 72.6 ± 14.8          | 43                 | 72.0 ± 14.4          | 0.833                |
| 3 year OS from study entry | 80                 | 86.8 ± 7.8           | 37                   | 83.4 ± 12.4          | 43                 | 89.8 ± 9.8           | 0.404                |
| 3 year CI of relapse from study entry | 80             | 20.2 ± 9.5           | 37                   | 16.6 ± 12.6          | 43                 | 23.3 ± 13.9          | 0.615                |
| 0.5 year TRM from study entry | 80             | 5.3 ± 5.2            | 37                   | 5.8 ± 8.2            | 43                 | 4.7 ± 6.5            | 0.748                |
| 3 year DFS from end of Induction II | 76            | 74.9 ± 10.3          | 36                   | 74.6 ± 14.7          | 40                 | 75.5 ± 14.3          | 0.708                |
| 3 year OS from end of Induction II | 76            | 88.9 ± 7.4           | 36                   | 85.8 ± 11.8          | 40                 | 91.8 ± 9.1           | 0.397                |
| 0.5 year TRM from end of Induction II | 76          | 21.0 ± 36.7          | 36                   | 24.7 ± 44.2          | 40                 | 2.5 ± 5.0            | 0.816                |
|                                | Overall | Arm A     | Arm B     | p-value |
|--------------------------------|---------|-----------|-----------|---------|
| **CBF (t(8;21) or inv(16))**   |         |           |           |         |
| **patients only**              |         |           |           |         |
| **3 year EFS from study entry**| 280     | 64.8 ± 5.9| 141       | 60.9 ± 8.6 | 139     | 68.8 ± 8.0 | 0.219 |
| **3 year OS from study entry** | 280     | 85.6 ± 4.4| 141       | 83.9 ± 6.8 | 139     | 87.2 ± 5.8 | 0.736 |
| **3 year CI of relapse from**  | 280     | 30.9 ± 5.7| 141       | 34.8 ± 8.5 | 139     | 26.9 ± 7.7 | 0.185 |
| **study entry**                | 280     | 30.9 ± 5.7| 141       | 34.8 ± 8.5 | 139     | 26.9 ± 7.7 | 0.185 |
| **1 year TRM from study entry**| 280     | 6.0 ± 3.8 | 141       | 4.8 ± 3.9  | 139     | 7.1 ± 6.5  | 0.960 |
| **3 year DFS from end of Induction II** | 262 | 67.9 ± 6.0 | 133 | 63.1 ± 8.8 | 129 | 72.8 ± 8.0 | 0.129 |
| **3 year OS from end of Induction II** | 262 | 52.5 ± 6.4 | 133 | 55.5 ± 9.4 | 129 | 55.5 ± 9.4 | 0.431 |
| **3 year CI of relapse from**  | 280     | 32.1 ± 5.8| 147       | 31.8 ± 7.9 | 133     | 32.5 ± 8.4 | 0.507 |
| **study entry**                | 280     | 32.1 ± 5.8| 147       | 31.8 ± 7.9 | 133     | 32.5 ± 8.4 | 0.507 |
| **1 year TRM from study entry**| 280     | 9.6 ± 8.0 | 147       | 9.1 ± 9.8  | 133     | 10.1 ± 13.1| 0.919 |
| **3 year DFS from end of Induction II** | 221 | 37.9 ± 6.7 | 117 | 38.0 ± 9.2 | 104 | 37.8 ± 9.2 | 0.675 |
| **3 year OS from end of Induction II** | 221 | 58.8 ± 7.1 | 117 | 56.2 ± 9.7 | 104 | 61.6 ± 10.5| 0.539 |
| **0.5 year TRM from end of Induction II** | 221 | 7.1 ± 8.8 | 117 | 6.1 ± 8.8 | 104 | 7.2 ± 14.5 | 0.346 |
Supplemental Table 4: Outcome by Age Group:

| Age Group | Overall | Arm A | Arm B | P-value |
|-----------|---------|-------|-------|---------|
|           | N       | % ± 2 SE% | N       | % ± 2 SE% | N       | % ± 2 SE% |
| 0-1 yrs   |         |         |        |         |         |         |
| 3-year EFS from study entry | 237 | 39.1 ± 6.6 | 107 | 34.4 ± 9.8 | 130 | 42.8 ± 8.9 | 0.326 |
| 3-year OS from study entry | 237 | 55.9 ± 7.1 | 107 | 53.2 ± 10.6 | 130 | 58.2 ± 9.5 | 0.390 |
| 2-10 yrs  |         |         |        |         |         |         |
| 3-year EFS from study entry | 372 | 42.3 ± 5.4 | 189 | 42.6 ± 7.5 | 183 | 41.9 ± 7.8 | 0.672 |
| 3-year OS from study entry | 372 | 64.4 ± 5.4 | 189 | 64.7 ± 7.6 | 183 | 64.0 ± 7.7 | 0.587 |
| 11-15 yrs |         |         |        |         |         |         |
| 3-year EFS from study entry | 273 | 49.0 ± 6.3 | 139 | 46.6 ± 8.8 | 134 | 51.4 ± 9.0 | 0.360 |
| 3-year OS from study entry | 273 | 70.0 ± 5.9 | 139 | 64.3 ± 8.7 | 134 | 75.8 ± 7.6 | 0.054 |
| ≥16 yrs   |         |         |        |         |         |         |
| 3-year EFS from study entry | 215 | 56.0 ± 7.3 | 107 | 57.2 ± 10.0 | 108 | 54.7 ± 10.5 | 0.747 |
| 3-year OS from study entry | 215 | 71.5 ± 6.9 | 107 | 70.9 ± 9.7 | 108 | 72.2 ± 9.7 | 0.858 |
### Supplemental Table 5. Univariable Analyses and Multivariable Analyses from End of Induction II

| Univariable analyses                  | OS from study entry | EFS from study entry | TRM from study entry |
|--------------------------------------|---------------------|----------------------|----------------------|
|                                      | N   | HzR  | 95% CI | P-value | HzR  | 95% CI | P-value | HzR  | 95% CI | P-value |
| **Treatment Arm**                    |     |      |        |         |      |        |         |      |        |         |
| Arm A                                | 542 | 1    | 1      | 1       | 1    | 1      | 1       |
| Arm B                                | 555 | 0.91 | 0.74 - 1.12 | 0.356 | 0.91 | 0.77 - 1.07 | 0.236 | 0.85 | 0.49 - 1.49 | 0.577 |
| **Age at diagnosis, years**          |     |      |        |         |      |        |         |      |        |         |
| 2-10                                 | 372 | 1    | 1      | 1       | 1    | 1      | 1       |
| 0-1                                  | 237 | 1.25 | 0.95 - 1.63 | 0.106 | 1.20 | 0.97 - 1.49 | 0.098 | 0.68 | 0.28 - 1.63 | 0.385 |
| ≥11                                  | 488 | 0.80 | 0.63 - 1.02 | 0.068 | 0.76 | 0.63 - 0.92 | **0.004** | 1.10 | 0.60 - 2.02 | 0.769 |
| **WBC at diagnosis, µL**             |     |      |        |         |      |        |         |      |        |         |
| ≤ 100,000                            | 916 | 1    | 1      | 1       | 1    | 1      | 1       |
| > 100,000                            | 178 | 1.43 | 1.10 - 1.86 | **0.007** | 1.66 | 1.35 - 2.03 | <**0.001** | 2.00 | 1.06 - 3.76 | **0.033** |
| **Race**                             |     |      |        |         |      |        |         |      |        |         |
| Non-black                            | 835 | 1    | 1      | 1       | 1    | 1      | 1       |
| Black                                | 137 | 1.31 | 0.97 - 1.76 | 0.077 | 1.03 | 0.80 - 1.33 | 0.796 | 1.87 | 0.95 - 3.67 | 0.069 |

| Univariable analyses                  | OS from end induction II | DFS from end induction II | RR from end induction II | TRM from end induction II |
|--------------------------------------|--------------------------|---------------------------|--------------------------|---------------------------|
|                                      | N   | HzR  | 95% CI | P-value | HzR  | 95% CI | P-value | HzR  | 95% CI | P-value | HzR  | 95% CI | P-value |
| **Treatment Arm**                    |     |      |        |         |      |        |         |      |        |         |      |        |         |
| Arm A                                | 453 | 1    | 1      | 1       | 1    | 1      | 1       |
| Arm B                                | 457 | 0.91 | 0.71 - 1.17 | 0.454 | 0.93 | 0.76 - 1.13 | 0.444 | 0.96 | 0.79 - 1.18 | 0.727 | 0.70 | 0.34 - 1.45 | 0.335 |
| **Age at diagnosis, years**          |     |      |        |         |      |        |         |      |        |         |      |        |         |
| 2-10                                 | 304 | 1    | 1      | 1       | 1    | 1      | 1       |
| 0-1                                  | 185 | 1.28 | 0.92 - 1.79 | 0.143 | 1.17 | 0.91 - 1.52 | 0.225 | 1.25 | 0.95 - 1.65 | 0.108 | 0.43 | 0.09 - 2.01 | 0.285 |
| ≥11                                  | 421 | 0.87 | 0.65 - 1.17 | 0.357 | 0.84 | 0.67 - 1.05 | 0.122 | 0.77 | 0.61 - 0.98 | **0.029** | 1.57 | 0.69 - 3.59 | 0.283 |
| **WBC at diagnosis, µL**             |     |      |        |         |      |        |         |      |        |         |      |        |         |
| ≤ 100,000                            | 781 | 1    | 1      | 1       | 1    | 1      | 1       |
| > 100,000                            | 128 | 0.92 | 0.63 - 1.34 | 0.662 | 1.42 | 1.10 - 1.83 | **0.008** | 1.54 | 1.18 - 2.02 | **0.002** | 0.47 | 0.11 - 1.96 | 0.298 |
| **Race**                             |     |      |        |         |      |        |         |      |        |         |      |        |         |
| Non-black                            | 696 | 1    | 1      | 1       | 1    | 1      | 1       |
| Black                                | 107 | 1.19 | 0.81 - 1.73 | 0.375 | 0.87 | 0.64 - 1.20 | 0.402 | 0.81 | 0.57 - 1.15 | 0.239 | 1.11 | 0.38 - 3.24 | 0.846 |
| Risk group | Low   | 769  | 1     | 1     | 1     | 1     | 1 |
|------------|-------|------|-------|-------|-------|-------|---|
|            | High  | 141  | 2.80  | 2.12 - 3.71 | <0.001 | 1.60 | 1.25 - 2.04 | <0.001 | 1.19 | 0.89 - 1.59 | 0.238 | 1.12 | 0.44 - 2.85 | 0.809 |

### Multivariable analyses

| Multivariable analyses | OS from end induction II | DFS from end induction II | RR from end induction II | TRM from end induction II |
|------------------------|--------------------------|---------------------------|---------------------------|---------------------------|
|                        | N | HzR | 95% CI | P-value | HzR | 95% CI | P-value | HzR | 95% CI | P-value | HzR | 95% CI | P-value |
| Treatment Arm          |   |     |        |         |     |        |         |     |        |         |     |        |         |
| Arm A                  | 401 | 1 |       |         | 1.01 | 0.82 - 1.24 | 0.957 | 1.03 | 0.83 - 1.28 | 0.807 | 0.82 | 0.38 - 1.79 | 0.623 |
| Arm B                  | 401 | 0.95 | 0.73 - 1.24 | 0.723 | 1.22 | 0.93 - 1.62 | 0.158 | 1.26 | 0.93 - 1.69 | 0.132 | 0.55 | 0.11 - 2.69 | 0.458 |
| Age at diagnosis, years|   |     |        |         |     |        |         |     |        |         |     |        |         |
| ≤ 10 - 10              | 258 | 1 |       |         | 1 |       |         | 1 |       |         | 1 |       |         |
| > 0 - 1                | 162 | 1.37 | 0.95 - 1.96 | 0.090 | 1.22 | 0.93 - 1.62 | 0.158 | 1.26 | 0.93 - 1.69 | 0.132 | 0.55 | 0.11 - 2.69 | 0.458 |
| ≥ 11                   | 382 | 0.95 | 0.70 - 1.30 | 0.760 | 0.87 | 0.69 - 1.10 | 0.244 | 0.78 | 0.61 - 1.00 | 0.046 | 1.98 | 0.79 - 4.96 | 1.145 |
| WBC at diagnosis, µL   |   |     |        |         |     |        |         |     |        |         |     |        |         |
| ≤ 100,000              | 683 | 1 |       |         | 1 |       |         | 1 |       |         | 1 |       |         |
| > 100,000              | 119 | 0.88 | 0.59 - 1.30 | 0.507 | 1.34 | 1.02 - 1.76 | 0.035 | 1.46 | 1.10 - 1.95 | 0.010 | 0.44 | 0.10 - 1.89 | 0.268 |
| Race                   |   |     |        |         |     |        |         |     |        |         |     |        |         |
| Non-black              | 695 | 1 |       |         | 1 |       |         | 1 |       |         | 1 |       |         |
| Black                  | 107 | 1.14 | 0.78 - 1.65 | 0.509 | 0.84 | 0.61 - 1.16 | 0.294 | 0.79 | 0.55 - 1.13 | 0.195 | 1.19 | 0.41 - 3.47 | 0.745 |
| Risk group             |   |     |        |         |     |        |         |     |        |         |     |        |         |
| Low                    | 682 | 1 |       |         | 1 |       |         | 1 |       |         | 1 |       |         |
| High                   | 120 | 2.81 | 2.08 - 3.80 | <0.001 | 1.67 | 1.28 - 2.17 | <0.001 | 1.16 | 0.84 - 1.59 | 0.377 | 1.39 | 0.53 - 3.66 | 0.502 |

Abbreviations: OS, overall survival; EFS, event-free survival; TRM, treatment-related mortality; DFS, disease-free survival; RR, relapse risk; HzR, hazard ratio; CI, confidence interval; WBC, white blood cell count
Supplemental Table 6. Treatment-Related Mortality (TRM) by Treatment Phase

|                      | Induction I | Induction II | Intensification I | Intensification II |
|----------------------|-------------|--------------|-------------------|-------------------|
|                      | Arm A       | Arm B        | Arm A             | Arm B             |
| Patients (N)         | 540         | 552          | 506               | 509               |
| Patients with TRM (N)| 9           | 11           | 1                 | 0                 |
| % TRM                | 1.7%        | 2.0%         | 0.2%              | 0.0%              |
|                      | Arm A       | Arm B        | Arm A             | Arm B             |
| Patients (N)         | 453         | 456          | 372               | 360               |
| Patients with TRM (N)| 3           | 3            | 9                 | 6                 |
| % TRM                | 0.7%        | 0.7%         | 2.4%              | 1.7%              |
Supplemental Table 7. Ejection Fraction/Shortening Fraction by Course and Treatment Arm

| Arm A: Lowest EF and SF in Each Course | Induction I | Induction II | Intensification I | Intensification II |
|--------------------------------------|-------------|--------------|------------------|-------------------|
| EF SF                                | Patients (N) | 574 518 460 373 | ECHO result (N) | 475 525 395 430 359 401 278 300 |
| Mean                                 | Mean 64.3 35.8 62.57 34.61 61.1 33.68 59.07 32.72 | Median 65 35.4 63 34.1 62 33.5 61 32.82 |
| Arm B: Lowest EF and SF in Each Course | Induction I | Induction II | Intensification I | Intensification II |
| EF SF                                | Patients (N) | 580 529 469 361 | ECHO result (N) | 483 539 408 464 384 418 284 307 |
| Mean                                 | Mean 63.45 35.3 61.34 33.6 59.58 32.44 58.37 31.37 | Median 64 34.9 62 33.9 61 32.9 59.8 32 |

Abbreviations: EF, Ejection fraction; SF, Shortening fraction
Supplemental Table 8. Toxicities in Arm B by Age Category

| Toxicity                                      | Treatment Arm                                      | Arm B                                      |
|-----------------------------------------------|---------------------------------------------------|--------------------------------------------|
|                                               | Age group                                         | Age: 0-1 yrs | Age: 2-10 yrs | Age: 11-15 yrs | Age: ≥16 yrs | P-value     |
|                                               | N                                                 | 81          | 115           | 91             | 74           |             |
| Cardiac                                       | Heart Failure                                     | 1           | 10            | 4              | 4            | 0.139       |
|                                               | EF Decreased                                      | 1.2%        | 8.7%          | 4.4%           | 5.4%         | 0.044       |
|                                               | Cardiac LVSD                                      | 0.0%        | 8.7%          | 9.9%           | 6.8%         |             |
|                                               |                                                   | 1.2%        | 9.6%          | 5.5%           | 13.5%        | 0.021       |
| Neurologic                                    | Peripheral Neuropathy/Paresthesia/Neuralgia       | 2           | 7             | 12             | 16           | <0.001      |
|                                               | Seizure                                           | 2.5%        | 6.1%          | 13.2%          | 21.6%        |             |
| Pulmonary                                     | ARDS                                              | 0           | 5             | 2              | 3            | 0.269       |
|                                               | Hypoxia                                           | 0.0%        | 4.3%          | 2.2%           | 4.1%         |             |
|                                               |                                                   | 13.0%       | 12.2%         | 22.0%          | 18.9%        | 0.289       |
|                                               | Respiratory Failure                               | 2.5%        | 7.0%          | 3.3%           | 10.8%        | 0.096       |
| Renal                                         | Acute kidney injury                               | 0           | 0             | 4              | 7            | <0.001      |
|                                               | Creatinine increased                              | 0.0%        | 0.0%          | 4.4%           | 9.5%         |             |
|                                               |                                                   | 0.0%        | 0.0%          | 2.2%           | 5.4%         | 0.020       |
| Microbiologically documented sterile site    | Viridans group                                    | 25          | 60            | 36             | 31           | 0.026       |
| infections (at least 1 occurrence)           | Streptococcus                                     | 30.9%       | 52.2%         | 39.6%          | 41.9%        |             |
|                                               |                                                   | 41.9%       | 52.2%         | 39.6%          | 41.9%        |             |
|                                               | Gram Negative Bacilli                             | 19          | 23            | 30             | 18           | 0.192       |
|                                               |                                                   | 23.5%       | 20.0%         | 33.0%          | 24.3%        |             |
|                                               | Fungi                                             | 4           | 1             | 4              | 3            | 0.353       |
|                                               |                                                   | 4.9%        | 0.9%          | 4.4%           | 4.1%         |             |
|                                               | Dose Reductions                                   | 12          | 31            | 22             | 26           | 0.033       |
|                                               |                                                   | 14.8%       | 27.0%         | 24.2%          | 35.1%        |             |
| PICU Admissions | 34  | 56  | 54  | 47  |
|-----------------|-----|-----|-----|-----|
|                 | 42.0% | 48.7% | 59.3% | 63.5% | 0.023 |
Induction I: All patients
Cytarabine 100 mg/m² BID x 10 days
Daunorubicin 50 mg/m² daily on days 1, 3 and 5
Etoposide 100 mg/m² daily on days 1-5

Induction II: Low Risk patients
Cytarabine 100 mg/m² BID x 8 days
Daunorubicin 50 mg/m² daily on days 1, 3 and 5
Etoposide 100 mg/m² daily on days 1-5

High Risk patients
Cytarabine 1000 mg/m² daily on days 1-4
Mitoxantrone 12 mg/m² daily on days 3-6

Intensification I: All patients
Cytarabine 1000 mg/m² BID x 5 days
Etoposide 150 mg/m² daily on days 1-5

Intensification II: Low Risk patients
Cytarabine 1000 mg/m² daily on days 1-4
Mitoxantrone 12 mg/m² daily on days 3-6

High Risk patients
Best allogenic donor stem cell transplant
Methods:

This was an open-label multi-center randomized trial conducted by COG in the United States, Canada, Australia and New Zealand. AAML1031 included patients aged 0 to 29.5 years who had previously untreated primary AML. Data were entered through the COG Web portal by each enrolling institution and were frozen December 31, 2017. No minimal performance status was required. Exclusion criteria were prior chemotherapy (except intrathecal cytarabine and hydroxyurea), acute promyelocytic leukemia [t(15;17)], juvenile myelomonocytic leukemia, bone marrow failure syndromes, or secondary AML. Pathologic (84.3%) and cytogenetic findings (97.6%) were centrally reviewed. The National Cancer Institute’s central institutional review board and institutional review boards at each enrolling center (n = 184) approved the study; patients and their families provided informed consent or assent as appropriate. The trial was conducted in accordance with the Declaration of Helsinki. The trial was registered at www.clinicaltrials.gov as NCT01371981.

Patients were randomly assigned at enrollment to either standard AML treatment or standard treatment with bortezomib given in each chemotherapy course. The allocation sequence was computer generated and randomization was conducted in blocks of 4. For those allocated to the intervention arm, bortezomib was given at a dose 1.3 mg/m² administered once on days 1, 4, and 8 of each chemotherapy course.

Patients with high allelic ratio FLT3 ITD were offered enrollment in a Phase I sorafenib treatment arm if that arm was open. Patients with HAR FLT3 ITD who declined
enrollment in the sorafenib arm or who enrolled while the arm was suspended continued to receive treatment according to their initial randomization. These patients were included in safety analyses but were excluded from all efficacy analyses.

Patients were classified as low or high risk after Induction I (defined below). Low risk patients received four courses of chemotherapy. Patients classified as high risk received three courses of chemotherapy followed by allogeneic SCT. Choice of alternative donors were at the transplantation center’s discretion and included matched or 1-antigen mismatched unrelated donors, 4-to-6 antigen matched cord blood, or mismatched family donor with at least one haplotype match or 5-of-6 antigen phenotypic match. High risk patients without an appropriate donor received four courses of chemotherapy.

Supplemental table 9 presents protocol mandated chemotherapy courses and doses. Targeted toxicity monitoring for infectious and other toxicities was employed as previously described. In addition, an echocardiogram was mandated prior to each course of protocol therapy and values for the lowest shortening fraction and ejection fraction in each course were submitted by treating centers.

Patients were classified as either low risk or high risk based on diagnostic cytogenetic and molecular risk features and disease response after Induction I. Low risk was defined by the presence of t(8;21)(q22;q22), inv(16)(p13.1q22), or t(16;16)(p13.1;q22), NPM, or CEBPA mutations. Low risk was also defined by negative minimal residual
disease (MRD) in the bone marrow specimen at the end of Induction I in patients with uninformative cytogenetic and molecular features (MRD level < 0.1%). (2, 3) MRD detection was performed in a centralized lab using a “different from normal” algorithm employed as previously reported. (3) High risk was defined by the presence of monosomy 7, monosomy 5/5q deletion, or uninformative cytogenetic/molecular features with MRD >0.1% after Induction I. Pathologic and cytogenetic findings were centrally reviewed. Cytogenetics and molecular features outweighed minimal residual disease in risk classification, (4) and FLT3-ITD HAR outweighed favorable cytogenetics. (5)

Refractory disease was defined as the persistence of CNS disease after Induction I, or the presence of morphologic bone marrow blasts ≥5% or any extramedullary disease at the end of Induction II. Patients with refractory disease were removed from protocol therapy.

The primary endpoint was EFS from study entry. EFS was defined as the time from study entry until death, refractory disease, or relapse of any type, whichever occurred first. The secondary endpoints were OS, remission rates, relapse risk, post induction disease free survival (DFS), and treatment-related mortality (TRM). OS was defined as time from study entry until death. Relapse risk was defined as the time from the end of Induction II for patients in complete remission (CR) to relapse, where deaths without a relapse were considered competing events. DFS was defined as the time from end of Induction II for patients in CR until relapse or death. TRM was defined as the time from either study entry, or from end of Induction II for patients in CR, to deaths without a
relapse with relapses considered as competing events. Patients without an event were
censored at their date of last known contact. However for TRM analyses, patients were
censored 30 days post end of therapy or 200 days post SCT.

Because of the limited data on the safety of combining bortezomib with standard AML
chemotherapy in pediatric patients at the time of study initiation, an interim toxicity
analyses was performed after 100 patients had been randomized to receive bortezomib.
Specifically, rates of TRM and adult respiratory distress syndrome (ARDS) were
compared against predetermined rates that would require study closure and the rates of
other targeted toxicities were compared between treatment arms.

Statistical Analysis: The study had a goal to enroll 1,200 eligible patients and was
designed with 1-sided testing and 2.5% type I error rate and 80% power to detect a 9%
difference in EFS plateaus (52% vs. 61%, hazard ratio = 0.78) between patients without
HAR FLT3 ITD randomized to standard therapy versus bortezomib/standard
combination therapy. The study was monitored by a data safety monitoring committee.
The alpha-spending function $\alpha t^2$ (truncated at three standard deviations) and 2.5% type
I error was used to monitor OS and EFS while futility monitoring was performed by
testing the alternative hypothesis at the 0.005 level.

The significance of observed difference in proportions was tested using the chi-squared
test and Fisher’s exact test when data were sparse. The Kruskal-Wallis test was used to
determine the significance between differences in medians of groups. Estimates of OS,
EFS, and DFS were calculated using the Kaplan-Meier procedure along with corresponding two Greenwood SEs. The significance of predictor variables was tested with the log-rank statistic for OS, EFS, DFS and with Gray’s statistic for RR and TRM. Cox proportional hazards models were used to estimate hazard ratios (HzR) for univariable and multivariable analyses of OS, EFS, and DFS. Competing risk regression models were used to estimate the subgroup HzR for univariable and multivariable analyses of RR and TRM. A landmark analysis comparing 3 year point estimates was used for any analyses that did not satisfy the proportional hazards assumption. All p values are two-sided.

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