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List of where the study has been presented in part elsewhere: Schultz KR, Bowman P, Slayton WB, Aledo A, Devidas M, Sather H, Borowitz M, Davies S, Trigg M, Pasut B, Jorstad D, Eslinger T, Burden L, Wang CG, Rutledge R, Camitta B, Gaynon P, Carroll A, Heerema NA, Winick N, Hunger S, Carroll WL. Philadelphia chromosome-negative (Ph^-) very high-risk (VHR) acute lymphoblastic leukemia (ALL) in children and adolescents: the impact of intensified chemotherapy on early event-free survival (EFS) in Children's Oncology Group (COG) Study AALL0031. ASH 6–8 December 2008.

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Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

OPEN

Low-dose lenalidomide plus cytarabine induce complete remission that can be predicted by genetic profiling in elderly acute myeloid leukemia patients

Leukemia (2014) **28,** 967–970; doi:10.1038/leu.2014.31

The outcome for elderly patients with acute myeloid leukemia (AML) is extremely poor. Intensive induction chemotherapy is often unsuitable.[1] High-dose lenalidomide is effective in AML, alone[2,3] or in combination with azacitidine.[4-6] Biomarkers that are able to predict response to lenalidomide would be extremely useful.

We report a single-arm, prospective, phase II study of a novel combination therapy with low-dose lenalidomide plus low-dose cytarabine. Patients aged $\geqslant$70 years with a World Health Organization (WHO) diagnosis of AML (*de novo*, treatment-related or transformed MDS), without isolated 5q abnormalities, were eligible. Additional inclusion criteria were as follows: WHO performance status $\leqslant$2; white blood cells $\leqslant$50 000/mm^3 at the time of enrolment; adequate hepatic function (total bilirubin concentration less than 2.5 times the upper normal limit (UNL), with AST and ALT concentration less than 3.5 times the UNL); adequate renal function (creatinine concentration $<$1.5 UNL); and negative HIV serology. Patients with acute promyelocytic leukemia or central nervous system leukemia, previously treated for AML, or eligible for standard therapy, were excluded.

Lenalidomide (10 mg) was administered orally once daily (days 1–21); cytarabine (20 mg/m^2 twice daily) was administered subcutaneously (days 1–15). Therapy was repeated every 6 weeks in the absence of disease progression or unacceptable toxicity, up to 6 cycles. Bone marrow evaluation was performed after 1, 2, 4 and 6 cycles. Responding patients experiencing a non-hematological toxicity $>$2 WHO received reduced courses (lenalidomide (10 mg) once daily (days 1–14), and cytarabine (10 mg, subcutaneously) twice daily (days 1–10)). All patients were hospitalized for the first cycle. Toxicities were scored using the NCI's Common Terminology Criteria for Adverse Events, version 3.[7] Responses were assessed according to the LeukemiaNet guidelines.[8] Patients who completed

Accepted article preview online 20 January 2014; advance online publication, 11 February 2014

one course of therapy were evaluable. Cytogenetic risk was assessed by the SWOG criteria.[9] The primary end point was the complete remission (CR) rate, according to the MiniMax statistical plan (Figure 1a and Supplementary information).

Thirty-three AML patients (median age 76 years, range 70–85) were enrolled. Fifteen patients had an intermediate karyotype (13 normal, 1 (-Y), 1 i(17)q), sixteen patients had an unfavorable karyotype (14 complex, 2 (+8)) and two were not evaluable. Thirteen patients had a *de novo* AML, and 20 had a secondary AML (Supplementary Table 1). Non-hematological toxicities were mild (Supplementary Table 2). After the first cycle of therapy, 4/33 patients died in documented aplasia, owing to infectious complications. One patient died during cycle 1 (acute heart failure). The cumulative induction-period mortality rate was 15% (5/33), in line with the induction-period mortality reported with other combinations classified as 'low intensity'.[10]

According to intent-to-treat, the CR rate was 33% (11/33 patients). Among 28 patients evaluable after the first cycle, 11 (39.2%) obtained a CR. Overall survival (OS) was statistically longer in patients obtaining CR (559 vs 52 days, $P<0.0001$, Figure 1b). Even if the small sample size restricts such analysis, we identified bone marrow blasts $<30\%$ at diagnosis as the only factor possibly influencing CR rate ($P=0.03$, Supplementary Table 3). After a median follow-up of 18 months (2–33), 5/11 responding patients are in continuous CR. Two patients died in CR after the second and the third cycle of therapy, because of multiorgan failure. One patient relapsed after 4 months and died; 3 patients who relapsed after 8,14 and 22 months are alive with stable disease (Supplementary Table 4). The other 17 patients who completed the first cycle were refractory and died.

Our results compare favorably with other 'low-intensity' therapies previously applied in older AML (low-dose cytarabine alone, azacitidine, tipifarnib, decitabine and vorinostat plus GO).[10] Reported CR rates with these approaches were, respectively, 15, 8, 18, 25 and 19%, with a median OS ranging from 3.6 to 25 months and induction-period mortality ranging from 10 to 25%.[10]

Our data confirm the efficacy of lenalidomide in AML patients. Fehninger showed that high-dose lenalidomide (HDL, 50 mg/day), when administered up to two sequential 28-day cycles, is able to induce CR/CRi in 30% of untreated, older AML patients.[2] However, responses were restricted to patients with low circulating blast count ($<1000\,\mu l$) at diagnosis, limiting this schedule to a minority.[2] Pollyea tested the efficacy of fixed dosage of azacitidine (75 mg/m^2/day i.v. for 7 days) followed by sequential lenalidomide (5, 10, 25 and 50 mg/day orally for 21 days) in a 42-day cycle, in 42 AML patients aged $\geqslant 60$ years. CR rate was 28%, with 4 patients alive and disease-free after 88 weeks of median follow-up.[4,5] Ramsingh *et al.*[6] reported on a prospective trial combining concomitant HDL and azacitidine (days 1–5 three-dose cohorts: 25, 50, 75 mg/m^2), followed by maintenance therapy with lenalidomide (10 mg) and azacitidine (75 mg/m^2, up to 1^2 cycles). CR/CRi rate was 30.8%. These data confirmed that HDL has clinical activity in AML, generating interest on possible combinations.[11,12] However, neither paper described a biomarker that is able to predict response or correlations between methylation and response. Furthermore, the reported CR rate was obtained with HDL, leaving the question about the activity of low-dose lenalidomide unanswered.

As no cytogenetic or known molecular abnormality (including *FLT3,* NPM1 or *CEBPA* mutations) showed any significant correlation with therapy response in our series, we aimed at identifying a potential biomarker, predictive of treatment response, by studying the global gene expression profile (GEP). AML blasts were collected from bone marrow before treatment. GEPs were generated and analyzed as previously reported using the Affymetrix Human Transcriptome 2.0 microarray[13] (see Supplementary Information). We analyzed 15 patients for whom AML cells collected at diagnosis were available and for whom a clear-cut clinical outcome (CR vs no-CR) could be defined. First, we applied an unsupervised approach that failed to discriminate any consistent subgroup: at principal component analysis, cases with different clinical outcome were quite mixed up, the variance explained by the three components being only 54% (Figure 2a). Similarly, by unsupervised hierarchical clustering, we could not identify major clinico-biological meaningful groups (Figure 2b). We then compared by supervised analysis (two-tailed *T*-test, $P<0.05$; fold change >2 and false discovery rate according to Benjamini-Hockeberg) cases who obtained ($N=7$) or did not obtain ($N=8$) a CR, and we identified 114 genes differentially expressed in the two groups (Figure 2c; Supplementary Table 5). On the basis of the expression of such genes, the samples could be successfully clustered into two groups that actually reflected the treatment response (Figure 2d). Interestingly, when we looked for specific biological functions (defined by GeneOntology) possibly enriched (i.e., significantly overrepresented) in the panel, we found blood vessel formation/angiogenesis, immune response and cell cycle regulation (Figure 2e; Supplementary Table 6). Such processes were indeed biologically sound with the proposed therapy, as lenalidomide is an antiangiogenic and immunomodulatory agent and both cytarabine and lenalidomide do interfere with the cell cycle.

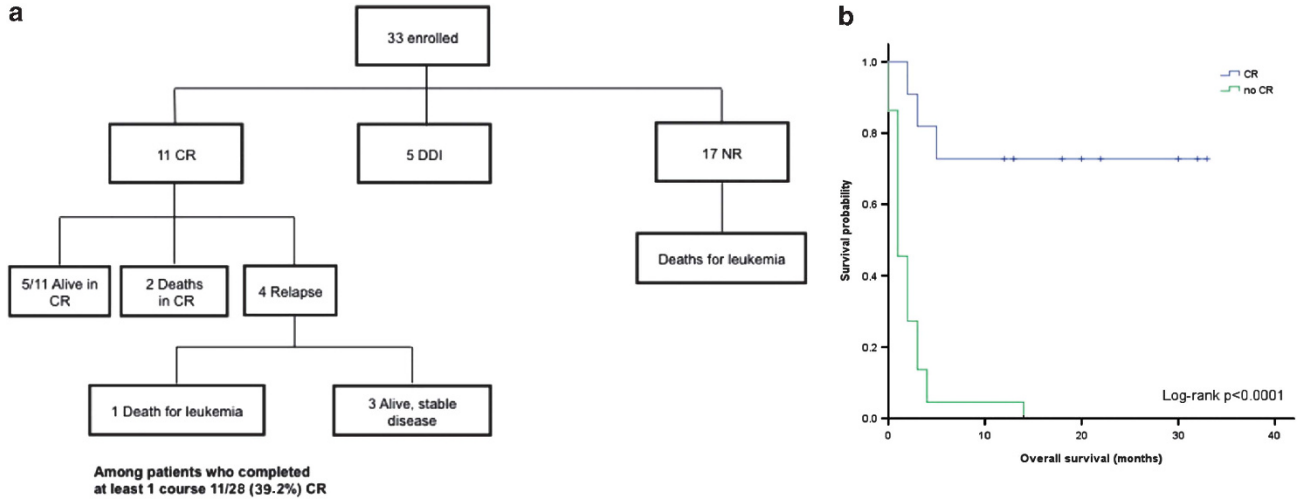

Figure 1. Study design with response to treatment (**a**). Overall survival according to response (**b**).

To make the biomarker easy to be applied in routine diagnostics, we applied a linear discriminant analysis and reduced the gene signature to as low as five genes (*CXorf40A, MAD1L1, PI4KA, PRRG4* and *SULT1C4*). We then investigated the ability of the minimal gene set (MGS) to predict treatment response in AML patients. As the limited number of samples did not allow to apply the test to an independent validation panel, we adopted a support vector machine (SVM) approach with the leave-one-out method, which ensured to reclassify each sample after having excluded it from the generation of the classifier. Remarkably, our assay correctly classified 13/15 AML samples (87%) with very high diagnostic accuracy (Supplementary Table 7). The analysis was conducted according to REMARK guidelines (Supplementary Table 8), and respected the evidence-based rules. However, as the number of cases that we could evaluate was relatively small, although the adopted algorithm (SVM with leave-one-out) partially biased this issue, it is definitely warranted to further test this assay in additional future cases.

In this report, we show for the first time that low-dose lenalidomide has clinical activity when coupled with low-dose cytarabine in AML patients aged $\geqslant$70 years, unfit for standard therapy. CR rate was 39.2% among patients evaluable after the first cycle. Patients achieving CR had a significantly longer median OS than non-responders (559 vs 52 days, $P<0.0001$). By studying the GEP, we identified a molecular signature, including 114 genes belonging to relevant functional categories (angiogenesis, cell-cycle regulation, immune response), associated with clinical response (CR versus no-CR). On the basis of the expression of five genes, we developed an algorithm to predict treatment response, successfully validated by showing an 87% overall accuracy. In this regard, it should be underlined that a major goal when testing new drugs or combinations would be to identify reliable biomarkers that are able to predict which patients are more likely to achieve clinical responses. This would allow to prevent undesirable toxicity in patients with less chance to obtain a benefit and to optimize the costs.

In conclusion, our data support, for the first time, the prospective use of a GEP-driven therapy in a cohort of hard-to-treat AML patients with an extremely poor prognosis. In the age of massive genome surveys, and after the completion of the AML-sequencing project, which demonstrated the genomic

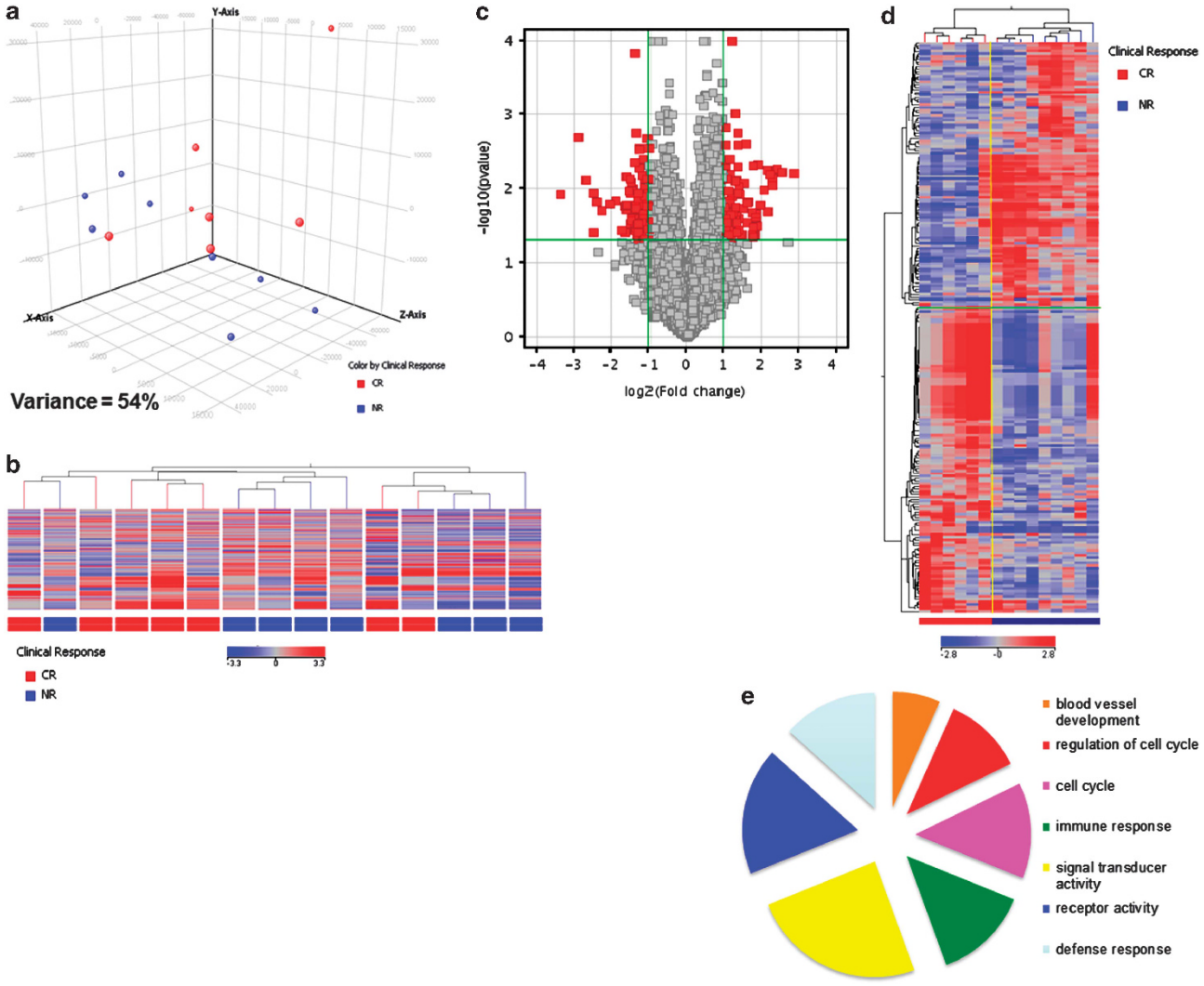

Figure 2. Unsupervised analysis (**a**, **b**). Principal component analysis (**a**) indicated an overall homogeneity in the patients' data set. Unsupervised hierarchical clustering (**b**) failed to distinguish patients according to their sensitivity/resistance to lena-ara. Supervised analysis (**c**) identified 114 genes differentially expressed in patients obtaining or not obtaining a CR ($P<0.05$; fold change >2). Based on their expression, samples were clearly distinct in the hierarchical clustering according to the clinical response (**d**). Interestingly, such genes turned out to belong to functional categories significantly overrepresented ($P<0.05$), including angiogenesis, regulation of cell cycle and immune response that might be related to the activity of lenalidomide (**e**). Specific genes are differentially expressed in patients obtaining or not obtaining a complete remission.

complexity of AML,[14] this is a step forward to an easier and highly efficacious GEP-driven therapeutic strategy.[15]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Celgene provided lenalidomide free of charge. This study was supported in part by AIL Pesaro Onlus, AIRC (5xMille 10007 and IG10519) and FIRB Futura 2011.

AUTHORS' CONTRIBUTIONS

GV, FF and AI designed the research and wrote the manuscript; FDR, MRC, CR and GS treated the patients, collected the data and commented on the manuscript; FL and AV collected and analyzed the data and commented on the manuscript; PPP and MR performed the statistical analysis. AG, MR, and MAL generated GEP; FF performed GEP data analysis; SP performed EBM diagnostic accuracy analysis; PPP, SAP, AI and GV designed the molecular analysis and funded it, analyzed GEP data and wrote the manuscript.

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Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)

MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease

Leukemia (2014) **28,** 970–973; doi:10.1038/leu.2014.41

Approximately 35–50% of the patients receiving matched related or unrelated allogeneic stem cell transplantation, develop severe forms of graft-versus-host disease (GvHD; Grade II–IV) that cannot be controlled with corticosteroids in up to 50% of the GvHD patients.[1] Owing to the lack of confirmed treatment options Le Blanc *et al.*[2] introduced mesenchymal stem cells (MSCs) as a strategy to treat severe therapy-refractory acute GvHD. Since then, a number of different studies have addressed the impact of MSC administration on GvHD with different outcomes.[3] New data suggest that the beneficial effects of MSCs rather derive from secreted, immune response-modulating factors than from their tissue intercalation themselves.[3] On the basis of a preclinical myocardial infarction model, evidence was provided that the immune-modulating factors of MSCs are also secreted *ex vivo* and

Accepted article preview online 21 January 2014; advance online publication, 21 February 2014