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Research paper

Low-dose lenalidomide plus cytarabine in very elderly, unfit acute myeloid leukemia patients: Final result of a phase II study

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ABSTRACT

Outcome for elderly patients with acute myeloid leukemia (AML) is extremely poor. Intensive induction chemotherapy is often unsuitable. Sixty-six newly diagnosed AML patients (median age: 76 years), ineligible for standard therapy, were consecutively treated with low-dose lenalidomide (10 mg/day orally, days 1–21) plus 10 mg/m^2 low-dose cytarabine, subcutaneously, twice a day (days 1–15) every six weeks, up to 6 cycles. Complete remission (CR) rate was 36.3% according to intention-to-treat. Responding patients had a longer median overall survival than non-responders (517 vs. 70 days, $P < 0.001$). The achievement of CR was not predicted by bone marrow blast count, cytogenetics, molecular markers, prior MDS, white blood cell count. Conversely, by studying the global gene expression profile, we identified a molecular signature, including 309 genes associated with clinical response (CR versus no CR). Based on the expression of a minimal set of 16 genes, we developed an algorithm to predict treatment response, that was successfully validated by showing an overall accuracy of 88%. We met the primary endpoint of the study, by beating the estimated successful CR rate (P1) fixed at 30%. Moreover, CR induced by this 2-drug combo was efficiently predicted by genetic profiling, identifying a biomarker that warrants validation in independent series.

1. Introduction

The incidence of acute myeloid leukemia (AML) increases with age, and outcome for elderly patients remains extremely poor [1]. These patients have a poor prognosis, with median overall survival rates of less than 1 year, and further duration of survival in patients aged seventy or more [2,3]. Intensive induction chemotherapy is often unsuitable for elderly patients and can result in significant periods of inpatient care [3,4]. Furthermore, no currently available treatment option for older patients with AML has shown any significant survival advantage compared to any other. Thus, novel therapeutic agents are urgently needed for older AML patients, in particular drugs with reduced toxicity and a specific mechanism of action, if compared to standard chemotherapy [5]. In this regard, a major goal when testing new drugs or combinations is to identify reliable biomarkers able to predict which patients are more likely to achieve clinical response [6].

Cytosine arabinoside is a mainstay in the treatment of acute myeloid leukemia. The cytotoxic effect depends on the conversion to its triphosphate form in leukemic cells, which is close related to the balance between phosphorylating and dephosphorylating enzymes [7]. In addition, the sensitivity to cytarabine-triphosphate-induced citotoxicity is dependent on the balance between pro and antiapoptotic signals in the leukemic cell [7].

Lenalidomide has shown to be effective in del(5q)-associated myelodisplasia (MDS) or AML [8,9], and in AML without del(5q) when administered at high doses [10,11]. A recent phase I–II study demonstrated the activity of lenalidomide in combination with azacitidine in AML patients older than 60 years [12,13]. However, up to now, no report identified reliable biomarkers able to predict which AML patients are more likely to respond to lenalidomide, either when administered

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alone at high-doses, or when combined with other drugs. Given these premises, the preliminary results of this trial, already reported elsewhere, showed the opportunity of predicting CR by studying the gene expression profile of AML blasts at diagnosis [14]. However, considering the small sample size of the first stage of our trial, those results needed to be confirmed in the entire patient population, as clearly stated in the manuscript [14].

Here we report the final results of a phase II study conducted on 66 elderly AML patients, aged seventy years or more, not eligible for standard chemotherapy, without deletion 5q, treated with low-dose lenalidomide coupled with low-dose cytarabine. Furthermore, based on the hypothesis that the genetic features might influence treatment response, we aimed at confirming the capability of predicting CR of the previously identified gene dataset [14] by studying the global gene expression profiles.

2. Materials & methods

2.1. Study design and patients

We did a prospective, multicentre, single arm study of a low-dose chemotherapy regimen combining lenalidomide and cytarabine in very elderly AML. Patients 70 years of age or older with a World Health Organization diagnosis of AML (*de novo*, treatment-related, or transformed MDS) were eligible for this study. Inclusion criteria were: previously untreated disease; no evidence of acute promyelocytic leukemia; no eligibility for standard chemotherapy; WHO performance status ≤ 2; white blood cells (WBC) $\leq 50.000/mm^3$ at the time of enrolment; normal hepatic function, defined as total bilirubin concentration less than 2.5 times the maximum normal concentration (MNC) with AST and ALT concentration less than 3.5 times the MNC; normal renal function, defined as creatinine concentration less than 1.5 the MNC, unless leukemia-related, and negative HIV serology test before enrolment. Exclusion criteria were: any prior chemotherapy for AML; concurrent therapy for another malignancy; at least 6 months since prior chemotherapy or radiotherapy for another malignancy; patients with uncontrolled insulin-dependent diabetes mellitus or uncompensated major thyroid or adrenal dysfunction; eligibility to receive standard chemotherapy for AML.

Patients signed written informed consent before enrolment. The study was approved by an independent research Ethics Committee and was done in accordance with the International Conference on Harmonisation Good Clinical Practice Guidelines, the Declaration of Helsinki (1996), and local regulatory requirements and laws.

The first stage of this study, with data available on 33 patients, was previously reported elsewhere. [14]

The study was registered at EMA with EUDRA-CT code number 2008-006790-33.

2.2. Procedures

Patients were treated with low-dose lenalidomide (10 mg) administered orally once daily (days 1–21) and low-dose cytarabine (10 mg/m^2) administered subcutaneously twice daily (days 1–15). Chemotherapy courses were repeated every 6 weeks in the absence of disease progression or unacceptable toxicity, up to 6 cycles, without the evidence of progressive disease. Bone marrow evaluation was performed after 1, 2, 4 and 6 cycles of chemotherapy. In responding patients who experienced a non-hematological toxicity > 2 according to WHO criteria, both drugs were administered at the same dosages but for a shorter period of time: lenalidomide (10 mg, orally) once daily (days 1–14) cytarabine (10 mg, subcutaneously) twice daily (days 1–10).

Treatment was intended to be administered in an outpatients basis. However, all patients required hospitalization for the first cycle of therapy. Standard antimicrobial prophylaxis and supportive care measures were as reported elsewhere [15].

Toxicities were scored using the NCI's Common Terminology Criteria for Adverse Events, version 3 [16]. A serious AE was an AE that resulted in death or immediate risk of death, prolonged hospitalization or substantial disability. Responses were assessed according to the LeukemiaNet guidelines [17]. Patients who completed one full cycle of treatment were considered evaluable. Cytogenetic risk was assessed by SWOG criteria [18].

Gene expression profile procedures, previously described elsewhere [19,20], are reported in details in the supplementary material section.

2.3. Statistical analysis

With a one-arm sample size, we calculated the sample size on the basis of a comparison to a fixed reference level. The study was designed according to the MiniMax design. The primary outcome was the complete remission rate. Fixing the lowest acceptable rate (P0) as 17% and the successful rate (P1) as 30%, with a significance level $\alpha = 0.05$ and a power $1\text{-}\beta = 0.90$, the sample size was estimated to be 66 patients, 33 during the first phase and 33 during the second phase. If 5 or fewer responses were observed during the first stage, the trial would have been stopped; if 16 of fewer responses are observed by the end of the trial, then no further investigations should be warranted, and the treatment rejected.

Statistical analysis was performed according to the intention to treat approach. The primary efficacy analysis was performed using all patients who received at least one cycle of oral lenalidomide and subcutaneous cytarabine. For further details, see supplementary materials.

2.4. Role of the funding source

This was an investigator-driven study. Celgene provided lenalidomide free of charge, without having any role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Clinical results

Fig. 1 shows the trial profile. Sixty-six AML patients with a median age of 76 years were consecutively enrolled in the study. The characteristics of patients are listed in Table 1.

All the most relevant toxicities are reported in Table 2. Briefly, 8/66 patients (12%) died in documented aplasia after having received the first cycle of therapy, due to infectious complications. One additional death occurred also during cycle 1, after 8 days of combined therapy, due to acute heart failure. Overall, CR rate was 36.3% (24/66 patients), and PR rate 3% (2/66) according to intention-to-treat. According to the study design, we met the primary endpoint of the study by achieving a 36.3% CR rate, better than the 30% fixed as the successful rate (P1). Five out of 26 responding patients (19%) are still in CR after a median follow-up time of 693 days (388–2092). Six patients died while in CR/PR without completing the 6-cicles program (1 lung cancer, 4 due to a multi-organ failure while receiving treatment, 1 unknown). Fifteen out of 26 responding patients relapsed after a median time of 330 days (range: 111–1559). At present, 11/15 relapsing patients died of leukemia, whereas 4/15 are still alive with active disease. The achievement of CR was not predicted by any clinical or biological prognostic factor widely used, such as bone marrow blasts, cytogenetics, molecular markers, prior MDS, white blood cell at diagnosis.

Fig. 2 shows cumulative DFS, cumulative OS and OS according to response. As expected, responding patients had a longer OS with respect to patient who did not respond. Nevertheless, the median OS for patients achieving a CR was 517 days [95% CI 415–1085], significantly higher ($p = 0.01$) with respect to patients who did not achieve a CR (70

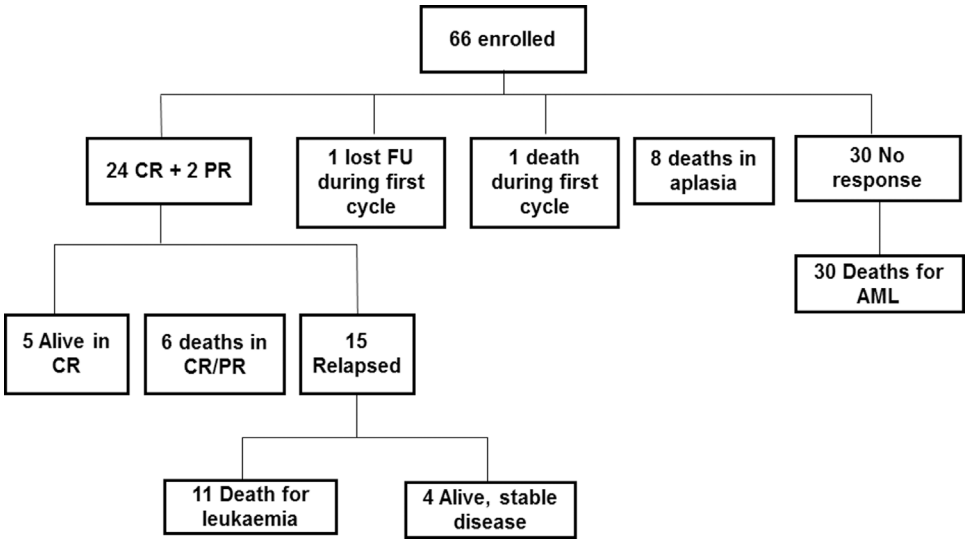


Fig. 1. Trial profile.

Table 1
Baseline characteristics (N = 66).

Characteristic	No (%) of patients	Value
Sex		
Male	37 (56)	
Female	29 (44)	
Median Age, years (range)		76 (70–85)
WHO performance status		
0–1	34 (52)	
2–3	32 (48)	
AML type		
De novo	28 (42)	
Secondary, n	38 (58)	
After MDS	29	
After Solid Tumor	3	
After CMPD	4	
After myelofibrosis	2	
Blasts, %		
Median, range		60 (20–95)
20–30	19 (29)	
31–50	11 (17)	
> 50	36 (54)	
Median haemoglobin level, g/dL (range)		9.1 (5.6–14.6)
Median platelet count, $\times 10^9$/L (range)		31 (3–339)
Median WBC count, $\times 10^9$/L (range)		3.42 (0.4–49.7)
Cytogenetic risk group[a], n		
Not evaluable	4 (6)	
Intermediate Karyotype (30/37 normal karyotype)	37 (56)	
Unfavourable Karyotype (20/25 complex karyotype)	25 (38)	

[a] Defined according to SWOG criteria (Slovack et al., Blood 2000) CMPD, chronic myeloproliferative disease; MDS, myelodysplastic syndromes; WHO, world health organization.

Table 2
Toxicities.

Event	Number of patients (%)	
Hematological Toxicity	Grade 1–2	Grade 3–4
Trombocytopenia	2 (3%)	47 (71%)
Neutropenia	0 (0%)	40 (61%)
Neutropenic fever	0 (0%)	12 (18%)
Anemia	26 (39%)	20 (30%)
Event	**Number of patients (%)**	
Non Hematological Toxicity	Grade 1–2	Grade 3–4
Epatobiliary disorders	6 (9%)	2 (3%)
Mucositis	6 (9%)	0 (0%)
Skin disorders	6 (9%)	0 (0%)
Cardiac disorders	3 (5%)	1 (2%)
Sepsis	2 (3%)	0 (0%)

The severity of adverse events was graded on a scale of 1–5 according to the NCI Common Terminology Criteria for Adverse Events (NCI-CTCAE) v4.0.

[95% CI 38–82] days).

3.2. Molecular profiling discriminated patients according to clinical response

Based on the clinical results and, specifically, on the remarkable CR rate, we next tried to identify a potential biomarker predictive of treatment response. As no cytogenetic or known molecular abnormality (including FLT3, NMP1, or CEBPA mutations) showed any significant correlation with therapy response, we decided to study the global gene expression profile (GEP). We analysed 26 patients for whom peripheral blood or marrow AML cells collected at diagnosis were still available and for whom a clear-cut clinical outcome (i. e. CR vs. no-CR) could be defined. First we applied an unsupervised approach that failed to discriminate any consistent subgroup of patients; in particular, based on a principal component analysis (PCA), cases with different clinical outcome were quite mixed up. Similarly, by unsupervised hierarchical clustering we could not identify major clinical-biological meaningful groups (data not shown). 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 = 14) or not (N = 12) a CR and we identified 309 genes differentially expressed in the two groups (Fig. 3A; Supplementary Table 1). Among others, we found several genes known to be associated with a malignant phenotype including *CXCR4, FOS, IRF2, IRF7, LEF1, LYN, MYD88, NFKBIA, PBX1,* and *RHOA*.

Based on the expression of such 309 genes, the samples could be successfully clustered into two groups that reflected the treatment response (χ^2, p = 0.04; Fig. 3A). Interestingly, when we looked for specific biological functions, as defined by the GeneOntology, possibly enriched (i.e. significantly over-represented) in the panel, we found genes related to signal transduction, cellular macromolecule metabolic process, regulation of gene expression, apoptosis, regulation of transcription, intracellular signalling cascade, protein kinase cascade, immune response, RHO protein signal transduction and IKB kinase/

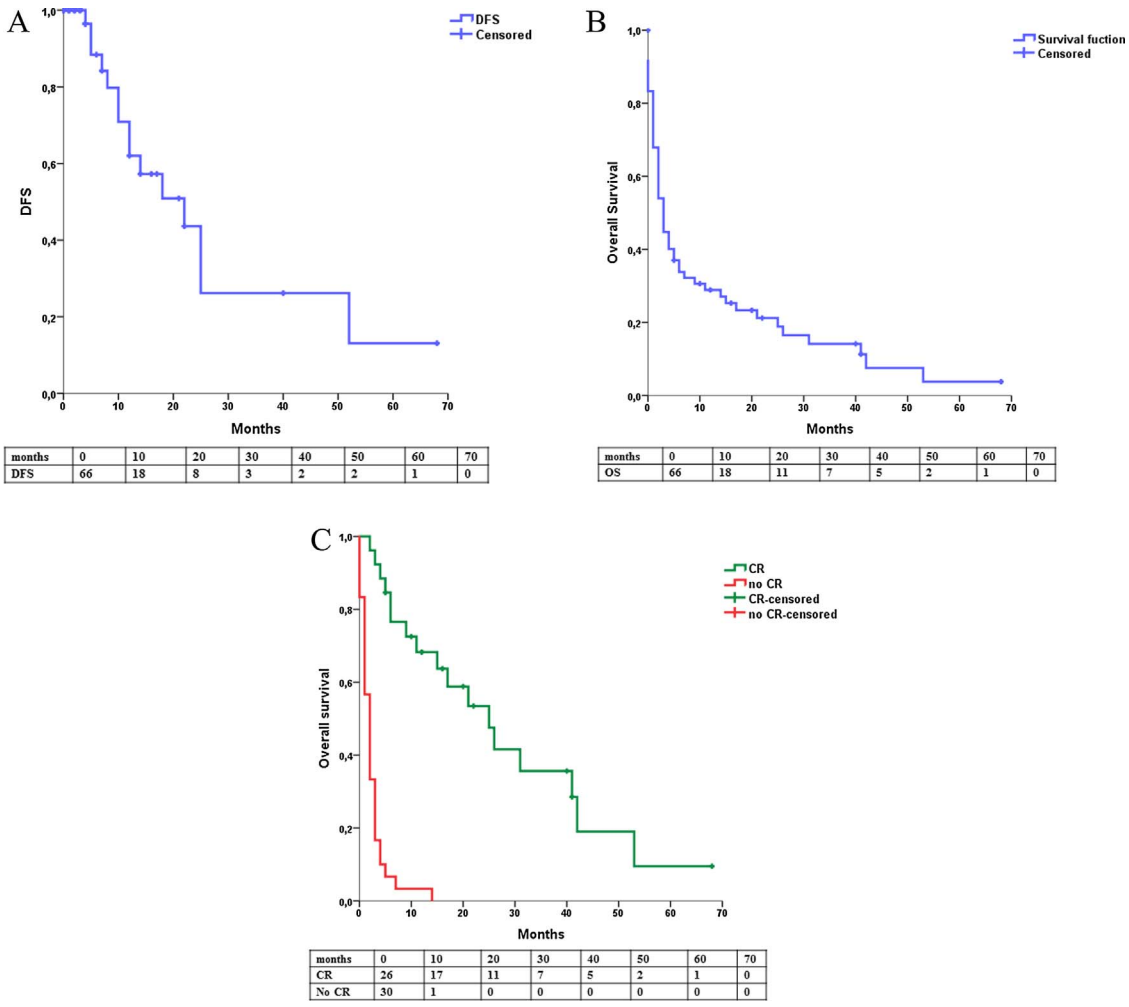


Fig. 2. A–C. Cumulative DFS (A), cumulative OS (B) and OS according to response (C).

nuclear factor K-B cascade (Fig. 3B; Supplementary Table 2). In addition, the 309 genes turned out to be significantly enriched in pathways such as AML with NPM1 mutated, MLL signature 1 and 2, EZH2 targets, targets of HOXA9 and MEIS1, ATM PCC network, reactome adaptive immune system, hematopoietic stem cell, myeloid cell development, bound by PML-RARA fusion that are either related to well characterized signalling associated to AML or, more generally, to the myeloid system and the immune response (Fig. 3C; Supplementary Table 3). Furthermore, when pathways relying on specific oncogenes were investigated, RELA/NFkB, VEGFA, HOXA9, CCND1, MTOR, PDGF and STK33 did significantly emerge (Fig. 3D; Supplementary Table 4). Of interest, such processes were indeed biologically sound with the proposed therapy as lenalidomide is an antiangiogenic and immunomodulatory agent.

We then aimed to identify an easily reproducible assay able to potentially recognize AML patients according to the sensitivity to lenalidomide and cytarabine before treatment administration. To do this, we first applied a linear discriminant analysis (based on the 309 genes) to identify a minimal gene set (MGS) of genes still capable to discriminate the two groups. We found that 16 genes (*ATF3, CHMP6, HCP5, HLA-E, HOMER3, MGC21881, MID1IP1, MLF2, PAX5, PGLS, PGM1, RHOA, RTN3, TMEM44, UBE2L3, VAMP8*) were sufficient to recapitulate the entire gene signature in terms of discriminant capability. Indeed, based on the expression of such 16 genes PCA could discriminate cases according to the clinical response (Fig. 4A).

We then investigated the ability of the MGS to predict treatment response in AML patients. As the relatively limited number of available samples did not allow to apply the test to an independent validation panel of cases, we adopted a support vector machine (SVM) approach with leave-one out method, that ensured to reclassify each sample after having excluded it from the generation of the classifier. Remarkably, our assay correctly classified 23/26 AML samples, with very high diagnostic accuracy (overall accuracy, 88%, Table 3).

To test the potential clinical impact of the assay, we compared the OS of patients classified as “predicted responder” or “predicted non-responder”. We found that the first group behaved significantly better (mean OS 16.24 months [95% CI 9.64–22.85] vs. 2.36 months [95% CI 1.06–3.67]; $p < 0.001$; Fig. 4B). Interestingly, the predicted OS curves generated with our gene set were superimposable to the OS curves of the patients treated with our combo and split according to response to therapy (CR vs. no CR).

4. Discussion

Despite the increasing knowledge in molecular pathways involved in AML development and progression, this has still not led to novel, successful therapies available in the clinical setting [21]. In this report, we showed for the first time that low-dose lenalidomide and cytarabine combination has clinical activity in elderly AML patients, aged 70 years or more, unfit for standard induction therapy. Remarkably, a specific molecular signature, independent from the cytogenetic or known molecular abnormalities already described (including FLT3, NMP1, or CEBPA mutations), discriminated patients according to treatment response.

Recent understanding of leukemia stem cell cycling suggests that

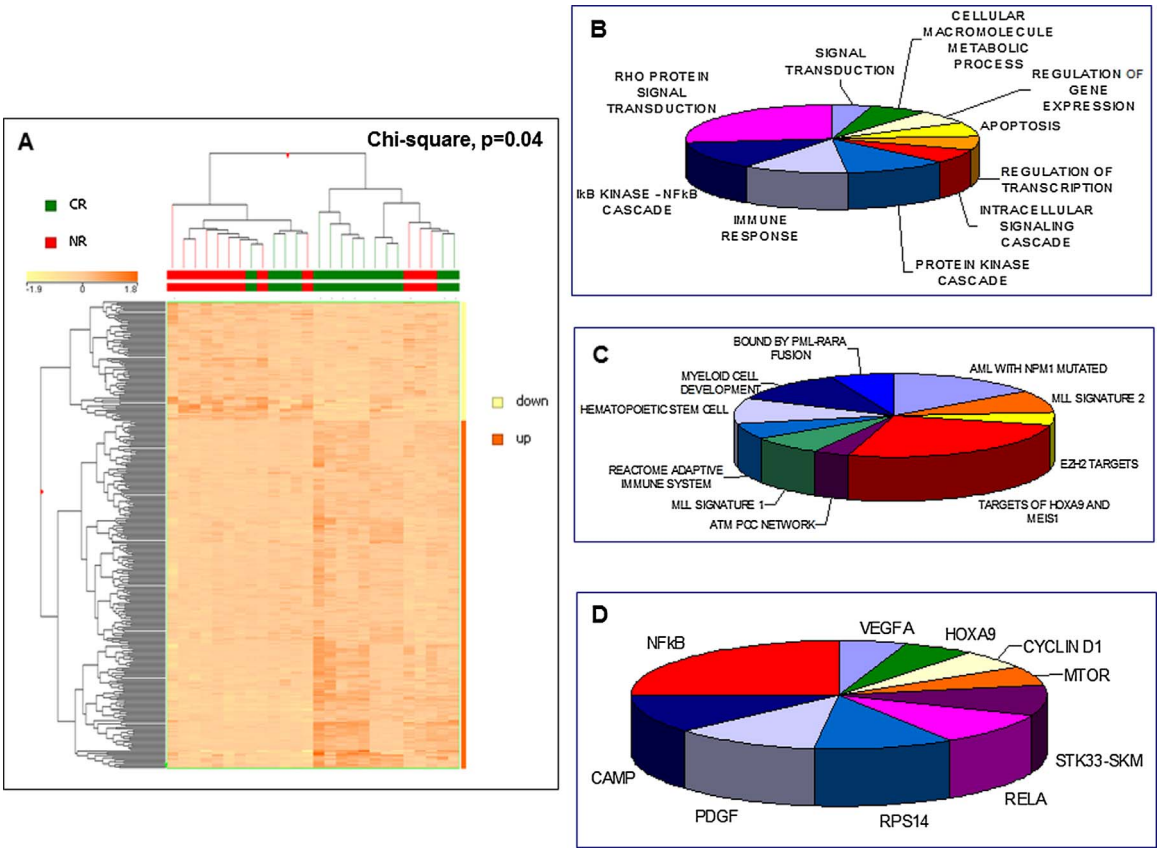
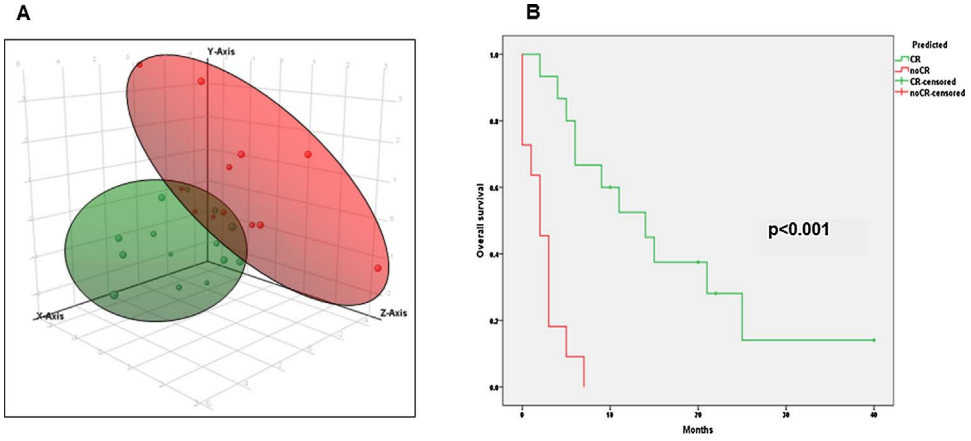


Fig. 3. A–D. A) Supervised hierarchical clustering based on genes differentially expressed in cases achieving (CR) or not (NR) a complete remission. Such genes were significantly enriched in cancer/leukemia related pathways (B), relevant biological processes (C) and pathways mastered by known oncoprotein (D).

prolonged cytotoxic exposure (14–21 days) could provide a more valuable anti-leukemic effect than the typical 3–7 days schedule. This could be due to a major number of leukemic stem cells able to undergo cell division in a longer period of time. Low-dose cytarabine has been widely used in AML treatment. The mechanism of action of low-dose cytarabine is still debated; however, even if differentiation induction has been claimed to be important, the majority of clinical studies supports the view of a preponderant cytotoxic effect on leukemia cells. In this view, the prolonged use of low-dose cytarabine (15 days) could be necessary to exert a cytotoxic effect ("debulking"), but it is surely not enough to explain the 36.3% CR rate of our study. In fact, low-dose cytarabine is active in AML, but the percentage of responses does not generally exceed 15–18% and the duration of response is short. Furthermore, the addition of Gemtuzumab Ozogamicin (GO) to low-dose cytarabine improved the CR rate, but only to 21% [22].

High-dose lenalidomide (50 mg/day) was able to induce CR/CRi in 30% of untreated, older AML patients [6] and in 16% of relapsed/refractory patients [5]. However, responses were reported almost exclusively in patients with low circulating blast count (< 1000 μl) at diagnosis, limiting this schedule to a minority of elderly AML patients. Pollyea and co-workers tested in a phase I–II study the efficacy of azacitidine followed by sequential lenalidomide at increasing doses in 42 AML patients aged 60 years or older. CR rate was 28%, with 4 patients being alive and disease-free after a median follow-up for



months	0	10	20	30	40
CR	15	8	4	1	0
No CR	11	0	0	0	0

Fig. 4. A–B. A) Linear discriminant analysis was then used to reduce the number of genes able to predict the clinical response up to 16. B) Overall survival of patients predicted to achieve a CR was significantly better than in patients predicted for NR (mean OS 24,37 mo vs. 1,36 mo; p < 0.001).

Table 3
Evidence based analysis of diagnostic accuracy of the 5-genes based molecular classifier.

	Lena-AraC Sensitivity		
	Present	Absent	
Test result			Likelihood Ratios
Positive	13	2	5.57 (1.56–19.91)
Negative	1	10	0.09 (0.01–0.58)
Sensitivity	93%; CI: 79–100		
Specificity	83%; CI: 62–100		
Positive Predictive Value	87%; CI: 69–100		
Negative Predictive Value	91%; CI: 74–100		

CatMaker, Centre for Evidence Based Medicine, Oxford University, http://www.cebm.net.

surviving patients of 88 weeks. However, the Authors did neither describe a reliable biomarker able to predict response nor a correlation between methylation and response.

The debulking effect of cytarabine could favourably couple with the principal mechanism of action of lenalidomide, operating not only in chronic lymphocytic leukemia, but even interacting with AML cells. In fact, recent evidence [23] shows a close cross-talk between leukemia cells and immune elements, with lenalidomide exerting a direct effect on the leukemic microenvironment, as well as modifying and improving cell to cell connections. This latter effect of lenalidomide is most probably favouring the cytotoxic effects of low-dose cytarabine, giving a preliminary, possible explanation of the promising results arising from our trial.

In our study, induction-period mortality was 12% (8/66). This is in line with the induction-period mortality reported from other drug combinations classified as “low-intensity”, and supports the classification of our combined schedule as a “low-intensity” therapy. Moreover, our results compare favourably with other “low-intensity” therapies that have been evaluated in older AML including low-dose cytarabine alone [24], azacitidine [25], tipifarnib [26], decitabine [27,28] and vorinostat plus GO [29]. Reported CR rates with low-dose cytarabine alone, azacitidine, tipifarnib, decitabine and vorinostat plus GO were, respectively, 15%, 8%, 18%, 25% and 19%, with a median OS of 3.6–25 months and induction-period mortality of 10–25%.

In this study, whereas the conventional molecular-genetic analysis failed to provide significant prognostic information, we were able to identify, with GEP, few relevant genes differentially expressed in patients achieving or not a CR. This confirmed that, beside the major genetic events occurring in AML cells, activation and silencing of pathways play a significant role. Furthermore, it highlights the intriguing possibility that specific treatment schedules, thought not directly targeted (e.g anti-ABL1, anti PML/RARA), might revert the prognostic impact of certain genotypes. Actually, we found that the 309 differentially expressed genes were representative of known pathways aberrantly regulated in malignant cells. Notably, some of them are known targets of lenalidomide, including VEGFA and more generally angiogenesis, the immune response and the NFkB pathway. Based on these findings, we also tried to evaluate whether targeted GEP might be regarded as a useful pre-treatment assessment to identify patients more likely to achieve a CR and a prolonged survival. We identified 16 genes that appeared to be promising in this sense. In fact, based on their expression patterns, we could clearly separate patients who achieved or not CR. Certainly, independent validation in future studies with lenalidomide and cytarabine are warranted.

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, unfit for standard therapy, 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 complexity of AML [30], this is a step forward to an easier and highly active GEP-driven therapeutic strategy.

Conflict-of-interest statement

The authors declare no competing financial interests.

Authors’ contributions

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

Ethics committee approval

The study was approved by an independent research Ethics Committee and was done in accordance with the International Conference on Harmonisation Good Clinical Practice Guidelines, the Declaration of Helsinki (1996), and local regulatory requirements and laws.

Role of funding source

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

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.leukres.2017.09.019.

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