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1 Article

2 DNA Repair Expression Profiling to Identify 3 High-Risk Cytogenetically Normal Acute Myeloid 4 Leukemia and Define New Therapeutic Targets

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17 **Abstract:** Cytogenetically normal acute myeloid leukemias (CN-AML) represent about 50% of total
18 adult AML. Despite the well-known prognosis role of gene mutations such as *NPM1* mutations or
19 *FLT3* internal tandem duplication (*FLT3*-ITD), clinical outcomes remain heterogeneous in this
20 subset of AML. Given the role of genomic instability in leukemogenesis, expression analysis of
21 DNA repair genes might be relevant to sharpen prognosis evaluation in CN-AML. Publicly
22 available gene expression profile dataset from two independent cohorts of patients with CN-AML
23 were analyzed (GSE12417). We investigated the prognostic value of 175 genes involved in DNA
24 repair. Among these genes, 23 were associated with a prognostic value. The prognostic information
25 provided by these genes was summed in a DNA repair score to consider connection of DNA repair
26 pathways. DNA repair score allowed to define a group of patients (n=87; 53,7%) with poor median
27 overall survival (OS) of 233 days (95% CI: 184-260). These results were confirmed in the validation
28 cohort (median OS: 120 days; 95% CI: 36-303). In multivariate Cox analysis, the DNA repair score,
29 *NPM1* and *FLT3*-ITD mutational status remained independent prognosis factors in CN-AML.
30 Combining these parameters allowed the identification of three risk groups with different clinical
31 outcomes in both training and validation cohorts. Combined with *NPM1* and *FLT3* mutational
32 status, our GE-based DNA repair score might be used as a biomarker to predict outcomes for
33 patients with CN-AML. DNA repair score has the potential to identify CN-AML patients whose
34 tumor cells are dependent on specific DNA repair pathways to design new therapeutic avenues.

35 **Keywords:** acute myeloid leukemia; normal karyotype; DNA repair; risk score; precision medicine

37 1. Introduction

38 Acute myeloid leukemia (AML) is the most frequent type of adult leukemia. When analyzed
39 with conventional cytogenetics, about 40-50% of AML exhibit no chromosomal abnormalities, and
40 are defined as “cytogenetically normal AML” (CN-AML)[1]. Recurrent mutated genes in CN-AML
41 were identified, such as *NPM1*, signal transduction genes (*FLT3*) or myeloid transcription factor
42 genes (*CEBPA*, *RUNX1*)[2]. Based on presence, absence and allelic ratio of these mutations, CN-AML
43 may be classified in favorable, intermediate or adverse prognosis, illustrating the high heterogeneity
44 of clinical outcomes in this AML subset[3]. Yet, a wide diversity of gene mutations occurring in

45 CN-AML were revealed by deep sequencing techniques, such as mutations of DNA modification,
 46 cohesin or tumor-suppressor genes, suggesting the wide heterogeneity of molecular mechanisms
 47 involved in leukemogenesis[4-6].

48 Even if the study of mutational landscape by new DNA sequencing technologies demonstrated
 49 a low mutation frequency in AML compared to others cancers[7], genomic instability remains a
 50 well-described leukemogenesis mechanism, illustrated by the high frequency of AML with
 51 non-random cytogenetics abnormalities or with complex karyotype[8, 9]. Therefore, the role of DNA
 52 damage response (DDR) in the AML field has been widely studied. Polymorphic variants of genes
 53 involved in several DNA repair pathways had been associated with the onset of AML, such as
 54 XPD-Lys751Gln, involved in the nucleotide excision repair mechanism[10]. Recurrent AML fusion
 55 transcripts such as RUNX1-RUNX1T1 or PML-RARA has also been demonstrated to downregulate
 56 the expression of genes implied in DDR[11-14]. Moreover, children or young adults AML are often
 57 associated with hereditary diseases due to DNA repair gene mutations, such as Fanconi disease[15],
 58 Bloom syndrome or Werner syndrome[16]. Finally, dysregulation in DDR also contribute to
 59 increased resistance to conventional chemotherapy by several mechanisms, such as paradoxical
 60 increased expression of DDR or cell cycle check-point genes[17-19].

61 In the current study, we investigate the prognostic value of genes related to the major DNA
 62 repair pathways. The data reveals specific patterns of gene expression in CN-AML that have
 63 prognostic value. Therefore, the expression analysis of DNA repair genes might be relevant in the
 64 context of CN-AML to sharpen prognosis evaluation of this heterogeneous AML subset.

65 2. Results

66 2.1. Linking Expression of DNA Repair Genes and AML Patient Overall Survival

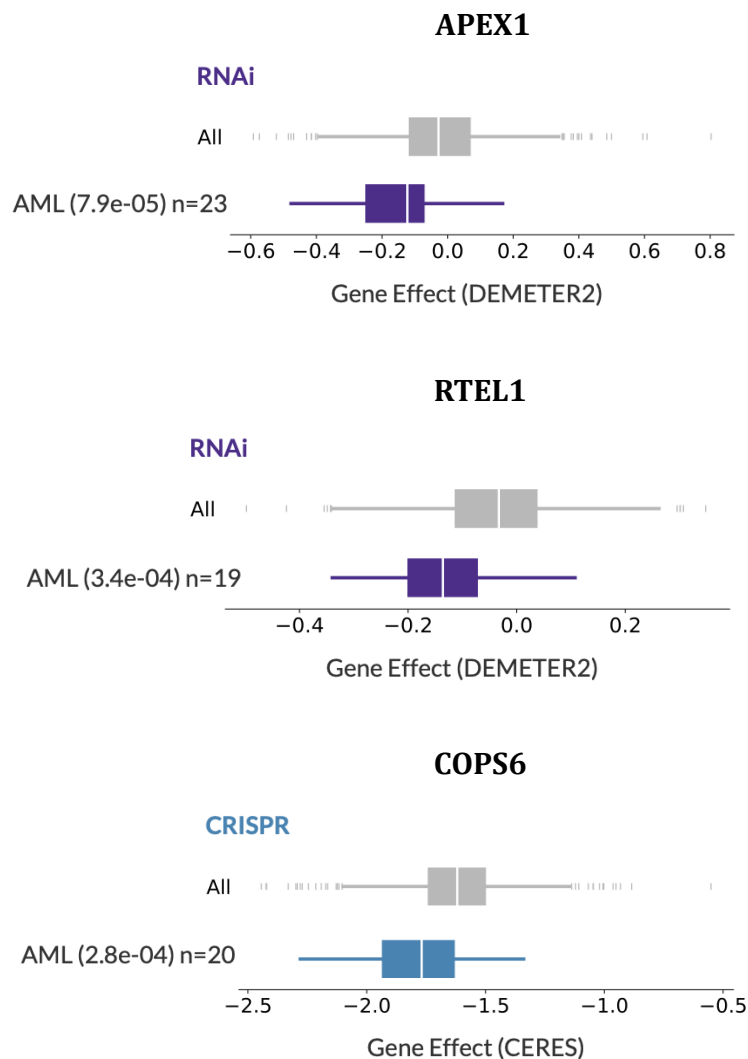
67 Considering the important role of DNA repair in drug resistance and adaptation to replication
 68 stress in cancer cells, we first aimed to identify the DNA repair genes associated with overall
 69 survival in CN-AML. A list set of 175 genes involved in six major DNA repair pathways (base
 70 excision repair (BER), NER, mismatch repair (MMR), homologous recombination repair (HRR), non-
 71 homologous end joining (NHEJ) and FANC pathways) was defined using the REPAIRtoire database
 72 (<http://repairtoire.genesilico.pl>) and review of the literature (Supplementary Table S1). Using the
 73 MaxStat R function, we identified 23 out of the 175 genes which level of expression had a prognostic
 74 value in the two independent cohorts. Nineteen genes were associated with poor prognosis and 4
 75 genes with good prognosis (**Table 1**). No statistically significant prognostic value was found for any
 76 gene involved in NHEJ pathway.

77 **Table 1. List of the 23 probe sets associated with good or bad prognosis in CN-AML.**
 78 Corresponding DNA repair pathway, gene symbol, adjusted p-value, hazard ratio and prognosis
 79 significance are provided for each gene.

DNA repair pathway	Probe set	Gene symbol	Benjamini Hochberg corrected p-value	Hazard ratio	Prognosis
Base Excision Repair pathway (BER)	210027_s_at	APEX1	0.02	1.6	Bad
	209731_at	NTHL1	0.0016	1.9	Bad
	202330_s_at	UNG	0.0095	2	Bad
	203655_at	XRCC1	0.022	1.6	Bad
Fanconi pathway (FANC)	209902_at	ATR	0.0048	1.8	Bad
	214727_at	BRCA2	0.0049	0.58	Good
	203719_at	ERCC1	0.0037	1.9	Bad
	203678_at	FAN1	0.0028	1.8	Bad
	221206_at	PMS2 ///	0.024	1.8	Bad
	219317_at	PMS2CL POLI	0.0016	1.9	Bad
Homologous Recombination Repair	214727_at	BRCA2	0.0049	0.58	Good
	205395_s_at	MRE11A	0.015	1.8	Bad

pathway (HRR)	205647_at	RAD52	0.044	1.9	Bad
	206092_x_at	RTEL1	0.00047	2.5	Bad
	212275_s_at	SRCAP	0.014	0.6	Good
	207598_x_at	XRCC2	0.007	1.7	Bad
Mismatch Repair pathway (MMR)	205887_x_at	MSH3	0.000043	2.8	Bad
	221206_at	PMS2 ///	0.024	1.8	Bad
	1053_at	PMS2CL RFC2	0.023	1.6	Bad
Nucleotide Excision Repair pathway (NER)	201405_s_at	COPS6	0.011	1.7	Bad
	213579_s_at	EP300	0.019	0.59	Good
	203719_at	ERCC1	0.0037	1.9	Bad
	205162_at	ERCC8	0.04	1.5	Bad
	223758_s_at	GTF2H2	0.033	1.5	Bad
	201046_s_at	RAD23A	0.0067	0.53	Good
	205672_at	XPA	0.0035	1.8	Bad
203655_at	XRCC1	0.022	1.6	Bad	

80 To further corroborate gene expression data on a functional level, we studied CRISPR or RNAi
 81 screening publicly available data (Dependency Map data, Broad Institute, www.depmap.org)[20,
 82 21]. Interestingly, among the 19 genes associated with a poor outcome, *APEX1* (BER), *RTEL1* (HRR)
 83 and *COPS6* (NER) were identified as significant essential AML genes ($p = 7.9e-05$, $3.4e-04$ and $2.8e-04$
 84 respectively) (Figure 1).



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Figure 1. Silencing of APEX1, RTEL1 and COPS6 impairs AML cell growth. Using CRISPR or RNAi screening publicly available data (Dependency Map data, Broad Institute, www.depmap.org),

88 dependency scores of APEX1, RTEL1 and COPS6 underline their specific importance for AML cell
89 survival compared to all cell lines tested.

90 2.2. GEP-Based DNA Repair Score for Predicting CN-AML Patients' Survival

91 Then, we searched to combine the prognostic information of these genes in a GE-based DNA
92 repair risk score. The 23 DNA repair genes associated with a prognostic value included 4 coding
93 genes for BER pathway, 6 genes for FANC pathway, 6 genes for HRR pathway, 3 genes for MMR
94 pathway and 8 genes for NER pathway (**Table 1**). Four out of these 23 probesets (BRCA2, ERCC1,
95 PMS2//PMS2CL and XRCC1) were involved in two different pathways. A specific GE-based risk
96 score was established for BER, FANC, HRR, MMR and NER DNA repair pathways. GE-based DNA
97 repair scores were defined by the sum of the beta coefficients of the Cox model for each prognostic
98 gene, weighted by +1 or -1 according to the patient signal above or below / equal the probe set
99 MaxStat value as previously described[22, 23]. Using Maxstat R function, high BER, FANC, HRR,
100 MMR and NER score values were significantly associated with poor prognosis in the training cohort
101 (**Supplementary Figure S1**).

102 In Cox multivariate analysis, only HRR and NER scores remained associated with overall
103 survival in the training cohort (**Table 2**). Therefore, a global DNA repair score was established,
104 incorporating the prognostic value of HRR and NER scores. To this aim, CN-AML patients were
105 split in three subgroups: group I included patients with low NER and HRR risk score values (n=20),
106 group III included patients with high NER and HRR risk scores (n=87) and group II included
107 patients with NER or HRR high-risk score value (n=55).

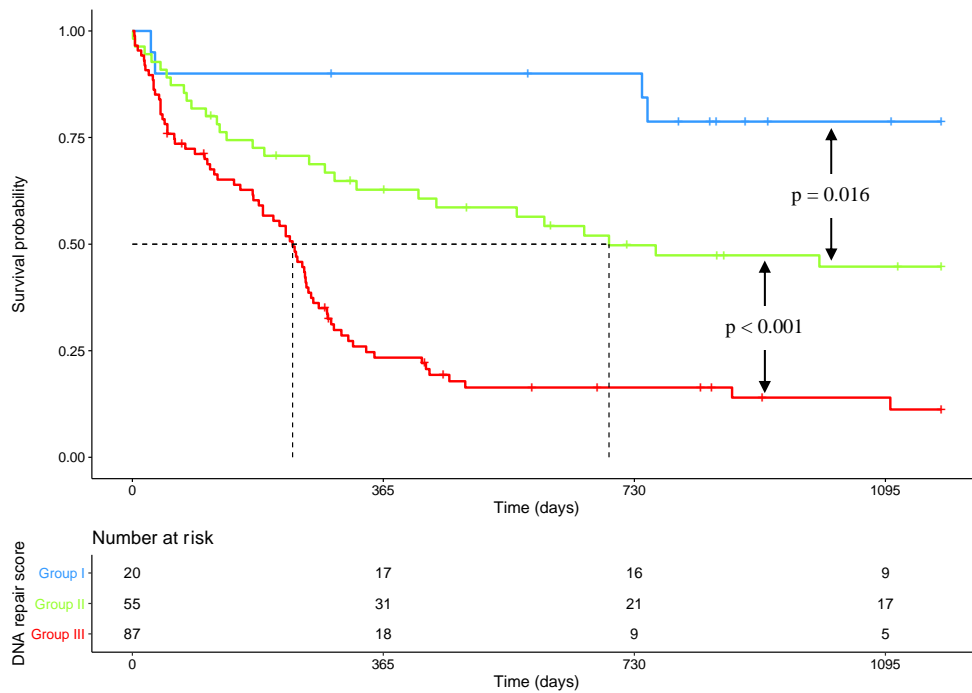
108 **Table 2. Cox analysis of overall survival in CN-AML training cohort (n=162) according to DNA**
109 **repair pathway scores.** Hazard ratio (HR) and p-values are shown for each DNA repair pathway
110 score in univariate and multivariate Cox analysis. NS: not significant.

DNA repair pathway score	Univariate Cox analysis		Multivariate Cox analysis	
	HR	p-value	HR	p-value
BER score	1.97	1.44e-03	0.93	NS
FANC score	2.32	2.98e-05	1.30	NS
HRR score	3.23	2.16e-07	2.36	5.89e-04
MMR score	2.80	1.59e-04	1.58	NS
NER score	3.83	2.90e-04	2.54	1.66e-02

111 After a median follow-up of 1176 days (95% CI: 916-NR), median overall survival (OS) was 293
112 days (95% CI: 252-461) for the whole training cohort (**Supplementary Figure S2a**). One-year OS was
113 45.2% (95% CI: 38.0-53.8). According to risk groups determined by the DNA repair score, median OS
114 was not reached (95% CI: NR-NR), 693 days (95% CI: 414-NR) and 233 days (95% CI: 184-260)
115 respectively for patients in groups I, II and III (**Figure 2a**). Median OS were statistically different
116 between each risk group (log-rank test; p = 0.016 between group I and II; p < 0.001 between group II
117 and III).

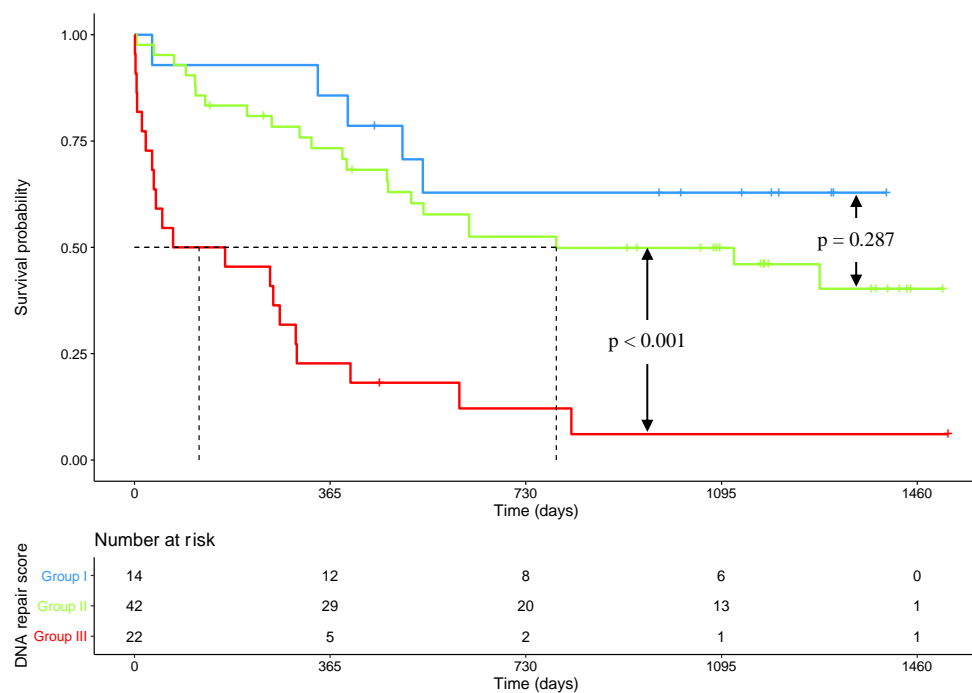
118 We searched to validate these results in an independent cohort of 78 patients. HRR and NER
119 scores computed with training cohort parameters were also prognostic in this validation cohort
120 (**Supplementary Table S2**). The global DNA repair score was also computed. In the validation set,
121 risk groups included 14, 42 and 22 patients respectively in groups I, II and III. After a median
122 follow-up of 1183 days (95% CI: 1092-1383), median overall survival (OS) was 538 days (95% CI:
123 388-1278) for the whole validation cohort (**Supplementary Figure S2b**). One-year OS was 61.1%
124 (95% CI: 51.1-73.0). According to risk groups determined by the DNA repair score, median OS was
125 not reached (95% CI: 538-NR), 787 days (95% CI: 473-NR) and 120 days (95% CI: 36-303) respectively
126 for patients in groups I, II and III (**Figure 2b**). Even if survival analysis failed to demonstrate a
127 statistical difference between groups I and II (log-rank test; p = 0.287), OS was still statistically
128 different between risk groups II and III (log-rank test; p < 0.001). Altogether, these data underlined
129 the identification of high-risk CN-AML patients characterized by DNA repair dysregulation and
130 that could benefit from DNA repair targeted treatment.

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Figure 2. Kaplan-Meier survival curves according to risk stratification determined by DNA repair score. (a) Kaplan-Meier survival curve in the training cohort (n=162). Median OS was not reached (95% CI: NR-NR), 693 days (95% CI: 414-NR) and 233 days (95% CI: 184-260) respectively for patients in groups I (low DNA repair score), II (medium DNA repair score) and III (high DNA repair score). One-year OS was 90.0% (95% CI: 77.7-100) in group I, 62.8% (95% CI: 51.1-77.2) in group II, and 23.4% (95% CI: 15.8-34.7) in group III. **(b)** Kaplan-Meier survival curve in the validation cohort (n=78). Median OS was not reached (95% CI: 538-NR), 787 days (95% CI: 473-NR) and 120 days (95% CI: 36-303) respectively for patients in groups I (low DNA repair score), II (medium DNA repair score) and III (high DNA repair score). One-year OS was 85.7% (95% CI: 69.2-100) in group I, 73.3% (95% CI: 60.9-88.2) in group II, and 22.7% (95% CI: 10.5-49.1) in group III. P-values are determined with log-rank test. NR: not reached.

146 2.3. DNA Repair Score and NPM1 / FLT3 Mutational Status Combination as Prognosis Factors in CN-AML

147 Because NPM1 mutations and FLT3-ITD (internal tandem duplications) are well-described
 148 prognosis factors in CN-AML, we conducted another Cox analysis to determine whether our DNA
 149 repair score provides additional prognostic information. Prognostic classification according to
 150 NPM1 and FLT3 mutational status was established in both cohort according to actual
 151 recommendations[3]: patients with only NPM1 mutation were classified as “better outcome”,
 152 patients with only FLT3-ITD were classified as “adverse prognosis” and patients with both or none
 153 of these mutations were classified as “intermediate prognosis”. Kaplan-Meier survival curves
 154 according to NPM1 and FLT3 mutational status are presented in **Supplementary Figure S3** for both
 155 cohorts.

156 Using multivariate Cox analysis, our DNA repair score and NPM1/FLT3 mutation classification
 157 remained independently associated with survival (**Table 3 & Supplementary Table S3**). Therefore,
 158 we investigated the interest of combining DNA repair score and NPM1 / FLT3 mutational status to
 159 predict CN-AML outcome. Patients were classified according to prognosis value of DNA repair
 160 score (0 point for group I; 1 for group II; 2 for group III), and NPM1 / FLT3 mutational status (0 point
 161 if NPM1 mutated without FLT3-ITD; 2 points if FLT3-ITD without NPM1 mutation; 1 point in other
 162 situations). The sum of the prognostic information was computed for all patients, allowing to
 163 separate patients in three new prognostic groups: group A including patients with 0 or 1 point,
 164 group B for patients with 2 points and group C for patients with 3 or 4 points. (**Table 4**).

165 **Table 3.** Cox analysis of overall survival in CN-AML training cohort (n=162) according to DNA
 166 repair score, and NPM1 & FLT3 mutational status. Hazard ratio (HR) and p-values are shown for each
 167 parameter in univariate and multivariate Cox analysis. ITD: internal tandem duplication.

Scores	Univariate Cox analysis		Multivariate Cox analysis	
	HR	p-value	HR	p-value
DNA repair score	2.76	1.49e-08	2.66	5.1e-08
NPM1 mutation / FLT3-ITD classification	1.81	1.18e-04	1.76	6.2e-04

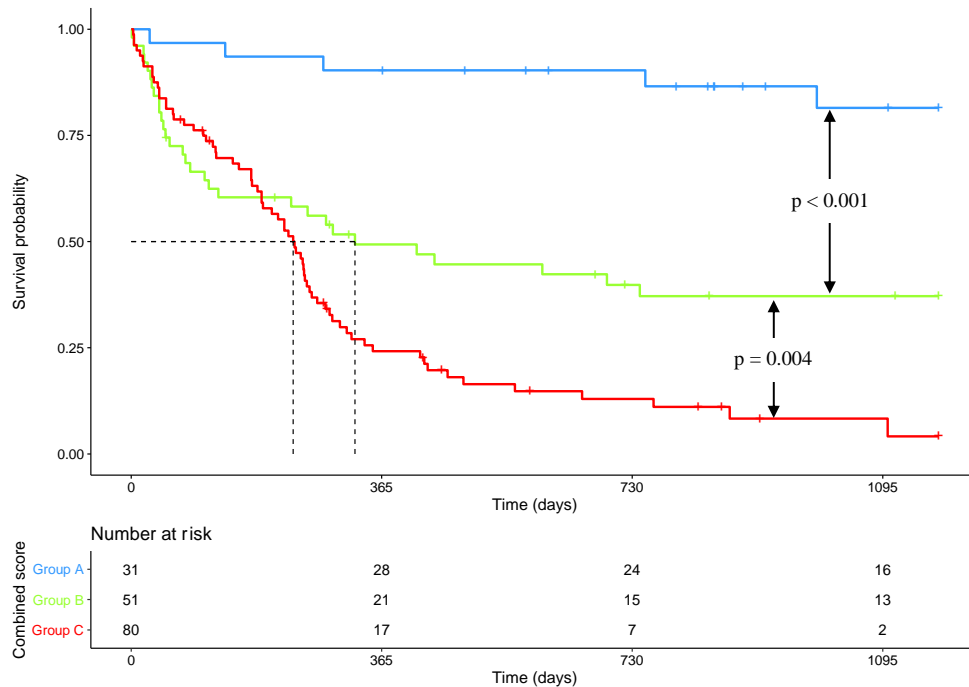
168 **Table 4.** DNA repair score and NPM1 / FLT3 mutational status combination in order to establish a
 169 global prognosis score in CN-AML. Patients were classified according to DNA repair score risk
 170 group (I, II or III) and NPM1 / FLT3 mutational status. Patients with NPM1 mutation and FLT3-ITD
 171 are respectively designated by NPM1+ and FLT3-ITD+. Patients without NPM1 mutation or
 172 FLT3-ITD are respectively designated by NPM1- and FLT3-ITD-. Points were attributed as described
 173 in the table. Patients with 0 or 1 point were grouped in group A (green), patients with 2 points were
 174 grouped in group B (yellow), and patients with 3 or 4 points were grouped in group C (red). ITD:
 175 internal tandem duplication.

		Classification according to DNA repair score		
		Group I 0 point	Group II 1 point	Group III 2 points
NPM1 and FLT3 mutational status	NPM1+ and FLT3-ITD- 0 point	0	1	2
	NPM1+ and FLT3-ITD+ or NPM1- and FLT3-ITD- 1 point	1	2	3
	NPM1- and FLT3-ITD+ 2 points	2	3	4

176 In the training cohort, median OS was not reached (95% CI: NR-NR), 326 days (95% CI: 127-NR)
 177 and 236 days (95% CI: 190-263) respectively for patients in groups A, B and C. One-year OS was
 178 90.3% (95% CI: 80.5-100) in group A, 49.3% (95% CI: 37.1-65.7) in group B, and 24.2% (95% CI:
 179 16.2-36.2) in group C. These results were confirmed in the validation cohort where median OS was
 180 not reached (95% CI: 1278-NR), 516 days (95% CI: 308-NR) and 253 days (95% CI: 52-403) for patients

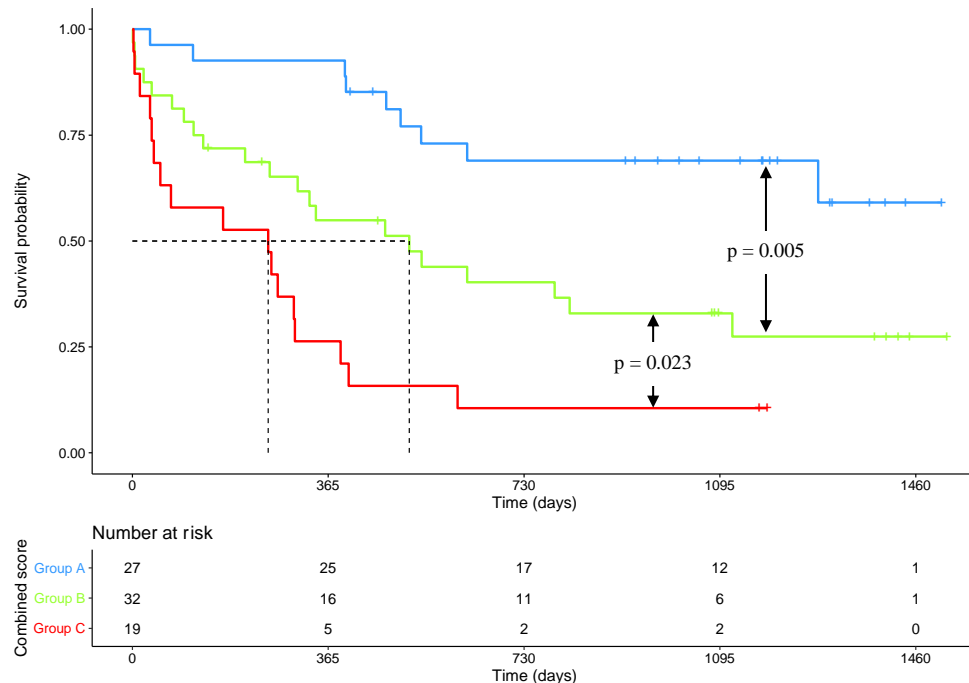
181 respectively in groups A, B and C. One-year OS was 92.6% (95% CI: 83.2-100) in group A, 54.9% (95%
 182 CI: 39.8-75.7) in group B, and 26.5% (95% CI: 12.4-55.8) in group C. OS was statistically different
 183 between groups A, B and C in both training and validation cohorts (**Figure 3**). Altogether, these data
 184 underlined the interest of GEP-based DNA repair deregulations, alone or in combination with
 185 *NPM1* and *FLT3* mutational status to identify high-risk CN-AML patients.
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Figure 3. Kaplan-Meier survival curves according to risk groups determined by combined score incorporating DNA repair score and *NPM1/FLT3* mutational status. (a) Kaplan-Meier survival curve in the training cohort (n=162). Median OS was not reached (95% CI: NR-NR), 326 days (95% CI: 127-NR) and 236 days (95% CI: 190-263) respectively for patients in groups A, B and C. One-year OS was 90.3% (95% CI: 80.5-100) in group A, 49.3% (95% CI: 37.1-65.7) in group B, and 24.2% (95% CI: 16.2-36.2) in group C. (b) Kaplan-Meier survival curve in the validation cohort (n=78). Median OS was not reached (95% CI:

196 1278-NR), 516 days (95% CI: 308-NR) and 253 days (95% CI: 52-403) respectively for patients in groups A, B
197 and C. One-year OS was 92.6% (95% CI: 83.2-100) in group A, 54.9% (95% CI: 39.8-75.7) in group B, and
198 26.5% (95% CI: 12.4-55.8) in group C. P-values are determined with log-rank test.

199 3. Discussion

200 Despite improvement in prognosis classification, mostly based on the identification of gene
201 mutations such as *NPM1*, *FLT3* or *CEBPA*, outcomes in CN-AML remain heterogeneous, underlying
202 the wide diversity of this AML subset. In this study, we developed a GE-based score using data from
203 genes involved in DNA damage response. Our model succeeded to predict poor outcomes in two
204 independent cohorts of adult patients with CN-AML treated with intensive chemotherapy.
205 Combining DNA repair score with *NPM1* and *FLT3*-ITD mutational status allows to distinguish
206 three prognostic groups including a low-risk group with a not reached median OS after a median
207 follow-up of more than 3 years in both cohorts, a high-risk group with a median OS of about 8
208 months in both cohorts, and an intermediate risk-group. This model may therefore be used for risk
209 stratification in CN-AML.

210 Among the GEP-based defined DNA-repair scores built in our study, HRR and NER scores
211 remained independent prognostic factors in CN-AML. HRR pathway is a process involved in DNA
212 double-strand break (DSB) repair, in which complementary sister chromatid is used as a template
213 for an error-free repair of DNA sequence[24, 25]. Among the prognostic factors composing the DNA
214 repair score, MRE11A is a nuclease involved in the MRN complex (for MRE11 - RAD50 - NBS1)
215 which acts as a sensor for DSB damage[26, 27]. RAD52, BRCA2, XRCC2 are proteins directly
216 involved in the DNA repair process[25, 28], and RTEL1 and SRCAP are regulators of HRR[29, 30].
217 NER pathway is involved in recognition and repair of lesions that disrupt DNA double helix, such
218 as adducts or inter-strand crosslinks (ICL)[31, 32]. RAD23A and COPS6 are involved in DNA
219 damage recognition. The recruitment of the DNA incision complex, in which ERCC1, ERCC8 and
220 GTF2H2 are involved, is mediated by XPA[31, 33]. XRCC1 and EP300 are respectively involved in
221 DNA final ligation process and NER regulation[34, 35]. Several polymorphisms in genes involved in
222 HRR and NER have been correlated with AML onset and outcome. RAD51 is a key protein in HRR
223 pathway. Its polymorphic variant *RAD51-G135C* has been suggested to be correlated with the onset
224 of therapy-related AML by several case-control studies, even if two meta-analysis seem to dismiss
225 the role of this polymorphism in *de novo* AML onset[36-39]. XPD is involved in NER pathway, and its
226 polymorphism XPD-Lys751Gln has been shown to be a risk factor for AML onset[10, 36, 37]. One
227 study also suggested that this polymorphism worsens the AML prognosis[40]. These data highlight
228 the role of DNA repair pathways in leukemogenesis, and suggest their role in chemotherapy
229 resistance.

230 Interestingly, when compared using multivariate analysis, the DNA repair score and
231 *NPM1/FLT3* mutational status remained statistically associated with outcome in CN-AML. *FLT3* and
232 *NPM1* have also been shown to play a role in DNA damage response in AML. *FLT3*-ITD mutations,
233 occurring in about 20-25% of CN-AML, leads to a constitutive activation of *FLT3*, and therefore
234 confers a growth advantage to leukemic cells. Several studies showed that the level of reactive
235 oxygen species (ROS) was increased in *FLT3*-ITD mutated AML cells, and correlated with high
236 levels of DSB and lower efficiency of NHEJ repair pathway[41]. Moreover, the use of tyrosine-kinase
237 inhibitors may reduce both ROS and DSB levels, and increase DNA repair efficiency, overcoming the
238 chemo-resistance of these cells[41, 42]. Other mechanisms have been suggested to explain the role of
239 *FLT3*-ITD in DNA damages and acquired drug resistance of AML cells, such as telomere-related
240 genome instability[43], or paradoxical up-regulation of *RAD51*[44]. *NPM1* is the most commonly
241 mutated gene in CN-AML, with more than 50 described mutations. The prognostic significance of
242 these mutations and co-mutations in other genes has been widely studied[45]. The role of *NPM1* in
243 DNA damage response and maintenance of genome stability is less clear. *NPM1* is involved in
244 regulation of centrosome duplication during cell cycle[46], or is recruited in its phosphorylated form
245 (*NPM1*-pT199) on DSB foci, even if its role in DSB repair remains discussed[47]. *NPM1* is also
246 involved in regulation of key DNA repair factors, such as *APEX1* or *p53*[48, 49]. Therefore, *NPM1*

247 mutations in AML result in APEX1 abnormal cytoplasmic accumulation, and impaired BER
248 activity[50], potentially explaining a chemotherapy improved response in *NPM1*-mutated AML.

249 Intensive chemotherapy for CN-AML patients usually includes cytarabine and anthracyclines
250 (daunorubicine or idarubicine)[51]. Cytarabine, a nucleoside analog, incorporates into DNA and
251 interferes with DNA synthesis during the phase S of the cell cycle, leading to genomic instability[52].
252 Anthracyclines are DNA topoisomerase II inhibitors that induce DNA damages such as DSB,
253 adducts and ICL[52]. Therefore, overexpression of HRR or NER pathway genes could be associated
254 with chemotherapy resistance, but a better understanding of the functional role of DNA repair
255 pathways in the pathogenesis and drug resistance of CN-AML is needed[53]. Gene silencing
256 approaches by sh-RNA or CRISPR-Cas9 strategies could be of particular interest. Of particular
257 interest, CRISPR-Cas9 or RNAi screening revealed that *APEX1* (BER), *RTEL1* (HRR) and *COPS6*
258 (NER) are essential AML genes. Among these genes, *COPS6* overexpression is associated with poor
259 outcome in many solid tumors. Interestingly, *COPS6* depletion showed *in vivo* efficacy against
260 glioblastoma[54], cervical cancer[55] or papillary thyroid carcinoma[56], through regulation of
261 several signaling pathways. However, the biological function of *COPS6* in leukemogenesis and AML
262 drug-resistance, remains largely unknown.

263 Therefore, inhibiting DNA repair might be a promising strategy to improve the efficacy of
264 genotoxic drugs and overcome drug resistance, according to the principle of “synthetic lethality” [57,
265 58]. *APEX1* inhibitor has demonstrated a promising toxicity on primary AML cells *in vitro*, alone or
266 in association with hypomethylating agent decitabine or PARP (poly(ADP-Ribose) polymerase)
267 inhibitor talazoparib. Even if *APEX1* expression levels did not significantly differ between
268 responding and non-responding AML cells, *APEX1* inhibitor appeared promising in normal
269 karyotype AML (83% of the *APEX1* inhibitor “responders”)[59]. Our data support the potential
270 therapeutic interest of DNA damage signaling and DNA repair inhibitors in CN-AML.

271 4. Materials and Methods

272 4.1. Patients and Gene Expression Data

273 Gene expression microarray data from two independent cohorts of adult patients diagnosed
274 with CN-AML were used. The first cohort (training set) included 162 patients and the second one
275 (validation set) 78 patients. At least 20 metaphases were analyzed for each patient to confirm the
276 normal karyotype. At the beginning of treatment, median age was 58 years in the training cohort
277 and 62 years in the validation cohort. Pretreatment clinical characteristics of patients have been
278 described previously[60]. *NPM1* and *FLT3* mutational status were kindly provided for each patient
279 by Metzeler *et al*[60]. All patients were treated with intensive chemotherapy.

280 Affymetrix gene expression data are publicly available *via* the online Gene Expression Omnibus
281 (<http://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE12417. They were performed using
282 Affymetrix HG-U133 A&B microarrays for first cohort and Affymetrix HG-U133 P 2.0 microarrays
283 for the second one. Normalization of microarray data was performed using the variance stabilizing
284 normalization algorithm, and probe set signals calculated by the median polish method[60, 61].
285 Quality control consisted of visual inspection of the array image for artifacts, assessment of RNA
286 degradation plots, and inspection of rank-vs-residual plots after normalization and probe set
287 summarization.

288 4.2. Selection of Prognostic Genes

289 DNA repair gene list was defined using the REPAIRtoire database
290 (<http://repairtoire.genesilico.pl>) and review of the literature (**Supplementary Table S1**)[62]. To
291 establish gene expression (GE)-based risk scores, we selected probe sets whose expression values
292 were significantly associated with overall survival, using MaxStat R function and Benjamini
293 Hochberg multiple testing correction (adjusted p-value < 0.05)[22].

294 4.3. Building DNA Repair Gene Expression-Based Risk Score

295 For each pathway, a GE-based risk score was created as the sum of the beta coefficients
 296 weighted by +1 or -1 according to the patient signal above or below / equal the probe set MaxStat
 297 value as previously reported[22, 23]. Patients from the training cohort were ranked according to
 298 increased prognostic score and for a given score value X, the difference in survival of patients with a
 299 prognostic score $\leq X$ or $> X$ was computed using MaxStat analysis.

300 Cox proportional hazards model was performed to determine statistically significant pathway
 301 scores in multivariate analysis. A global DNA repair score was calculated based on the pathway
 302 scores which remained statistically significant in this analysis. Survival analyses were assessed using
 303 Kaplan-Meier method, and survival curves were compared using log-rank test.

304 4.4. Validation of the DNA Repair Score on Validation Cohort

305 Pathway and DNA repair scores were individually calculated in the validation cohort, using
 306 the cutoff values determined for the training cohort. Survival analyses were assessed using
 307 Kaplan-Meier method, and survival curves were compared using log-rank test.

308 4.5. Statistical Analyses

309 All statistical tests were two-tails and Alpha-risk was fixed at 5%. Analyses were performed
 310 using R.3.6.0. and SPSS Statistics version 23.0.0.0 for Mac.

311 5. Conclusions

312 The DNA repair score may be useful to identify high-risk CN-AML patients and define the best
 313 DNA repair inhibitor to use in combination with conventional treatment to improve patients'
 314 outcome. The DNA repair score could also be valuable for adapting targeted treatment according to
 315 the drug resistance mechanisms selected during clonal evolution of relapsing AML. These advances
 316 may improve the survival of CN-AML patients, and limit the side effects of treatment, improving
 317 compliance with dosing regimens and overall quality of life.

318 **Author Contributions:** L.G performed research, data analyses and participated in the writing of the paper. G.B
 319 and G.C participated in the research and in the writing of the paper. J.M and C.B supervised the research and
 320 the writing of the paper.

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324 **Conflicts of Interest:** The authors declare no conflict of interest.

325 Appendix A - Supplementary Tables & Figures

326 **Supplementary Table S1. Genes coding for proteins involved in DNA repair.** Gene symbols are
 327 provided with corresponding probe sets for each DNA repair pathway.

Base Excision Repair (BER) pathway					
210027_s_at	APEX1	226585_at	NEIL2	212836_at	POLD3
204408_at	APEX2	219502_at	NEIL3	202996_at	POLD4
218527_at	APTX	209731_at	NTHL1	216026_s_at	POLE
204767_s_at	FEN1	205301_s_at	OGG1	233852_at	POLH
204883_s_at	HUS1	208644_at	PARP1	221049_s_at	POLL
202726_at	LIG1	215773_x_at	PARP2	218685_s_at	SMUG1
207348_s_at	LIG3	201202_at	PCNA	203743_s_at	TDG
214048_at	MBD4	218961_s_at	PNKP	202330_s_at	UNG
203686_at	MPG	203616_at	POLB	203655_at	XRCC1
207727_s_at	MUTYH	203422_at	POLD1		
219396_s_at	NEIL1	201115_at	POLD2		

Fanconi (FANC) pathway					
213454_at	APITD1	1557217_a_at	FANCB	205024_s_at	RAD51
208442_s_at	ATM	205189_s_at	FANCC	206066_s_at	RAD51C
209902_at	ATR	223545_at	FANCD2	218428_s_at	REV1
1552937_s_at	ATRIP	220255_at	FANCE	218979_at	RMI1
205733_at	BLM	222713_s_at	FANCF	226456_at	RMI2
204531_s_at	BRCA1	203564_at	FANCG	201529_s_at	RPA1
214727_at	BRCA2	213008_at	FANCI	201756_at	RPA2
221800_s_at	C17orf70	218397_at	FANCL	209507_at	RPA3
214816_x_at	C19orf40	242711_x_at	FANCM	218317_x_at	SLX1
205394_at	CHEK1	202520_s_at	MLH1	233334_x_at	SLX1A
203229_s_at	CLK2	218463_s_at	MUS81	239687_at	SLX4
234464_s_at	EME1	219530_at	PALB2	214299_at	TOP3A
203719_at	ERCC1	221206_at	PMS2	202633_at	TOPBP1
228131_at	ERCC1/ASE1	209805_at	PMS2///PMS2CL	202412_s_at	USP1
235215_at	ERCC4	233852_at	POLH	65591_at	WDR48
203678_at	FAN1	219317_at	POLI		
203805_s_at	FANCA	242804_at	POLN		
Homologous Recombination Repair (HRR) pathway					
208442_s_at	ATM	227286_at	INO80E	204146_at	RAD51AP1
205345_at	BARD1	214258_x_at	KAT5	210255_at	RAD51B
205733_at	BLM	202726_at	LIG1	206066_s_at	RAD51C
204531_s_at	BRCA1	224320_s_at	MCM8	37793_r_at	RAD51D
214727_at	BRCA2	219673_at	MCM9	205647_at	RAD52
214816_x_at	C19orf40	205395_s_at	MRE11A	219494_at	RAD54B
210416_s_at	CHEK2	210533_at	MSH4	203344_s_at	RBBP8
208386_x_at	DMC1	210410_s_at	MSH5///MSH5- SAPCD1///SAPCD1	221686_s_at	RECQL5
234464_s_at	EME1			201529_s_at	RPA1
1569868_s_at	EME2	218463_s_at	MUS81	201756_at	RPA2
204603_at	EXO1	202907_s_at	NBN	209507_at	RPA3
224683_at	FBXO18	219530_at	PALB2	206092_x_at	RTEL1
228286_at	GEN1	203422_at	POLD1	212275_s_at	SRCAP
225357_s_at	INO80	201115_at	POLD2	214299_at	TOP3A
65133_i_at	INO80B/// INO80B-WBP1	212836_at 202996_at	POLD3 POLD4	207598_x_at 216299_s_at	XRCC2 XRCC3
1559716_at	INO80C	208393_s_at	RAD50		
227931_at	INO80D	205024_s_at	RAD51		
Mismatch Repair (MMR) pathway					
204603_at	EXO1	1554742_at	PMS1	208021_s_at	RFC1
202726_at	LIG1	221206_at	PMS2	1053_at	RFC2
202520_s_at	MLH1	209805_at	PMS2///PMS2CL	204127_at	RFC3
204838_s_at	MLH3	203422_at	POLD1	204023_at	RFC4
209421_at	MSH2	201115_at	POLD2	203209_at	RFC5
205887_x_at	MSH3	212836_at	POLD3	201529_s_at	RPA1
202911_at	MSH6	202996_at	POLD4	209507_at	RPA3
201202_at	PCNA	216026_s_at	POLE		
Nucleotide Excision Repair (NER) pathway					
204093_at	CCNH	235215_at	ERCC4	216026_s_at	POLE
211297_s_at	CDK7	202414_at	ERCC5	202725_at	POLR2A
209194_at	CETN2	207347_at	ERCC6	201046_s_at	RAD23A
202467_s_at	COPS2	205162_at	ERCC8	201222_s_at	RAD23B
202078_at	COPS3	202451_at	GTF2H1	218117_at	RBX1
218042_at	COPS4	223758_s_at	GTF2H2	208021_s_at	RFC1
201652_at	COPS5	222104_x_at	GTF2H3	201529_s_at	RPA1
201405_s_at	COPS6	203577_at	GTF2H4	201756_at	RPA2

209029_at	COPS7A	213357_at	GTF2H5	209507_at	RPA3
219997_s_at	COPS7B	200943_at	HMG1N1	216241_s_at	TCEA1
236204_at	COPS8	202726_at	LIG1	203919_at	TCEA2
201423_s_at	CUL4A	207348_s_at	LIG3	226388_at	TCEA3
208619_at	DDB1	202167_s_at	MMS19	233893_s_at	UVSSA
203409_at	DDB2	203565_s_at	MNAT1	218110_at	XAB2
213579_s_at	EP300	201202_at	PCNA	205672_at	XPA
203719_at	ERCC1	203422_at	POLD1	209375_at	XPC
228131_at	ERCC1/ASE1	201115_at	POLD2	203655_at	XRCC1
213468_at	ERCC2	212836_at	POLD3		
202176_at	ERCC3	202996_at	POLD4		

Non-Homologous End Joining (NHEJ) pathway					
241379_at	APLF	209940_at	PARP3	1569098_s_at	TP53BP1
208442_s_at	ATM	218961_s_at	PNKP	205667_at	WRN
235478_at	DCLRE1C	221049_s_at	POLL	205072_s_at	XRCC4
205436_s_at	H2AFX	222238_s_at	POLM	232633_at	XRCC5
206235_at	LIG4	210543_s_at	PRKDC	200792_at	XRCC6
219418_at	NHEJ1	206554_x_at	SETMAR		
210470_x_at	NONO	201585_s_at	SFPQ		

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Supplementary Table S2. Cox analysis of overall survival in CN-AML validation cohort (n=78) according to DNA repair pathway scores. Hazard ratio (HR) and p-values are shown for each HRR and NER repair pathway scores (computed with training cohort parameters) in univariate Cox analysis.

DNA repair pathway score	Univariate Cox analysis	
	HR	p-value
HRR score	3.73	1.32e-05
NER score	2.83	0.028

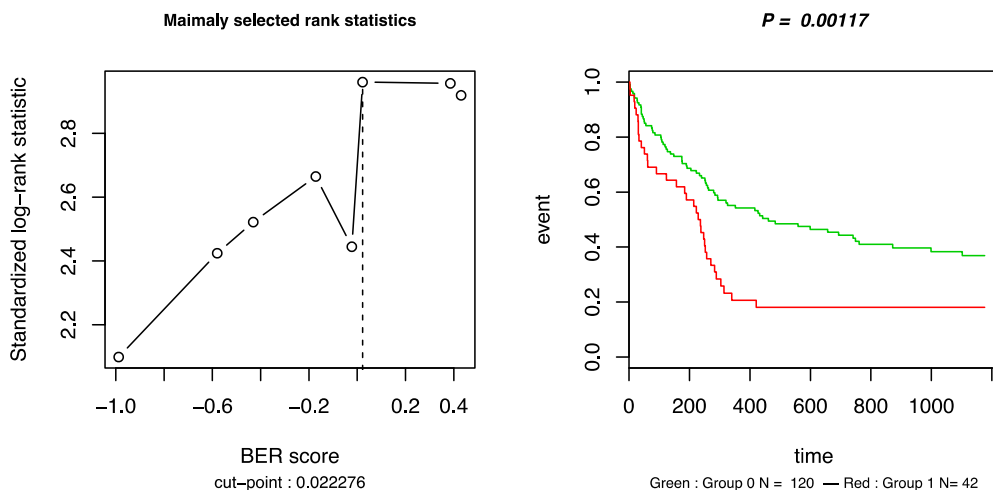
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Supplementary Table S3. Cox analysis of overall survival in CN-AML validation cohort (n=78) according to DNA repair score, and NPM1 & FLT3 mutational status. Hazard ratio (HR) and p-values are shown for each parameter in univariate and multivariate Cox analysis. NS: not significant. ITD: internal tandem duplication.

Scores	Univariate Cox analysis		Multivariate Cox analysis	
	HR	p-value	HR	p-value
DNA repair score	3.04	1.01e-05	3.07	1.4e-05
NPM1 mutation / FLT3-ITD classification	1.71	0.020	1.67	0.03

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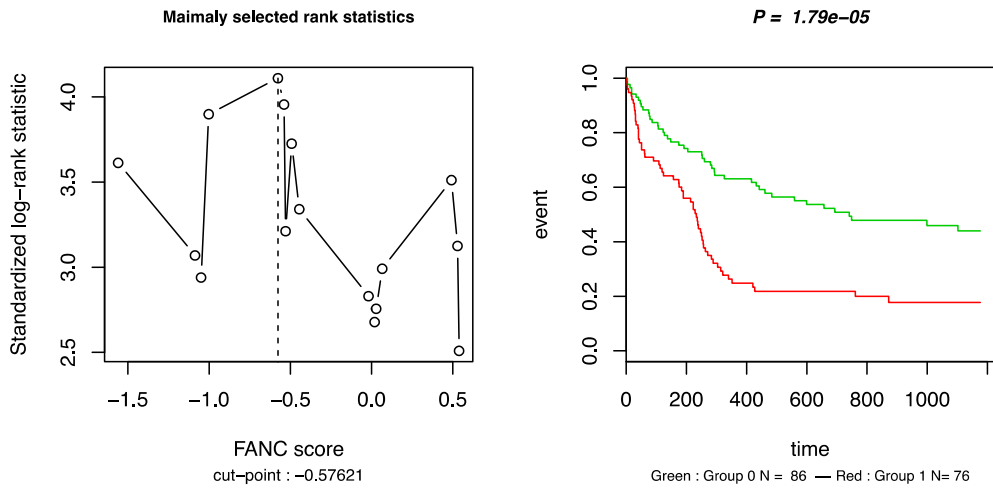
a)



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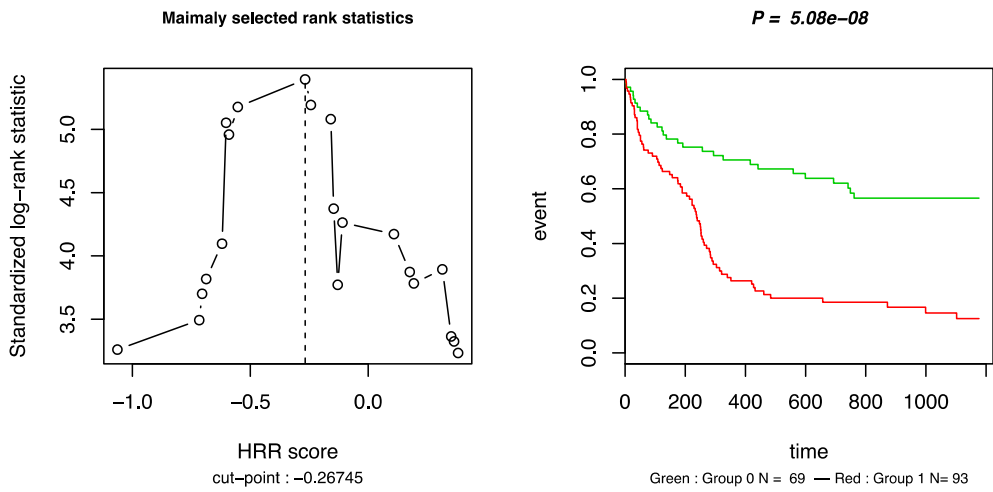
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b)



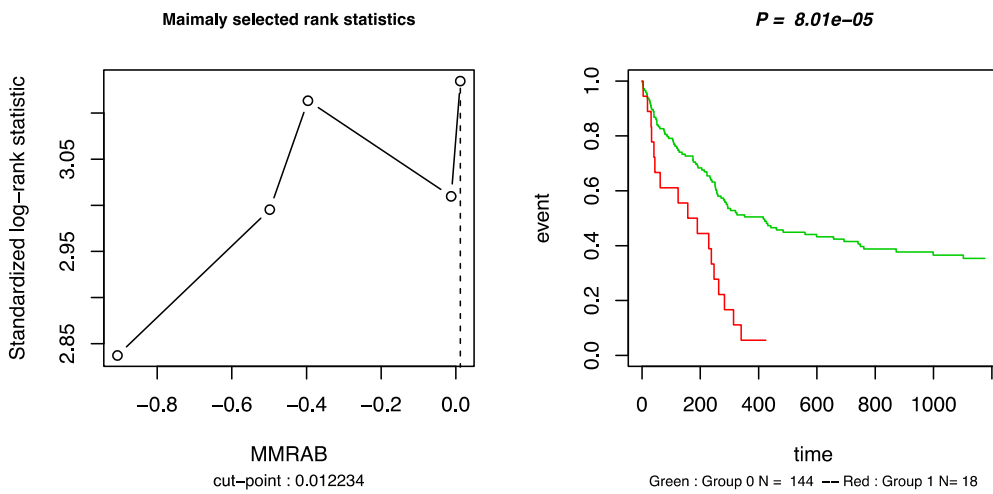
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c)



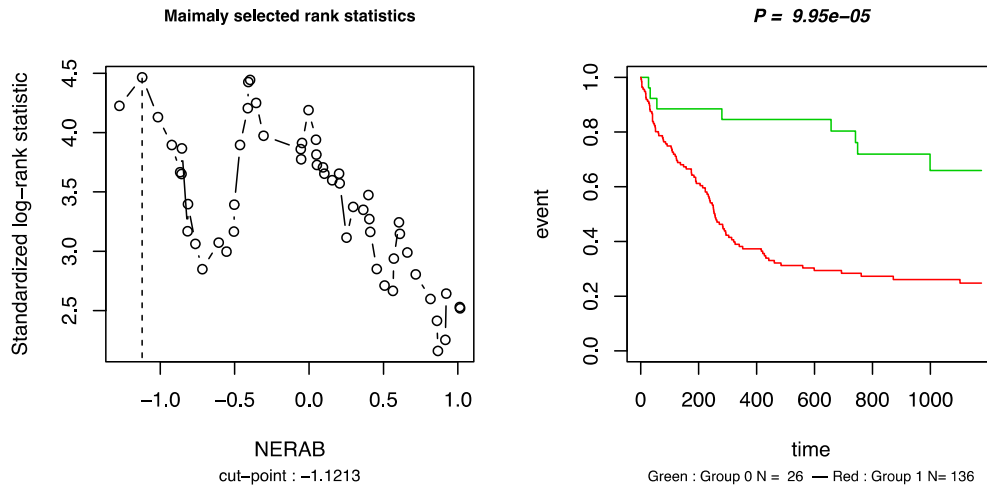
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d)



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e)



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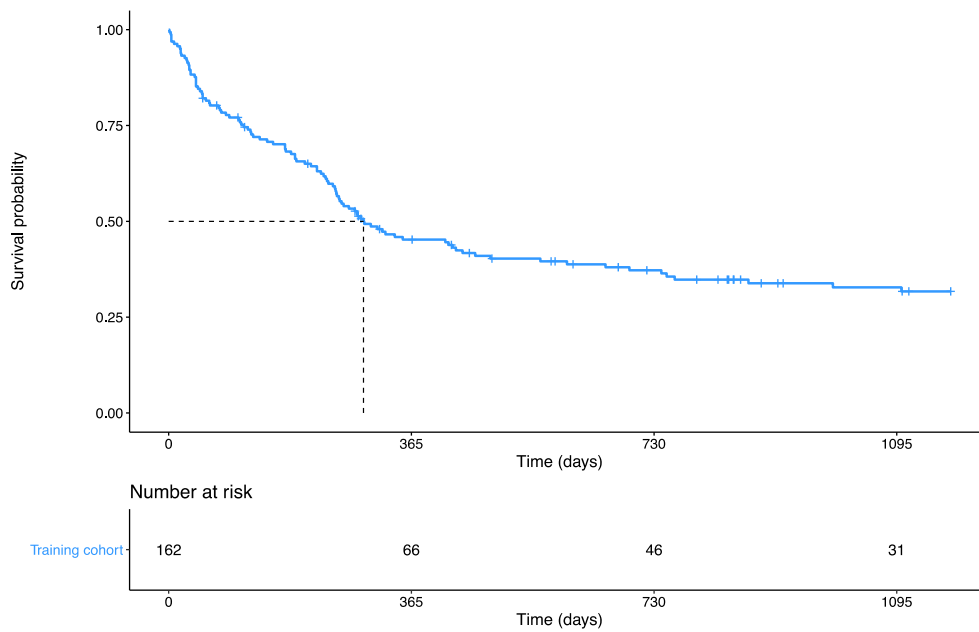
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Supplementary Figure S1. Prognostic value of DNA repair pathway scores in CN-AML patients of the training cohort. Patients of the training cohort (n=162) were ranked according to increasing BER (a), FANC (b), HRR (c), MMR (d) and NER (e) scores and a maximum difference in OS was obtained using MaxStat R function. Green survival curves represent patients whose score is inferior or equal to the MaxStat determined cut-point. Red survival curves designate patients whose score is strictly superior to the MaxStat determined cut-point.

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a)

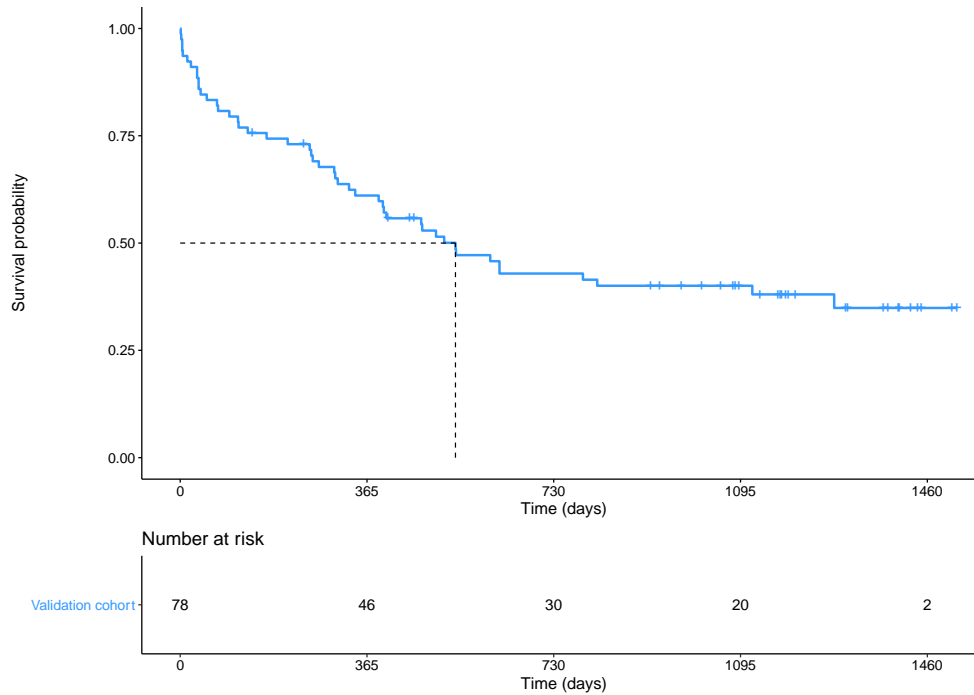


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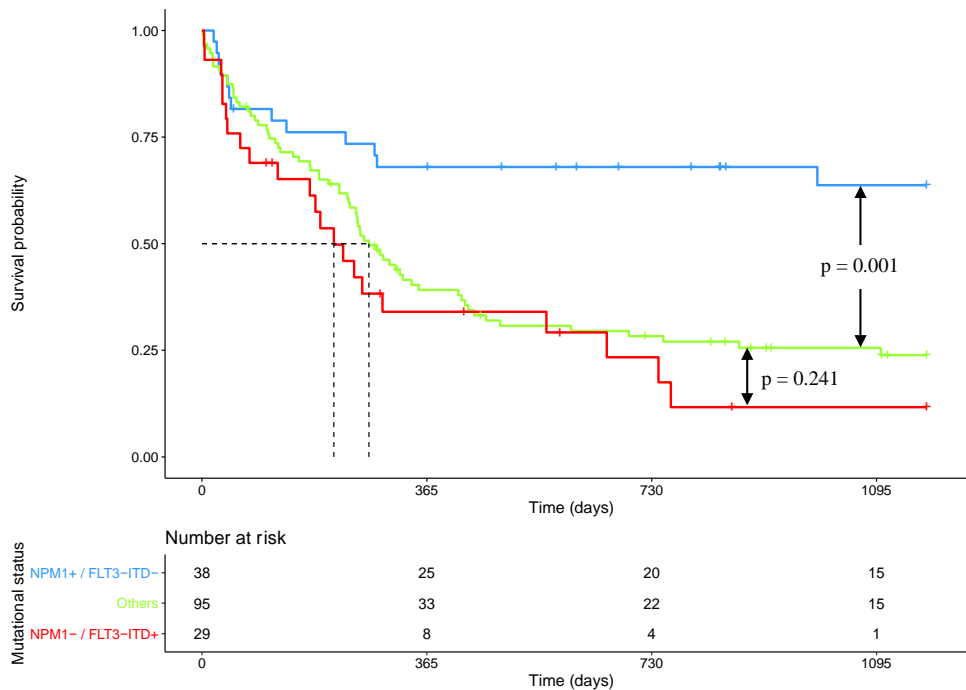
b)



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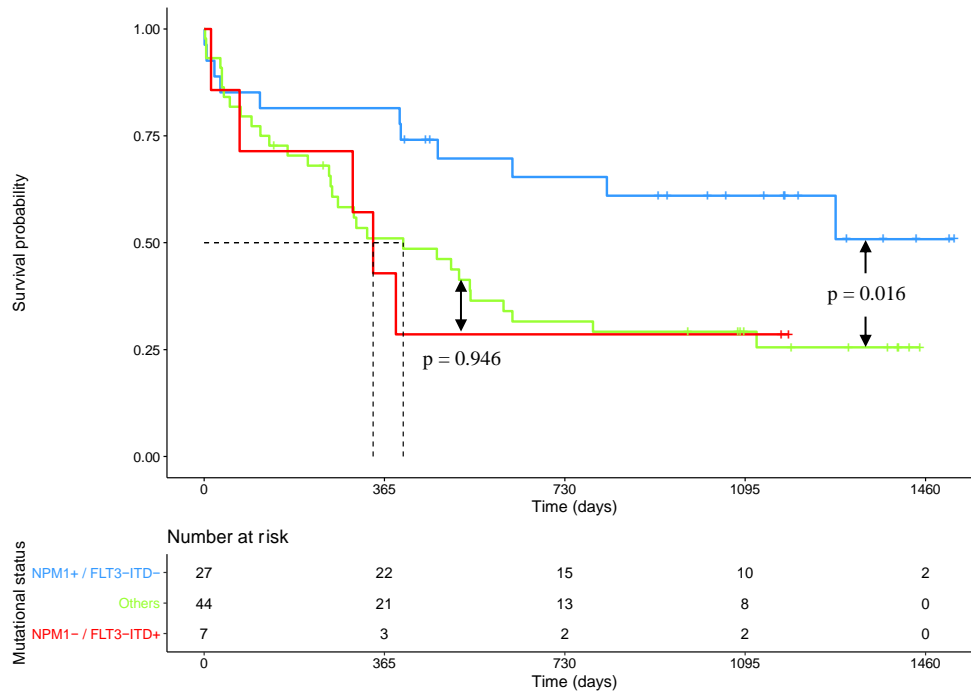
361 **Supplementary Figure S2.** Kaplan-Meier survival curves for training and validation cohorts. (a)
 362 Kaplan-Meier survival curve for the whole training cohort (n=162). After a median follow-up of 1176
 363 days (95% CI: 916-NR), median overall survival (OS) was 293 days (95% CI: 252-461) for the whole training
 364 cohort. (b) Kaplan-Meier survival curve for the whole validation cohort (n=78). After a median follow-up
 365 of 1183 days (95% CI: 1092-1383), median overall survival (OS) was 538 days (95% CI: 388-1278) for the
 366 whole validation cohort.

367 a)



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b)



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Supplementary Figure S3. Kaplan-Meier survival curves according to *NPM1/FLT3* mutational status. (a) Kaplan-Meier survival curve for the training cohort (n=162). Median OS was not reached (95% CI: 999-NR) for patients with *NPM1+/FLT3-ITD-* mutational status, 271 days (95% CI: 240-416) for patients with *NPM1+/FLT3-ITD+* or *NPM1-/FLT3-* mutational status (“Others”) and 214 days (95% CI: 123-657) for patients with *NPM1-/FLT3-ITD+* mutational status. (b) Kaplan-Meier survival curve for the validation cohort (n=78). Median OS was not reached (95% CI: 624-NR) for patients with *NPM1+/FLT3-ITD-* mutational status, 403 days (95% CI: 259-624) for patients with *NPM1+/FLT3-ITD+* or *NPM1-/FLT3-* mutational status (“Others”) and 342 days (95% CI: 72-NR) for patients with *NPM1-/FLT3-ITD+* mutational status. P-values are estimated with log-rank test.

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