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

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

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

1. Introduction

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

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

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

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

2. Results

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

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

Table 1. List of the 23 probe sets associated with good or bad prognosis in CN-AML. Corresponding DNA repair pathway, gene symbol, adjusted p-value, hazard ratio and prognosis 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 /// PMS2CL	0.024	1.8	Bad
	219317_at	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 /// PMS2CL	0.024	1.8	Bad
	1053_at	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

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

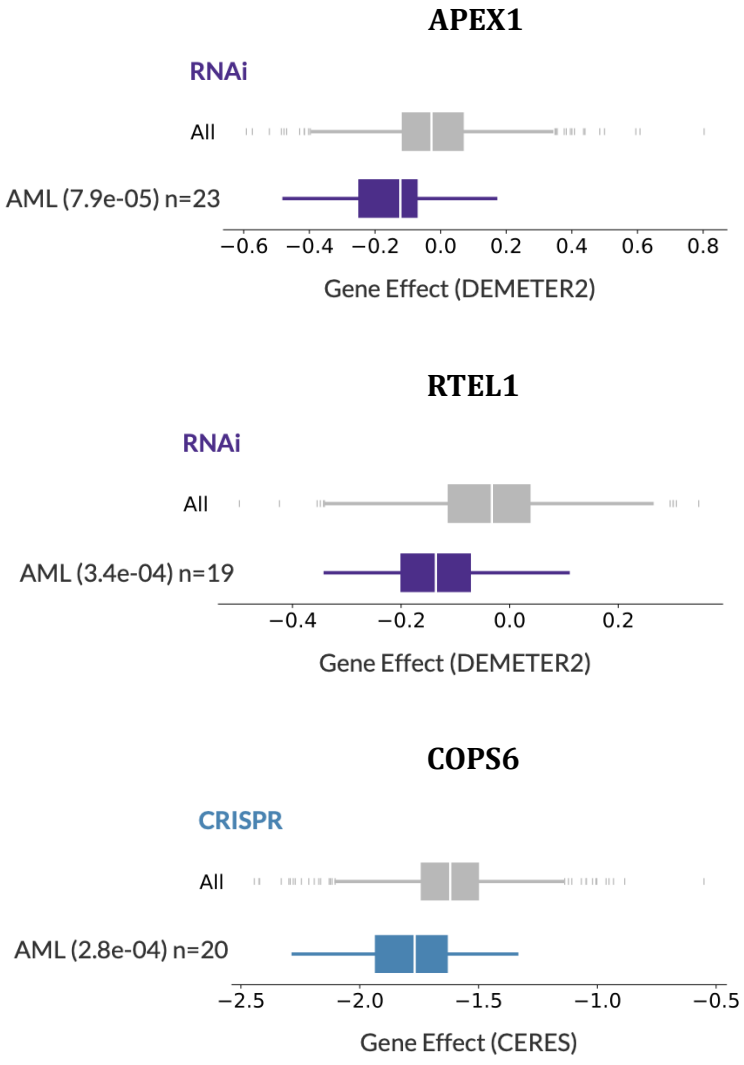


Figure 1. Silencing of APEX1, RTEL1 and COPS6 impairs AML cell growth. Using CRISPR or RNAi screening publicly available data (Dependency Map data, Broad Institute, *www.depmap.org*),

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

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

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

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

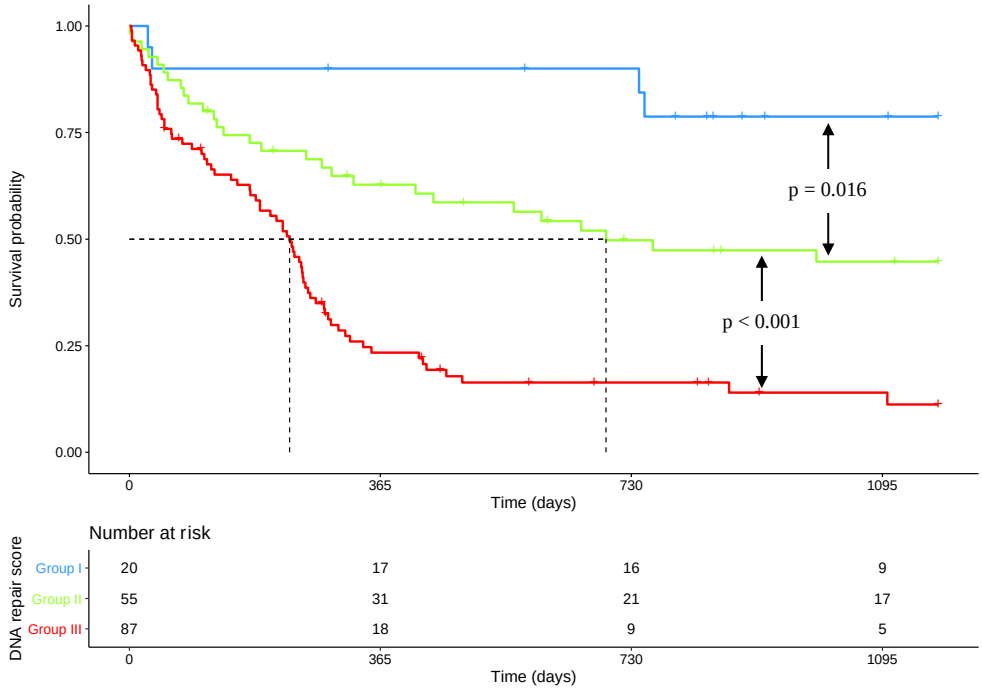
Table 2. Cox analysis of overall survival in CN-AML training cohort (n=162) according to DNA repair pathway scores. Hazard ratio (HR) and p-values are shown for each DNA repair pathway score in univariate and multivariate Cox analysis. NS: not significant.

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

After a median follow-up of 1176 days (95% CI: 916-NR), median overall survival (OS) was 293 days (95% CI: 252-461) for the whole training cohort (**Supplementary Figure S2a**). One-year OS was 45.2% (95% CI: 38.0-53.8). According to risk groups determined by the DNA repair score, 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, II and III (**Figure 2a**). Median OS were statistically different between each risk group (log-rank test; $p = 0.016$ between group I and II; $p < 0.001$ between group II and III).

We searched to validate these results in an independent cohort of 78 patients. HRR and NER scores computed with training cohort parameters were also prognostic in this validation cohort (**Supplementary Table S2**). The global DNA repair score was also computed. In the validation set, risk groups included 14, 42 and 22 patients respectively in groups I, II and III. After a median follow-up of 1183 days (95% CI: 1092-1383), median overall survival (OS) was 538 days (95% CI: 388-1278) for the whole validation cohort (**Supplementary Figure S2b**). One-year OS was 61.1% (95% CI: 51.1-73.0). According to risk groups determined by the DNA repair score, 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, II and III (**Figure 2b**). Even if survival analysis failed to demonstrate a statistical difference between groups I and II (log-rank test; $p = 0.287$), OS was still statistically different between risk groups II and III (log-rank test; $p < 0.001$). Altogether, these data underlined the identification of high-risk CN-AML patients characterized by DNA repair dysregulation and that could benefit from DNA repair targeted treatment.

a)



b)

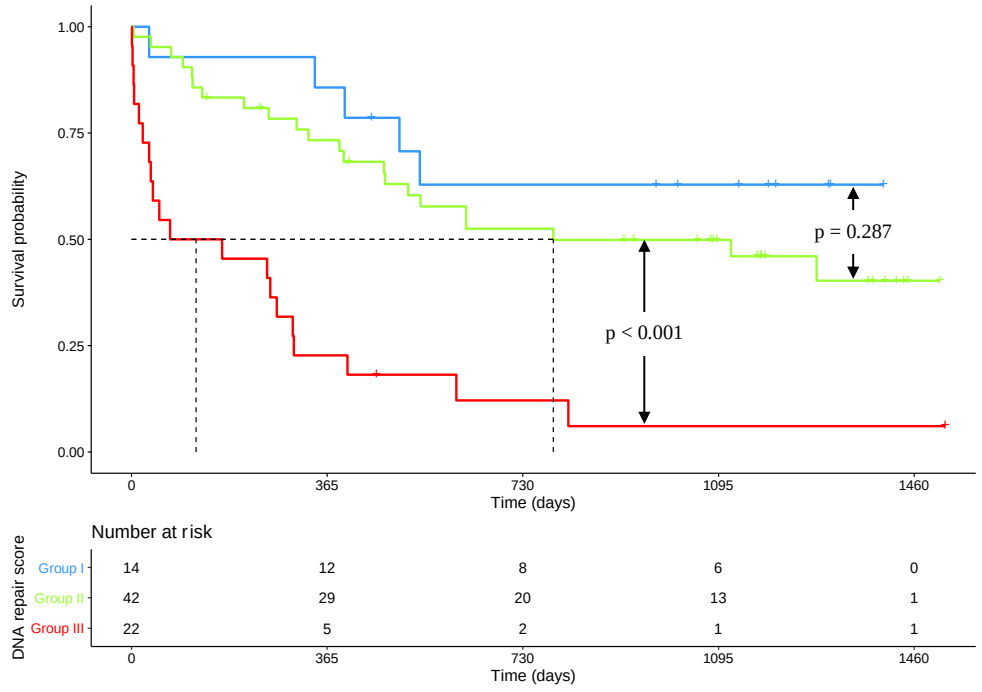


Figure 2. Kaplan-Meier survival curves according to risk stratification determined by DNA repair score. (a) Kaplan-Meier survival curve in the training cohort (n=162). Median OS was not reached (95% CI: NR-NR), 693 days (95% CI: 414-NR) and 233 days (95% CI: 184-260) respectively for patients in groups 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.

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

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

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

Table 3. Cox analysis of overall survival in CN-AML training cohort (n=162) 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. 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

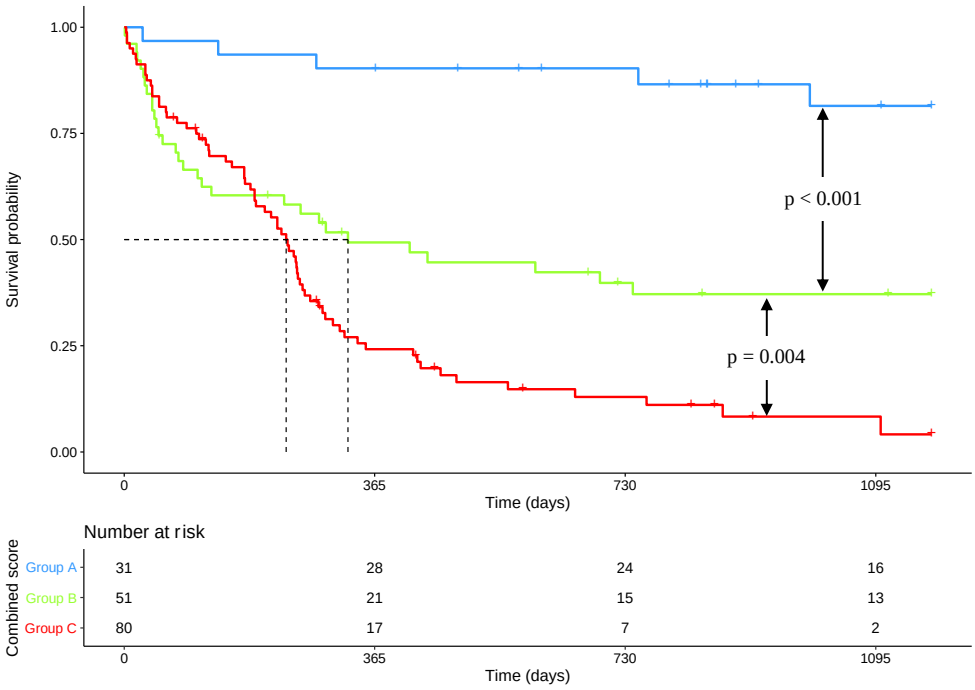
Table 4. DNA repair score and NPM1 / FLT3 mutational status combination in order to establish a global prognosis score in CN-AML. Patients were classified according to DNA repair score risk group (I, II or III) and NPM1 / FLT3 mutational status. Patients with NPM1 mutation and FLT3-ITD are respectively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

In the training cohort, 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. These results were confirmed in the validation cohort where median OS was not reached (95% CI: 1278-NR), 516 days (95% CI: 308-NR) and 253 days (95% CI: 52-403) for patients

respectively in groups A, B 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 26.5% (95% CI: 12.4-55.8) in group C. OS was statistically different between groups A, B and C in both training and validation cohorts (**Figure 3**). Altogether, these data underlined the interest of GEP-based DNA repair deregulations, alone or in combination with *NPM1* and *FLT3* mutational status to identify high-risk CN-AML patients.

a)



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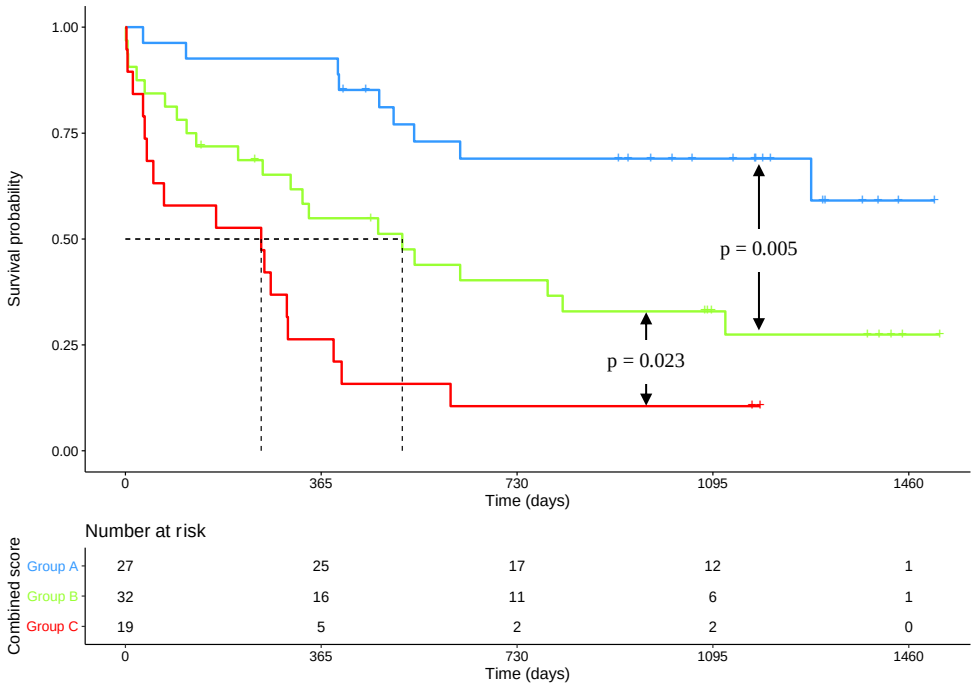

Figure 3. Kaplan-Meier survival curves according to risk groups determined by combined score incorporating DNA repair score and *NPM1*/*FLT3* mutational status. (a) Kaplan-Meier survival curve in the training cohort (n=162). *Median OS was not reached (95% CI: NR-NR), 326 days (95% CI: 127-NR) and 236 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*

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

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

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

4. Materials and Methods

4.1. Patients and Gene Expression Data

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

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

4.2. Selection of Prognostic Genes

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

4.3. Building DNA Repair Gene Expression-Based Risk Score

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

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

4.4. Validation of the DNA Repair Score on Validation Cohort

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

4.5. Statistical Analyses

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

5. Conclusions

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

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

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

Appendix A - Supplementary Tables & Figures

Supplementary Table S1. Genes coding for proteins involved in DNA repair. Gene symbols are 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-	221686_s_at	RECQL5
234464_s_at	EME1		SAPCD1///SAPCD1	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///	212836_at	POLD3	207598_x_at	XRCC2
	INO80B-WBP1	202996_at	POLD4	216299_s_at	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	HMGN1	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		

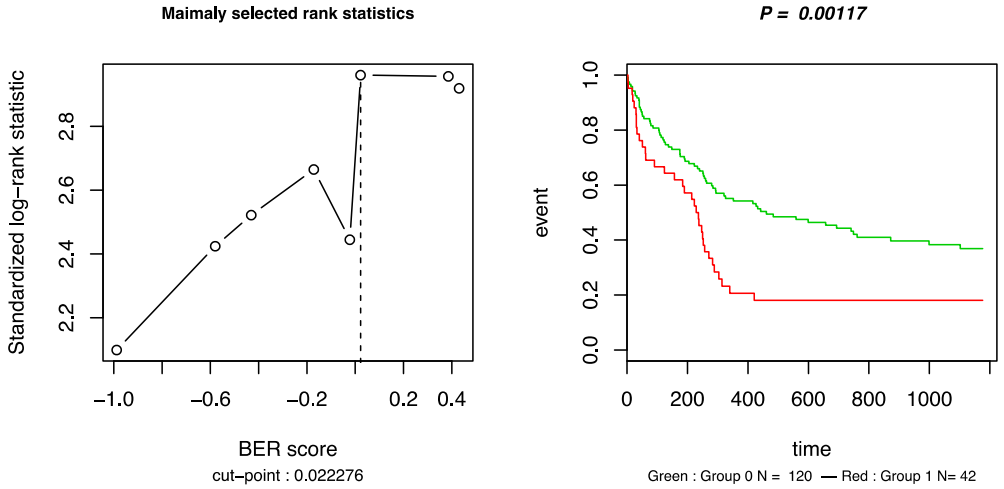
Supplementary Table S2. Cox analysis of overall survival in CN-AML validation cohort (n=78) according to DNA repair pathway scores. *Hazard ratio (HR) and p-values are shown for each HRR and NER repair pathway scores (computed with training cohort parameters) in univariate Cox analysis.*

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

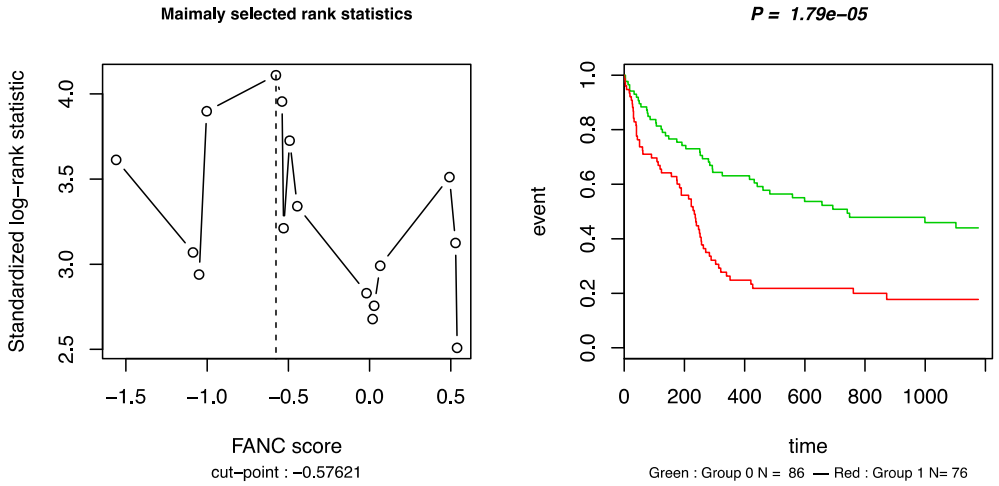
Supplementary Table S3. Cox analysis of overall survival in CN-AML validation cohort (n=78) according to DNA repair score, and *NPM1* & *FLT3* mutational status. *Hazard ratio (HR) and p-values are shown for each parameter in univariate and multivariate Cox analysis. NS: not significant. ITD: internal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

a)



b)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Standardized log−rank statistic
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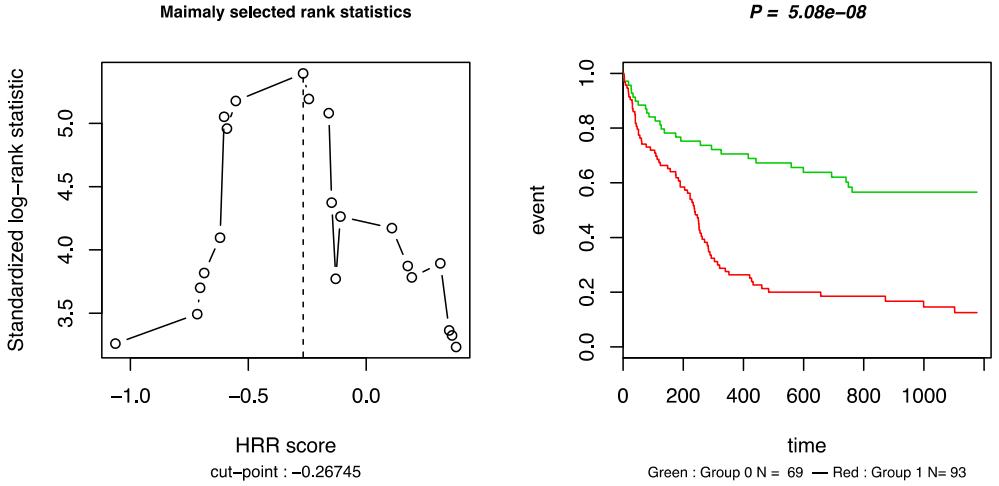
cut−point : −0.57621
P = 1.79e−05
event
time
Green : Group 0 N = 86 — Red : Group 1 N= 76

c)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event
time
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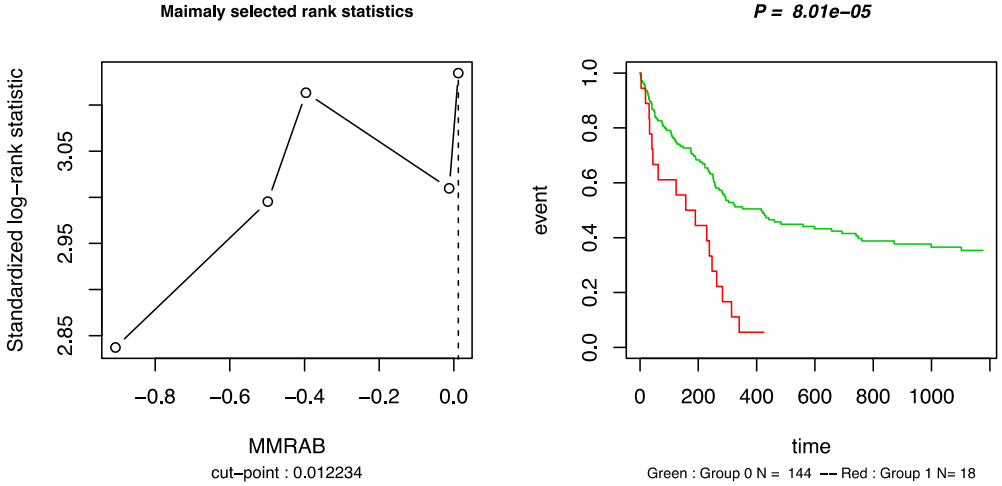
d)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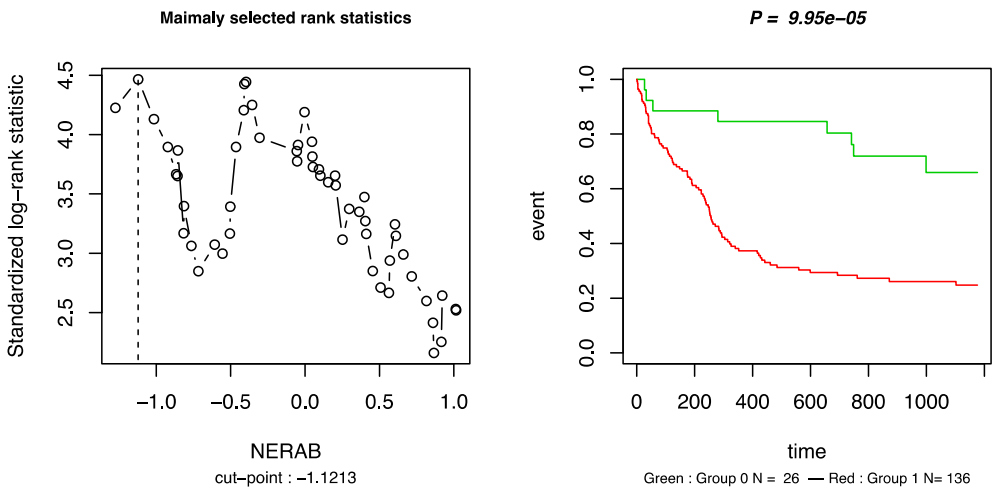
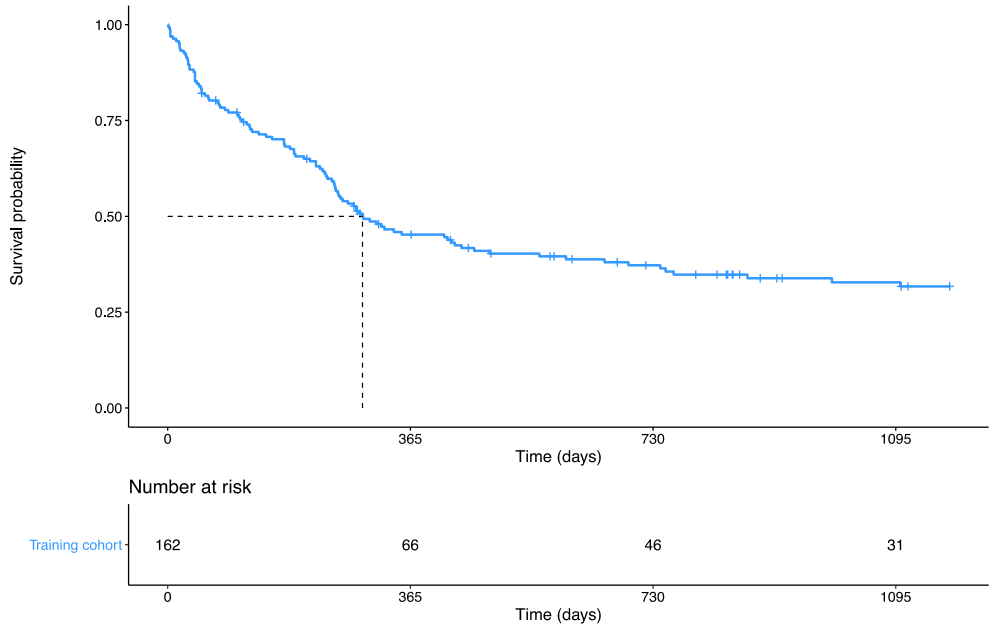
Standardized log−rank statistic
MMRAB
cut−point : 0.012234
P = 8.01e−05
event
time
Green : Group 0 N = 144 — Red : Group 1 N= 18

e)

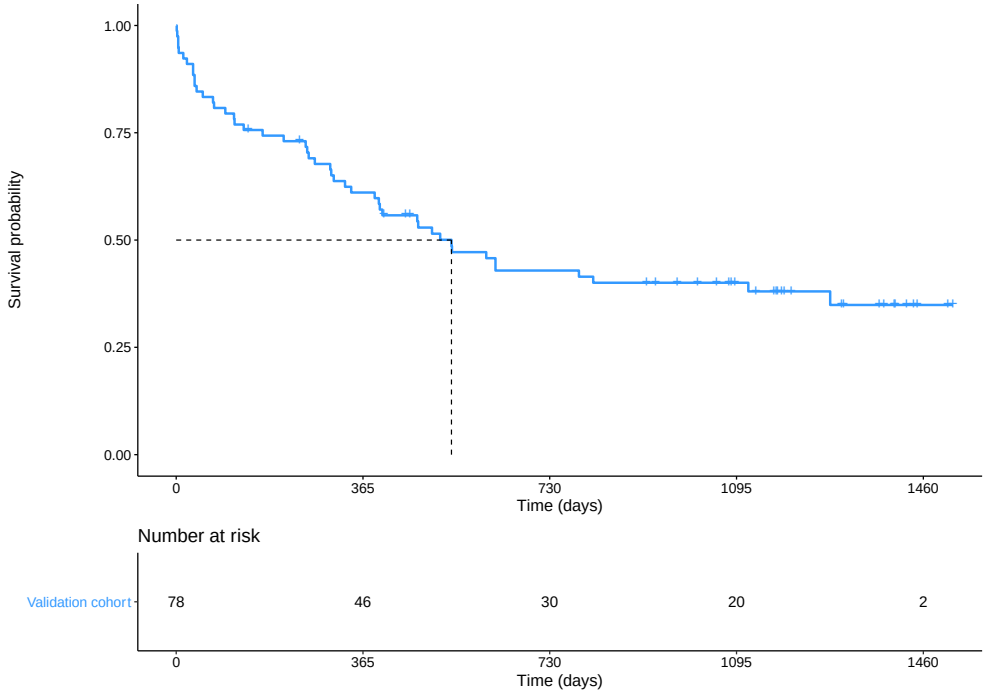


Supplementary Figure S1. Prognostic value of DNA repair pathway scores in CN-AML patients of the training cohort. Patients of the training cohort (n=162) were ranked according to increasing BER (a), FANC (b), HRR (c), MMR (d) and NER (e) scores and a maximum difference in OS was obtained using MaxStat 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.

a)

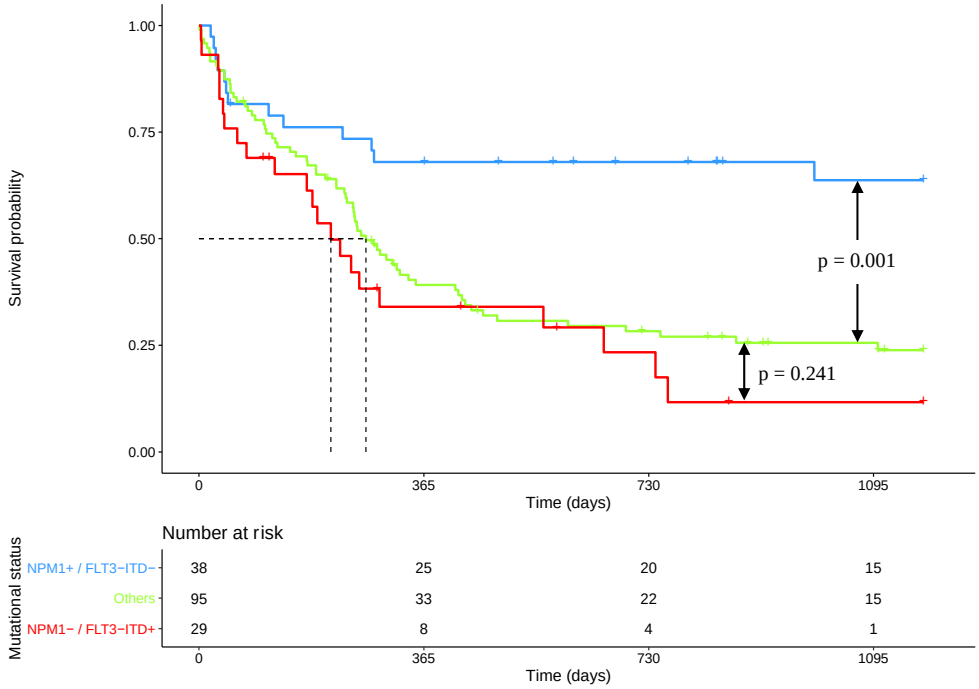


b)



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

a)



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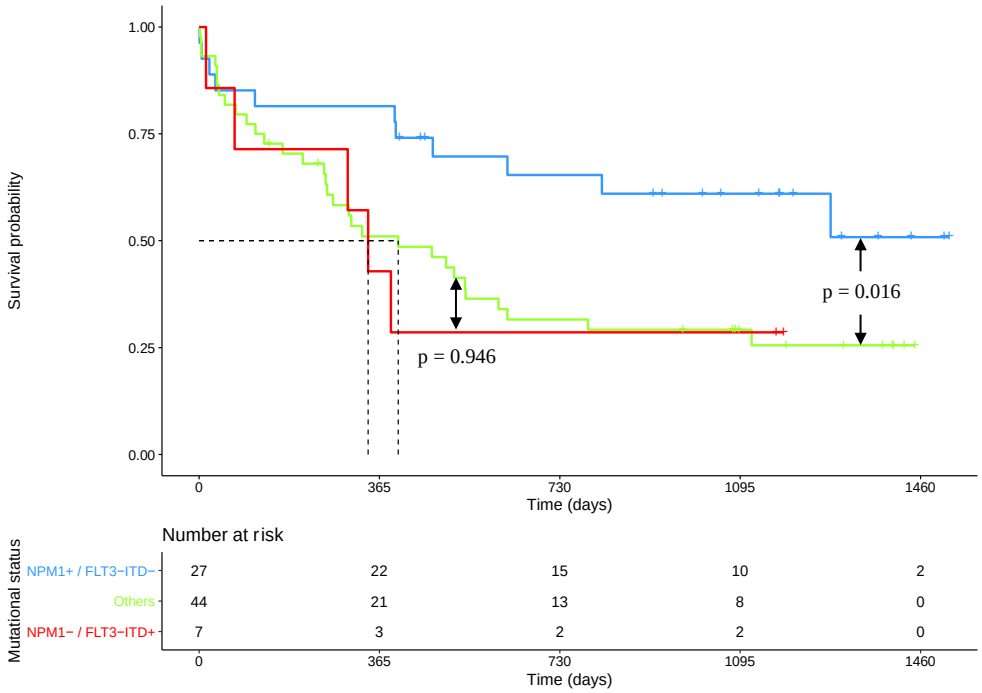

Supplementary Figure S3. Kaplan-Meier survival curves according to *NPM1/FLT3* mutational status. (a) Kaplan-Meier survival curve for the training cohort (n=162). *Median OS was not reached (95% CI: 999-NR) for patients with NPM1+/FLT3-ITD- mutational status, 271 days (95% CI: 240-416) for patients with 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.*

References

1. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010;116(3):354-65. Epub 2010/04/14. doi: 10.1182/blood-2009-11-254441. PubMed PMID: 20385793.
2. Port M, Bottcher M, Thol F, Ganser A, Schlenk R, Wasem J, et al. Prognostic significance of FLT3 internal tandem duplication, nucleophosmin 1, and CEBPA gene mutations for acute myeloid leukemia patients with normal karyotype and younger than 60 years: a systematic review and meta-analysis. Ann Hematol. 2014;93(8):1279-86. Epub 2014/05/08. doi: 10.1007/s00277-014-2072-6. PubMed PMID: 24801015.
3. Dohner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Buchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-47. Epub 2016/11/30. doi: 10.1182/blood-2016-08-733196. PubMed PMID: 27895058; PubMed Central PMCID: PMCPMC5291965.
4. Cancer Genome Atlas Research N, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059-74. Epub 2013/05/03. doi: 10.1056/NEJMoa1301689. PubMed PMID: 23634996; PubMed Central PMCID: PMCPMC3767041.
5. Ibanez M, Carbonell-Caballero J, Such E, Garcia-Alonso L, Liquori A, Lopez-Pavia M, et al. The modular network structure of the mutational landscape of Acute Myeloid Leukemia. PLoS One. 2018;13(10):e0202926. Epub 2018/10/12. doi: 10.1371/journal.pone.0202926. PubMed PMID: 30303964; PubMed Central PMCID: PMCPMC6179200.
6. Grossmann V, Tiacci E, Holmes AB, Kohlmann A, Martelli MP, Kern W, et al. Whole-exome sequencing identifies somatic mutations of BCOR in acute myeloid leukemia with normal karyotype. Blood. 2011;118(23):6153-63. Epub 2011/10/21. doi: 10.1182/blood-2011-07-365320. PubMed PMID: 22012066.
7. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013;502(7471):333-9. Epub 2013/10/18. doi: 10.1038/nature12634. PubMed PMID: 24132290; PubMed Central PMCID: PMCPMC3927368.
8. Kayser S, Dohner K, Krauter J, Kohne CH, Horst HA, Held G, et al. The impact of therapy-related acute myeloid leukemia (AML) on outcome in 2853 adult patients with newly diagnosed AML. Blood. 2011;117(7):2137-45. Epub 2010/12/04. doi: 10.1182/blood-2010-08-301713. PubMed PMID: 21127174.
9. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med. 2016;374(23):2209-21. Epub 2016/06/09. doi: 10.1056/NEJMoa1516192. PubMed PMID: 27276561; PubMed Central PMCID: PMCPMC4979995.
10. Liu D, Wu D, Li H, Dong M. The effect of XPD/ERCC2 Lys751Gln polymorphism on acute leukemia risk: a systematic review and meta-analysis. Gene. 2014;538(2):209-16. Epub 2014/02/04. doi: 10.1016/j.gene.2014.01.049. PubMed PMID: 24486506.
11. Alcalay M, Meani N, Gelmetti V, Fantozzi A, Fagioli M, Orleth A, et al. Acute myeloid leukemia fusion proteins deregulate genes involved in stem cell maintenance and DNA repair. J Clin Invest. 2003;112(11):1751-61. Epub 2003/12/09. doi: 10.1172/JCI17595. PubMed PMID: 14660751; PubMed Central PMCID: PMCPMC281638.
12. Krejci O, Wunderlich M, Geiger H, Chou FS, Schleimer D, Jansen M, et al. p53 signaling in response to increased DNA damage sensitizes AML1-ETO cells to stress-induced death. Blood. 2008;111(4):2190-9. Epub 2007/11/03. doi: 10.1182/blood-2007-06-093682. PubMed PMID: 17975013; PubMed Central PMCID: PMCPMC2234055.
13. Yeung PL, Denissova NG, Nasello C, Hakhverdyan Z, Chen JD, Brenneman MA. Promyelocytic leukemia nuclear bodies support a late step in DNA double-strand break repair by homologous recombination. J Cell Biochem. 2012;113(5):1787-99. Epub 2012/01/04. doi: 10.1002/jcb.24050. PubMed PMID: 22213200; PubMed Central PMCID: PMCPMC3337353.
14. van der Kouwe E, Staber PB. RUNX1-ETO: Attacking the Epigenome for Genomic Instable Leukemia. Int J Mol Sci. 2019;20(2). Epub 2019/01/19. doi: 10.3390/ijms20020350. PubMed PMID: 30654457; PubMed Central PMCID: PMCPMC6358732.

15. Alter BP. Fanconi anemia and the development of leukemia. Best Pract Res Clin Haematol. 2014;27(3-4):214-21. Epub 2014/12/03. doi: 10.1016/j.beha.2014.10.002. PubMed PMID: 25455269; PubMed Central PMCID: PMCPMC4254647.
16. Quinn E, Nichols KE. Cancer predisposition syndromes associated with myeloid malignancy. Semin Hematol. 2017;54(2):115-22. Epub 2017/06/24. doi: 10.1053/j.seminhematol.2017.04.003. PubMed PMID: 28637615.
17. Schoch C, Kern W, Kohlmann A, Hiddemann W, Schnittger S, Haferlach T. Acute myeloid leukemia with a complex aberrant karyotype is a distinct biological entity characterized by genomic imbalances and a specific gene expression profile. Genes Chromosomes Cancer. 2005;43(3):227-38. Epub 2005/04/23. doi: 10.1002/gcc.20193. PubMed PMID: 15846790.
18. Cavelier C, Didier C, Prade N, Mansat-De Mas V, Manenti S, Recher C, et al. Constitutive activation of the DNA damage signaling pathway in acute myeloid leukemia with complex karyotype: potential importance for checkpoint targeting therapy. Cancer Res. 2009;69(22):8652-61. Epub 2009/10/22. doi: 10.1158/0008-5472.CAN-09-0939. PubMed PMID: 19843865.
19. Wang P, Ma D, Wang J, Fang Q, Gao R, Wu W, et al. INPP4B-mediated DNA repair pathway confers resistance to chemotherapy in acute myeloid leukemia. Tumour Biol. 2016;37(9):12513-23. Epub 2016/10/27. doi: 10.1007/s13277-016-5111-1. PubMed PMID: 27342972.
20. Meyers RM, Bryan JG, McFarland JM, Weir BA, Sizemore AE, Xu H, et al. Computational correction of copy number effect improves specificity of CRISPR-Cas9 essentiality screens in cancer cells. Nat Genet. 2017;49(12):1779-84. Epub 2017/10/31. doi: 10.1038/ng.3984. PubMed PMID: 29083409; PubMed Central PMCID: PMCPMC5709193.
21. Dempster JM, Rossen, J., Kazachkova, M., Pan, J., Kugener, G., Root, D. E., & Tsherniak, A. . Extracting Biological Insights from the Project Achilles Genome-Scale CRISPR Screens in Cancer Cell Lines. BioRxiv. 2019. doi: 10.1101/720243.
22. Kassambara A, Hose D, Moreaux J, Walker BA, Protopopov A, Reme T, et al. Genes with a spike expression are clustered in chromosome (sub)bands and spike (sub)bands have a powerful prognostic value in patients with multiple myeloma. Haematologica. 2012;97(4):622-30. Epub 2011/11/22. doi: 10.3324/haematol.2011.046821. PubMed PMID: 22102711; PubMed Central PMCID: PMCPMC3347668.
23. Herviou L, Kassambara A, Boireau S, Robert N, Requirand G, Muller-Tidow C, et al. PRC2 targeting is a therapeutic strategy for EZ score defined high-risk multiple myeloma patients and overcome resistance to IMiDs. Clin Epigenetics. 2018;10(1):121. Epub 2018/10/05. doi: 10.1186/s13148-018-0554-4. PubMed PMID: 30285865; PubMed Central PMCID: PMCPMC6171329.
24. Hartlerode AJ, Scully R. Mechanisms of double-strand break repair in somatic mammalian cells. Biochem J. 2009;423(2):157-68. Epub 2009/09/24. doi: 10.1042/BJ20090942. PubMed PMID: 19772495; PubMed Central PMCID: PMCPMC2983087.
25. Wright WD, Shah SS, Heyer WD. Homologous recombination and the repair of DNA double-strand breaks. J Biol Chem. 2018;293(27):10524-35. Epub 2018/03/31. doi: 10.1074/jbc.TM118.000372. PubMed PMID: 29599286; PubMed Central PMCID: PMCPMC6036207.
26. D'Amours D, Jackson SP. The Mre11 complex: at the crossroads of dna repair and checkpoint signalling. Nat Rev Mol Cell Biol. 2002;3(5):317-27. Epub 2002/05/04. doi: 10.1038/nrm805. PubMed PMID: 11988766.
27. Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science. 2005;308(5721):551-4. Epub 2005/03/26. doi: 10.1126/science.1108297. PubMed PMID: 15790808.
28. Esposito MT, So CW. DNA damage accumulation and repair defects in acute myeloid leukemia: implications for pathogenesis, disease progression, and chemotherapy resistance. Chromosoma. 2014;123(6):545-61. Epub 2014/08/13. doi: 10.1007/s00412-014-0482-9. PubMed PMID: 25112726.
29. Uringa EJ, Youds JL, Lisaingo K, Lansdorp PM, Boulton SJ. RTEL1: an essential helicase for telomere maintenance and the regulation of homologous recombination. Nucleic Acids Res. 2011;39(5):1647-55. Epub 2010/11/26. doi: 10.1093/nar/gkq1045. PubMed PMID: 21097466; PubMed Central PMCID: PMCPMC3061057.
30. Dong S, Han J, Chen H, Liu T, Huen MSY, Yang Y, et al. The human SRCAP chromatin remodeling complex promotes DNA-end resection. Curr Biol. 2014;24(18):2097-110. Epub 2014/09/02. doi: 10.1016/j.cub.2014.07.081. PubMed PMID: 25176633.
31. Spivak G. Nucleotide excision repair in humans. DNA Repair (Amst). 2015;36:13-8. Epub 2015/09/22. doi: 10.1016/j.dnarep.2015.09.003. PubMed PMID: 26388429; PubMed Central PMCID: PMCPMC4688078.

32. Hashimoto S, Anai H, Hanada K. Mechanisms of interstrand DNA crosslink repair and human disorders. Genes Environ. 2016;38:9. Epub 2016/06/29. doi: 10.1186/s41021-016-0037-9. PubMed PMID: 27350828; PubMed Central PMCID: PMCPMC4918140.
33. Vonarx EJ, Tabone EK, Osmond MJ, Anderson HJ, Kunz BA. Arabidopsis homologue of human transcription factor IIH/nucleotide excision repair factor p44 can function in transcription and DNA repair and interacts with AtXPD. Plant J. 2006;46(3):512-21. Epub 2006/04/21. doi: 10.1111/j.1365-313X.2006.02705.x. PubMed PMID: 16623910.
34. London RE. The structural basis of XRCC1-mediated DNA repair. DNA Repair (Amst). 2015;30:90-103. Epub 2015/03/22. doi: 10.1016/j.dnarep.2015.02.005. PubMed PMID: 25795425; PubMed Central PMCID: PMCPMC5580684.
35. Pietrzak J, Ploszaj T, Pulaski L, Robaszkiewicz A. EP300-HDAC1-SWI/SNF functional unit defines transcription of some DNA repair enzymes during differentiation of human macrophages. Biochim Biophys Acta Gene Regul Mech. 2019;1862(2):198-208. Epub 2018/11/12. doi: 10.1016/j.bbagrm.2018.10.019. PubMed PMID: 30414852.
36. Seedhouse C, Faulkner R, Ashraf N, Das-Gupta E, Russell N. Polymorphisms in genes involved in homologous recombination repair interact to increase the risk of developing acute myeloid leukemia. Clin Cancer Res. 2004;10(8):2675-80. Epub 2004/04/23. PubMed PMID: 15102670.
37. Jawad M, Seedhouse CH, Russell N, Plumb M. Polymorphisms in human homeobox HLX1 and DNA repair RAD51 genes increase the risk of therapy-related acute myeloid leukemia. Blood. 2006;108(12):3916-8. Epub 2006/08/12. doi: 10.1182/blood-2006-05-022921. PubMed PMID: 16902145.
38. Li C, Liu Y, Hu Z, Zhou Y. Genetic polymorphisms of RAD51 and XRCC3 and acute myeloid leukemia risk: a meta-analysis. Leuk Lymphoma. 2014;55(6):1309-19. Epub 2013/08/28. doi: 10.3109/10428194.2013.835404. PubMed PMID: 23978154.
39. Wu L, Long ZG, Dai ZS. 135G/C polymorphism in the RAD51 gene and acute myeloid leukemia risk: a meta-analysis. Genet Mol Res. 2016;15(2). Epub 2016/05/14. doi: 10.4238/gmr.15027383. PubMed PMID: 27173193.
40. Allan JM, Smith AG, Wheatley K, Hills RK, Travis LB, Hill DA, et al. Genetic variation in XPD predicts treatment outcome and risk of acute myeloid leukemia following chemotherapy. Blood. 2004;104(13):3872-7. Epub 2004/09/02. doi: 10.1182/blood-2004-06-2161. PubMed PMID: 15339847.
41. Sallmyr A, Fan J, Datta K, Kim KT, Grosu D, Shapiro P, et al. Internal tandem duplication of FLT3 (FLT3/ITD) induces increased ROS production, DNA damage, and misrepair: implications for poor prognosis in AML. Blood. 2008;111(6):3173-82. Epub 2008/01/15. doi: 10.1182/blood-2007-05-092510. PubMed PMID: 18192505.
42. Seedhouse CH, Hunter HM, Lloyd-Lewis B, Massip AM, Pallis M, Carter GI, et al. DNA repair contributes to the drug-resistant phenotype of primary acute myeloid leukaemia cells with FLT3 internal tandem duplications and is reversed by the FLT3 inhibitor PKC412. Leukemia. 2006;20(12):2130-6. Epub 2006/10/27. doi: 10.1038/sj.leu.2404439. PubMed PMID: 17066094.
43. Aalbers AM, Calado RT, Young NS, Zwaan CM, Wu C, Kajigaya S, et al. Telomere length and telomerase complex mutations in pediatric acute myeloid leukemia. Leukemia. 2013;27(8):1786-9. Epub 2013/02/22. doi: 10.1038/leu.2013.57. PubMed PMID: 23426163; PubMed Central PMCID: PMCPMC4163790.
44. Bagrintseva K, Geisenhof S, Kern R, Eichenlaub S, Reindl C, Ellwart JW, et al. FLT3-ITD-TKD dual mutants associated with AML confer resistance to FLT3 PTK inhibitors and cytotoxic agents by overexpression of Bcl-x(L). Blood. 2005;105(9):3679-85. Epub 2005/01/01. doi: 10.1182/blood-2004-06-2459. PubMed PMID: 15626738.
45. Alpermann T, Schnittger S, Eder C, Dicker F, Meggendorfer M, Kern W, et al. Molecular subtypes of NPM1 mutations have different clinical profiles, specific patterns of accompanying molecular mutations and varying outcomes in intermediate risk acute myeloid leukemia. Haematologica. 2016;101(2):e55-8. Epub 2015/10/17. doi: 10.3324/haematol.2015.133819. PubMed PMID: 26471486; PubMed Central PMCID: PMCPMC4938334.
46. Okuda M, Horn HF, Tarapore P, Tokuyama Y, Smulian AG, Chan PK, et al. Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. Cell. 2000;103(1):127-40. Epub 2000/10/29. doi: 10.1016/s0092-8674(00)00093-3. PubMed PMID: 11051553.

47. Koike A, Nishikawa H, Wu W, Okada Y, Venkitaraman AR, Ohta T. Recruitment of phosphorylated NPM1 to sites of DNA damage through RNF8-dependent ubiquitin conjugates. Cancer Res. 2010;70(17):6746-56. Epub 2010/08/18. doi: 10.1158/0008-5472.CAN-10-0382. PubMed PMID: 20713529.
48. Lirussi L, Antoniali G, Vascotto C, D'Ambrosio C, Poletto M, Romanello M, et al. Nucleolar accumulation of APE1 depends on charged lysine residues that undergo acetylation upon genotoxic stress and modulate its BER activity in cells. Mol Biol Cell. 2012;23(20):4079-96. Epub 2012/08/25. doi: 10.1091/mbc.E12-04-0299. PubMed PMID: 22918947; PubMed Central PMCID: PMCPMC3469522.
49. Colombo E, Marine JC, Danovi D, Falini B, Pelicci PG. Nucleophosmin regulates the stability and transcriptional activity of p53. Nat Cell Biol. 2002;4(7):529-33. Epub 2002/06/25. doi: 10.1038/ncb814. PubMed PMID: 12080348.
50. Vascotto C, Lirussi L, Poletto M, Tiribelli M, Damiani D, Fabbro D, et al. Functional regulation of the apurinic/apyrimidinic endonuclease 1 by nucleophosmin: impact on tumor biology. Oncogene. 2014;33(22):2876-87. Epub 2013/07/09. doi: 10.1038/onc.2013.251. PubMed PMID: 23831574.
51. Lichtman MA. A historical perspective on the development of the cytarabine (7days) and daunorubicin (3days) treatment regimen for acute myelogenous leukemia: 2013 the 40th anniversary of 7+3. Blood Cells Mol Dis. 2013;50(2):119-30. Epub 2012/11/17. doi: 10.1016/j.bcmd.2012.10.005. PubMed PMID: 23154039.
52. Murphy T, Yee KWL. Cytarabine and daunorubicin for the treatment of acute myeloid leukemia. Expert Opin Pharmacother. 2017;18(16):1765-80. Epub 2017/10/12. doi: 10.1080/14656566.2017.1391216. PubMed PMID: 29017371.
53. Bret C, Klein B, Moreaux J. Nucleotide excision DNA repair pathway as a therapeutic target in patients with high-risk diffuse large B cell lymphoma. Cell Cycle. 2013;12(12):1811-2. Epub 2013/05/28. doi: 10.4161/cc.25115. PubMed PMID: 23708513; PubMed Central PMCID: PMCPMC3735686.
54. Hou J, Deng Q, Zhou J, Zou J, Zhang Y, Tan P, et al. CSN6 controls the proliferation and metastasis of glioblastoma by CHIP-mediated degradation of EGFR. Oncogene. 2017;36(8):1134-44. Epub 2016/08/23. doi: 10.1038/onc.2016.280. PubMed PMID: 27546621.
55. Gao S, Fang L, Phan LM, Qdaisat A, Yeung SC, Lee MH. COP9 signalosome subunit 6 (CSN6) regulates E6AP/UBE3A in cervical cancer. Oncotarget. 2015;6(29):28026-41. Epub 2015/09/01. doi: 10.18632/oncotarget.4731. PubMed PMID: 26318036; PubMed Central PMCID: PMCPMC4695042.
56. Wen D, Liao T, Ma B, Qu N, Shi RL, Lu ZW, et al. Downregulation of CSN6 attenuates papillary thyroid carcinoma progression by reducing Wnt/beta-catenin signaling and sensitizes cancer cells to FH535 therapy. Cancer Med. 2018;7(2):285-96. Epub 2018/01/18. doi: 10.1002/cam4.1272. PubMed PMID: 29341469; PubMed Central PMCID: PMCPMC5806103.
57. Shaheen M, Allen C, Nickoloff JA, Hromas R. Synthetic lethality: exploiting the addiction of cancer to DNA repair. Blood. 2011;117(23):6074-82. Epub 2011/03/29. doi: 10.1182/blood-2011-01-313734. PubMed PMID: 21441464.
58. Curtin NJ. Inhibiting the DNA damage response as a therapeutic manoeuvre in cancer. Br J Pharmacol. 2013;169(8):1745-65. Epub 2013/05/21. doi: 10.1111/bph.12244. PubMed PMID: 23682925; PubMed Central PMCID: PMCPMC3753833.
59. Kohl V, Flach J, Naumann N, Brendel S, Kleiner H, Weiss C, et al. Antileukemic Efficacy in Vitro of Talazoparib and APE1 Inhibitor III Combined with Decitabine in Myeloid Malignancies. Cancers (Basel). 2019;11(10). Epub 2019/10/19. doi: 10.3390/cancers11101493. PubMed PMID: 31623402; PubMed Central PMCID: PMCPMC6826540.
60. Metzeler KH, Hummel M, Bloomfield CD, Spiekermann K, Braess J, Sauerland MC, et al. An 86-probe-set gene-expression signature predicts survival in cytogenetically normal acute myeloid leukemia. Blood. 2008;112(10):4193-201. Epub 2008/08/22. doi: 10.1182/blood-2008-02-134411. PubMed PMID: 18716133; PubMed Central PMCID: PMCPMC2954679.
61. Huber W, von Heydebreck A, Sultmann H, Poustka A, Vingron M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. Bioinformatics. 2002;18 Suppl 1:S96-104. Epub 2002/08/10. doi: 10.1093/bioinformatics/18.suppl_1.s96. PubMed PMID: 12169536.
62. Bret C, Klein B, Cartron G, Schved JF, Constantinou A, Pasero P, et al. DNA repair in diffuse large B-cell lymphoma: a molecular portrait. Br J Haematol. 2015;169(2):296-9. Epub 2014/11/06. doi: 10.1111/bjh.13206. PubMed PMID: 25369781.

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