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DNA repair in diffuse large B-cell lymphoma: a molecular portrait

Diffuse large B-cell lymphoma (DLBCL) accounts for 30–40% of adult non-Hodgkin lymphomas. Most DLBCL patients achieve long-term remission after treatment, but a third relapse after conventional Rituximab (R)-based chemotherapy regimens, such as CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) (Siegel *et al*, 2012).

Cancer cells are exposed to chronic replication stress, which impedes the duplication of their genome and induces mitotic catastrophe (Shaheen *et al*, 2011). Functional DNA repair pathways are therefore important for the survival of cancer cells. This dependence can be exploited therapeutically to hamper repair of the intrinsic DNA damage occurring during replication or to exacerbate DNA damage induced by chemotherapy (Shaheen *et al*, 2011). Furthermore, high-risk DLBCL patients overexpress genes potentially involved in resistance to CHOP-based regimens, such as genes of the nucleotide excision repair (NER) pathway (Bret *et al*, 2012, 2013).

This study aimed to identify deregulated DNA repair pathways in DLBCL tumour samples in order to develop novel therapeutic strategies that exploit the concept of synthetic lethality and overcome drug resistance.

Gene expression microarray data from two independent cohorts of patients diagnosed with DLBCL were used ($n = 233$ treated with R-CHOP; $n = 181$ treated with CHOP) (Lenz *et al*, 2008). (Gene Expression Omnibus; accession number GSE10846).

A list set of 176 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 (Table SI). The Maxstat R function and Benjamini Hochberg multiple testing correction showed that 126 out of the 176 genes have a prognostic value (92 genes with poor and 34 with good prognostic values) (Table SII).

For each pathway, a gene expression profile (GEP)-based risk score was created as the sum of the beta coefficients weighted by ± 1 according to the patient signal above or below the probe set Maxstat value as previously reported (Kassambara *et al*, 2012). For each pathway, patients were ranked according to increased prognostic score and for a given score value X, the difference in survival of patients with a prognostic score $\leq X$ or $> X$ was computed using Maxstat analysis. High FANC, NER, HRR, BER, NHEJ and MMR scores were significantly associated with poor prognosis in the two cohorts of patients (Figure S1A–F and Table SIII–SVIII).

The NER, HRR, BER, NHEJ or MMR scores were significantly higher ($P < 0{\cdot}01$) in the activated B-cell like (ABC) molecular subgroup compared to the germinal centre B cell (GCB) subgroup whereas no significant differences were observed for FANC score (Figure S2). Interestingly, FANC, NER, HRR, BER, NHEJ and MMR scores had prognostic value in both GCB and ABC molecular subgroups (Figure S3). Cox analysis was used to determine whether the different DNA repair pathway scores provided additional

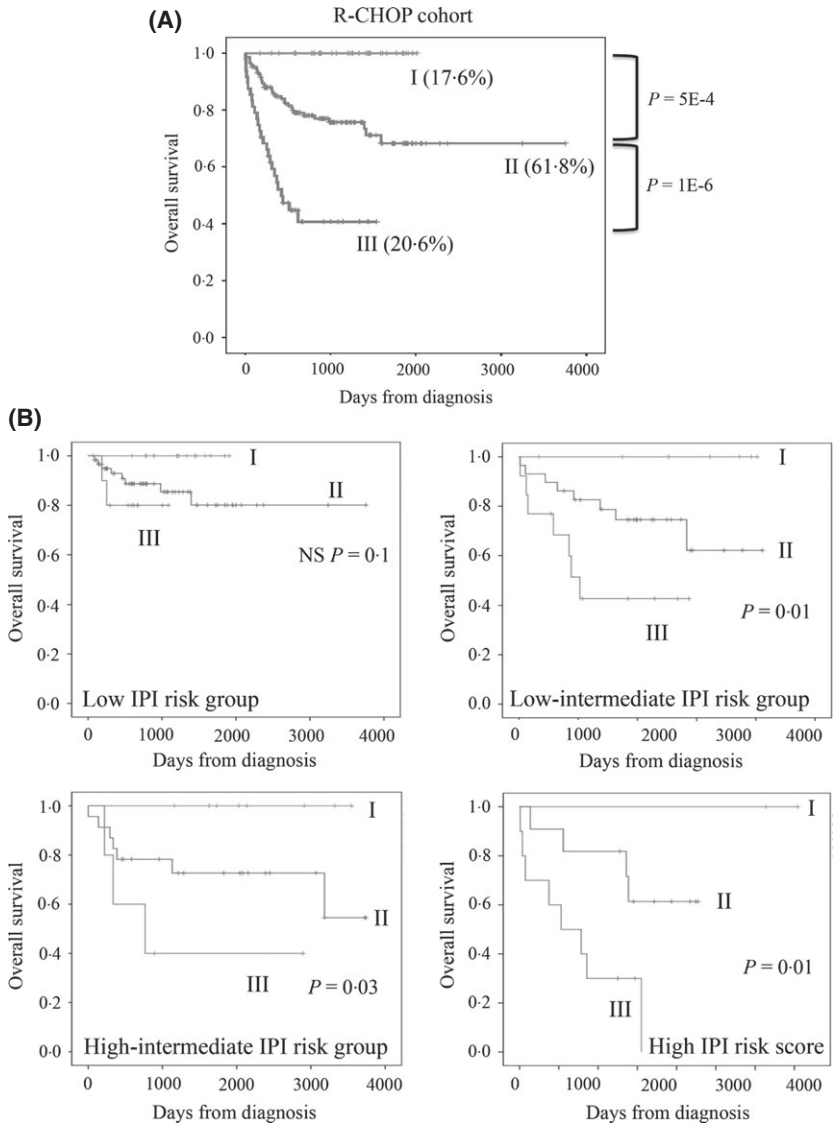

Fig 1. (A) Combination of the prognostic information of FANC, NHEJ and MMR scores in a DNA repair score. Patients of the R-CHOP cohort (n = 233) were ranked according to increasing DNA repair score and separated in three groups using Maxstat R function. (B) Prognostic value of DNA repair score for subgroups of DLBCL patients defined by international prognostic index (IPI). DLBCL patients within low, low-intermediate, high-intermediate or high-risk IPI groups were divided using DNA repair score. IPI groups: low risk group/IPI score 0 or 1 (n = 89), low-intermediate risk group/IPI score 2 (n = 49), high-intermediate risk group/IPI score 3 (n = 36) and high risk group/IPI score 4 or 5 (n = 23). DLBCL, diffuse large B-cell lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; GERS, gene expression-based risk score; GCB, germinal centre B cell-like; ABC, activated B cell-like; IPI, International Prognostic Index.

prognostic information compared to previously identified poor outcome-related factors. When all parameters were tested together, only the gene expression-based risk score (GERS), FANC, NHEJ and MMR scores maintained prognostic value (Table SIX).

Given that the FANC, NHEJ and MMR scores displayed independent prognostic information, these three DNA repair scores were combined to create a new DNA repair score.

Using Maxstat, the RCHOP cohort was classified into three groups according to the combined DNA repair score. Groups I (low DNA repair score; n = 40) and II (intermediate DNA repair score; n = 144) had not reached a median overall survival (OS) although Group I had significantly better OS than Group II (P = 0·0005). Group III (high DNA repair score; n = 48) had the worst prognostic value with a median OS of 13·9 months (Fig 1A): 28% of Group III patients were in the low International Prognostic Index (IPI) risk group, 33% were low-intermediate IPI, 13% were high-intermediate IPI and 26% were high risk IPI; 58% of patients were ABC subtype, 33% in GCB and 9% were unclassified. The prognostic value of the DNA repair score was validated in the CHOP cohort (Figure S4). Comparing the DNA repair score with other poor outcome-related factors, such as GERS score, GCB or ABC subtype and the IPI in multivariate COX analysis, only DNA repair score retained prognostic value (Table I). We investigated the prognostic value of the DNA repair score for subgroups of DLBCL patients defined by IPI. DNA repair score allowed all IPI subgroups to be divided into three groups (Fig 1B). The prognostic value of the DNA repair score was not significant in the low IPI risk group (P = 0·1) but segregated DLBCL patients with low-intermediate, high-intermediate and high IPI risk into three significantly different prognostic groups (P = 0·01, P = 0·03 and P = 0·01 respectively) (Fig 1B).

Several DNA repair inhibitors are being tested in clinical cancer trials (Shaheen *et al*, 2011). DLBCL treatments

Table I. Cox univariate and multivariate analysis of overall survival in DLBCL patient treated with R-CHOP (n = 233) including DNA repair score. The prognostic factors were tested as single variable (A) or multivariables (B) using Cox-model. *P*-values and Hazard Ratios (HR) are shown.

	Overall survival (n = 233)	
Prognostic variable	HR	*P* value
A.		
GERS	4·62	<0·00001
Age (>60 years)	2·2	<0·0001
GCB-ABC subtype	2·75	<0·0001
IPI	1·79	<0·0001
DNA repair score	3·8	<0·0001
B.		
GERS	1·99	NS
Age (>60 years)	0·93	NS
GCB-ABC subtype	1·72	NS
IPI	1·19	NS
DNA repair score	2·26	0·008

DLBCL, diffuse large B-cell lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; GERS, gene expression-based risk score; GCB, germinal centre B cell-like; ABC, activated B cell-like; IPI, International Prognostic Index; NS, not significant at a 5% threshold.

include cyclophosphamide, a nitrogen mustard derivate that induces interstrand crosslinks (ICLs), and doxorubicin, a DNA topoisomerase inhibitor that induces DNA double-strand breaks, DNA adducts and formaldehyde-dependent ICL formation (Bret *et al*, 2013). Inhibiting DNA repair is a promising strategy to improve the efficacy of genotoxic drugs and overcome drug resistance (Curtin, 2013). Our data support the view that inhibitors of DNA damage signalling and DNA repair have potential therapeutic interest in DLBCL.

Despite overall improvements in the treatment of DLBCL, including the use of rituximab, approximately one-third of patients fail to achieve complete remission or experience relapse. This remains a major cause of morbidity and mortality. The DNA repair scores could be useful to identify high-risk DLBCL patients and define the best DNA repair inhibitor to employ in combination with conventional treatment. Accordingly, it will be important to evaluate the association of DNA repair scores with event-free survival or time to first relapse. Furthermore, these DNA repair scores could be useful at different times of treatment, particularly at relapse, to define targeted therapies that have greater effectiveness and render resistant tumours responsive to treatment. Recent data indicate that DLBCL relapse may result from multiple different evolutionary mechanisms (Redmond *et al*, 2013). The DNA repair scores could be valuable for adapting targeted treatment according to the drug resistance mechanisms selected during clonal evolution. As GEP is not included in the current routine diagnostic work-up, efforts should be made to identify substitutes involving immunohistochemistry (Curtis *et al*, 2010) or quantitative measurement of alterations in DNA repair pathways using single cell network profiling by flow cytometry (Rosen *et al*, 2014). Furthermore, understanding of the functional role of these DNA repair pathways in the pathogenesis and drug resistance of DLBCL is needed. These advances may limit the side effects of treatment, improving compliance with dosing regimens and overall quality of life.

Acknowledgement

This work was supported by grants from ANR emergence (ETTMM), FEDER (141786 & 42667) and Languedoc Roussillon CRLR (R14026FF).

Author contributions

CB performed research, bioinformatic studies and participated in the writing of the paper. BK participated in the research and in the writing of the paper. GC participated in clinical data analysis and participated in the writing of the paper. JFS participated in the writing of the paper. AC participated in the research and in the writing of the paper. PP participated in the research and in the writing of the paper. JM supervised the research, bioinformatic studies and the writing of the paper.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Keywords: diffuse large B-cell lymphoma, gene expression profile, DNA repair, prognosis

First published online 5 November 2014
doi: 10.1111/bjh.13206

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig S1. Prognostic value of DNA repair scores in DLBCL patients.

Fig S2. FANC, NER, HRR, BER, NHEJ, and MMR scores in ABC and GCB molecular subgroups.

Fig S3. Prognostic prediction applying FANC, NER, HRR, BER, NHEJ and MMR scores in ABC/GCB subgroups of DLBCL patients.

Fig S4. The prognostic value of the DNA repair score was validated on an independent cohort of 181 patients treated with CHOP regimen.

Table SI. Genes coding for proteins involved in DNA repair pathways.

Table SII. Identification of DNA repair genes whose expression is associated with a prognostic value in DLBCL patients.

Table SIII. Identification of Fanconi pathway genes whose expression associated with a prognostic value in DLBCL patients.

Table SIV. Identification of NER genes whose expression associated with a prognostic value in DLBCL patients.

Table SV. Identification of homologous recombination repair genes whose expression associated with a prognostic value in DLBCL patients.

Table SVI. Identification of base excision repair genes whose expression associated with a prognostic value in DLBCL patients.

Table SVII. Identification of NHEJ genes whose expression associated with a prognostic value in DLBCL patients.

Table SVIII. Identification of MMR genes whose expression associated with a prognostic value in DLBCL patients.

Table SIX. Cox univariate and multivariate analysis of OS in DLBCL patient's R-CHOP cohort (n = 233).

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Up-front therapy for LCH: is it time to test an alternative to vinblastine/prednisone?

Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasia (Berres *et al*, 2014) occurring most commonly in children that can be fatal if 'risk' organs (liver, spleen, bone marrow) are involved. Long-term consequences including endocrinopathies, sclerosing cholangitis and debilitating neurodegeneration remain problematic (McClain *et al*, 2011). Vinblastine plus prednisone is standard of care for children with *de novo* multisystem disease and has been the primary backbone utilized in 30 years of multinational trials (Gadner *et al*, 2013). While overall survival has steadily improved, outcomes for patients with LCH remain suboptimal. Approximately half of patients will fail to be cured with this approach, requiring extended or alternate therapy. The estimated 5-year mortality in patients with risk organ disease remains approximately 15%, and the highest risks of death or disease complications are in patients who fail initial therapy (Gadner *et al*, 2013). Toxicities of vinblastine and prednisone include excessive weight gain, growth retardation and peripheral neuropathy. In our view, these outcomes with vinblastine/prednisone leave room for improvement.