



HAL
open science

The negative influence of baseline cell-free DNA on long-term survival in DLBCL depends on frontline treatment intensity

Fabienne Desmots-Loyer, Delphine Rossille, Mikael Roussel, Céline Pangault, Laetitia Louarn, Mylène de Saint Jore, Steven Le Gouill, Krimo Bouabdallah, V Delwail, Rémy Gressin, et al.

► To cite this version:

Fabienne Desmots-Loyer, Delphine Rossille, Mikael Roussel, Céline Pangault, Laetitia Louarn, et al.. The negative influence of baseline cell-free DNA on long-term survival in DLBCL depends on frontline treatment intensity. *Clinical Cancer Research*, 2023, 29 (12), pp.2280-90. 10.1158/1078-0432.CCR-22-2964 . hal-04125745

HAL Id: hal-04125745

<https://hal.science/hal-04125745>

Submitted on 13 Jun 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 ***The negative influence of baseline cell-free DNA on long-term survival in***
2 ***DLBCL depends on frontline treatment intensity***

3

4 **Running title:** cfDNA and DLBCL overall survival

5

6 **Authors:** Fabienne Desmots^{1,2}, PhD §, Delphine Rossille^{1,2}, PhD §, Mikael Roussel^{1,}
7 ², MD, PhD, Céline Pangault^{1, 2}, PhD, Laetitia Louarn ^{1, 2}, Mylène de Saint Jore²,
8 Steven Le Gouill³, MD, PhD, Krimo Bouabdallah⁴, MD, Vincent Delwail⁵, MD, Remy
9 Gressin⁶, MD, Jérôme Cornillon⁷, MD, Gandhi Damaj^{8, 9}, MD, Hervé Maisonneuve¹⁰,
10 MD, Diane Damotte¹¹, MD, PhD, Françoise Kraeber-Bodere¹², MD, PhD, Thierry
11 Lamy, MD, PhD ^{1, 13}, Marie-Cécile Parrens¹⁴, MD, PhD, Noël Milpied⁴, MD § and
12 Thierry Fest, MD, Ph.D^{1,2}§.

13

14 §) These authors contributed equally

15 \$) co-seniorship

16 **Funding:** This trial was funded by the French government's PHRC program
17 (reference: #20.05); cfDNA analysis was funded by Carnot/CALYM and Laboratoire
18 d'Hématologie du CHU de Rennes.

19 The GOELAMS 075 trial was registered at ClinicalTrials.gov (NCT00561379)

20 **Affiliations:** 1) Université de Rennes 1, INSERM, Établissement Français du Sang
21 de Bretagne, UMR_S1236, Rennes, F-35043, France; 2) Laboratoire d'Hématologie,
22 Pôle de Biologie, Centre Hospitalier Universitaire, Rennes, France; 3) Service
23 d'hématologie, Centre Hospitalier Universitaire, Inserm, CIRCINA, Nantes, France; 4)
24 Service d'hématologie et de thérapie cellulaire, Centre Hospitalier Universitaire,
25 Bordeaux, France ; 5) Service d'oncologie hématologique et de thérapie cellulaire,
26 Centre Hospitalier Universitaire, INSERM, CIC 1402, Poitiers, Centre d'Investigation
27 Clinique, Poitiers, France; 6) Service d'onco-hématologie, Centre Hospitalier
28 Universitaire and Institute for Advanced Biosciences, INSERM U1209/CNRS UMR
29 5309, Grenoble, France; 7) Département d'Hématologie Clinique, Centre Hospitalier
30 Universitaire, Saint-Etienne, France; 8) Service d'Hématologie, Centre Hospitalier
31 Universitaire, Amiens, France; 9) Current address: Institut d'hématologie, Centre

32 Hospitalier Universitaire, Caen, France; 10) Service d'hématologie, Centre
33 Hospitalier Départemental Vendée, La Roche-sur-Yon, France; 11) Département de
34 pathologie, Assistance Public Hôpitaux de Paris, Paris, France; 12) Service de
35 médecine nucléaire, Centre Hospitalier Universitaire, Inserm, CIRCINA, Nantes,
36 France; 13) Service d'hématologie, Centre Hospitalier Universitaire, Rennes, France;
37 14) Département de pathologie, Centre Hospitalier Universitaire, Bordeaux, France.

38

39 **Corresponding author:** Thierry Fest, MD, PhD, INSERM U1236, Faculté de
40 Médecine, 2 avenue du Pr Léon Bernard, CS 34317, F-35043 Rennes cedex,
41 France. Phone: +33-299-284-127. E-mail: thierry.fest@univ-rennes1.fr

42

43 **Regular article: scientific category Lymphoma**

44 Abstract word count: 236 words

45 Main text word count: 3,827 words

46 Figure/Table count: 6

47 Reference count: 29

48 Supplemental Methods, Tables & Figures

49

50 **Keywords:** cell-free DNA, DLBCL, R-CHOP, high-dose regimens, ASCT in DLBCL

51

52

53 **TRANSLATIONAL RELEVANCE**

54 *Tumor-derived plasma cell free DNA (cfDNA), as a surrogate marker of disease*
55 *burden and early predictor of response to therapy, offers opportunities for therapeutic*
56 *intervention, particularly in DLBCL. The gold standard treatment for de novo DLBCL*
57 *is R-CHOP, but some studies have shown benefit for patients with dose escalation in*
58 *first-line therapy. In our study, we took advantage of a single randomized clinical trial*
59 *comparing R-CHOP with conventional high-dose chemotherapy plus autologous*
60 *stem cell transplantation (ASCT) for first-line treatment of advanced DLBCL to*
61 *explore the effect of cfDNA on patients' clinical outcome. We confirmed that cfDNA*
62 *concentration at diagnosis was an adverse prognostic factor for patients in the R-*
63 *CHOP arm but not for those in the high-dose arm. Interestingly, patients with positive*
64 *PETscan at the interim evaluation who underwent salvage chemotherapy*
65 *supplemented with ASCT had significantly improved survival compared with good*
66 *responders at the interim evaluation but treated with R-CHOP and with high cfDNA*
67 *levels at diagnosis. Our results demonstrate that high-dose first-line therapy mitigates*
68 *the negative influence of high cfDNA levels at diagnosis. Moreover, once CR was*
69 *achieved, for 11 out of 24 R-CHOP patients, the cfDNA concentration did not fall*
70 *back to normal levels. Overall, these results raise the question of whether R-CHOP*
71 *should be maintained as first-line therapy for DLBCL patients with elevated cfDNA*
72 *levels at diagnosis; alternative treatments could be considered, including high-dose*
73 *chemotherapy combined with ASCT.*

74

75

76

77

78 **ABSTRACT**

79 Purpose: In this study, we address the question of the effect of first-line treatment
80 intensity in de novo DLBCL on the impact of baseline cfDNA on long-term survival.

81

82 Experimental Design: The GOELAMS 075 randomized clinical trial compared R-
83 CHOP with high-dose R-chemotherapy plus autologous stem cell transplantation (R-
84 HDT) for patients aged ≤ 60 . An interim PET assessment was used to refer patients
85 for salvage therapy. With a median follow-up of more than 5.8 years, we analyzed
86 the effects of the treatment arm, salvage therapy, and cfDNA level at diagnosis on
87 overall survival (OS).

88

89 Results: In a representative group of 123 patients, a high cfDNA concentration
90 ($>55\text{ng/ml}$) at diagnosis was associated with poor clinical prognostic factors and
91 constituted a prognostic marker, independently of the age-adjusted International
92 Prognostic Index. A cfDNA level above a threshold value of 55 ng/ml at diagnosis
93 was associated with significantly worse OS. In an intention-to-treat analysis, high-
94 cfDNA R-CHOP patients (but not high-cfDNA R-HDT patients) had worse OS (hazard
95 ratio [95% confidence interval]: $3.99 [1.98-10.74]$, $P=0.006$). In patients with high
96 cfDNA levels, salvage therapy and transplantation were associated with a
97 significantly higher OS rate. Among 50 patients with complete response 6 months
98 after the end of treatment, for 11 out of 24 R-CHOP patients, the cfDNA did not fall
99 back to normal values.

100

101 Conclusions: In this randomized clinical trial, intensive regimens mitigated the
102 negative influence of high cfDNA levels in de novo DLBCL, relative to R-CHOP.

103 **INTRODUCTION**

104 The first-line treatment for diffuse large B-cell lymphoma (DLBCL) combines
105 rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone. This R-
106 CHOP regimen is the standard of care, regardless of the cancer's
107 immunohistochemical profile or molecular subtype ¹. However, 30 to 40% of patients
108 are refractory to this treatment or relapse within 2 years of an initial response. After
109 relapse, the overall cure rate ranges from 25 to 35% ¹. Refractory patients have a
110 poor prognosis, with a median overall survival (OS) time of around 6 months ². It is
111 therefore very important to provide the most appropriate treatment as soon as *de*
112 *novo* DLBCL has been diagnosed. In the early 2000s (i.e., before the rituximab era),
113 we showed that first-line high-dose chemotherapy with autologous stem cell
114 transplantation (ASCT) was associated with longer disease-free survival in
115 transplant-eligible patients with advanced DLBCL ^{3,4} and an OS benefit in a subset of
116 patients ³. Since then, it has been shown that a combination of rituximab with high-
117 dose conventional chemotherapy prolongs survival in young adults with a low-
118 intermediate level of risk (according to the International Prognostic Index (IPI)) ⁵. At
119 present, primary staging is based on the IPI and positron emission tomography-
120 computed tomography (PET-CT) findings, which also allow assessment of the
121 treatment response ⁶. In 2005, we initiated a French, open-label, randomized, Phase
122 3 trial (GOELAMS 075, NCT00561379) of R-CHOP vs. a combination of rituximab,
123 high-dose conventional chemotherapy and ASCT (hereafter referred to as R-HDT)
124 for the first-line treatment of advanced DLBCL adult patients aged ≤60. The median
125 follow-up period after randomization was >5.8 years (70.4 months; 95% confidence
126 interval (CI), 55.0 – 88.9).

127 The bloodstream of cancer patients usually contains circulating, cell-free DNA
128 (cfDNA) released from tumor cells after apoptotic, caspase-dependent cleavage. The

129 predominant DNA peak is 166 base-pairs (bp) and is accompanied by a series of
130 smaller peaks with a periodicity of 10 bp ⁷. Although tumor-specific alterations can
131 have diagnostic and prognostic value, not all patients have detectable changes ⁸.
132 The circulating cfDNA concentration varies significantly from one healthy individual to
133 another and is higher in patients with lymphoma ⁹. The proportion of circulating tumor
134 (ct) DNA is especially high in patients with aggressive lymphomas ¹⁰. The concept of
135 ctDNA as a surrogate marker of disease burden and an early predictor of the
136 treatment response offers opportunities for therapeutic intervention. Although the
137 optimal ctDNA measurement time points and the thresholds for quantitative changes
138 have not been validated extensively, a post-treatment fall in detectable ctDNA level is
139 a good indicator of clinical benefit for various tumors and treatment contexts ¹¹.
140 In the present study of patients with newly diagnosed, aggressive DLBCL, we
141 examined the potential prognostic value of the cfDNA concentration in a 90-to-320 bp
142 window. We also investigated the putative impact of the type of first-line therapy on
143 the cfDNA concentration. To this end, we took advantage of the GOELAMS 075
144 study, in which plasma samples were collected (i) at diagnosis for 123 patients and
145 (ii) at the time of a complete response (CR) 6 months after the end of treatment
146 (EOT) for 50 of these 123 patients. The median follow-up time was 6.9 years. Our
147 results revealed that the cfDNA concentration at diagnosis was an unfavorable
148 prognostic factor for patients in the R-CHOP arm but not for those in the R-HDT arm.
149 The achievement of CR parallels a clearance of cfDNA, except in a number of R-
150 CHOP patients who maintained elevated levels of cfDNA.

151

152 **METHODS**

153

154 **Patients**

155 The GOELAMS 075 study (referred to hereafter as the G075 cohort study) is a
156 multicenter trial in which adults aged 18 to 60 with untreated, histologically-proven,
157 CD20-positive DLBCL were randomly assigned to receive several courses of R-
158 CHOP or R-HDT (supplemental Figure 1A). Patients were required to have an Ann
159 Arbor stage of I or II with bulk >7 cm or a stage of III or IV with zero to three adverse
160 prognostic factors, as defined by the age-adjusted IPI (aa-IPI). Patients with
161 transformed low-grade, lymphoblastic, mantle cell or Burkitt's lymphoma were
162 excluded. Diagnostic slides were reviewed centrally by two hematopathologists (D.D.
163 and M-C.P.). The trial was approved by the investigational review board at Nantes
164 University Medical Center (Nantes, France), and all patients gave their written,
165 informed consent. The study was conducted in accordance with the guidelines of the
166 Declaration of Helsinki.

167 Patients with DLBCL (N=178; median follow-up time: 44 months) randomized into the
168 GAINED trial (NCT01659099) were used as an independent validation cohort for the
169 OS analysis ⁶. The GAINED study is a multicenter randomized phase 3 trial
170 comparing Obinutuzumab to Rituximab in association with a chemotherapy
171 administered every 14 days (doxorubicin, cyclophosphamide, prednisone plus
172 vindesine, bleomycin [ACVBP] or vincristine [CHOP]) using an interim PET-scan
173 driven strategy (supplemental Figure 1B). Transplant-eligible patients (18-60 years,
174 n=670), enrolled between 2012 and 2017, were diagnosed with a de novo DLBCL
175 with an age-adjusted International Prognostic Index (aaIPI) score ≥ 1 . The median
176 follow-up time of the GAINED cohort was 35.4 months.

177

178 **Data and tissue samples collected in the G075 cohort**

179 Plasma samples collected at the time of diagnosis (i.e. before any treatment) were
180 available for 123 of the 340 patients enrolled in the trial. Furthermore, post-treatment
181 plasma samples were available for 50 of the 123 patients who had maintained a CR
182 six months after the EOT (detailed information for CR patients in **Supplemental table**
183 **1**). For 178 of 670 patients of the GAINED cohort, plasma samples were collected at
184 randomization and control plasma was obtained from healthy volunteer (HV) blood
185 donors at the French National Blood Agency (age, 18-60). Plasma was collected in
186 BD™ P100® tubes (BD Diagnostics, Le Pont-De-Claix, France), shipped at room
187 temperature within 24 hours of collection to the core lab in Rennes (France),
188 centrifuged, and stored at -80°C.

189 For all G075 patients, baseline clinical data and treatment responses were obtained
190 during inclusion, interim and final assessments. For the 50 patients with a CR 6
191 months after the EOT, clinical data were obtained 7 to 10 years after inclusion.
192 Interim and final assessment responses were based on the PET-CT and standard CT
193 findings. The interim PET results were based on visual criteria and not the Deauville
194 criteria, which were established after the G075-cohort study had started. As per
195 protocol, patients with a positive interim imaging result received salvage therapy. The
196 collection of data and tissue samples for all patients in the present study had been
197 approved by the French national investigational review board.

198

199 ***cfDNA extraction, quantification, and size distribution analysis***

200 Samples from both trials were processed in exactly the same way and a maximum of
201 2 ml of plasma was reserved for this cfDNA study (**supplemental Figure 2A**). In our
202 study, the concentration of cfDNA corresponds to the amount of DNA detected in the
203 90- to 320-bp window (**supplementary Figure 2B**), and the median DNA fragment

204 size was significantly smaller for patients than for HVs (supplementary Figure 2C).
205 We established the best threshold above which patient samples at diagnosis were
206 considered cfDNA-enriched using two different approaches. First, we applied the
207 maximum selected rank statistics (maxstat R package¹²) to determine the threshold
208 that predicts the most significant differences in OS of patients independently in the
209 GOELAMS 075 and GAINED cohort (supplementary Figure 2D). Next, the threshold
210 was defined as 10 times the upper limit of cfDNA concentration in HV, this value is
211 clinically relevant and easily applicable to a patient population (supplementary Figure
212 2E). Because the maxstat and HV cohort results were close in value (49.2 and 52.1
213 vs. 54.9 ng/ml, respectively), the cutoff was set at 55 ng/ml for the entire study,
214 defining high and low cfDNA levels as above and below 55 ng/ml, respectively
215 (supplementary Methods).

216

217 **Statistical analysis**

218 Analysis was performed on an intention-to-treat (ITT) basis, for evaluable patients.
219 Descriptive statistics were quoted as the median [interquartile range (IQR)] or the
220 number (percentage). Groups were compared using a chi-squared test, the Mann-
221 Whitney U-test, Wilcoxon's signed-rank test, or the Kruskal-Wallis test, depending on
222 the type of data and the number of groups.

223 OS (defined as the time from randomization to the date of last follow-up or death
224 from any cause) was estimated using the Kaplan–Meier method. Differences in
225 survival curves were assessed in a log-rank test. A Cox proportional hazards model
226 was used to determine hazard ratios (HRs) and their 95%CI. All statistical tests were
227 two-sided, and the threshold for statistical significance was set to $P < 0.05$. Statistical

228 analyses were performed using GraphPad Prism 8.0 software (GraphPad Software
229 Inc., La Jolla, CA) and R software (version 4.1.1, R Core Team 2021).

230 Raw data for this study were generated at Hematology Laboratory of Rennes
231 University Hospital. Derived data supporting the findings of this study are available
232 from the corresponding author upon request.

233

234 RESULTS

235

236 *R-HDT is not superior to standard R-CHOP with regard to OS*

237 Between May 2005 and August 2010, 340 patients were enrolled in the G075 cohort.
238 Seventeen patients were deemed to be ineligible, and the remaining 323 were
239 randomized to receive either R-CHOP (n=161) or R-HDT (n=162). The two groups
240 were similar, except for performance status (supplemental Table 2). There were no
241 differences in demographic and clinical characteristics between the parent cohort and
242 the 123 patients with cfDNA assay data (supplemental Table 2). Of the 123 patients,
243 68 were assigned to the R-CHOP and 55 were assigned to the R-HDT arm (Figure
244 1A); the median follow-up time after randomization was 69 months (95%CI, 55.3-
245 88.0). With the exception of the sex ratio, the R-CHOP and R-HDT arms were well
246 balanced with regard to their demographic and clinical characteristics (Table 1). Fifty-
247 nine patients (87%) from the R-CHOP arm achieved a confirmed or unconfirmed
248 complete response (CR) or partial response at the EOT assessment, compared with
249 51 (93%) in the R-HDT arm. There was no difference in 5-year OS between the
250 randomization arms for the 123 patients (80.0% [70.1-91.3] for R-HDT vs. 77.7%
251 [68.3-88.3] for R-CHOP; $P=0.57$) or for the 323 patients in the parent cohort (85.5%
252 [80.2-91.2] for R-HDT vs. 81.1% [75.3-87.5] for R-CHOP; $P=0.39$) (Figures 1B-C).

253 Bone marrow involvement, the presence of more than one extranodal site, the aa-IPI
254 as well as age >50 were found to be significantly associated with poor OS
255 (supplemental Table 3).

256

257 ***The cfDNA concentration is elevated in patients with adverse prognostic***
258 ***factors***

259 The cfDNA fragment distribution in the 90- to 320-bp range of patients differed from
260 that of HVs, with a higher proportion of fragments shorter than 150 bp⁷. The median
261 cfDNA concentration was significantly higher in patients (17.8 ng/ml, IQR 8.4-56.8)
262 than in HVs (1.04 ng/ml, IQR 0.2-3.9) (Figure 2A), but did not differ significantly when
263 comparing the two treatment arms (Table 1). The cfDNA concentration at diagnosis
264 did not differ significantly when comparing patients with or without salvage therapy,
265 or with negative or positive iPET. Patients with high-risk clinical factors at diagnosis
266 (e.g., more than one extranodal site, a poor Revised-IPI (R-IPI), and a high-risk aa-
267 IPI) had significantly higher cfDNA levels (Figures 2B, C and D).

268

269 ***A high cfDNA level at diagnosis is associated with worse outcomes***

270 Based on the 55 ng/ml threshold for cfDNA, 21 of 55 (38%) R-HDT patients had high
271 cfDNA levels compared to 14 of 68 (21%) R-CHOP patients ($P=0.05$) (Table 1). The
272 OS rate at 60 months was significantly worse for the 35 high-cfDNA patients than for
273 the 88 low-cfDNA patients (65.7%, 95%CI, 51.7-83.5 vs. 83.9%, 95%CI, 76.5-92.0,
274 respectively; HR=2.4, 95%CI, 1.1-5.2) (Figure 3A). Progression-Free Survival (PFS),
275 taking progression, relapse or death from lymphoma as the first event, was
276 performed on 118 of 123 patients. Indeed, of the 27 patients who died, five died in
277 CR from a cause unrelated to the lymphoma (supplemental Table 4). Of the 118

278 patients, the PFS showed a better result for patients with a cfDNA below the
279 threshold value of 55ng/ml, with a *P* value at the limit of significance (*P*=0.076),
280 which was not the case in the GAINED cohort (supplemental Figure 4A-B).

281 Similar results were observed in patients from the GAINED cohort. The median DNA
282 fragment size was 174 bp (IQR 167-231), and median [IQR] cfDNA levels were
283 significant higher in patients than in HVs (9.24 ng/ml [4.1-31] vs. 1.04 [0.2-3.9],
284 respectively; supplementary Figure 2E). Twenty-six of the 178 GAINED patients were
285 in the high-cfDNA group and had a significantly worse OS at 48 months (73.1%,
286 95%CI, 57.9-92.3 vs. for low-cfDNA 88.5%, 95%CI, 83.3-94.1; HR=2.67, 95%CI,
287 1.11-6.53) (Figure 3B). Interestingly, the 55ng/ml cutoff is close to the optimal
288 threshold at 52ng/ml obtained for the GAINED cohort using the maxstat method
289 (supplementary Figure 2F).

290 As for known risk clinical factors, when combined with the randomization arm,
291 abnormal serum LDH, age \geq 50 years, and aalPI=2 or 3 had significantly worse OS
292 compared with their respective low-risk counterpart (LDH, *P*=0.038; age, *P*=0.019;
293 aalPI=2, *P*=0.036; aalPI=3, *P*=0.011). These significant differences were observed
294 only in the R-CHOP arm, as for cfDNA. Only high-risk (HR) aalPI (=3) distinguished
295 patients with a low OS at 60 months by 50%, as did cfDNA, but did not differentiate
296 R-HDT patients from R-CHOP patients in the HR aalPI category (supplemental
297 Figure 4C).

298 In a multivariate Cox analysis, the cfDNA levels (in ng/ml) and the aa-IPI risk still had
299 statistically significant relationships with OS in the 075 cohort: HR [95%CI] =5.48
300 [1.53-19.56] for high- vs. low-risk aa-IPI (*P*=0.0088) and 7.33 [2.33-23.09] for cfDNA
301 (*P*=0.00066), whereas the intermediate vs. low risk aa-IPI (HR=2.91, 95%CI, 0.96-
302 8.75, *P*=0.057) was not significant.

303

304 **High-cfDNA patients in the R-CHOP arm had significantly worse survival**

305 With regard to the randomization arm (Figure 4A), patients in the R-CHOP arm had
306 significantly worse OS when they were in the high-cfDNA category (HR=3.99,
307 95%CI, 1.48-10.74, $P=0.006$); this was not the case for patients in the R-HDT arm
308 (HR=1.40, 95%CI, 0.43-4.59, $P=0.58$). This finding is consistent with the fact that
309 high-cfDNA patients in the R-HDT arm tended to do better than high-cfDNA patients
310 in the R-CHOP arm (HR=2.74, 95%CI, 0.87-8.69, $P=0.084$). Overall, long-term OS
311 was worse among the 14 high-cfDNA R-CHOP patients than the other subgroups,
312 with a plateau of 5-year survival at 50% [29.6-84.4], vs. 76.2% [60-96.8] for high-
313 cfDNA R-HDT, 82.4% [70.5-96.2] for low-cfDNA R-HDT, and 82.9% [73.3-93.8] for
314 low-cfDNA R-CHOP.

315

316 **Intensive treatment mitigates the negative outcome expected in patients with**
317 **high cfDNA levels**

318 In the parent cohort (n=323), the OS rate at 60 months was 76.9% [69.5-85.1] for the
319 114 interim PET-positive patients and 89.0% [84.7-93.6] for the 196 PET-negative
320 patients ($P=0.009$).

321 In the studied G075-cohort, only for iPET-negative patients at the interim
322 assessment, those with high cfDNA had a statistically significantly shorter OS than
323 low-cfDNA patients (HR=3.48, 95%CI, 0.68-5.63, $P=0.02$) (supplementary Figure
324 5A).

325 When considering the 82 patients (regardless of the randomization arm) who did not
326 receive salvage therapy after the interim assessment, OS was significantly worse for
327 high-cfDNA patients than for low-cfDNA patients (HR=3.82, 95%CI, 1.55-9.43,

328 $P=0.0036$) (Figure 4B). In contrast, for individuals receiving salvage therapy, high-
329 cfDNA patients had much the same OS rate as low-cfDNA patients. Thus, for high-
330 cfDNA patients and regardless of randomization arm, those who did not receive
331 salvage therapy at the interim assessment tended to survive for a shorter time than
332 those who received salvage therapy (HR=3.08, 95%CI, 0.83-11.41, $P=0.092$). The
333 outcome was particularly unfavorable for the nine high-cfDNA patients who received
334 8 R-CHOP cycles, (supplemental Figure 5B). Six of the nine died, resulting in a 60-
335 month OS rate of 33.3% (HR=5.70, 95%CI, 2.87-14.22, $P=0.0002$) (supplemental
336 Figure 5C). As with OS, analysis of PFS showed that high-cfDNA patients who did
337 not receive salvage treatment had a statistically significantly worse outcome than
338 low-cfDNA patients with no salvage ($P=0.003$) and high-cfDNA patients who did
339 receive salvage treatment ($P=0.026$) (supplemental Figure 5D).

340

341 Lastly, we looked at the effect of ASCT in our study. ASCT was performed in 69 of
342 the 123 patients, either as an integral part of the R-HDT protocol or as elective
343 salvage treatment. We found that ASCT abolished the unfavorable outcome
344 associated with the high-cfDNA category because the 60-month OS rate among the
345 24 high-cfDNA patients with ASCT was 83.3% (95%CI: 69.7-99.7), which is similar to
346 that observed in the low-cfDNA subgroup. In contrast, only three of the 11 high-
347 cfDNA patients who did not undergo ASCT were still alive after 36 months of follow-
348 up with an OS of 27.3% (95%CI: 10.4 – 41.6) (Figure 4C).

349

350 ***High plasma cfDNA levels may persist at CR in R-CHOP patients***

351 The 50 patients with a CR 6 months after the EOT and for whom we had a plasma
352 sample were evenly distributed between the two randomization arms (24 in R-CHOP

353 and 26 in R-HDT) and did not differ from the study population as a whole with regard
354 to their clinical characteristics (Table 1 and Supplemental Table 1). New follow-up
355 time points were assessed, giving a median documented follow-up time of 83 months
356 (6.9 years). Three of the 50 patients experienced a late relapse of lymphoma 5.5,
357 8.8, and 10.6 years after the initial diagnosis, all were treated with eight courses of R-
358 CHOP. One died as a result of the relapse.

359 In contrast to the R-CHOP patients, the R-HDT patients showed a significant
360 decrease in cfDNA levels (Figure 5A and Supplemental Figure 3). We used the log₂
361 ratio of cfDNA concentration measured at CR to that measured at baseline. We thus
362 identified three subgroups of patients: “A” with a log₂ ratio > 1, “B” with a log₂ ratio
363 between -1 and 1 and “C” with a log₂ ratio < 1 (Figure 5B). These three subgroups
364 had similar clinical characteristics, with the exception of subgroup B, which had
365 proportionally smaller tumors than the other two (supplemental Table 5). The
366 distribution between the two randomization arms of subgroup A and subgroups B+C
367 was significantly different (Fisher test, $P=0.045$). Thus, of the 7 patients in subgroup
368 A, 6 were randomized to the R-CHOP arm and, with the exception of one, none of
369 them were referred for catch-up treatment at the midterm assessment. More
370 importantly, the three patients who relapsed are in subgroup A and thus had higher
371 cfDNA concentrations at CR than at baseline. Finally, two of 26 patients in the R-
372 HDT arm and 11 of 24 patients in the R-CHOP arm showed an increase in cfDNA
373 concentration at 6Mo_CR compared to diagnosis (Supplemental Figure 3).

374

375 **DISCUSSION**

376 This study shows that the level of cfDNA measured between 90 and 320 bp at
377 diagnosis is a reliable and robust marker (independent of the aa-IPI) of aggressive *de*

378 *novo* DLBCL in adults under 60. Firstly, a cfDNA concentration >55 ng/ml was
379 associated with a significantly worse clinical outcome. Secondly, in an intention-to-
380 treat analysis, R-HDT was not superior to standard R-CHOP with regard to OS and
381 so should not be recommended for young adults with DLBCL, in whom conventional
382 R-CHOP is equally effective, less toxic, and resource-saving – as shown previously
383 in other studies ^{13–15}. Nevertheless, our present results showed that the R-HDT
384 regimen mitigates the harmful influence of a cfDNA level ≥ 55 ng/ml at diagnosis.
385 Thirdly, we found that cfDNA levels fell significantly after a CR had been achieved
386 but only in patients in the R-HDT arm.

387

388 Today's techniques for measuring tumor-derived cfDNA are based on genotyping by
389 deep DNA sequencing ¹⁶; this technique requires specific preprocessing steps and a
390 sensitive analytical platform ^{10,17}. The GOELAMS 075 multicenter clinical trial began
391 in 2005, and we obtained 1 to 2 ml of plasma from a BDTM P100 blood collection tube
392 that contains potassium EDTA anticoagulant and proprietary protein stabilizers. This
393 unique sample collection provided reliable information on cfDNA levels and disease
394 status, with the expected distribution pattern (in terms of the median DNA fragment
395 size and cfDNA concentration) relative to HVs ¹⁸. Higher cfDNA concentrations were
396 significantly associated with adverse clinical factors in DLBCL. Overall, our results
397 suggest that cfDNA is a relevant, simple, cost-effective biomarker of aggressive
398 DLBCL in newly diagnosed patients. We established 55 ng/ml cfDNA to be the
399 optimal cutoff for the G075 cohort and independently confirmed it on the GAINED
400 cohort. We show that patients with cfDNA >55 ng/ml had a significantly worse
401 outcome only when in the standard R-CHOP arm.

402

403 The clinical trial's interim assessment included a visual interpretation of the PET
404 data. Depending on the randomization arm, PET-negative patients received either
405 four additional courses of R-CHOP or a BEAM regimen with ASCT. PET-positive
406 patients were offered salvage therapy (a combination of rituximab and intensive
407 chemotherapy) and then (if there was a disease response) the same BEAM regimen
408 with ASCT. In line with previous reports ^{19,20}, our analysis of 323 patients confirmed
409 that BEAM with ASCT was effective and compared favorably with the two- and three-
410 year OS rates of 61% and 62% reported respectively for the continuation of CHOP
411 and R-CHOP in patients with a PET-positive interim assessment ^{21,22}.

412

413 Our present results show that patients with cfDNA<55 ng/ml had a good OS rate
414 (over 70%), whatever their treatment. Similarly good outcomes were achieved by
415 patients with cfDNA>55 ng/ml at diagnosis receiving an intensive treatment regimen
416 (R-HDT, salvage therapy and/or ASCT). However, patients with cfDNA>55 ng/ml who
417 did not undergo intensive treatment (i.e., those having received eight cycles of R-
418 CHOP) had a significantly worse outcome. Our results demonstrate that high-dose
419 first-line treatment mitigates the negative influence of high cfDNA levels at diagnosis.
420 Furthermore, in contrast to aaIPI, cfDNA at baseline only identified R-CHOP>55ng/ml
421 patients with statistically worse OS.

422

423 Overall, cfDNA assays have shown significant benefits in clinical settings, and the
424 large range of applications continues to expand ²³. The present study was based on
425 a clinical trial with a long follow-up time, although the number of patients was low and
426 the interim PET assessment did not include calculation of the Standardized Uptake
427 Value ²⁴⁻²⁶. The results showed that for patients who do not undergo salvage

428 therapy, R-CHOP is much less effective than R-HDT in reducing the risk of relapse
429 when the cfDNA concentration at diagnosis exceeds 55 ng/ml. Furthermore, the
430 results for patients with a CR 6 months after EOT showed that R-CHOP was
431 associated with persistence in some patients with high plasma cfDNA levels.
432 However, a lack of material prevented us from assessing the tumor's
433 molecular/biological characteristics at diagnosis – characteristics that are known to
434 be of value when evaluating DLBCL ^{24–26}. Overall, the results showed that the cfDNA
435 concentration was a prognostic factor (like the IPI) for newly diagnosed DLBCL
436 patients in a clinical trial and that this biomarker could identify patients requiring
437 additional drugs to the standard R-CHOP regimen ²⁷, intensive therapies such as
438 ASCT (in young, fit, chemosensitive patients) ²⁸ or T-cell immunotherapy (in elderly,
439 chemoresistant patients) ²⁷. However, these results need to be confirmed in
440 independent PET-driven clinical cohorts and using technical aspects that are now
441 more widely used at the preanalytical and molecular level. For example, the
442 availability of large amounts of material allows personalized cancer profiling by deep
443 sequencing in B-cell lymphoma ^{16,29}. New studies should include extensive
444 comparisons between cfDNA measured in the 90-320 bp window and ctDNA to
445 carefully determine whether cfDNA concentration may reflect tumor burden. In
446 addition, characterization of plasma DNA genotyping at the time of CR after
447 cessation of all treatments would also be critical to determine whether maintenance
448 of elevated cfDNA is associated with mutations detected in the tumor and/or plasma
449 at diagnosis. These new investigations would also provide insight into minimal
450 residual disease and the prediction of late relapse or possibly a second cancer.

451

452 **ACKNOWLEDGMENTS**

453 This work was funded by an internal grant from the Laboratoire d'Hématologie at the
454 CHU de Rennes. We are indebted to the Centre de Ressources Biologiques (CRB)-
455 Santé (BB-0033-00056, <http://www.crbsante-rennes.com>) at the CHU de Rennes for
456 help with processing biological samples, the LYSA-P pathology facility for extracting
457 DNA from tumor tissues, and the Lymphoma Study Association (LYSA) for its
458 support of the study. We also thank the patients and their clinicians for providing
459 samples for research purposes. David Fraser at Biotech Communication for editing
460 services. Lastly, we thank C. Gisselbrecht and M.-H. Delfau for reading the
461 manuscript in detail.

462

463 **AUTHOR CONTRIBUTIONS**

464 F.D. designed the study, developed methodologies, performed analysis and wrote
465 the manuscript; D.R. developed methodologies for database analysis and statistical
466 approaches and wrote the manuscript; M.R. helped with the database analysis; C.P.
467 collected samples; L.L. provided technical assistance with sequencing; M de S. J.
468 analyzed plasma DNA; S. Le G. was the principal investigator of the GAINED study;
469 V.D., R.G., J.C. G.D., H.M., T.L. recruited patients; D.D. and M-C. P. supervised the
470 hematopathology analysis; F. K-B. performed PET analysis; N. M. was the principal
471 clinical investigator of the GOELAMS 075 trial; T.F. (the principal investigator of the
472 biological part of the GOELAMS 075 trial) designed and supervised the presented
473 study and wrote the manuscript.

474

475 **COMPETING INTERESTS**

476 F.D., D.R., M.R., C.P., L.L., M. de S.J., D.D., J.C., M-C.P., R.G., H.M., D.D., V.D., F.
477 K-B., T.L., M-C.P. and T.F. have no conflicts of interest to declare. N. M. reports

478 advisory board from Gilead and travel funds from Gilead and Roche; K.B. advisory
479 board from Kite/Gilead and Takeda, travel funds from Kite/Gilead and Takeda,
480 Honoraria from Kite/Gilead, Takeda, Sandoz, Incyte; G.D. reports advisory board
481 from blueprint medicines, Takeda, Roche & Thermo fischer, travel funds from Gilead,
482 BMS, Takeda, Pfizer; S.L.G. received grants, personal fees, or nonfinancial support
483 from Roche Genentech, during the conduct of the study; personal fees from Celgene;
484 and grants and personal fees from Janssen-Cilag, Gilead/Kite, and Servier outside of
485 the submitted work.

486

487 **FIGURE LEGENDS**

488

489 **Figure 1:** Clinical results of the GOELAMS 075 trial. **A)** Study flow chart. R-CHOP:
490 rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CEEP/MC:
491 rituximab, cyclophosphamide, epirubicin, vindesine, prednisone/methotrexate,
492 cytarabine; ASCT: autologous stem cell transplantation (also referred to as rituximab
493 high dose therapy (R-HDT)); CR: complete remission. Plasma was collected at
494 baseline and 6 months after the EOT (6Mo_CR). **B)** Overall survival curves (in an
495 intent-to-treat analysis) for the 123 patients. **C)** Overall survival curves (in an intent-
496 to-treat analysis) for the parent cohort of 323 patients.

497

498 **Figure 2:** The cfDNA biomarker. **A)** Measurement of cfDNA in plasma from 22 HVs
499 and in plasma collected at the time of diagnosis from 123 patients in the 075 cohort.
500 The threshold of 10 times the maximum value found in HVs was used to classify the
501 cfDNA levels as high or low. **B-D)** The cfDNA concentration as a function of the
502 number of extranodal sites, the R-IPI, and the aa-IPI.

503

504 **Figure 3:** Clinical outcomes of patients, as a function of the 55 ng/ml threshold value
505 for cfDNA. **A)** Overall survival curves as a function of the cfDNA concentration for all
506 123 patients. **B)** Overall survival curves as a function of the cfDNA concentration, for
507 the 178 patients from the GAINED cohort.

508

509 **Figure 4:** Survival curves for the 123 patients in the 075 cohort, as a function of the
510 55 ng/ml cutoff value for cfDNA. **A)** Overall survival, analyzed on an intention-to-treat
511 basis. **B)** Overall survival, taking into account the administration or not of salvage
512 treatment. **C)** Overall survival of patients, depending on whether or not they had
513 undergone ASCT.

514

515 **Figure 5:** cfDNA concentration in plasma of 50 patients at diagnosis and at CR 6
516 months after the end of treatment (6Mo_CR). **A)** Plasma cfDNA concentrations for 24
517 patients in the R-CHOP arm and 26 patients in the R-HDT arm. **B)** Ratio of Log 2
518 cfDNA concentrations measured at 6Mo_CR to that measured at baseline; patients
519 were classified into three subgroups: A: $\log_2 \text{ratio} > 1$; B: $-1 \leq \log_2 \text{ratio} \leq 1$; C: \log_2
520 $\text{ratio} > 1$.

521 **REFERENCES**

522

- 523 1. Sehn LH, Salles G. Diffuse Large B-Cell Lymphoma. *New Engl J Med.*
524 2021;384(9):842–858.
- 525 2. Gisselbrecht c, Crump m, Neelapu SS. Outcomes in refractory diffuse large B-cell
526 lymphoma: results from the international SCHOLAR-1 study. *Blood.*
527 2017;130(16):1800–1808.
- 528 3. Milpied N, Deconinck E, Gaillard F, et al. Initial Treatment of Aggressive
529 Lymphoma with High-Dose Chemotherapy and Autologous Stem-Cell Support. *New*
530 *Engl J Medicine.* 2004;350(13):1287–1295.
- 531 4. Haioun C, Lepage E, Gisselbrecht C, et al. Survival Benefit of High-Dose Therapy
532 in Poor-Risk Aggressive Non-Hodgkin’s Lymphoma: Final Analysis of the Prospective
533 LNH87–2 Protocol—A Groupe d’Etude des Lymphomes de l’Adulte Study. *J Clin*
534 *Oncol.* 2000;18(16):3025–3030.
- 535 5. Récher C, Coiffier B, Haioun C, et al. Intensified chemotherapy with ACVBP plus
536 rituximab versus standard CHOP plus rituximab for the treatment of diffuse large B-
537 cell lymphoma (LNH03-2B): an open-label randomised phase 3 trial. *Lancet.*
538 2011;378(9806):1858–1867.
- 539 6. Gouill SL, Ghesquières H, Oberic L, et al. Obinutuzumab vs rituximab for
540 advanced DLBCL: a PET-guided and randomized phase 3 study by LYSA. *Blood.*
541 2021;137(17):2307–2320.
- 542 7. Jiang P, Lo YMD. The Long and Short of Circulating Cell-Free DNA and the Ins
543 and Outs of Molecular Diagnostics. *Trends Genet.* 2016;32(6):360–371.
- 544 8. Phallen J, Sausen M, Adleff V, et al. Direct detection of early-stage cancers using
545 circulating tumor DNA. *Sci Transl Med.* 2017;9(403):eaan2415.
- 546 9. Hohaus S, Giachelia M, Massini G, et al. Cell-free circulating DNA in Hodgkin’s
547 and non-Hodgkin’s lymphomas. *Ann Oncol.* 2009;20(8):1408–1413.
- 548 10. Roschewski M, Rossi D, Kurtz DM, Alizadeh AA, Wilson WH. Circulating Tumor
549 DNA in Lymphoma: Principles and Future Directions. *Blood Cancer Discov.* 2021;
- 550 11. Cescon DW, Bratman SV, Chan SM, Siu LL. Circulating tumor DNA and liquid
551 biopsy in oncology. *Nature Cancer.* 2020;1(3):276–290.
- 552 12. T. H. maxstat: Maximally Selected Rank Statistics version 0.7.25. 2017;
- 553 13. Cortelazzo S, Tarella C, Gianni AM, et al. Randomized Trial Comparing R-CHOP
554 Versus High-Dose Sequential Chemotherapy in High-Risk Patients With Diffuse
555 Large B-Cell Lymphomas. *J Clin Oncol.* 2016;34(33):4015–4022.

- 556 14. Gisselbrecht C, Lepage E, Molina T, et al. Shortened First-Line High-Dose
557 Chemotherapy for Patients With Poor-Prognosis Aggressive Lymphoma. *J Clin*
558 *Oncol.* 2002;20(10):2472–2479.
- 559 15. Gouill SL, Milpied NJ, Lamy T, et al. First-line rituximab (R) high-dose therapy (R-
560 HDT) versus R-CHOP14 for young adults with diffuse large B-cell lymphoma:
561 Preliminary results of the GOELAMS 075 prospective multicenter randomized trial. *J*
562 *Clin Oncol.* 2011;29(15_suppl):8003–8003.
- 563 16. Scherer F, Kurtz DM, Newman AM, et al. Distinct biological subtypes and
564 patterns of genome evolution in lymphoma revealed by circulating tumor DNA. *Sci*
565 *Transl Med.* 2016;8(364):364ra155-364ra155.
- 566 17. Kurtz DM, Scherer F, Jin MC, et al. Circulating Tumor DNA Measurements As
567 Early Outcome Predictors in Diffuse Large B-Cell Lymphoma. *Journal of clinical*
568 *oncology : official journal of the American Society of Clinical Oncology.*
569 2018;36(28):2845–2853.
- 570 18. Mouliere F, Chandrananda D, Piskorz AM, et al. Enhanced detection of
571 circulating tumor DNA by fragment size analysis. *Sci Transl Med.*
572 2018;10(466):eaat4921.
- 573 19. Gisselbrecht C, Glass B, Mounier N, et al. Salvage Regimens With Autologous
574 Transplantation for Relapsed Large B-Cell Lymphoma in the Rituximab Era. *J Clin*
575 *Oncol.* 2010;28(27):4184–4190.
- 576 20. Crump M, Kuruvilla J, Couban S, et al. Randomized Comparison of Gemcitabine,
577 Dexamethasone, and Cisplatin Versus Dexamethasone, Cytarabine, and Cisplatin
578 Chemotherapy Before Autologous Stem-Cell Transplantation for Relapsed and
579 Refractory Aggressive Lymphomas: NCIC-CTG LY.12. *J Clin Oncol.*
580 2014;32(31):3490–3496.
- 581 21. Haioun C, Itti E, Rahmouni A, et al. [18F]fluoro-2-deoxy-D-glucose positron
582 emission tomography (FDG-PET) in aggressive lymphoma: an early prognostic tool
583 for predicting patient outcome. *Blood.* 2005;106(4):1376–1381.
- 584 22. Safar V, Dupuis J, Itti E, et al. Interim [18F]Fluorodeoxyglucose Positron
585 Emission Tomography Scan in Diffuse Large B-Cell Lymphoma Treated With
586 Anthracycline-Based Chemotherapy Plus Rituximab. *J Clin Oncol.* 2011;30(2):184–
587 190.
- 588 23. Cisneros-Villanueva M, Hidalgo-Pérez L, Rios-Romero M, et al. Cell-free DNA
589 analysis in current cancer clinical trials: a review. *Brit J Cancer.* 2022;126(3):391–
590 400.
- 591 24. Wright GW, Huang DW, Phelan JD, et al. A Probabilistic Classification Tool for
592 Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications.
593 *Cancer cell.* 2020;37(4):551-568.e14.

- 594 25. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell
595 lymphoma are associated with distinct pathogenic mechanisms and outcomes.
596 *Nature medicine*. 2018;24(5):679–690.
- 597 26. Casasnovas R-O, Meignan M, Berriolo-Riedinger A, et al. SUVmax reduction
598 improves early prognosis value of interim positron emission tomography scans in
599 diffuse large B-cell lymphoma. *Blood*. 2011;118(1):37–43.
- 600 27. Xu P-P, Huo Y-J, Zhao W-L. All roads lead to targeted diffuse large B-cell
601 lymphoma approaches. *Cancer Cell*. 2022;40(2):131–133.
- 602 28. Stiff PJ, Unger JM, Cook JR, et al. Autologous Transplantation as Consolidation
603 for Aggressive Non-Hodgkin's Lymphoma. *New Engl J Medicine*.
604 2013;369(18):1681–1690.
- 605 29. Rossi D, Diop F, Spaccarotella E, et al. Diffuse large B-cell lymphoma genotyping
606 on the liquid biopsy. *Blood*. 2017;129(14):1947–1957.
- 607

Table 1

Characteristics	Cohort 123 patients (n = 123)	R-HDT (n = 55)	R-CHOP (n = 68)	P-value between both arms	Cohort 50 patients (n = 50)	P-value betw. Cohort 123 vs Cohort 50
	No. of patients (%)	No. of patients (%)	No. of patients (%)		No. of patients (%)	
At diagnosis						
Intention To Treat						
R-CHOP	68 (55)				24 (48)	0.48
R-HDT	55 (45)				26 (52)	
Age, years						
median (IQR)	49.9 (41.5-55.5)	51.4 (42.9-55.5)	47.6 (40.8-55.5)	0.32	47.4 (40.3-53.8)	0.47
< 50 years old	62 (50)	24 (44)	38 (56)	0.24	32 (64)	0.14
≥ 50 years old	61 (50)	31 (56)	30 (44)		18 (36)	
Gender						
Male	75 (61)	40 (73)	35 (51)	0.027	29 (58)	0.85
Female	48 (39)	15 (27)	33 (49)		21 (42)	
ECOG PS						
< 2	108 (88)	46 (84)	62 (91)	0.32	43 (86)	0.94
≥ 2	15 (12)	9 (16.4)	6 (8.8)		7 (14)	
Symptoms						
A	74 (60)	34 (62)	40 (59)	0.88	33 (66)	0.59
B	49 (40)	21 (38)	28 (41)		17 (34)	
Bulky disease						
< 7 cm	54 (45)	20 (38)	34 (51)	0.22	19 (38)	0.5
≥ 7 cm	66 (55)	33 (62)	33 (49)		31 (62)	
Bone marrow involvement						
Not involved	100 (83)	43 (80)	57 (86)	0.46	44 (92)	0.25
Involved	20 (17)	11 (20)	9 (14)		4 (8.3)	
Extranodal sites						
< 2	77 (63)	33 (60)	44 (65)	0.73	36 (72)	0.32
≥ 2	46 (37)	22 (40)	24 (35)		14 (28)	
Ann Arbor Stage						
I-II	24 (20)	12 (22)	12 (18)	0.73	11 (22)	0.87
III-IV	99 (80)	43 (78)	56 (82)		39 (78)	
Serum LDH						
Normal	32 (26)	10 (19)	22 (32)	0.13	14 (28)	0.96
Elevated	90 (74)	44 (81)	46 (68)		36 (72)	
aalPI score						
0-1	45 (37)	18 (33)	27 (40)	0.64	20 (40)	0.92
2	65 (53)	30 (55)	35 (51)		25 (50)	
3	13 (11)	7 (12.7)	6 (8.8)		5 (10)	
R-IPI score						
0	10 (8.1)	3 (5.5)	7 (10.3)	0.43	3 (6)	0.54
1-2	70 (57)	30 (55)	40 (59)		33 (66)	
3-5	43 (35)	22 (40)	21 (31)		14 (28)	
Histology						
de novo DLBCL	87 (71)	42 (76)	45 (66)	0.47	32 (64)	0.48
PMBL	22 (18)	8 (15)	14 (21)		13 (26)	
others	14 (11)†	5 (9.1)††	9 (13.2)†††		5 (10)†††	
Cell-of-origin subtype (IHC)						
GCB	31 (39)	17 (42)	14 (36)	0.71	11 (35)	0.88
NGC	48 (61)	23 (58)	25 (64)		20 (65)	
cfDNA						
median (IQR)	17.8 (8.4-56.8)	34.2 (9.7-94.4)	13.0 (6.8-35.7)	0.51	19.6 (8.9-36.6)	0.16
< cutoff	88 (72)	34 (62)	54 (79)	0.05	41 (82)	0.22
> cutoff	35 (28)	21 (38)	14 (21)		9 (18)	

Characteristics	Cohort 123 patients (n = 123)	R-HDT (n = 55)	R-CHOP (n = 68)	P-value	Cohort 50 patients (n = 50)
	No. of patients (%)	No. of patients (%)	No. of patients (%)		No. of patients (%)
At intermediate evaluation					
[18]FDG-PET scan response				0.64	
Negative PET	75 (62)	32 (59)	43 (65)		34 (68)
Positive PET	45 (38)	22 (41)	23 (35)		16 (32)
Salvage therapy				0.65	
No	82 (67)	35 (64)	47 (69)		35 (70)
Yes	41 (33)	20 (36)	21 (31)		15 (30)
Autologous stem cell				< 0.0001	
No	54 (44)	4 (7.3)	50 (73.5)		17 (34)
Yes	69 (56)	51 (93)	18 (26)		33 (66)
At end of treatment (EOT)					
[18]FDG-PET scan response				0.57	
Negative PET	82 (80)	37 (84)	45 (78)		38 (90)
Positive PET	20 (20)	7 (16)	13 (22)		4 (10)
Final response				0.38	
CR, unCR, PR	110 (89)	51 (93)	59 (87)		50 (100)
SD, PD, Death	13 (11)	4 (7)	9 (13)		0
Survival data					
Status				0.80	
Alive	96 (78)	44 (80)	52 (76)		50 (100%)
Dead	27 (22)	11 (20)	16 (24)		0
Overall Survival				0.60	
36-months estimates (95% CI)	83.7 (77.4-90.5)	87.3 (78.9-96.5)	80.7 (71.8-90.7)		100.0
60-months estimates (95% CI)	78.7 (71.7-86.3)	80.0 (70.1-91.3)	77.7 (68.3-88.3)		100.0

Table 1 (cont'n)

Table 1. Baseline demographic, clinical and survival characteristics of the patients.

Abbreviations: R-HDT, rituximab plus high dose therapy; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone; ECOG PS, Eastern Cooperative Oncology Group performance status, ECOG PS ranges from 0-4, where higher score indicates greater degree of impairment; LDH, lactate dehydrogenase; aaIPI, age-adjusted International Prognostic Index; R-IPI, revised IPI; Cell-of-origin, IHC algorithm according to Hans classification (Hans *et al.* Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* **103**, 275–282 (2004)); GCB, germinal center B-cell like; NGC, nongerminal center B-cell like; gated-cfDNA, circulating tumor DNA, cutoff defined as equal to 10 times the maximal value of cfDNA in the healthy cohort; IQR, interquartile range. Histology: † secondary DLBCL, n=3; DLBCL rich in T cells, n=7; CD25+ DLBCL, n=1, non determined (ND), n=1; †† secondary

DLBCL, n=1; DLBCL rich in T, n=3; ND, n=1; +++ secondary DLBCL, n=2; DLBCL rich in T, n=4; CD25+ DLBCL, n=1; ND, n=2; ++++ secondary DLBCL, n=1; DLBCL rich in T, n=3; ND, n=1.

Figure 1

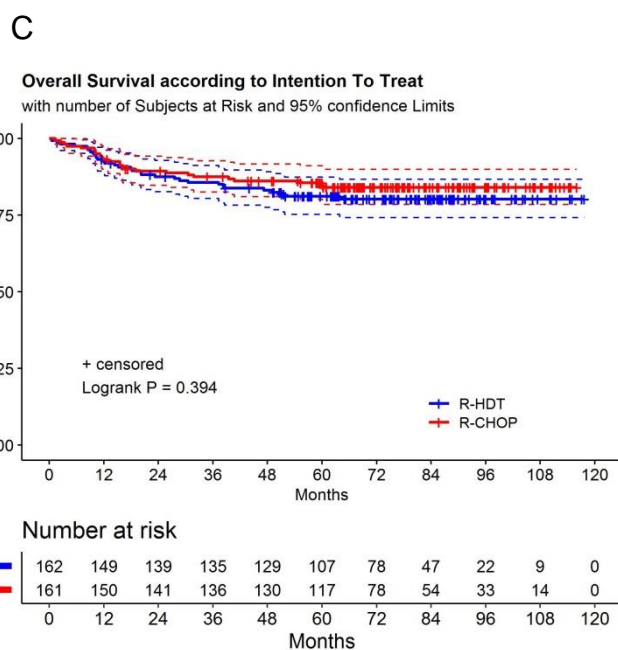
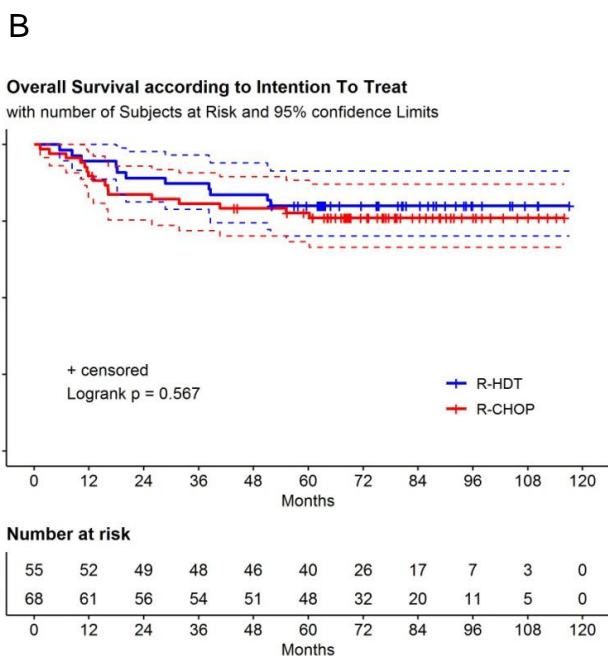
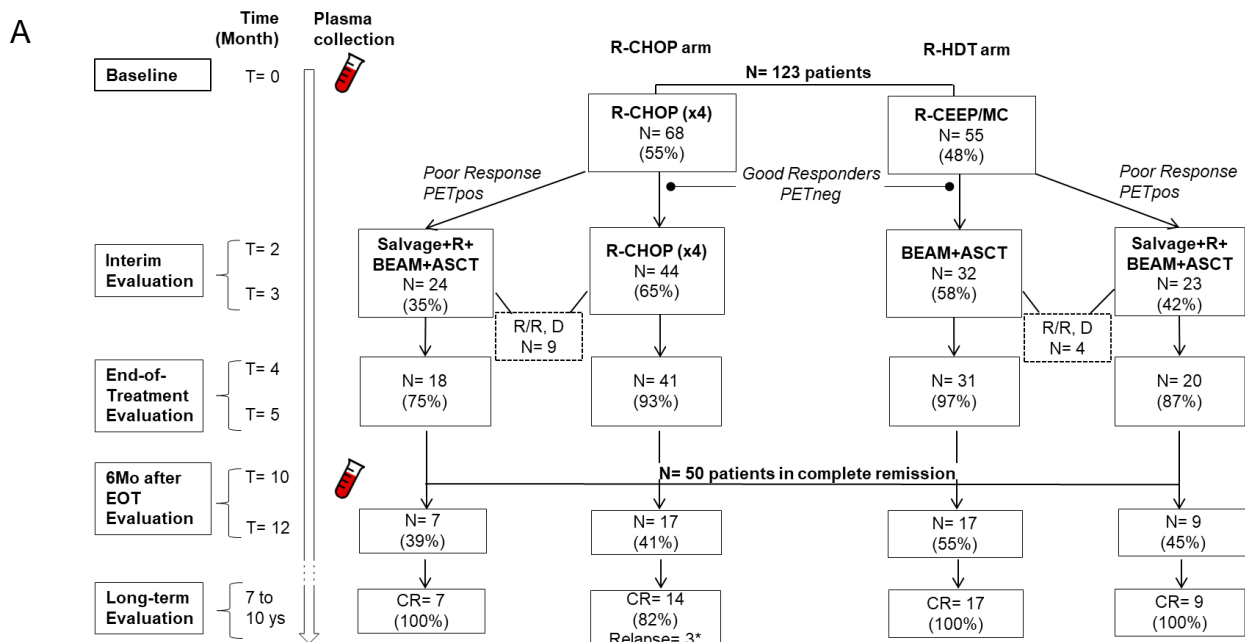


Figure 2

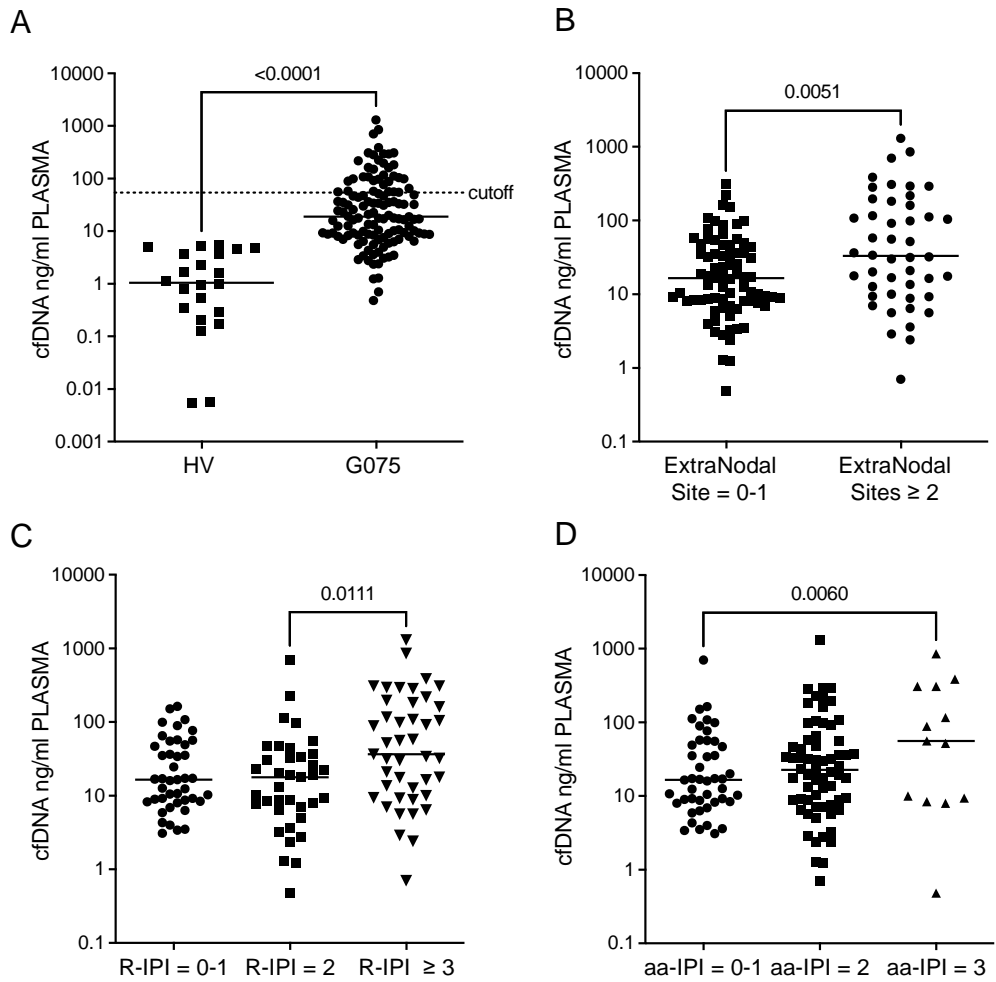
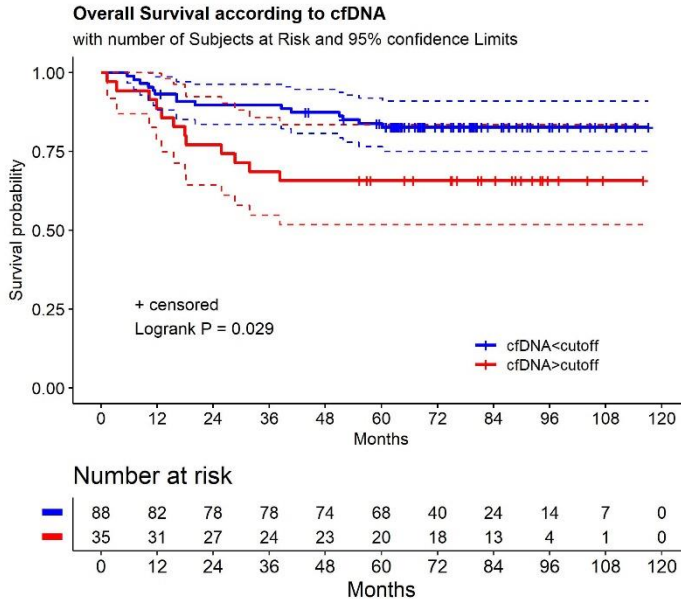


Figure 3

A



B

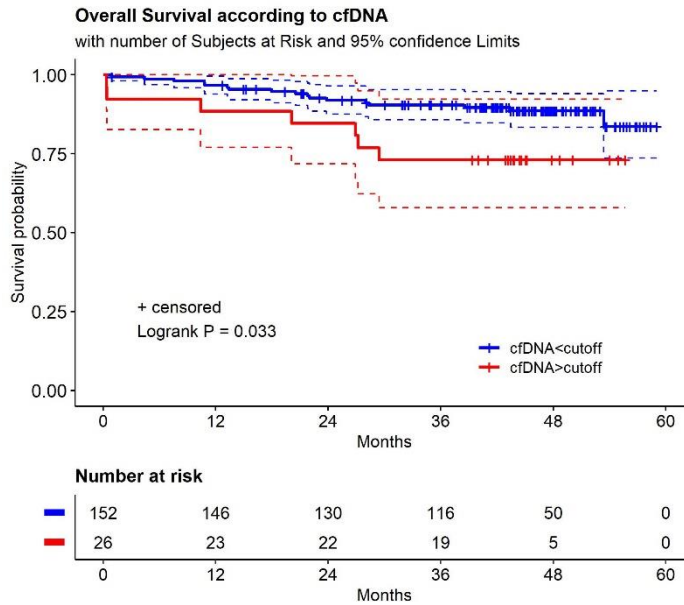
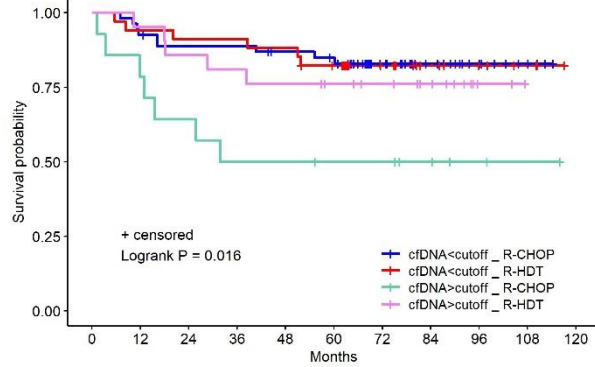


Figure 4

A

OS according to cfDNA + Intention To Treat
with number of Subjects at Risk

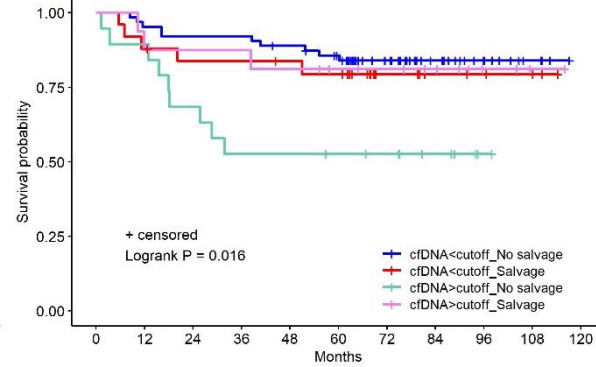


Number at risk

	0	12	24	36	48	60	72	84	96	108	120
cfDNA<cutoff _ R-CHOP	54	50	47	47	44	42	26	16	9	4	0
cfDNA<cutoff _ R-HDT	34	32	31	31	30	26	14	8	5	3	0
cfDNA>cutoff _ R-CHOP	14	11	9	7	6	6	4	2	1	0	0
cfDNA>cutoff _ R-HDT	21	20	18	17	16	14	12	9	2	0	0

B

OS according to cfDNA + Salvage therapy
with number of Subjects at Risk

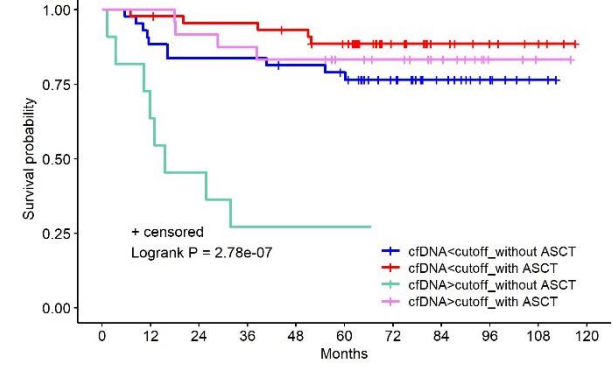


Number at risk

	0	12	24	36	48	60	72	84	96	108	120
cfDNA<cutoff _ No salvage	63	60	58	58	55	50	32	19	10	4	0
cfDNA<cutoff _ Salvage	25	22	20	20	19	18	8	5	4	3	0
cfDNA>cutoff _ No salvage	19	17	13	10	10	9	8	5	1	0	0
cfDNA>cutoff _ Salvage	16	14	14	14	13	11	10	6	3	1	0

C

Overall Survival according to cfDNA + ASCT
with number of Subjects at Risk



Number at risk

	0	12	24	36	48	60	72	84	96	108	120
cfDNA<cutoff _ without ASCT	43	38	36	36	34	32	23	14	8	3	0
cfDNA<cutoff _ with ASCT	45	44	42	42	40	36	17	10	6	4	0
cfDNA>cutoff _ without ASCT	11	7	5	3	3	3	3	2	1	0	0
cfDNA>cutoff _ with ASCT	24	24	22	21	20	17	15	11	3	1	0

		Cox analysis	
		HR (95% CI)	P-value
cfDNA>cutoff _ R-CHOP versus	cfDNA<cutoff _ R-CHOP	3.99 (1.98-10.74)	0.006
cfDNA>cutoff _ R-HDT versus	cfDNA<cutoff _ R-HDT	1.40 (0.43-4.59)	0.58
cfDNA>cutoff _ R-CHOP versus	cfDNA>cutoff _ R-HDT	2.74 (0.87-8.69)	0.084
cfDNA<cutoff _ R-CHOP versus	cfDNA<cutoff _ R-HDT	0.98 (0.34-2.73)	0.95
cfDNA>cutoff _ R-CHOP versus	cfDNA<cutoff _ R-HDT	3.86 (0.23-11.51)	0.0154
cfDNA<cutoff _ R-CHOP versus	cfDNA>cutoff _ R-HDT	1.45 (0.49-4.33)	0.51

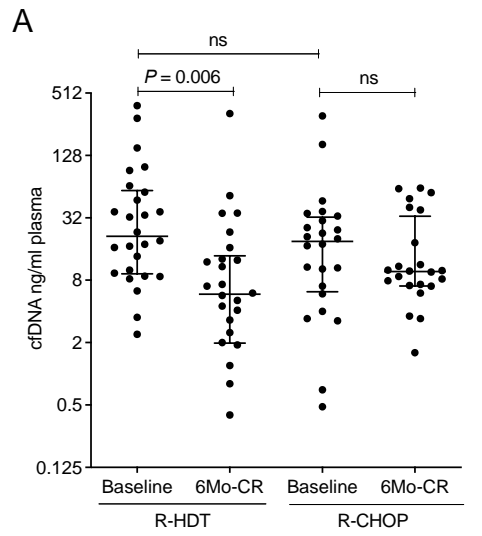
		Cox analysis	
		HR (95% CI)	P-value
cfDNA>cutoff _ No salvage versus	cfDNA<cutoff _ No salvage	3.82 (1.55-9.43)	0.0036
cfDNA>cutoff _ Salvage versus	cfDNA<cutoff _ Salvage	0.89 (0.21-3.73)	0.87
cfDNA>cutoff _ No salvage versus	cfDNA>cutoff _ Salvage	3.08 (0.83-11.41)	0.092
cfDNA<cutoff _ No salvage versus	cfDNA<cutoff _ Salvage	0.72 (0.25-2.10)	0.55
cfDNA>cutoff _ No salvage versus	cfDNA>cutoff _ Salvage	2.74 (0.92-8.21)	0.071
cfDNA<cutoff _ No salvage versus	cfDNA>cutoff _ Salvage	0.81 (0.22-2.93)	0.74

		Cox analysis	
		HR (95% CI)	P-value
cfDNA>cutoff _ without ASCT versus	cfDNA<cutoff _ without ASCT	5.36 (2.09-13.73)	0.00046
cfDNA>cutoff _ with ASCT versus	cfDNA<cutoff _ with ASCT	1.52 (0.41-5.66)	0.53
cfDNA>cutoff _ without ASCT versus	cfDNA>cutoff _ with ASCT	7.99 (2.39-26.75)	0.00075
cfDNA<cutoff _ without ASCT versus	cfDNA<cutoff _ with ASCT	2.26 (0.77-6.62)	0.14
cfDNA>cutoff _ without ASCT versus	cfDNA<cutoff _ with ASCT	12.13 (3.93-37.47)	0.000014
cfDNA<cutoff _ without ASCT versus	cfDNA>cutoff _ with ASCT	1.49 (0.47-4.75)	0.50

	Negative iPET	Positive iPET	NA
cfDNA<cutoff _ No salvage	60	2	1
cfDNA<cutoff _ Salvage	0	24	1
cfDNA>cutoff _ No salvage	15	4	1
cfDNA>cutoff _ Salvage	0	15	0

	60-month OS (95% CI)
cfDNA>cutoff _ without ASCT	27.3 (10.4-41.6)
cfDNA>cutoff _ with ASCT	83.3 (69.7-99.7)
cfDNA<cutoff _ without ASCT	79.0 (67.7-92.2)
cfDNA<cutoff _ with ASCT	88.6 (79.6-98.5)

Figure 5



Baseline (50)		6Mo_CR (50)	
R-HDT (N=26)	R-CHOP (N=24)	R-HDT (N=26)	R-CHOP (N=24)
cfDNA (ng/ml plasma) Median [IQR]			
21.2	18.9	5.8	9.7
[9.2-58.6]	[6.1-32.4]	[1.9-13.7]	[7.0-33.1]

