GAPDH Expression Predicts the Response to R-CHOP, the Tumor Metabolic Status, and the Response of DLBCL Patients to Metabolic Inhibitors

Johanna Chiche, Julie Reverso-Meinietti, Annabelle Mouchotte, Camila
Rubio-Patino, Rana Mhaidly, Elodie Villa, Jozef P. Bossowski, Emma Proics,
Manuel Grima-Reyes, Agnes Paquet, et al.

1 **GAPDH expression predicts the response to R-CHOP, the tumor metabolic**
2 **status and the response of DLBCL patients to metabolic inhibitors**

3 Johanna Chiche[1], Julie Reverso-Meinietti[1,2], Annabelle Mouchotte[1], Camila Rubio-Patiño[1], Rana
4 Mhaidly[1], Elodie Villa[1], Jozef P. Bossowski[1], Emma Proics[1], Manuel Grima-Reyes[1], Agnès Paquet[3],
5 Konstantina Fragaki[4,5,6], Sandrine Marchetti[1], Josette Briere[7], Damien Ambrosetti[2], Jean-François
6 Michiels[2,4], Thierry Jo Molina[8], Christiane Copie-Bergman[9], Jacqueline Lehmann-Che[10], Isabelle
7 Peyrottes[11], Frederic Peyrade[11], Eric De Kerviler[12], Bruno Taillan[13], Georges Garnier[13], Els
8 Verhoeyen[1], Véronique Paquis-Flucklinger[4,5,6] Laetitia Shintu[14], Vincent Delwail[15], Celine Delpech-
9 Debiais[16], Richard Delarue[17], André Bosly[18], Tony Petrella[19], Gabriel Brisou[20], Bertrand Nadel[20],
10 Pascal Barbry[3], Nicolas Mounier[21], Catherine Thieblemont[7,22,*] and Jean-Ehrland Ricci[1,23,*]

11 1: Université Côte d'Azur, INSERM, C3M, Nice, France.
12 2: Centre Hospitalier Universitaire de Nice, Département d'anatomo-pathologie, Nice, France,
13 Laboratoire Central d'AnatomocytoPathologie (LCAP), CHU de Nice
14 3: Université Côte d'Azur, CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, 06560 Sophia
15 Antipolis, France
16 4: Université Côte d'Azur, Nice, France
17 5: IRCAN, UMR CNRS 7284/INSERM U1081/UNS, School of Medicine, Nice Sophia-Antipolis
18 University, France
19 6: Department of Medical Genetics, National Centre for Mitochondrial Diseases, Nice Teaching
20 Hospital, France
21 7: APHP Hopital Saint-louis, Hemato-oncologie – Université Paris Diderot, Sorbonne Paris cité, Paris
22 France
23 8: Département de Pathologie, Hôpital Necker, AP-HP, EA 7324, Université Paris Descartes,
24 Sorbonne Paris Cité, Paris, France
25 9: IMRB-Inserm U955, AP-HP Hôpital Henri Mondor, Créteil, France.
26 10: AP-HP, Hôpital Saint Louis, Unité d'oncologie moléculaire, Univ Paris Diderot, Sorbonne Paris
27 Cité, 75010 Paris, France
28 11: Centre Antoine-Lacassagne, Nice, France
29 12: APHP, Hopital Saint-Louis. Service de Radiologie, Université Paris Diderot, Sorbonne Paris Cité,
30 F-75010 Paris, France
31 13: Centre Hospitalier Princesse Grace de Monaco, Monaco
32 14: Aix Marseille Univ, CNRS, Centrale Marseille, Institut des Sciences Moléculaires de Marseille
33 (ISM2), Marseille, France
34 15: Service d'Oncologie Hématologique et de Thérapie Cellulaire, CHU de Poitiers, INSERM, CIC
35 1402, Centre d'Investigation Clinique, Université de Poitiers, France
36 16: Department of Pathology, CHU/Université de Poitiers, Poitiers, France
37 17: Department of Hematology, AP-HP Hôpital Necker, Paris, France.
38 18: CHU Dinant Godinne, UcL Namur, Yvoir, Belgium.
39 19: Department of Pathology, Hôpital Maisonneuve-Rosemont, Montréal, Quebec, Canada.
40 20 : Aix Marseille Univ, CNRS, INSERM, CIML, Marseille, France.
41 21: Centre Hospitalier Universitaire de Nice, Département d'Onco-Hématologie, Nice, France.
42 22: NF-kappaB, Différenciation et Cancer, EA7324, Université Paris Descartes, Paris France.
43 23 Lead contact : Jean-Ehrland Ricci, Ph.D.
44
45 *: Co-corresponding authors
46 • Jean-Ehrland Ricci, Ph.D, Inserm U1065, équipe 3, 151 route de Ginestière, BP 2 3194, 06204
47 Nice Cedex 03, France. Phone: +33 4 89 06 43 04; Fax +33 4 89 06 42 21, email:
48 ricci@unice.fr
49 • Catherine Thieblemont, M.D., Ph.D, AP-HP-Hôpital Saint-Louis, Service d'hémato-
50 Oncologie, Paris, email : catherine.thieblemont@sls.aphp.fr
51
52

1   **Summary**

2   Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease treated with anti-CD20-based

3   immuno-chemotherapy (R-CHOP). We identified that low levels of GAPDH predict a poor response

4   to R-CHOP treatment. Importantly, we demonstrated that GAPDH^{low} lymphomas use OxPhos

5   metabolism and rely on mTORC1 signaling and glutaminolysis. Consistently, disruptors of OxPhos

6   metabolism (phenformin) or of glutaminolysis (L-asparaginase) induce cytotoxic responses in

7   GAPDH^{low} B cells and improve GAPDH^{low} B lymphoma-bearing mice survival, while they are low/no

8   efficient on GAPDH^{high} B lymphomas. Ultimately, we selected four GAPDH^{low} DLBCL patients, that

9   were refractory to all anti-CD20-based therapies and targeted DLBCL metabolism using L-

10   asparaginase (K), mTOR inhibitor (T) and metformin (M) (called KTM therapy). Three out of the four

11   patients presented a complete response upon one cycle of KTM. These findings establish that GAPDH

12   expression level predicts DLBCL patients' response to R-CHOP treatment and their sensitivity to

13   specific metabolic inhibitors.

14

15   **Keywords:** DLCBL, R-CHOP, metabolism, OxPhos, glycolysis, mTOR, predictive marker, L-

16   asparaginase

17

18

19

1   Introduction

2   Diffuse large B-cell lymphoma (DLBCL) is a genetically heterogeneous group of tumors and the most

3   common type of lymphoid malignancies (Swerdlow et al., 2016). DLBCLs represent highly

4   metabolically active tumors previously treated with a polychemotherapy CHOP (cyclophosphamide,

5   hydroxydaunorubicin, Oncovin® and prednisone) (Fisher et al., 1993). Since 1998, immunotherapy

6   anti-CD20 (Rituximab) combined with CHOP (referred as R-CHOP) became the standard treatment

7   for this disease, as it significantly improved the survival of patients (Coiffier et al., 2002). However,

8   approximately 40% of patients with DLBCL still experience therapeutic failure upon R-CHOP (Sehn

9   and Gascoyne, 2015). DLBCLs molecular heterogeneity is considered a major factor influencing the

10   response to R-CHOP therapy (Lenz and Staudt, 2010; Lenz et al., 2008). The cell-of-origin (COO)

11   classification delineates DLBCL subsets into distinct transcriptional profiles: the Germinal Center B-

12   cell (GCB)-, the Activated B-cell (ABC)- and the Unclassified-DLBCL subtypes (Alizadeh et al.,

13   2000). Unlike GCB-DLBCLs, ABC-DLBCLs are associated with a poor outcome upon R-CHOP

14   treatment (Lenz et al., 2008). Comparison of other gene signatures highlighted three other DLBCL

15   clusters that are recapitulated in the consensus cluster classification (CCC): i) the B cell receptor

16   (BCR)/proliferation (BCR-DLBCL) up-regulates genes encoding BCR signaling components; ii) the

17   oxidative phosphorylation (OxPhos) cluster (OxPhos-DLBCL) is enriched in genes involved in

18   electron transport chain (ETC) complexes, OxPhos metabolism and in other mitochondrial functions

19   and iii) the host response (HR) cluster is characterized by a host inflammatory infiltrate (Monti et al.,

20   2005). Importantly, there is no link between the COO and the CCC classifications (Caro et al., 2012;

21   Monti et al., 2005). Further characterization demonstrated that OxPhos-DLBCL cell lines lack the B

22   cell receptor and are insensitive to inhibition of the BCR signaling pathway (Chen et al., 2008). In

23   contrast, BCR-DLBCL cell lines express a functional BCR and primarily rely on glycolysis to produce

24   energy (Caro et al., 2012; Chen et al., 2008). Altogether those studies have identified, for the first

25   time, the metabolic heterogeneity within the same tumor entity (Caro et al., 2012; Monti et al., 2005;

26   Norberg et al., 2017). However, whether distinct metabolic fingerprints influence DLBCL response to

27   R-CHOP remains unknown. Moreover, it is unclear whether targeting DLBCL metabolic specificity

1 might be a valuable therapeutic approach in the clinic, in particular for R-CHOP low responder

2 patients. In this study, we addressed two unmet clinical needs: can we identify, at the diagnosis, which

3 DLCBL patients is likely to be a low responder to R-CHOP? and can we foretell if those patients will

4 be likely to respond to specific metabolic inhibitors according to their DLBCL's metabolic status?

5 Using unbiased analysis, we identified the glyceraldehyde-3-phosphate dehydrogenase, GAPDH, as

6 the only glycolytic enzyme able to predict overall survival of patients with DLBCL treated with R-

7 CHOP. Moreover, we established *in vivo* as well as in the clinic, that GAPDH holds potential as a

8 marker of DLBCL metabolic status and of patients's response to specific metabolic inhibitors.

9

10 **Results**

11

12 **GAPDH expression predicts overall survival of patients with DLBCL treated with R-CHOP and**

13 **DLBCL metabolic status.**

14 Unbiased analysis of expression profile data set from 233 newly diagnosed DLBCLs (Lenz et al.,

15 2008) allowed us to identify 587 probesets (p<0.05), corresponding to 524 unique genes associated

16 with differences in overall survival (OS) upon R-CHOP treatment (Table S1, Fig S1A). Analysis of

17 molecular functions associated with OS predicting genes, identified classical pathways involved in

18 resistance to chemotherapies (cell survival, proliferation, DNA repair…) and highlighted the

19 mitochondrial energetic function (Table S1 and Fig S1B). In contrast, only one gene involved in

20 glycolysis, the glyceraldehyde-3-phosphate dehydrogenase (*gapdh)* predicts a favorable outcome

21 when highly expressed (3 independent probesets: HR= 0.53, 0.53 and 0.54 and corresponding p

22 value= 0.01573, 0.01771 and 0.01929; Table S1). Of note, we also observed that lactate

23 dehydrogenase b subunit (*ldhb*) is significantly associated with a poor outcome (Table S1). Though,

24 LDH-B converts lactate to pyruvate, a source for OxPhos metabolism or for gluconeogenesis (Doherty

25 and Cleveland, 2013), we focused our attention on *gapdh*.

26

When subgrouping DLBCLs according to *gapdh* mRNA levels (high or low, Fig 1A), patients with DLBCL-*gapdh*^{low} have a lower OS upon R-CHOP than those with DLBCL-*gapdh*^{high} (Fig 1B). To further determine whether GAPDH protein expression predicts differences in OS upon R-CHOP, we set up an automated immuno-histochemical (IHC) staining of GAPDH and a scoring system to quantify its expression in 43 newly diagnosed DLBCL (training cohort, Table S2). As for *gapdh* mRNA (Fig 1B), DLBCLs expressing low levels of GAPDH protein (GAPDH^{low}, score $\leq$ 150) are associated with a poorer OS upon R-CHOP than DLBCLs expressing high levels of GAPDH (GAPDH^{high}, score > 150) (Figs 1D and 1E, see also STAR methods for determination of GAPDH IHC score and cutoff of 150). This finding was confirmed in a larger cohort of 294 *de novo* DLBCLs treated with R-CHOP (validation cohort, Table S2) (Delarue et al., 2013) by staining and scoring GAPDH from paraffin-embedded tissue microarray (TMA) using the previously established IHC score cutoff (Figs 1F and 1G).

Interestingly, GAPDH expression is not associated with clinical factors such as age-adjusted International Prognostic Index (aaIPI), COO or Hans classification (Hans et al., 2004), expression of bad prognosis markers, double hit (*MYC/BCL2)* or triple hit (*MYC/BCL2/BCL6*) translocations previously validated in DLBCLs (Horn et al., 2013; Johnson et al., 2012) (Table 1). Of note, there is no difference in OS noted on the basis of BCL6, or MYC or MYC-BCL2 expressions in the validation cohort. However, BCL2 expression significantly impacts the OS (Petrella et al., 2017). Multivariate analysis showed that GAPDH^{high} expression remained a significant marker predicting improved OS (HR=0.603, p=0.0371), independently of BCL2 (HR=1.686, p=0.0213). When compared to the aaIPI, multivariate analysis showed that GAPDH expression also remained a significant marker predicting improved OS in the training and validation cohorts (Table S3).

Altogether, these results demonstrate that the predictive value of GAPDH expression levels is independent of known biomarkers/factors of poor prognosis.

According to the CCC classification, we observed that 69% of DLBCL-*gapdh*^{low} (n=49/71) are OxPhos-DLBCLs, while 61% of DLBCL-*gapdh*^{high} (n=51/82) belong to the BCR/proliferation cluster

1   associated with glycolysis (two-sided p=0.00019; analysis from (Lenz et al., 2008) cohort). OxPhos-

2   DLBCLs significantly express lower levels of *gapdh* mRNA than BCR-DLBCLs (Fig 1H). In

3   addition, we did not observe association between GAPDH expression levels and specific COO

4   subtypes in the training and validation cohorts (Table S2), consistent with the absence of correlation

5   between the CCC and the COO classification (Caro et al., 2012; Monti et al., 2005). Moreover,

6   patients with BCR- or OxPhos-DLBCL display the same outcome upon CHOP treatment (Monti et al.,

7   2005). Accordingly, distinct *gapdh* mRNA levels display similar OS when DLBCL are treated with

8   the polychemotherapy CHOP only (Fig S1C).

9   Arguments from the literature suggest that OxPhos-DLBCL- might be less sensitive than BCR-

10   DLBCL to Rituximab due to the lack of Syk (Spleen Tyrosine Kinase)-dependent BCR signaling

11   (Kheirallah et al., 2010). Compared to BCR-DLBCL cell lines (SU-DHL-4 and SU-DHL-6), i.e

12   glycolytic lymphoma cells expressing a functional BCR (IgM or IgG) (Chen et al., 2008), OxPhos-

13   DLBCL cell lines (Toledo and Karpas 422) express lower levels of GAPDH, secrete less lactate,

14   express neither IgM nor IgG and are less sensitive to Rituximab-induced death (Figs S2A to S2D). Of

15   note, GAPDH expression (mRNA and protein) is not influenced upon BCR activation with anti-IgM

16   in SU-DHL-6 (Fig S2E to S2G). In another approach, we investigated the energetic metabolic status of

17   malignant B cells harvested from fresh micro-biopsies of primary DLBCL tumors, according to the

18   level of GAPDH. DLBCL-GAPDH^{low} cells produce less glycolytic ATP than DLBCL-GAPDH[high]

19   cells and there is a significant correlation between GAPDH expression level, and the percentage ATP

20   generated from glycolysis (Fig 1I).

21   We concluded that DLBCL-GAPDH[low] are low responders to R-CHOP treatment and mainly rely on

22   OxPhos metabolism to fulfill their energetic demand.

23

24   **Mouse primary B lymphomas expressing low levels of GAPDH are sensitive to the inhibition of**

25   **mitochondrial ETC complex I.**

Identification of a novel biomarker predicting DLBCL sensitivity to current treatment is only of clinical interest if we can propose new therapeutic options. Since R-CHOP low responders, i.e. DLBCL-GAPDH^{low}, primarily rely on OxPhos metabolism (Fig 1H, 1I), we investigated their sensitivity to inhibitors of mitochondrial energetics.

Using the pre-clinical Eμ-*Myc* mouse model that spontaneously develop Non-Hodgkin (NH) B-cell lymphomas (Adams et al., 1985), we wondered whether GAPDH expression levels could influence the metabolic status of B lymphomas and the response to disruptors of mitochondrial metabolism *in vitro* and *in vivo*. We previously characterized the expression of GAPDH in Eμ-*Myc* lymphomas and classified them according to the level of GAPDH (mRNA and protein) (GAPDH^{low} or GAPDH^{high}), while the expression of the other glycolytic enzymes is similar between the two groups ((Chiche et al., 2015) and Figs S3A to S3C). Of note, we did not observe any link between GAPDH and LDH (total or LDH-B) expression (Fig S3A). While the exact nature of GAPDH regulation remains to be found, *gapdh* mRNA was more stable in GAPDH^{high} than in GAPDH^{low} cells (Fig S3D). Cells from Eμ-*Myc*-GAPDH^{low} and Eμ-*Myc*-GAPDH^{high} lymphomas produce equivalent amount of ATP (Fig 2A). However, Eμ-*Myc*-GAPDH^{low} cells produce less glycolytic ATP (Fig 2B), as observed in human DLBCL cells (Fig 1H and 1I) and secrete less lactate than Eμ-*Myc*-GAPDH^{high} cells (Fig 2C). Accordingly, Eμ-*Myc*-GAPDH^{low} cells consume more O$_2$ (Fig 2D), while glucose transporters expression (Fig S3E) and the mitochondrial mass (Fig S3F) are similar between the two groups.

In vitro, the mitochondrial complex I inhibitor, phenformin (Wheaton et al., 2014) reduces baseline O$_2$ consumption driven by complex I and induces cytotoxic responses in Eμ-*Myc*-GAPDH^{low} cells but not in Eμ-*Myc*-GAPDH^{high} cells (Figs 2E and S3G to S3I). Importantly, *gapdh*-silencing in Eμ-*Myc*-GAPDH^{high} cells decreases lactate production and increases cell sensitivity to phenformin-induced death (Figs S4A to S4D). *In vivo,* phenformin treatment increases the survival of Eμ-*Myc*-GAPDH^{low} lymphoma-bearing mice, but not that of Eμ-*Myc*-GAPDH^{high} lymphoma-bearing mice (Figs 2 F and 2G), which demonstrates the causal correlation between low expression of GAPDH and phenformin sensitivity. We confirmed that phenformin was indeed active at the dose administrated *in vivo* (Figs S3J-and S3K).

Overall, Eμ-*Myc*-GAPDH[low] lymphomas are sensitive to the inhibition of mitochondrial metabolism, which represents a potential therapeutic strategy for patients with DLCBL-GAPDH[low] that are low responders to R-CHOP treatment.

Active mTORC1 signaling in GAPDH low lymphomas is implicated in OxPhos metabolism.

GAPDH was shown to modulate mTOR signaling through its binding to Rheb (Lee et al., 2009). We therefore speculated that mTORC1 could be active in GAPDH[low] B lymphomas. Indeed, we observed a high mTORC1 activity in Eμ-*Myc*-GAPDH[low] lymphomas, as shown by p70-S6K phosphorylation on threonine 389 (Fig 3A). This was confirmed in human DLBCL biopsies, for which 57% of DLBCL-GAPDH[low] stained positive for p70-S6K phosphorylation (indicating high mTORC1 activity), while 91% of DLBCL-GAPDH[high] do not express this form (indicating low mTORC1 activity) (Fig 3B).

Inhibition of mTORC1 signaling with rapamycin in Eμ-*Myc*-GAPDH^{low} cells, decreases basal O_2 consumption rate, maximal respiratory capacities and ATP-coupled OCR along with an increase in glycolytic ATP (Figs 3 C to 3G). Of note, the total amount of intracellular ATP was not modulated upon rapamycin treatment (Fig S5A). More specifically, rapamycin decreases O_2 consumption driven by complex I but not complex II (Figs S5B and S5C), due to the inhibition of complex I activity but not of complex II activity (Fig S5D). The exact reason of such effect remains to be determined. Similar results were obtained using an independent Eμ-*Myc*-GAPDH[low] lymphoma (Figs S5E to S5G). As previously reported in several cancer cell lines (Fruman and Rommel, 2014), rapamycin does not induce cytotoxic responses (Fig 3H), but rather cytostatic effects, as we observed (Fig S5H). Finally, *in vivo* inhibition of mTORC1 activity with Temsirolimus (Dancey, 2010) significantly increases the survival of OxPhos Eμ-*Myc*-GAPDH[low]–bearing mice (Figs 3I and 3J).

These results suggest that mTORC1 signaling is involved in the control of GAPDH[low] B lymphoma metabolism.

B lymphomas expressing low levels of GAPDH rely on glutamine metabolism and are sensitive to the hydrolysis of extracellular glutamine and asparagine with *E-Coli* L-asparaginase.

mTORC1 is a central sensor of amino acids, including Gln (Duran et al., 2012). Eμ-*Myc*-GAPDH[low] lymphomas display active mTORC1 signaling (Fig 3A) and consume more Gln than the Eμ-*Myc*-GAPDH^{high} cells (Fig 4A) due to a specific increase in Gln transport rates (Figs 4B, S6A and S6B). We next profiled metabolic differences between Eμ-*Myc*-GAPDH[low] and Eμ-*Myc*-GAPDH^{high} lymphoma cells using liquid chromatography–mass spectrometry (LC-MS)–based metabolomics with the intent to further characterize the intracellular fate of Gln (Fig 4C). The relative intracellular levels of metabolites involved in glycolysis (G6P, G3P, lactate) and in the non-oxidative arm of the pentose phosphate pathway (PPP) (Sedoheptulose-7-phosphate) are significantly decreased in Eμ-*Myc*-GAPDH^{low} cells (Fig 4C). Glutamine is metabolized through glutaminolysis to produce glutamate, α-ketoglutarate, the latter being used to replenish the TCA cycle. In favor of an active glutaminolysis, glutamate and α-ketoglutarate levels are higher in Eμ-*Myc*-GAPDH^{low} cells (Fig 4C), consistent with an increase expression of the glutaminase (GLS), the mitochondrial enzyme catalyzing the conversion of glutamine into glutamate and ammonia (Fig 4D). While fewer metabolites could be detected by ^{1}H HRMAS NMR spectroscopy, a significant increase in intracellular glutamate levels was also confirmed in Eμ-*Myc*-GAPDH^{low} cells (Figs S6C and S6D). Extracellular sources of Gln are required for Eμ-*Myc*-GAPDH^{low} cells to sustain basal O_2 consumption, maximal respiratory capacities and ATP-coupled OCR (Figs S6E to S6G). Pharmacologic hydrolysis of extracellular sources of L-glutamine and L-asparagine with *E-Coli* L-asparaginase (Fig 4E), reduces mTORC1 activity (Fig S6H, (Willems et al., 2013)) and induces cytotoxic responses in Eμ-*Myc*-GAPDH^{low} lymphoma cells (Fig 4F). In addition, *gapdh*-silenced Eμ-*Myc*-GAPDH^{high} lymphoma cells are more sensitive than control cells to *E-Coli* L-ase-induced death (Fig S4E). *In vivo*, plasma sources of Gln were depleted in *E-Coli* L-ase treated mice, regardless of the Eμ-*Myc* lymphoma injected (Fig 4G). However, only the survival of Eμ-*Myc*-GAPDH[low]-bearing mice was enhanced upon *E-Coli* L-ase treatment (Figs 4H and 4I). Of note, *E-Coli* L-ase treatment inhibited mTORC1 signaling *in vivo* (Figs S6I and S6J).

Specific overexpression of GAPDH reduces mTORC1 signaling and B lymphomas sensitivity to phenformin and L-asparaginase *in vivo*.

To further support our conclusions, we overexpressed GAPDH in Eμ-*Myc*-GAPDH^{low} cells (Fig 5A). GAPDH overexpression was sufficient to reduce mTORC1 signaling (Fig 5A) and to induce a metabolic switch toward glycolysis, as judged by the increase in glycolytic ATP, lactate secretion along with the reduction in baseline OCR and Gln consumption (Figs 5B to 5F). Equivalent observations were obtained upon GAPDH expression in the OxPhos-DLBCL cell line Karpas 422 (Figs S7A to S7C). Importantly, the metabolic switch induced by GAPDH overexpression prevented most of the beneficial effects brought by either phenformin or *E-Coli* L-asparaginase *in vivo* (Figs 5G to 5I).

Together we confirmed that GAPDH expression influences the metabolic state and the sensitivity of B lymphomas to phenformin or *E-Coli* L-asparaginase.

Treatment of DLBCL-GAPDH[low] with specific metabolic inhibitors demonstrates a significant benefit for patients.

We demonstrated that GAPDH[low] B lymphomas preferentially use OxPhos metabolism to produce energy and are sensitive to cell death induced by phenformin or *E-Coli* L-asparaginase. To ultimately validate our findings, we designed a clinical therapeutic intervention (called KTM), to interfere with DLBCL-GAPDH[low] metabolism (Fig 6A). KTM therapy consists in 4 weeks-cycles of treatment including *E-Coli* L-asparaginase (K, Kidrolase), mTOR inhibitor Temsirolimus (T, Torisel) and Metformin (Fig 6B). The first two weeks, patients receive *E-Coli* L-asaparaginase on days 1, 3, 5, 7, 9, 11, 13 and Temsirolimus on days 1, 7 and 14. This combination was not administered longer than 14 days since *E-Coli* L-asparaginase is not well tolerated in adults (Boissel and Sender, 2015). Instead, the last two weeks of each KTM cycle, patients received metformin to sustain a therapeutic pressure on mitochondrial metabolism (Fig 6B). It is important to consider that prior to KTM, patients were refractory to all R-based therapies and only supportive care was proposed.

1 Fig 6C illustrates the patient#1, a 24-year-old man who presented a DLBCL in therapeutic failure. At

2 diagnosis, clinical presentation was a cervical bulky mass corresponding to a *Myc*-translocated GC-

3 DLBCL (diameter 240 x 100 mm) with bone marrow and blood infiltration (Table S4). Following

4 diagnosis, the patient received four regimens of immuno-chemotherapy. Early tumor progression was

5 systematically observed before initiating each new cycle of Rituximab-based therapy. Verification of

6 GAPDH expression at diagnosis by IHC showed a low GAPDH IHC score (of 35). As we could not

7 offer any other standard therapeutic option, patient #1 was selected for KTM treatment. 7 and 15 days

8 after the beginning of KTM therapy, the tumor mass significantly decreased (Fig 6C). After 30 days of

9 KTM treatment, patient #1 had 83% reduction of the tumor mass (Fig 6D) and was negative at PET-

10 Scan analysis. He had a normal life for 4 months and then died upon local and central nervous system

11 relapse of the lymphoma. To demonstrate that KTM efficacy is not limited to one single patient

12 presenting a DLBCL-GAPDH^{low}, three other patients with identical eligible criteria (*Myc*$^+$-DLBCL-

13 GAPDH^{low} at diagnosis and treatments-refractory disease) were treated with KTM (Fig 6E). Three out

14 of four patients had a complete response after two cycles of treatment, and one patient had a

15 progressive disease due to early adverse events during the first cycle of treatment and KTM treatment

16 had to be discontinued (Fig 6F). Median duration of response was 6 months (4-6 mo).

17

18 **Discussion:**

19 Several metabolic inhibitors were developed with the intent to kill or to sensitize cancer cells to

20 chemotherapies (Galluzzi et al., 2013). So far, more than 100 clinical trials using metabolic inhibitors

21 are ongoing in the field of cancer (Meynet and Ricci, 2014). Unfortunately, to date, most of those

22 trials have failed to improve the outcome of patients (Kordes et al., 2015). This may not be linked to

23 the inefficacy of the compound to inhibit its target but rather to a subset of tumors with a distinct

24 metabolic dependence from the targeted metabolic pathway. Altogether, our study highlighted that

25 tumor heterogeneity within the same tumor entity has to be taken into consideration for ongoing

26 clinical trials using metabolic inhibitors.

While, more than 150'000 claimed biomarkers have been described in the literature, less than 100 are routinely used in the clinic (Poste, 2011) and we still lack a marker of the tumor metabolic status. Here, we identified GAPDH, as a clinically compatible biomarker of i) the conventional R-CHOP treatment and of ii) the metabolic status of DLBCLs, which help to further predict the efficacy of mitochondrial metabolism disruptors in the context of DLCBL-GAPDH[low] that are associated with a poor outcome of patients treated with R-CHOP.

GAPDH is a multifunctional protein harboring glycolytic and non-glycolytic functions (Colell et al., 2009). It is highly regulated at the transcriptional, translational and post-translational levels. Endogenous *gapdh* mRNA and protein are highly abundant in all mammalian cells and increased expression levels of *gapdh* by 30% or more, significantly impacts on cell survival and growth (Colell et al., 2009), particularly in B lymphomas (Chiche et al., 2015). We observed that human DLBCL expressed heterogeneous levels of *gapdh* mRNA and protein defining patients with DLBCL-GAPDH[low] or DLBCL-GAPDH[high]. As expected, when comparing healthy tissues (GCB cells from non-cancerous reactive lymph nodes) to cancer samples, DLBCL-GAPDH[low] express significantly higher levels of GAPDH (Fig 1D). Moreover, when compared to DLBCL-GAPDH[low], DLBCL-GAPDH[high] express approximatively two times more GAPDH, which also highlights GAPDH as a marker of tumor heterogeneity within the same tumor entity. Interestingly, *gapdh* mRNA stability was higher in Eμ-*Myc*-GAPDH^{high} cells (Fig S3D). As GAPDH is an RNA binding protein (Colell et al., 2009), we could speculate that it could bind and stabilize its own mRNA. However, the exact nature of *gapdh* regulation in DLBCL is still unclear and will be investigated in further studies.

Mechanistically, we observed a central role of GAPDH-dependent regulation of mTORC1 signaling in the control of DLBCL metabolic status. It was previously reported that *gapdh*-silenced HEK293 cells increase mTORC1 signaling by preventing GAPDH-Rheb binding (Lee et al., 2009). Using the Eμ-*Myc* model and newly diagnosed DLBCL samples we also observed an increase in mTORC1 signaling, glutamine uptake, glutaminolysis and sensitivity to L-asparaginase-induced death in

GAPDH[low] B lymphomas (Fig 3 and 4). Altogether our findings are consistent with the role of functional mTORC1 on glutamine uptake (Csibi et al., 2014) and the feedback role of glutaminolysis on the activation of Rag-mTORC1 signaling (Duran et al., 2012). How GAPDH-dependent control of mTORC1 regulates metabolism? mTORC1 is a known-regulator of HIF-1α and c-Myc translation, which in turn induce the expression of genes involved in glucose uptake and glycolysis (Gordan et al., 2007). In primary Eμ-*Myc* lymphomas, we did not observe differences in glucose transporters expression (Fig S3E) according to GAPDH expression levels, despite differences in mTORC1 signaling. In contrast, inhibition of mTORC1 reduces O_2 consumption of Eμ-*Myc* GAPDH^{low} cells, in agreement with previous studies (Cunningham et al., 2007; Morita et al., 2013). It seems therefore possible that Eμ-*Myc* lymphomas and human DLBCLs do not follow the classical Warburg scheme in which mTORC1 activates glycolysis. Instead, mTORC1 seems implicated in the regulation of OxPhos metabolism in this biological model (Fig 3). This notion is further supported by the increase in glutamine uptake, along with an increase in the relative intracellular levels of glutamate and α-ketoglutarate and L-asparaginase efficacy on reduced mTORC1 signaling *in vitro* and *in vivo* (Willems et al., 2013) (Figs 4 and S6).

Since IHC detection of GAPDH seemed to help for the determination of the tumor metabolic status, we selected four patients with Myc[+]-DLBCL-GAPDH[low]. All patients were refractory to R-based therapies. In the face of a therapeutic dead end, we ultimately target DLBCLs-GAPDH[low] metabolism with already available (FDA-approved) metabolic disruptors (KTM therapy) (Fig 6). Based on the observed cytostatic effect of Temsirolimus *in vitro* and *in vivo* (Figs 3I and S5H), we decided still to administer this mTOR inhibitor either with L-asparaginase (D1, D7 of a KTR cycle) or with Metformin (D14 of a KTR cycle) to maximize the potential beneficial effect of the co-treatment. However, knowing that rapalogs alone or in combination with R-CHOP did not provide significant benefits in randomized phase II and phase III trials for patients with NH B-cell lymphomas (Ricci and Chiche, 2018), it is unlikely that the rapid and complete responses obtained after one week of KTM treatment can be attributed to Temsirolimus. According to the beneficial effect observed in Eμ-*Myc*-

1 GAPDH^{low} lymphomas upon *E-Coli* L-ase (Kidrolase) and to the early reduction of the tumor burden

2 observed from D7 in patient #1 (Fig 6C), it is likely that most of the treatment efficacy can be

3 attributed to the L-ase activity. Unfortunately, in the clinic, *E-Coli* L-ase cannot be administrated

4 longer than two weeks in a row in adults, as it may provoke major toxicities (Boissel and Sender,

5 2015). We therefore sustained inhibition of mitochondrial energetics with metformin and mTOR

6 inhibitor for the last two weeks of KTM cycle of treatment (Fig 6B). Despite the drastic reduction of

7 the tumor mass, all patients finally relapsed, which very likely reflect the metabolic flexibility and

8 adaptation of the remaining malignant B cells during or after KTM therapy.

9

10 **Limitations of study**

11 A major limitation in the field of DLBCL is a lack of robust pre-clinical models (cell lines or *in vivo*

12 mouse models) that recapitulate the major aspects of the human disease. As human cancer cell lines

13 were isolated decades ago and since grown in high glucose, high oxygen media, it is possible that their

14 metabolism do not reflect the metabolic status of the initial tumor cells. We therefore decided to

15 characterize several independent murine primary NH B cell lymphomas harvested from the Eµ-*Myc*

16 mice model (Figs 2 to 5). While this model represents highly aggressive tumors (Ki67 100%) and is

17 largely used as a robust model to test new therapeutic options for NH B lymphomas, they lack key

18 features of human DLBCL such as marker of mature B cells. We therefore validated our findings in

19 humans, using malignant B cells immediately isolated from "fresh" DLBCL micro-biopsies and

20 ultimately by treating a few patients suffering from refractory disease with specific metabolic

21 inhibitors (Figs 1, 3 and 6). Another limitation of our work is the low number of patients included in

22 the KTM therapy. However, our study established the *proof-of-principle* that patients with DLBCL-

23 GAPDH^{low} are low responders to immuno-chemotherapy R-CHOP but are potential candidates for

24 KTM therapy. Finally, while GAPDH levels represent the first clinically compatible marker of

25 DLBCL metabolic heterogeneity, it remains to be determined if this marker can be extended to other

26 hematological malignances and to solid tumors.

27

1

2 **Acknowledgements**

20

21 **Author Contributions**

22 JC performed the majority of research described herein and was assisted by AM, C R-P, R M, E Vil.,

23 JPB, EP, MGR, KF, SM, E Ver., V P-F, LS, GB, BN. Clinical study and patient samples: J R-M, JB,

24 DA, J-FM, TJM, CC-B, JL-C, IP, FP, EDK, BT, GG, VD, CD-D, RD, AB, TP, NM, CT.

25 Bioinformatic analysis: AP, PB. J-E.R secured funding and supervised the study. CT supervised the

26 clinical study. JC and J-E.R designed research and wrote the manuscript.

1

2 **Declaration of Interests,**

3 CT is on the scientific board for Jazz Pharmaceuticals; Celgene; Roche, AbbVie; and Gilead.

4 The remaining authors declare no conflicts of interest.

5 Related patents:

6 WO2015/132163: A METHOD FOR PREDICTING THE RESPONSIVENESS A PATIENT TO A

7 TREATMENT WITH AN ANTI-CD20 ANTIBODY

8 WO2017/055484: METHODS FOR DETERMINING THE METABOLIC STATUS OF

9 LYMPHOMAS

10 EP17305294.5: A METHOD FOR PREDICTING THE RESPONSIVENESS OF A PATIENT TO A

11 TREATMENT WITH mTOR INHBITORS

12

13 **References**

14 Adams, J.M., Harris, A.W., Pinkert, C.A., Corcoran, L.M., Alexander, W.S., Cory, S., Palmiter, R.D.,
15 and Brinster, R.L. (1985). The c-myc oncogene driven by immunoglobulin enhancers induces
16 lymphoid malignancy in transgenic mice. Nature *318*, 533-538.

17 Alizadeh, A.A., Eisen, M.B., Davis, R.E., Ma, C., Lossos, I.S., Rosenwald, A., Boldrick, J.C., Sabet,
18 H., Tran, T., Yu, X., et al. (2000). Distinct types of diffuse large B-cell lymphoma identified by gene
19 expression profiling. Nature *403*, 503-511.

20 Boissel, N., and Sender, L.S. (2015). Best Practices in Adolescent and Young Adult Patients with
21 Acute Lymphoblastic Leukemia: A Focus on Asparaginase. J Adolesc Young Adult Oncol *4*, 118-128.

22 Caro, P., Kishan, A.U., Norberg, E., Stanley, I.A., Chapuy, B., Ficarro, S.B., Polak, K., Tondera, D.,
23 Gounarides, J., Yin, H., et al. (2012). Metabolic signatures uncover distinct targets in molecular
24 subsets of diffuse large B cell lymphoma. Cancer Cell *22*, 547-560.

25 Chen, L., Monti, S., Juszczynski, P., Daley, J., Chen, W., Witzig, T.E., Habermann, T.M., Kutok, J.L.,
26 and Shipp, M.A. (2008). SYK-dependent tonic B-cell receptor signaling is a rational treatment target
27 in diffuse large B-cell lymphoma. Blood *111*, 2230-2237.

28 Chiche, J., Pommier, S., Beneteau, M., Mondragon, L., Meynet, O., Zunino, B., Mouchotte, A.,
29 Verhoeyen, E., Guyot, M., Pages, G., et al. (2015). GAPDH enhances the aggressiveness and the
30 vascularization of non-Hodgkin's B lymphomas via NF-kappaB-dependent induction of HIF-1alpha.
31 Leukemia *29*, 1163-1176.

32 Coiffier, B., Lepage, E., Briere, J., Herbrecht, R., Tilly, H., Bouabdallah, R., Morel, P., Van Den
33 Neste, E., Salles, G., Gaulard, P., et al. (2002). CHOP chemotherapy plus rituximab compared with
34 CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med *346*, 235-242.

Colell, A., Green, D.R., and Ricci, J.E. (2009). Novel roles for GAPDH in cell death and carcinogenesis. Cell Death Differ *16*, 1573-1581.

Copie-Bergman, C., Gaulard, P., Leroy, K., Briere, J., Baia, M., Jais, J.P., Salles, G.A., Berger, F., Haioun, C., Tilly, H., et al. (2009). Immuno-fluorescence in situ hybridization index predicts survival in patients with diffuse large B-cell lymphoma treated with R-CHOP: a GELA study. J Clin Oncol *27*, 5573-5579.

Csibi, A., Lee, G., Yoon, S.O., Tong, H., Ilter, D., Elia, I., Fendt, S.M., Roberts, T.M., and Blenis, J. (2014). The mTORC1/S6K1 pathway regulates glutamine metabolism through the eIF4B-dependent control of c-Myc translation. Current biology : CB *24*, 2274-2280.

Cunningham, J.T., Rodgers, J.T., Arlow, D.H., Vazquez, F., Mootha, V.K., and Puigserver, P. (2007). mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. Nature *450*, 736-740.

Dancey, J. (2010). mTOR signaling and drug development in cancer. Nat Rev Clin Oncol *7*, 209-219.

Delarue, R., Tilly, H., Mounier, N., Petrella, T., Salles, G., Thieblemont, C., Bologna, S., Ghesquieres, H., Hacini, M., Fruchart, C., et al. (2013). Dose-dense rituximab-CHOP compared with standard rituximab-CHOP in elderly patients with diffuse large B-cell lymphoma (the LNH03-6B study): a randomised phase 3 trial. The Lancet. Oncology *14*, 525-533.

Doherty, J.R., and Cleveland, J.L. (2013). Targeting lactate metabolism for cancer therapeutics. J Clin Invest *123*, 3685-3692.

Dubois, S., Viailly, P.J., Bohers, E., Bertrand, P., Ruminy, P., Marchand, V., Maingonnat, C., Mareschal, S., Picquenot, J.M., Penther, D., et al. (2017). Biological and Clinical Relevance of Associated Genomic Alterations in MYD88 L265P and non-L265P-Mutated Diffuse Large B-Cell Lymphoma: Analysis of 361 Cases. Clin Cancer Res *23*, 2232-2244.

Duran, R.V., Oppliger, W., Robitaille, A.M., Heiserich, L., Skendaj, R., Gottlieb, E., and Hall, M.N. (2012). Glutaminolysis activates Rag-mTORC1 signaling. Mol Cell *47*, 349-358.

Fisher, R.I., Gaynor, E.R., Dahlberg, S., Oken, M.M., Grogan, T.M., Mize, E.M., Glick, J.H., Coltman, C.A., Jr., and Miller, T.P. (1993). Comparison of a standard regimen (CHOP) with three intensive chemotherapy regimens for advanced non-Hodgkin's lymphoma. N Engl J Med *328*, 1002-1006.

Frecha, C., Fusil, F., Cosset, F.L., and Verhoeyen, E. (2011). In vivo gene delivery into hCD34+ cells in a humanized mouse model. Methods Mol Biol *737*, 367-390.

Fruman, D.A., and Rommel, C. (2014). PI3K and cancer: lessons, challenges and opportunities. Nat Rev Drug Discov *13*, 140-156.

Galluzzi, L., Kepp, O., Vander Heiden, M.G., and Kroemer, G. (2013). Metabolic targets for cancer therapy. Nat Rev Drug Discov *12*, 829-846.

Gautier, L., Cope, L., Bolstad, B.M., and Irizarry, R.A. (2004). affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics *20*, 307-315.

Gordan, J.D., Thompson, C.B., and Simon, M.C. (2007). HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. Cancer Cell *12*, 108-113.

Green, T.M., Young, K.H., Visco, C., Xu-Monette, Z.Y., Orazi, A., Go, R.S., Nielsen, O., Gadeberg, O.V., Mourits-Andersen, T., Frederiksen, M., et al. (2012). Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol *30*, 3460-3467.

Hans, C.P., Weisenburger, D.D., Greiner, T.C., Gascoyne, R.D., Delabie, J., Ott, G., Muller-Hermelink, H.K., Campo, E., Braziel, R.M., Jaffe, E.S., et al. (2004). Confirmation of the molecular

classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood *103*, 275-282.

Haralambieva, E., Kleiverda, K., Mason, D.Y., Schuuring, E., and Kluin, P.M. (2002). Detection of three common translocation breakpoints in non-Hodgkin's lymphomas by fluorescence in situ hybridization on routine paraffin-embedded tissue sections. J Pathol *198*, 163-170.

Horn, H., Ziepert, M., Becher, C., Barth, T.F., Bernd, H.W., Feller, A.C., Klapper, W., Hummel, M., Stein, H., Hansmann, M.L., et al. (2013). MYC status in concert with BCL2 and BCL6 expression predicts outcome in diffuse large B-cell lymphoma. Blood *121*, 2253-2263.

Johnson, N.A., Slack, G.W., Savage, K.J., Connors, J.M., Ben-Neriah, S., Rogic, S., Scott, D.W., Tan, K.L., Steidl, C., Sehn, L.H., et al. (2012). Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. J Clin Oncol *30*, 3452-3459.

Kheirallah, S., Caron, P., Gross, E., Quillet-Mary, A., Bertrand-Michel, J., Fournie, J.J., Laurent, G., and Bezombes, C. (2010). Rituximab inhibits B-cell receptor signaling. Blood *115*, 985-994.

Kordes, S., Pollak, M.N., Zwinderman, A.H., Mathot, R.A., Weterman, M.J., Beeker, A., Punt, C.J., Richel, D.J., and Wilmink, J.W. (2015). Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial. The Lancet. Oncology *16*, 839-847.

Lee, M.N., Ha, S.H., Kim, J., Koh, A., Lee, C.S., Kim, J.H., Jeon, H., Kim, D.H., Suh, P.G., and Ryu, S.H. (2009). Glycolytic flux signals to mTOR through glyceraldehyde-3-phosphate dehydrogenase-mediated regulation of Rheb. Mol Cell Biol *29*, 3991-4001.

Lenz, G., and Staudt, L.M. (2010). Aggressive lymphomas. N Engl J Med *362*, 1417-1429.

Lenz, G., Wright, G., Dave, S.S., Xiao, W., Powell, J., Zhao, H., Xu, W., Tan, B., Goldschmidt, N., Iqbal, J., et al. (2008). Stromal gene signatures in large-B-cell lymphomas. N Engl J Med *359*, 2313-2323.

Lipton, A., Kostler, W.J., Leitzel, K., Ali, S.M., Sperinde, J., Weidler, J., Paquet, A., Sherwood, T., Huang, W., Bates, M., et al. (2010). Quantitative HER2 protein levels predict outcome in fluorescence in situ hybridization-positive patients with metastatic breast cancer treated with trastuzumab. Cancer *116*, 5168-5178.

Mackay, G.M., Zheng, L., van den Broek, N.J., and Gottlieb, E. (2015). Analysis of Cell Metabolism Using LC-MS and Isotope Tracers. Methods Enzymol *561*, 171-196.

Mareschal, S., Ruminy, P., Bagacean, C., Marchand, V., Cornic, M., Jais, J.P., Figeac, M., Picquenot, J.M., Molina, T.J., Fest, T., et al. (2015). Accurate Classification of Germinal Center B-Cell-Like/Activated B-Cell-Like Diffuse Large B-Cell Lymphoma Using a Simple and Rapid Reverse Transcriptase-Multiplex Ligation-Dependent Probe Amplification Assay: A CALYM Study. J Mol Diagn.

Meynet, O., and Ricci, J.E. (2014). Caloric restriction and cancer: molecular mechanisms and clinical implications. Trends in molecular medicine.

Molina, T.J., Canioni, D., Copie-Bergman, C., Recher, C., Briere, J., Haioun, C., Berger, F., Ferme, C., Copin, M.C., Casasnovas, O., et al. (2014). Young patients with non-germinal center B-cell-like diffuse large B-cell lymphoma benefit from intensified chemotherapy with ACVBP plus rituximab compared with CHOP plus rituximab: analysis of data from the Groupe d'Etudes des Lymphomes de l'Adulte/lymphoma study association phase III trial LNH 03-2B. J Clin Oncol *32*, 3996-4003.

Monti, S., Savage, K.J., Kutok, J.L., Feuerhake, F., Kurtin, P., Mihm, M., Wu, B., Pasqualucci, L., Neuberg, D., Aguiar, R.C., et al. (2005). Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. Blood *105*, 1851-1861.

Morita, M., Gravel, S.P., Chenard, V., Sikstrom, K., Zheng, L., Alain, T., Gandin, V., Avizonis, D., Arguello, M., Zakaria, C., et al. (2013). mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. Cell metabolism *18*, 698-711.

Nicholson, J.K., Foxall, P.J., Spraul, M., Farrant, R.D., and Lindon, J.C. (1995). 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. Analytical chemistry *67*, 793-811.

Norberg, E., Lako, A., Chen, P.H., Stanley, I.A., Zhou, F., Ficarro, S.B., Chapuy, B., Chen, L., Rodig, S., Shin, D., et al. (2017). Differential contribution of the mitochondrial translation pathway to the survival of diffuse large B-cell lymphoma subsets. Cell Death Differ *24*, 251-262.

Petrella, T., Copie-Bergman, C., Briere, J., Delarue, R., Jardin, F., Ruminy, P., Thieblemont, C., Figeac, M., Canioni, D., Feugier, P., et al. (2017). BCL2 expression but not MYC and BCL2 coexpression predicts survival in elderly patients with diffuse large B-cell lymphoma independently of cell of origin in the phase 3 LNH03-6B trial. Ann Oncol *28*, 1042-1049.

Poste, G. (2011). Bring on the biomarkers. Nature *469*, 156-157.

Ricci, J.E., and Chiche, J. (2018). Metabolic Reprogramming of Non-Hodgkin's B-Cell Lymphomas and Potential Therapeutic Strategies. Front Oncol *8*, 556.

Rustin, P., Chretien, D., Bourgeron, T., Gerard, B., Rotig, A., Saudubray, J.M., and Munnich, A. (1994). Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta *228*, 35-51.

Sehn, L.H., and Gascoyne, R.D. (2015). Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biologic heterogeneity. Blood *125*, 22-32.

Swerdlow, S.H., Campo, E., Pileri, S.A., Harris, N.L., Stein, H., Siebert, R., Advani, R., Ghielmini, M., Salles, G.A., Zelenetz, A.D., et al. (2016). The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood *127*, 2375-2390.

Wheaton, W.W., Weinberg, S.E., Hamanaka, R.B., Soberanes, S., Sullivan, L.B., Anso, E., Glasauer, A., Dufour, E., Mutlu, G.M., Budigner, G.S., et al. (2014). Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. Elife *3*, e02242.

Willems, L., Jacque, N., Jacquel, A., Neveux, N., Maciel, T.T., Lambert, M., Schmitt, A., Poulain, L., Green, A.S., Uzunov, M., et al. (2013). Inhibiting glutamine uptake represents an attractive new strategy for treating acute myeloid leukemia. Blood *122*, 3521-3532.

Wishart, D.S., Jewison, T., Guo, A.C., Wilson, M., Knox, C., Liu, Y., Djoumbou, Y., Mandal, R., Aziat, F., Dong, E., et al. (2013). HMDB 3.0--The Human Metabolome Database in 2013. Nucleic Acids Res *41*, D801-807.

Figure titles and legends

Figure 1. DLBCLs expressing low levels of GAPDH are associated with a poor response to R-CHOP treatment and with OxPhos metabolism.

A. Distribution of *gapdh* expression values (log2 mRNA intensity) in newly diagnosed DLBCLs characterized as DLBCLs-*gapdh*low (n=125) and DLBCLs-*gapdh*high (n=108) (analysis from (Lenz et al., 2008). p value from Wilcoxon rank sum.

B. Overall survival (OS) of R-CHOP-treated DLBCL patients, according to *gapdh*low (n=125) and *gapdh*high (n=108) mRNA levels in DLBCL biopsies at diagnosis (analysis from (Lenz et al., 2008). p value from log-rank test.

C. Illustration of IHC staining of GAPDH in germinal center (GC) B cells of a reactive lymph node (LN) (score = 80) and in *de novo* DLBCL expressing low (score = 80) or high (score = 260) levels of GAPDH protein. Magnification 20X (100 µm).

D. Distribution of GAPDH protein expression scored by IHC in GCB cells from human paraffin-embedded reactive lymph nodes (n=5) or newly diagnosed DLBCLs expressing low (score ≤ 150; n=15) or high (score > 150; n=28) GAPDH protein (Training cohort). p value from Wilcoxon rank sum.

E. OS of R-CHOP-treated DLBCL patients according to the levels of GAPDH expression determined by IHC in newly diagnosed DLBCLs (n=15 GAPDHlow and n=28 GAPDHhigh; Training cohort). p value from log-rank test.

F. Distribution of GAPDH protein expression scored by IHC staining of GAPDH in tissue-microarray corresponding to 294 paraffin-embedded *de novo* DLBCL biopsies. 204 DLBCLs-GAPDHlow and 90 DLBCLs-GAPDHhigh are identified (validation cohort). p value from Wilcoxon rank sum.

G. OS of 294 R-CHOP-treated patients with DLBCLs grouped on the basis of GAPDH expression levels presented in **F**. p value from log-rank test.

H. Transcript abundance (probe intensity) of *gapdh* mRNA according to the CCC classification (n=82 BCR-DLBCL and n=71 OxPhos-DLBCL) identified by genomic clustering (Monti et al., 2005), analysis from ((Lenz et al., 2008). p value from Wilcoxon test.

1   **I.** Correlation between GAPDH expression levels scored by IHC and glycolytic ATP in malignant B

2   cells harvested from patients with newly diagnosed DLBCLs. Blue dots represent DLBCLs-

3   GAPDH^{low} (n=3) and red dots represent DLBCL-GAPDH^{high} (n=8). R, Pearson correlation coefficient.

4   p value from correlation test.

5   See also Tables S1, S2, S3 and Figures S1 and S2, related to Figure 1.

6

7   **Figure 2. Mouse primary B lymphomas expressing low levels of GAPDH rely on OxPhos**

8   **metabolism and are sensitive to inhibition of the electron transport chain complex I activity.**

9   **A.** Quantification of intracellular ATP levels produced in Eµ-*Myc*-GAPDH^{low} and in Eµ-*Myc*-

10 GAPDH^{high} cells (n=5 independent lymphomas per group). Results are expressed as relative

11 luminescence units normalized by live cell number.

12 **B.** Glycolytic ATP production measured as the percentage of total ATP produced by Eµ-*Myc*-

13 GAPDH^{low} and Eµ-*Myc*-GAPDH^{high} cells (n=5 independent lymphomas per group). Data are

14 expressed as mean ± s.d (n=3 independent experiments).

15 **C.** Lactate secretion was determined in the supernatant of Eµ-*Myc*-GAPDH^{low} and of Eµ-*Myc*-

16 GAPDH^{high} cells (n=5 independent lymphomas per group), 24 hours after seeding (mean ± s.d; n=3

17 independent experiments).

18 **D.** Baseline oxygen consumption rate (OCR) of Eµ-*Myc*-GAPDH^{low} and of Eµ-*Myc*-GAPDH^{high} cells

19 was determined with Clarck electrode 24 hours after seeding (n=5 independent lymphomas/group).

20 Data are expressed as mean ± s.d (n=3 independent experiments).

21 **E.** Eµ-*Myc*-GAPDH^{low} and Eµ-*Myc*-GAPDH^{high} cells (n=3 independent lymphomas/group) were

22 seeded in the presence or absence (Ctl, DMSO) of phenformin (Phen, 300 µM) for 24 hours. Cell

23 death was determined by DAPI staining and analyzed by flow cytometry. Data are expressed as mean

24 ± s.d (n=4 independent experiments).

25 **F. G.** Kaplan–Meier curves for the survival of syngeneic C57BL/6 mice intravenously injected with

26 primary Eµ-*Myc*-GAPDH^{low} (**F.**) or Eµ-*Myc*-GAPDH^{high} (**G.**) cells and treated six days later, with

27 either vehicle (H$_2$O) or 100 mg/kg/day of Phenformin, orally and daily during 3 weeks (n=10

28 mice/group, p value from log-rank test, *** p< 0.001).

1   From panels **A.** to **E.,** ***p<0.001 indicated significance of the observed differences. *ns*, not

2   significant.

3   See also Figures S3 and S4, related to Figure 2.

4

5   **Figure 3. Active mTORC1 signaling in B lymphomas expressing low levels of GAPDH is**

6   **implicated in OxPhos metabolism.**

7   **A.** Whole-cell lysates prepared from Eμ-*Myc* cells harvested from independent Eμ-*Myc* lymphomas

8   were analyzed by immunoblot with the indicated antibodies. Each lane represents an independent

9   lymphoma. Erk2 is used as a loading control.

10 **B.** Representative immunohistochemistry slides of *de novo* DLBCL biopsies stained for GAPDH and

11 P-p70S6K (T389) proteins. Magnification 20X (100 μm). Table represents the summary of the

12 GAPDH and P-p70S6K (T389) stained slides for 18 *de novo* DLBCL biopsies. p value from Fisher

13 test.

14 **C.** Eμ-*Myc*-GAPDH^{low} cells (lymphomas #F) were seeded in the presence or absence (Ctl, DMSO) of

15 10 nM and 20 nM of Rapamycin for 15 hours. Whole-cell lysates were then analyzed for the indicated

16 proteins. Erk2 was used as a loading control.

17 **D-F.** Baseline OCR (**D.**), maximal respiration (**E.**) and ATP-coupled OCR (**F.**) of Eμ-*Myc*-GAPDH^{low}

18 cells (lymphoma #F) pre-treated or not (Ctl, DMSO) with indicated doses of Rapamycin for 15 hours

19 were determined with Seahorse XF96 Extracellular Flux Analyzer. Maximal respiration (**E.**) is

20 immediately determined after injection of 0.5 μM of FCCP. ATP-coupled OCR (**F.**) represents

21 oligomycin-sensitive respiration. Data are expressed as mean ± s.d. (n=3 independent experiments, in

22 quadruplicates).

23 **G.** Glycolytic ATP production measured as the percentage of total ATP in Eμ-*Myc*-GAPDH^{low} cells

24 (lymphoma #F) incubated in the presence or absence (Ctl, DMSO) of 10 nM and 20 nM of Rapamycin

25 for 15 hours, was quantified using a luciferin/luciferase-based assay. The presented data are expressed

26 as mean ± s.d. (n=2 independent experiments).

27 **H.** Eμ-*Myc*-GAPDH^{low} and Eμ-*Myc*-GAPDH^{high} cells (n=3 independent lymphomas/group) were

28 seeded in the presence or absence (Ctl, DMSO) of indicated doses of Rapamycin for 15, 24 and 48

1 hours. Cell death was determined by DAPI staining and analyzed by flow cytometry. Data are

2 expressed as mean ± s.d (n=2 independent experiments).

3 **I.** Survival of syngeneic C57BL/6 mice intravenously injected with primary Eμ-*Myc*-GAPDH^{low} cells

4 (isolated from lymphoma #F) and treated six days later with either vehicle (NaCl 0.9%-5%Tween-5%

5 PEG-200-1% EtOH) or Temsirolimus (10 mg/kg) intraperitoneally two times per week (n=6

6 mice/group, p value from log-rank, ***p< 0.001).

7 **J.** Whole-cell lysates prepared from axillary lymph node tumors of Vehicle or Temsirolimus

8 (10 mg/kg) treated Eμ-*Myc*-GAPDH^{low}-bearing mice were analyzed by immunoblot for the indicated

9 antibodies. Each lane represents an independent mouse. *n.s* means non-specific band. Erk2 is used as a

10 loading control. ***p<0.001 indicated significance of the observed differences.

11 See also Figures S5, related to Figure 3.

12

13 **Figure 4. Mouse primary B lymphomas expressing low levels of GAPDH rely on glutamine**

14 **metabolism and are sensitive to hydrolysis of extracellular glutamine by *E-Coli* L-asparaginase.**

15 **A.** Glutamine consumption was determined in the supernatant of Eμ-*Myc*-GAPDH^{low} and Eμ-*Myc*-

16 GAPDH^{high} cells cells (n=5 independent lymphomas per group), 24 hours after seeding.

17 **B.** Glutamine transport rates (in cpm) is measured in 5.10^6 Eμ-*Myc*-GAPDH^{low} or Eμ-*Myc*-GAPDH^{high}

18 cells (n=4 independent lymphomas/group) incubated for 30 minutes in an amino-acid-free, 20 mM

19 glucose containing DMEM supplemented with [^{14}C]-Glutamine. Data are expressed as mean ± s.d

20 (n=2 independent experiments in triplicate).

21 **C.** Heat map depicting significantly changed glycolytic/ non-oxidative pentose phosphate pathway

22 (PPP) and glutaminolysis/ tricarboxylic acid (TCA) cycle metabolite levels in Eμ-*Myc*-GAPDH^{low} and

23 in Eμ-*Myc*-GAPDH^{high} cells (n=3 independent lymphomas per group, four replicates per lymphoma)

24 24 hours after seeding, as analyzed by LC-MS. Red, increase; blue, decrease.

25 **D.** Whole-cell lysates prepared from Eμ-*Myc*-GAPDH high and low cells were analyzed by

26 immunoblot for the mitochondrial glutaminase (GLS). Erk2 is used as a loading control.

27 **E.** Concentration of glutamine (left panel) and of asparagine (right panel) in the supernatant of Eμ-

28 *Myc*-GAPDH high (in red) and low (in bleu) cells (n=4 independent lymphomas per group) treated or

1 not (Ctl) with *E-Coli* L-asparaginase (L-ase) at indicated doses for 4 hours. The presented data are

2 expressed as mean ± s.d (n=3 independent experiments).

3 **F.** Eμ-*Myc*-GAPDH[low] and Eμ-*Myc*-GAPDH^{high} cells (n=3 independent lymphomas/group) were

4 seeded in the presence or absence (Ctl) of *E-Coli* L-asparaginase (L-ase, at indicated doses) for 15

5 hours. Cell death was determined by DAPI staining and analyzed by flow cytometry (n=3 independent

6 experiments).

7 **G.** Plasma glutamine concentration 4 hours after the last *ip* administration of NaCl 0.9% (Ctl) or *E-*

8 *Coli* L-asparaginase (L-ase, 2500 IU/kg) in Eμ-*Myc*-GAPDH[low]- and in Eμ-*Myc*-GAPDH[high] -bearing

9 mice (n=4 mice per group).

10 **H. I.** Survival of syngeneic C57BL/6 mice intravenously injected with primary Eμ-*Myc*-GAPDH[low]

11 (**H.**) or Eμ-*Myc*-GAPDH^{high} cells (**I.**) and treated six days later with either vehicle (NaCl 0.9%) or

12 2500 IU/kg of *E-Coli* L-asparaginase (L-ase) intraperitoneally five times per week the first week and

13 three times per week the following weeks (n=6 mice/group, p value from log-rank, ***p< 0.001).

14 From panel **A.** to **G.**, **p< 0.01, ***p<0.001 indicated significance of the observed differences. *ns*,

15 not significant.

16 See also Figure S6, related to Figure 4.

17

18 **Figure 5. Specific overexpression of human GAPDH reduces mTORC1 signaling and decreases**

19 **B lymphomas sensitivity to phenformin or *E-Coli* L-asparaginase *in vivo*.**

20 **A.** Total cell extracts from mouse primary Eμ-*Myc*-GAPDH^{low} cells (lymphoma #F) stably transduced

21 with control (pMIG) or GAPDH-V5-encoding pMIG vectors were immunoblotted for the indicated

22 proteins. Erk2 is used as a loading control.

23 **B.** Intracellular ATP levels produced in Eμ-*Myc*-GAPDH^{low} cells stably overexpressing GAPDH-V5

24 or control vector (pMIG) were quantified using a luciferin/luciferase-based assay and results were

25 expressed as relative luminescence units normalized by cell number (n=3 independent experiments).

26 **C.** Glycolytic ATP production was measured as the percentage of total ATP in cells presented in **A.**

27 Data are presented as means ± s.d (n=3 independent experiments).

1   **D.** Lactate production was determined in the supernatant of mouse primary Eμ-*Myc*-GAPDH^{low} cells

2   (lymphoma #F) stably transduced with control (pMIG) or GAPDH-V5-encoding pMIG vectors, 24

3   hours after seeding. Data are presented as mean ± s.d (n=3 independent experiments).

4   **E.** Baseline OCR of cells presented in **A.** was determined with Clarck electrode, 24 hours after cell

5   seeding. Data are expressed as mean ± s.d (n=3 independent experiments).

6   **F.** Glutamine consumption was determined 24 hours after cell seeding in the supernatant of Eμ-*Myc*-

7   GAPDH^{low} cells (lymphoma #F) stably transduced with control (pMIG) or GAPDH-V5-encoding

8   pMIG vectors. Data are expressed as mean ± s.d (n=3 independent experiments).

9   **G.** Weight of inguinal (left) and axillary (right) lymphomas harvested from wild-type syngeneic

10 C57BL/6 mice twenty days after intravenous injection of primary Eμ-*Myc*-GAPDH^{low} cells stably

11 over-expressing or not (pMIG) GAPDH-V5. Phenformin (100 mg/kg/day) or vehicle (H$_2$O) were

12 administrated every day for the last thirteen days (pMIG-Vehicle, n=8; pMIG-Phenformin, n=9;

13 GAPDH-V5-Vehicle, n=10; GAPDH-V5-Phenformin, n=7).

14 **H. I.** Survival of syngeneic C57BL/6 mice intravenously injected with primary Eμ-*Myc*-GAPDH[low]

15 cells stably transduced with control pMIG vector (**H.**) or GAPDH-V5-encoding pMIG vectors (**I.**) and

16 treated six days later with either vehicle (NaCl 0.9%) or 2500 IU/kg of E-Coli L-asparaginase (L-ase)

17 intraperitoneally five times per week the first week and three times per week the following weeks

18 (n=10 mice/group, p value from log-rank, **$p< 0.01$; ***$p< 0.001$).

19 From panel **A.** to **G.**, *$p<0.05$, **$p<0.01$, ***$p<0.001$ indicated significance of the observed

20 differences. *ns*, not significant.

21 See also Figure S7, related for Figure 5.

22

23 **Figure 6. Patients with DLBCL-GAPDH[low] are sensitive to specific inhibitors of mitochondrial**

24 **metabolism (KTM treatment).**

25 **A.** Schematic representation of conventional therapeutic protocol for patients with DLBCL. Once

26 diagnosed, patients with DLBCL receive immuno-chemotherapy, R-CHOP. Approximately, 40% of

27 the patients will experience therapeutic failure (refractory or relapse). Other anti-CD20-based

28 therapies such as R-DHAP (Rituximab, dexamethasone-Ara-C (cytarabine)-Platinol (cisplatin)) or R-

ICE (Rituximab, Ifosfamide-Carboplatin-Etoposide) are then proposed. Another set of 40% of patients will not respond to those treatments and will be eligible for HDT/ASCT (High Dose Therapy with Autologous Stem Cell Transplantation). In the case of *de novo* DLBCL-GAPDH^{low} (low responders to R-CHOP), we propose a clinical protocol called KTM (Kidrolase-Torisel-Metformin), to interfere with tumor metabolism upon relapse of all anti-CD20-based therapies.

B. Schematic representation of one cycle of KTM therapy. 4 weeks-cycle of KTM consisted in the combination of *E-Coli* L-asparaginase (K, Kidrolase® 6000 UI/m^2) on days 1, 3, 5, 7, 9, 11, and 13, mTOR inhibitor temsirolimus (T, Torisel 75 mg D1, 7, 14) and Metformin (1000 mg/day) on day 14 to day 28. Four patients with DLBCL-GAPDH^{low} at diagnosis and refractory to all anti-CD20-based therapies, were eligible to KTM therapy.

C. Progressive regression of the patient #1's DLBCL (CD10$^+$, bcl2$^+$, Mum1$^-$, Ki67$^+$ 80%, MYC$^+$, Ann Arbor Stage IV) upon KTM treatment.

D. 3D modelization of Computed Tomography (CT) scan of patient #1 before (T=0) and after (T=30 days) two cycles of KTM treatment.

E. Illustration of GAPDH expression (and respective GAPDH IHC score) in DLBCL biopsies at diagnosis, from the four patients enrolled in the KTM study.

F. Duration of treatment and therapeutic response (by CT or PET) to KTM for the four individual patients presenting DLBCL-GAPDH^{low} at diagnosis (**E.**).

KTM, Kidrolase, Torisel and Metformin; CR, complete response; PR, partial response; PD, progressive disease; PAC, patient alive at last consultation; AE, adverse event.

See also Table S4 related to Figure 6.

Tables with titles and legends,

Table 1. GAPDH expression levels are not associated with other biological prognostic factors.

BCL2 ≥ 70% means that 70% (or more) of malignant B cells stained for BCL2 protein by IHC (Green et al., 2012; Petrella et al., 2017); BCL6 > 25% means up to 25% of malignant B cells stained for BCL6 by IHC (Horn et al., 2013); MYC ≥ 40% (Johnson et al., 2012) means that 40% (or more) of

1 malignant B cells stained for MYC protein by IHC. Double hit (*MYC/BCL2*) and triple hit

2 (*MYC/BCL2/BCL6*) were obtained by Fluorescence In Situ Hybridization (FISH).

3 *p* value from Fisher test. See also Tables S2 and S3, related to Table 1.

STAR Methods

CONTACT FOR REAGENT AND RESOURCE SHARING

7 Further information and requests for resources and reagents should be directed to and will be fulfilled

8 by the Lead Contact, Jean-Ehrland Ricci (ricci@unice.fr).

Materials and Methods

Experimental model and subject details

Mice and in vivo studies using Eμ-Myc cells

15 C57BL/6 Eμ-*Myc* transgenic mice were purchased from the Jackson Laboratory (#002728) and

16 housed in our local animal facility (C3M, Nice, France).

18 The lymphoma transfer of Eμ-*Myc* cells was performed into syngeneic, nontransgenic, 6-weeks-old

19 C57BL/6JOlaHsd females (Envigo) by intravenous injection (*iv*) of 0.1×10^6 viable Eμ-*Myc*

20 lymphoma cells per recipient mouse (in 150 µl of sterile PBS). Six days after cell injection, mice

21 received treatments. 100 mg/kg/day of Phenformin (P7045, Sigma-Aldrich) or vehicle (H_2O) (n=10

22 mice/group) were administrated by gavage (*p.o*) every day for three weeks. 2500 UI/kg/day of *E-Coli*

23 L-asparaginase (Kidrolase, L-ase, Jazz Pharmaceutical) or vehicle (NaCl 0.9%) was injected

24 intraperitoneally for five days the first week and three times per week the following weeks, for a total

25 of three weeks of treatment (n=6 mice/group). 10 mg/kg of Temsirolimus (PZ0020, Sigma-Aldrich) or

26 vehicle (NaCl 0.9%-5%Tween-5% PEG-200-1% EtOH) were intraperitoneally injected two times per

27 week for three weeks (n=6 mice per group). Tumor onset was determined by inguinal lymph node

28 palpation. Lymphoma-bearing animals were sacrificed by cervical dislocation. Phenformin-treated

mice were sacrificed two hours after the last gavage of phenformin to analyze effects of the compound on lymphoma cell signaling. *E-Coli* L-asparaginase (L-ase) treated mice were sacrificed four hours after the last *ip* injection of L-ase and plasma was immediately isolated to analyze plasma glutamine concentrations. Temsirolimus treated mice were sacrificed 24 hours after the last treatment to analyze *in vivo* efficacy of this compound within the lymphomas.

All mice were maintained in specific pathogen-free conditions and all experimental procedures were approved by the national Animal Care and Use Committee and the regional ethics committee (05111.02 from Direction Générale de la Recherche et de l'Innovation). Survival was determined as the time between the *i.v.* injection of the cells and the time when mice had to be sacrificed, as soon as they exhibit systemic signs of illness (apathy, hair loss, breathing problems, precipitous weight loss, and limited ability to reach food or water), in agreement with the guidelines of the Institutional Animal Care and Use Committee. Food was given *ad libitum*. Experiments using the Eµ-*Myc* cells transplantation in syngeneic C57BL/6 mice have shown statistical significance starting from 6 mice/group. No randomization was performed. Endpoint was evaluated by blinding.

Cell lines and culture conditions

Mouse primary Eµ-*Myc* lymphomas cells were isolated from different C57BL/6 Eµ-*Myc* transgenic mice as previously described (Chiche et al., 2015) and maintained in DMEM-GlutamaX (#31966047, Thermo Fisher Scientific) supplemented with 10% FBS, 50 µM of 2-mercaptoethanol (#31350010, Thermo Fisher Scientific), 0.37 mM of L-asparagine (A0884, Sigma-Aldrich) and 10 mM of HEPES pH 7.4 (#15630056, Thermo Fisher Scientific).

The human DLBCL cell lines Toledo, Karpas 422, SU-DHL-4 and SU-DHL-6 were tested mycoplasma-free. Cell line authentication was performed by DSMZ by DNA profiling using 8 different and highly polymorphic short tandem repeat (STR) loci. There was also no presence of mitochondrial DNA sequences from rodent cells as mouse, rat, Chinese and Syrian hamster. The

Karpas 422 cell line was from the ECACC (European Collection of Authenticated Cell cultures), the Toledo cell line was from ATCC (American Type Culture Collection), the SU-DHL-4 and SUD-HL-6 cell lines were from DSMZ (German Collection of Microorganisms and Cell Culture). Toledo, Karpas 422 and SU-DHL-4 cells were maintained in RPMI-GlutamaX (#61870044, Thermo Fisher Scientific), supplemented with 10% FBS. SU-DHL-6 cells were maintained in RPMI-GlutamaX supplemented with 20% FBS.

Microarray data analysis

Raw.CEL files and clinical information from the study of Lenz G. *et al*. (Lenz et al., 2008). were downloaded from the Gene Expression Omnibus database (GSE10846). The total number of samples included in this study was 414, 181 treated with CHOP and 233 with R-CHOP. Data were summarized, normalized and log2 transformed using the justRMA function from the Bioconductor package affy (version 1.40.0, (Gautier et al., 2004)). Follow-up time was limited to 5 years. Association between gene expression levels and overall survival was performed on samples from the R-CHOP arm only. Samples with low expression levels were filtered out (log2 intensity < 8). For each remaining probeset, samples were split into two groups (below and above the median) and difference in survival was assessed using Cox proportional hazards models. 587 candidate probesets were selected based on nominal p-value < 0.05 and an abs(log2 HR)>0.75.

Association of expression levels with OS was first tested by categorizing samples into high/low expression samples using median values. Then for *gapdh*, cutoff was optimized using positional scanning (Lipton et al., 2010). Survival curves were plotted following the Kaplan and Meier method, and statistical significance was assessed using the log-rank test. Survival analysis was performed using functions from the R package survival.

OxPhos and BCR classification were obtained from the supplemental data of (Caro et al., 2012). Statistical significance was assessed using Wilcoxon rank sum test. Enrichment in biological themes (Molecular function and canonical pathways) were performed using Ingenuity Pathway Analysis

1   software.

2

3   ***DLBCL patients: Training & Validation cohorts***

4   DLBCL tumors were required to be *de novo* and treated with Rituximab-CHOP (R-CHOP). All cases

5   were diagnosed and reviewed by experienced hematopathologists and patient clinical information

6   were obtained from the Hopital Saint Louis, Centre Hospitalier Princesse Grace and Centre Antoine

7   Lacassagne institutions. DLBCLs were classified according to the WHO classification. Clinical data

8   including gender, age, and age-adjusted International Prognosis Index (IPIaa) (scored (0-1, 2 and 3)

9   based on i) the Ann Arbor stage (II-II *vs* III-IV), ii) the level of lactate dehydrogenase in the serum

10   (LDH ≤ normal vs ≥ normal) and iii) the Performance status (PS 0-1 *vs* 2-4)) were collected in all

11   cases and are summarized in Table S2. These cases were anonymized and processed in accordance

12   with a protocol approved by scientific committee of the LYSA (The Lymphoma Study Association).

13   All patients gave written non-opposition consent. Patients samples were allocated to GAPDH high and

14   low groups according to their respective expression (see GAPDH IHC staining and scoring section).

15   *Training cohort.* A total of 43 patients aged >18 years (aged between 24-85 years) who had been

16   diagnosed with *de novo* DLBCL and then treated with Rituximab and CHOP between 2008 and 2014

17   at Onco-Hematology Department of Nice CHU (France) and Saint-Louis Hospital, APHP, Paris

18   (France) were included in this training set. The samples were prepared from the paraffin blocks of

19   lymph nodes that were recovered from the archives of the Pathology departments.

20   *Validation cohort.* All cases analyzed in the validation cohort were enrolled in the prospectively

21   randomized phase 3 multi-center GELA-LNH03-6B trial (Delarue et al., 2013) in which 602 patients

22   with untreated diffuse large B-cell lymphomas (DLBCLs) and at least one adverse prognostic factor

23   aged between 60-80 years had been randomly assigned to receive eight cycles of R-CHOP-14 or eight

24   cycles of R-CHOP-21. They did not show any difference in efficacy endpoints between dose-dense

25   and standard regimens of R-CHOP. 294 biopsies specimens at diagnosis were available for the tissue

26   microarray (TMA). TMA contains three representative 0.6-mm cores of routinely processed tissues

1 (Beecher Instruments, Silver Spring, MD).

2

3 *Clinical protocol KTM (Kidrolase-Torisel-Metformin)*

4 Four patients harboring *de novo Myc*-translocated-DLBCLs and selected for KTM treatment presented

5 i) a retrospective GAPDH^{low} levels (scored by IHC in diagnosed biopsies), ii) a therapeutic failure

6 after a median of 2 prior lines of Rituximab-based therapies and iii) adequate performance status and

7 organ functions. KTM treatment consisted in L-asparaginase (K, Kidrolase®, Jazz Pharamaceutical,

8 6000 UI/m^2) on days 1, 3, 5, 7, 9, 11, and 13, mTOR inhibitor (T, Torisel, Pfizer, 75 mg) on days 1, 7

9 and 14, and Metformin (Mylan, 1000 mg/day) on day 14 to day 28. The study was approved on Oct

10 16th, 2012 by the Scientific Committee of the LYSA (The Lymphoma Study Association, reference:

11 REMtor). All patients gave a written informed consent to receive the treatment. Consent to publish

12 patients' images was obtained. There was no clinical trial registration number because the therapeutic

13 protocol was conducted on a local cohort.

14

15 **Methods details**

16

17 *COO classification*

18 Frozen tissues were analyzed using GeneChip Human Genome HGU133 Plus 2.0 arrays (Affymetrix,

19 Santa Clara) as previously reported (Dubois et al., 2017). From the LNH03-6B TMA (validation

20 cohort), sixty DLBCLs had both COO molecular classification.

21 Reverse transcription-multiplex ligation-dependent probe amplification (RT-MLPA) was performed to

22 determine the COO on thirty-five formalin-fixed paraffin-embedded (FFPE) DLBCLs (training

23 cohort), using a targeted molecular gene expression approach as recently described (Mareschal et al.,

24 2015).

25

GAPDH immunohistochemical (IHC) staining and scoring.

Sections (4 µm) of formalin-fixed, paraffin-embedded DLBCL biopsies (training cohort) or tissue-microarray (TMA) from the LYSA group (validation cohort) were treated for deparaffinization, rehydration, and antigen retrieval using standard procedures (EnVision™ FLEX reagents, Agilent, Dako). Before immunohistochemistry, antigen retrieval was performed at pH 9.0, for 20 minutes at 97°C, according to the manufacturer's instructions (EnVision™ FLEX reagents, Agilent, Dako). After washing in diluted EnVision FLEX Wash Buffer, slides were incubated in EnVision FLEX Peroxidase-Blocking Reagent solution for 5 min to quench endogenous peroxidase. Slides were then washed, stained at room temperature on an automated system (Autostainer link 48, Dako) with diluted (EnVision FLEX antibody diluent) anti-GAPDH (rabbit polyclonal anti-human GAPDH, HPA40067, Sigma-Aldrich; dilution 1:500) for 20 minutes, washed, incubated 20 minutes with Dako EnVision™ FLEX /HRP), washed, incubated 8 minutes with EnVision FLEX Substrate Working solution and counter-stained with Hematowylin (EnVision™ FLEX Hematoxylin). Sections were then dehydrated and mounted with pertex (Histolab).

Cytoplasmic GAPDH IHC staining was blindly scored by two pathologists from the LCAP from Nice CHU (Dr D. Ambrosetti and Dr. J. Reverso-Meinietti) based on two parameters: the GAPDH IHC staining intensity in tumor cells (scored 0 for absence of staining, 1 for a weak staining, 2 for a medium staining and 3 for a strong staining) and the proportion of tumor cells displaying each intensity. Then GAPDH IHC score was obtained by multiplying each GAPDH staining intensity with the corresponding percentage of tumor cells stained (example: GAPDH IHC score= 0 (non-stained) x % tumor cells + 1 (weak intensity) x % tumor cells stained + 2 (medium intensity) x % tumor cells stained + 3 (strong intensity) x % tumor cells stained). For the training cohort, GAPDH IHC staining was scored on the whole slide. For the TMA, GAPDH IHC score represents the mean of three different tissue spots from the same tumor. GAPDH IHC cut off of 150 was determined using positional scanning (Lipton et al., 2010). Illustrations of GAPDH IHC staining were performed using SlidePath Digital Image software (Leica Biosystems).

1 *Immunohistochemistry and fluorescence in situ hybridization*

2 Sections (4 µm) of formalin-fixed, paraffin-embedded DLBCL biopsies (training cohort) were treated

3 for deparaffinization, rehydration, and antigen retrieval using standard procedures (EnVision™ FLEX

4 reagents, Agilent, Dako). Antibodies against CD5 (M3641, Agilent, Dako), CD79A (M7050, Agilent,

5 Dako), CD20 (M0755, Agilent, Dako), CD10 (GA64861-2, Agilent, Dako), BCL2 (M0887, Agilent,

6 Dako), BCL6 (M7211, Agilent, Dako), MUM1 (M7259, Agilent, Dako), c-MYC (ab32072, Abcam)

7 and phospho-p70S6K (T389) (rabbit monoclonal anti-phospho-p70S6K (T389), #9234, Cell Signaling

8 Technology) were used for immunostainings performed on an automated system (Autostainer link 48,

9 Dako). Antigen retrieval was performed at specific pH prior incubation with indicated antibodies: pH

10 6.0 prior anti-CD79A, anti-CD20, anti-MUM1 and pH 9.0 prior anti-CD5, anti-CD10, anti-BCL2,

11 anti-BCL6, anti-c-Myc and anti-phospho-p70S6K (T389). Primary antibodies were diluted in

12 EnVision FLEX antibody diluent (anti-CD5, anti-CD10, anti-CD20, anti-BCL2 are prediluted; anti-

13 BCL6, dilution 1:50; anti-phospho-p70S6K (T389) 1:50; anti-CD79A, dilution 1:100; anti-MUM1,

14 dilution 1:100; anti-c-Myc, dilution 1:100) (see IHC procedure in "GAPDH immunohistochemical

15 (IHC) staining and scoring" section).

16 Tissue-microarrays (TMA) from the LYSA group (validation cohort) were treated and

17 immunoassayed for the same proteins using standard procedures of the LYSA platform as previously

18 described (Molina et al., 2014). GCB vs non-GCB scoring according to the Hans algorithm (Hans et

19 al., 2004), as well as MYC and BCL2 were evaluated (Petrella et al., 2017). In 30 out of the 294 cases,

20 COO classification by IHC (Hans algorithm) could not be assessed. The thresholds employed were

21 40% for MYC and 70% for BCL2 as previously reported (Green et al., 2012; Johnson et al., 2012).

22 FISH analyses were performed on 3µm tissue sections using split signal FISH DNA probes for

23 cMYC/8q24 (Y5410, Dako), BCL2/18q21 (Y5407, Dako), BCL6/3q27 (Y5408, Dako) and slides

24 were analyzed as previously described (Copie-Bergman et al., 2009). Scoring of the hybridization

25 signals were performed according to the algorithm published by (Haralambieva et al., 2002).

26

ATP analysis

ATP was measured using the Cell Titer Glo kit (G7570, Promega). Briefly, 20.000 cells were resuspended in 80 µL of corresponding medium supplemented with 10% FBS and distributed in a 96 well plate. Cells were then treated in triplicates for one hour with PBS (control), or sodium iodoacetate (100 µM) to inhibit glycolysis, or a combination of 100 µM of iodoacetate (I9148, Sigma-Aldrich) and 10 µg/ml of oligomycin (O4876, Sigma-Aldrich) to obtain the residual amount of ATP. Short time treatment (1h) was chosen to avoid a metabolic switch upon inhibition of glycolysis and to avoid cell death upon inhibition of both metabolic pathways. 100 µL of Cell Titer Glo reaction mix was then added to each well for a final volume of 200 µL. Plates were analyzed for luminescence with a Luminoscan (Berthold Technologies). We verified that ATP measurements were in the linear range of the detection. The difference between total ATP production and the ATP produced under iodoacetate treatment results in glycolytic ATP contribution. Glycolytic ATP is represented by percentage of total ATP produced by the cells.

Lactate measurement

Lactate concentration in the supernatant was determined by an enzyme-based assay using 900 µM β-NAD$^+$ (N1636, Sigma-Aldrich), 175 µg/ml L-lactic dehydrogenase (L2500, Sigma-Aldrich) and 100 µg/ml glutamate-pyruvate transaminase (#10737127001, Roche) diluted in a 620 mM sodium carbonate (S2127, Sigma-Aldrich)-79 mM L-glutamate (G1626, Sigma-Aldrich) buffer adjusted to pH 10. Lithium lactate (L2250, Sigma-Aldrich) was used as a standard. Measurements were recorded from a microplate reader (excitation 355 nm, emission 612 nm) after 30 min incubation at 37 °C. Lactate secretion was expressed as mM and normalized to viable cell numbers (using Trypan blue exclusion method).

Lactate concentration in the supernatant was also determined electro-enzymatically using the YSI 2950 Biochemistry Analyzer (Yellow Springs Instruments) and normalized to viable cell number.

1

Glutamine consumption

Cells were grown in DMEM high glucose, no glutamine (#11960044, Thermo Fisher Scientific) supplemented with 2 mM of L-Glutamine (#25030081, Thermo Fisher Scientific), 10% FBS, 50 µM of 2-mercaptoethanol (#31350010, Thermo Fisher Scientific), 0.37 mM of L-asparagine (A0884, Sigma-Aldrich) and 10 mM of HEPES pH 7.4 (#15630056, Thermo Fisher Scientific) for 24 hours. The concentrations of L-Glutamine (mg/L) from cultured-cell supernatants were determined electro-enzymatically using the YSI 2950 Biochemistry Analyzer (Yellow Springs Instruments) and normalized to viable cell number.

L-[^{14}C]-Glutamine uptake

Cells ($5x10^6$, in triplicate for each lymphoma) were washed with PBS and incubated 5 min at 37°C in 80 µl of pre-warmed DMEM low glucose (5.5 mM), amino acid-, pyruvic acid- HCO_3^- -free (US Biological, D9800-13) supplemented to a final concentration of 15 mM D-glucose (G6152, Sigma-Aldrich), 20 mM HEPES (#15630056, Thermo Fisher Scientific) and adjusted to pH 7.4, prior addition of 1.25 µCi/ml of L-[^{14}C]-Glutamine (NEC451050UC, PerkinElmer,) for 30 min at 37°C in a CO_2-free incubator. Subsequently, cells were washed twice with PBS and lysed with 120 µl of 0.1N NaOH and mixed with 3 ml of Ultima Gold (#6013321, PerkinElmer). Radioactivity was measured using a β-scintillation counter. Inhibition experiments were performed in the presence of 10 mM of the system A transporter inhibitor α-(Methylamino)isobutyric acid (MeAIB, M2383, Sigma-Aldrich).

2-NBDG uptake

200.000 cells were incubated in glucose-free DMEM medium (#11966025, Thermo Fisher Scientific) containing 40 µM 2-NBDG (#23002-v, Peptide Institute) for 2 hours and analyzed by flow cytometry (Miltenyi Biotec) in the presence of 0.5 µg/ml of DAPI (D9542, Sigma-Aldrich) to represent glucose uptake (Mean Fluorescence intensity) in viable cells.

1

Measurement of the mitochondrial mass

The mitochondrial mass was measured by flow cytometry (Miltenyi Biotec) in viable (DAPI negative) cells), after 30 min incubation of cells at 37°C in the presence of 150 nM of Mitotracker green probe (M7514, Invitrogen).

6

Oxygen consumption

Polarographic studies: Intact cell respiration and mitochondrial substrates oxidation on 0.004% digitonin (D5628, Sigma-Aldrich)-permeabilized cells were carried out as previously described (Rustin et al., 1994). Proteins were measured according to Bradford microassay (#500-006, Bio-Rad). Results were expressed in nanomols of oxygen per minute per milligram of proteins or per cell number.

XF Seahorse Analyzer:

Oxygen consumption rate (OCR) was measured in real time using the XF96 extracellular flux analyzer (Agilent). Eμ-*Myc* cells were seeded on Cell-Tak (#10317081, Thermo Fisher Scientific)- coated XF96 plates at 0.12×10^6 cells/well in 180 µl of XF base medium minimal DMEM media (#102353-100, Agilent) supplemented with 20 mM D-glucose (G6152, Sigma-Aldrich), 1 mM sodium pyruvate (#11360088, Thermo Fisher Scientific), 2 mM L-glutamine (#25030081, Thermo Fisher Scientific) and adjusted to pH 7.4. The plates were spun at 200 g (breaks off) and incubated at 37°C for 20 min to ensure cell attachment. Measurements were obtained under basal conditions and in response to mitochondrial inhibitors, 1 µM oligomycin (O4876, Sigma-Aldrich), 0.5 µM of Carbonyl cyanide 4-(trifluoromethoxy)phenylhudrazone (FCCP, C2920, Sigma-Aldrich), and 1µM rotenone (R8875, Sigma-Aldrich) combined with 2µM antimycin A (A8674, Sigma-Aldrich).

24

Mitochondrial respiratory complex activities

Enzymatic spectrophotometric measurements of the OxPhos respiratory chain complex I, complex II and citrate synthase were performed at 37°C on cells according to standard procedures (Rustin et al., 1994). Proteins were measured according to Bradford microassay (#500-006, Bio-Rad). Results are expressed as activity ratios compared to the activity of citrate synthase.

Cell viability assays

Cells (0.3×10^6) were seeded in 96 well-plates in the presence or absence (Ctl, DMSO or NaCl 0.9%) of phenformin (P7045, Sigma-Aldrich), *E-Coli* L-asparaginase (Kidrolase®, Jazz Pharmaceutical), Rapamycin (#1292, Bio-techne) or anti-CD20 antibody Rituximab (Mabthera®, #9197719, Roche*)* at indicated concentration and period of time. Cells were then labeled with 0.5 µg/ml of DAPI (D9542, Sigma-Aldrich) and analyzed immediately by flow cytometry (Miltenyi Biotec).

Activation of the B-cell receptor

SU-DHL-6 cells (1.10^6 cells/ml) were incubated in the presence of 10 µg/ml of anti-IgM antibody (#109-006-129, Jackson ImmunoResearch) for 10 min and 18hours.

Isolation of human tumor cells from DLBCL core-needle biopsies

'Fresh' image-guided core-needle biopsies (non-frozen tumor biopsies) were harvested in the radiology departments of St Louis hospital in Paris (APHP, France), Centre Hospitalier Princesse Grace (CHPG, Monaco) and the Centre Antoine Lacassagne (CAL, Nice, France), anonymized and immediately sent to our laboratory (Nice, France) in accordance with the GlyPhome protocol approved by institutional review boards of APHP, CHPG and CAL and Commission Nationale de l'Informatique et des Libertés (CNIL) reference 912657 V1. All patients gave written non-opposition consent. Immediately upon receipt, tumor cells were dissociated in RPMI medium (#61870044, Thermo Fisher Scientific) supplemented with 2% of FBS, 10 µg/ml of DNAse (D4627, Sigma-Aldrich) and 0.1 mg/ml of collagenase (C9891, Sigma-Aldrich) 5 min at 37°C. Red blood cells were

1   lyzed (BD Pharm Lyse, #555899, BD Bioscience) 1 min at room temperature. After PBS washing,

2   cells were counted (by flow cytometry, using DAPI staining) and analyzed for glycolytic ATP

3   production in RPMI medium (#61870044, Thermo Fisher Scientific) supplemented with 10% FBS.

4   Diagnosis of the biopsies was provided by histopathology analyses.

5

6   ***Plasmids and generation of GAPDH-overexpressing cells and gapdh-silenced cells.***

7   The coding sequence of human *gapdh* (M33197) was first cloned by PCR into a pcDNA3.1/V5-His

8   TOPO TA expression plasmid (K480001, Invitrogen) following classical methods. *gapdh* was then

9   subcloned into a pMIG vector (9044, Addgene) for retroviral infection.

10 The shRNA targeting mouse *gapdh* was obtained using the oligonucleotide sequences forward 5'-

11 GATCCCCGTATGATGACATCAAGAAGTTCAAGAGACTTCTTGATGTCATCATACTTTTTA-

12 3'   and   reverse   5'-

13 AGCTTAAAAAGTATGATGACATCAAGAAGTCTCTTGAACTTCTTGATGTCATCATACGGG-

14 3' and inserted into the pSUPER-retro.neo+ gfp vector (VEC-PRT-0006, Oligoengine) following the

15 manufacturer's instruction. Self-inactivating retroviruses were generated by transient transfection of

16 293T cells (ATCC, #CRL-1573) and tittered as described previously (Frecha et al., 2011). Briefly 8.6

17 µg of plasmid of interest was co-transfected with 3 µg of envelope plasmid phCMV-VSV-G, 8.6 µg of

18 MLV-Gag-Pol (gift from Dr. Verhoeyen) using the classical calcium phosphate method. 72 hours after

19 transduction, GFP positive cells were sorted (FACS Aria, BD Biosciences).

20

21 ***RNA extraction and Real-time Quantitative PCR***

22 Total RNA was extracted from cells (RNeasy mini kit, #74104, Qiagen) according to the

23 manufacturer's instructions. 2µg of RNA were added to 20 µl reverse transcription-PCR using the

24 Omniscript RT kit (#205113, Qiagen). The relative mRNA levels of *gapdh* expression (mouse or

25 human) were obtained by real-time quantitative PCR (qPCR), using the TaqMan Fast Universal PCR

26 Master Mix (#4366072, Fisher Scientific) and TaqMan assay primer sets (mouse *gapdh*:

Mm99999915_g1; human *gapdh*: Hs02758991_g1, Thermo Fisher Scientific) on the Step One

(Applied Biosystems) according to the manufacturer's instructions. For mouse primary malignant B

cells, samples were normalized to mouse *rn18s* (Mm03928990_g1, Thermo Fisher Scientific). For

human DLBCL cell lines, samples were normalized to human *rplp0* (Hs99999902_m1, Thermo Fisher

Scientific).

To measure *gapdh* mRNA stability, cells were treated with 5 µg/ml of Actinomycin D (A9415,

Sigma-Aldrich) for indicated period of times.

Western blot analysis

Cells were washed in PBS and lysed in laemmli buffer. Tissue samples were collected and lysed in

Precellys 24 (Bertin Instruments) homogenizer (3x30 s, 6500g) in laemmli buffer. After quantification

(Pierce BCA Protein Assay kit, #23225, Thermo Fisher Scientific), 40 µg of proteins were separated

on 8% to 13% SDS polyacrilamide gels and transferred onto polyvinylidene difluoride membranes

(Millipore). Membranes were blotted with antibodies against indicated proteins. Immunoreactive

bands were detected with an anti-mouse (#7076, Cell Signaling Technology) or an anti-rabbit (7074S,

Cell Signaling Technology) IgG horseradish peroxidase (HRP)-linked antibodies. Immunoblots were

visualized (FUJIFILM LAS4000) by chemoluminescence using Pierce ECL Western Blotting

substrates (#32106, Pierce ECL, Thermo Fisher Scientific). When indicated, Western blot

quantification was performed using ImageJ software.

Anti-V5 (R96025) was purchased from Invitrogen. Anti-Erk2 (sc-1647) was purchased from Santa

Cruz. Anti-GAPDH (ab9485), anti-LDH (ab52488), anti-LDH-B (ab85319), anti-GLS (ab93434) were

purchased from Abcam. Anti-HKII (#2106), Anti-ENO1 (#3810), anti-PKM2 (#3198), anti-p53

(#2524), anti-phospho-ACC (Ser 79) (#11818), anti-ACC (3676), anti-phospho-p70-S6K (T389)

(#9234), anti-p70S6K (#9202), anti-phospho-Syk (Tyr 525/526) (#2710), anti-Syk (#2712) were

purchased from Cell Signaling Technology.

Flow cytometry analysis

Intracellular staining for GAPDH was performed using the Cytofix/Cytoperm kit (#554714, BD Biosciences), anti-GAPDH (ab9485, Abcam) and Alexa-Fluor 647 goat anti-rabbit IgG secondary antibody (ab150087, Abcam).

IgG and IgM expressions on the surface of DLBCL cell lines were analyzed using FITC-anti-IgG (#130-093-192, Miltenyi Biotec) and APC-anti-IgM (#130-093-076, Miltenyi Biotec).

Staining were analyzed by flow cytometry (Miltenyi Biotec). Analysis of the FACS data was performed using Macsquantify Version 2.11 (Miltenyi).

Targeted LC-MS metabolites analyses

Metabolites were extracted as described (Mackay et al., 2015). Briefly, 1.10^6 Eµ-Myc cells (4 replicates per clones) were used for extraction. Extraction solution was composed of 50% methanol, 30% ACN, and 20% water. The volume of extraction solution added was adjusted to the number of cells (0.5 ml/1x10^6 cells). After addition of extraction solution, samples were vortexed for 5 min at 4°C and then centrifuged at 16,000 g for 15 min at 4°C. The supernatants were collected and stored at −80°C until analyses. Liquid chromatography/Mass Spectrometry (LC/MS) analyses were conducted on a QExactive Plus Orbitrap mass spectrometer equipped with an Ion Max source and a HESI II probe and coupled to a Dionex UltiMate 3000 UPLC system (Thermo, USA). External mass calibration was performed using the standard calibration mixture every 7 days as recommended by the manufacturer. 5 µl of each sample was injected onto Zic-pHilic (150 mm × 2.1 mm i.d. 5 µm) with the guard column (20 mm × 2.1 mm i.d. 5 µm) (Millipore) for the liquid chromatography separation. Buffer A was 20 mM ammonium carbonate, 0.1% ammonium hydroxide (pH 9.2); buffer B was acetonitrile. The chromatographic gradient was run at a flow rate of 0.200 µl/min as follows: 0–20 min; linear gradient from 80% to 20% B; 20–20.5 min; linear gradient from 20% to 80% B; 20.5–28 min: hold at 80% B (Mackay et al., 2015). The mass spectrometer was operated in full scan, polarity switching mode with the spray voltage set to 2.5 kV, the heated capillary held at 320°C. The

1 sheath gas flow was set to 20 units, the auxiliary gas flow was set to 5 units, and the sweep gas flow

2 was set to 0 unit. The metabolites were detected across a mass range of 75–1,000 m/z at a resolution of

3 35,000 (at 200 m/z) with the AGC target at 106, and the maximum injection time at 250 ms. Lock

4 masses were used to insure mass accuracy below 5 ppm. Data were acquired with Thermo Xcalibur

5 software (Thermo Fisher Scientific). The peak area of metabolites was determined using Thermo

6 TraceFinder software (Thermo Fisher Scientific), identified by the exact mass of each singly charged

7 ion and by known retention time on the HPLC column. Statistical analyses were performed using

8 Metaboanalyst 4.0 software.

9

10 *^{1}H HRMAS NMR spectroscopy*

11 Cell pellets (10 million cells) were frozen at -80°C. After thawing cell pellets, 10 microliters of D_2O

12 were added. Solvent and cells were then placed into a 30 µl disposable insert introduced into a 4mm

13 ZrO_2 HRMAS rotor before NMR analysis. All the NMR experiments were recorded on a Bruker

14 Avance III spectrometer operating at 400 MHZ and 100MHZ for ^{1}H and ^{13}C, respectively, and

15 equipped with a ^{1}H/^{13}C/^{31}P HRMAS probe. All the spectra were recorded at 4000 Hz spinning rate and

16 a temperature of 277 K. For each sample, ^{1}H HRMAS NMR spectrum was acquired using the Carr-

17 Purcell-Meiboom-Gill NMR sequence, preceded by a water presaturation pulse during relaxation time

18 of 2 s. This sequence enables us to reduce the macromolecule and lipid signal intensities using a T2

19 filter of 50 ms synchronised with the rotor rotation speed. For each spectrum, 256 free induction

20 decays of 20 800 data points, were collected using 8000 Hz of spectral window. The FIDs were

21 multiplied by an exponential weighting function corresponding to a line broadening of 0.3 Hz and

22 zero-filled prior to Fourier transformation. The calibration on the alanine doublet (δ=1.47 ppm) and

23 the phase and baseline corrections were performed manually for all the spectra. The identification of

24 the NMR signals was carried out using ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC NMR experiments, online

25 databases (HMDB, BRMB) and literature (Nicholson et al., 1995; Wishart et al., 2013). Each

26 spectrum was divided into 0.005 ppm-width integrated buckets and normalized to total spectrum

27 intensity after having removed the presaturated water signal region (4.85 -5.10 ppm) using AMIX

1 software (Bruker Biospin GmbH, Karlsruhe, Germany). The water region must be removed due to

2 inconsistent signal suppression.

3 A matrix of 42 samples and 1700 buckets was then created and exported to the software Simca-P v13

4 (Umetrics, Umea, Sweden) for statistical analysis. Principal component analysis was first performed in

5 order to detect intrinsic clusters and outliers within the dataset. Since expected discrimination was not

6 achieved using PCA, the data were analyzed with orthogonalized projections to latent structures

7 discriminant analysis (OPLS-DA). The OPLS algorithm derives from basic partial least-squares (PLS)

8 regression and allows a more effective use of the relevant discriminating variables by removing

9 information orthogonal to the Y matrix (matrix containing the sample classes), i.e., not relevant for

10 this particular discrimination. The resulting scores and loading plots were used to visualize

11 respectively the samples and the NMR frequency signals (variables) in the predictive and orthogonal

12 reduced component frame. Resampling the model 999 times under the null hypothesis performed

13 OPLS-DA model validation, i.e. by generating 999 models for each between 50 to 100 % of the Y

14 matrix were permutated. The quality of the model was assessed by monitoring changes in goodness-

15 of-fit and predictive statistics, R2Y and Q2, respectively, between the models calculated from the

16 permuted Y-matrix and the original one.

17 The normalized signals of each discriminant metabolite were then integrated for each spectrum in

18 order to calculate their respective fold change between the two groups. The significance of the fold

19 change was tested using a two-tailed t-test with a significance level of 0.05.

20

21 Plasma samples (200 µl) were frozen at -80 C. After thawing, 400 µl of 0.9% saline solution in

22 D2O was added. Samples were then placed into a 5mm NMR tube and NMR experiments were

23 recorded on a Bruker Avance III spectrometer operating at 600 MHz and 150MHz for ^{1}H and ^{13}C,

24 respectively, and equipped with a ^{1}H/^{13}C TXI probe. Spectra were recorded at 300 K. A Carr-Purcell-

25 Meiboom-Gill (CPMG) NMR spin echo sequence $[90° — (\tau — 180° — \tau)n]$ with an effective spin

26 echo time of 75.6 ms, preceded by a water presaturation pulse during the relaxation time was

27 employed to reduce the signal intensities of lipids and macromolecules. A relaxation time of 33 s was

1 necessary to achieve full relaxation for all the metabolites in order to enable absolute quantification.

2 For each sample, 128 free induction decays (FID) of 32768 complex data points were collected using a

3 spectral width of 12 000 Hz. A sample of 1.67 mM of maleic acid was used as an external reference to

4 calibrate the ERETIC signal (electronic signal) used for the quantification of glutamine.

5
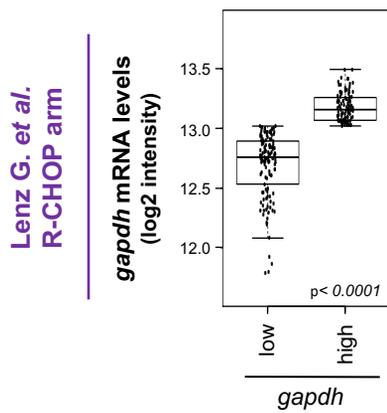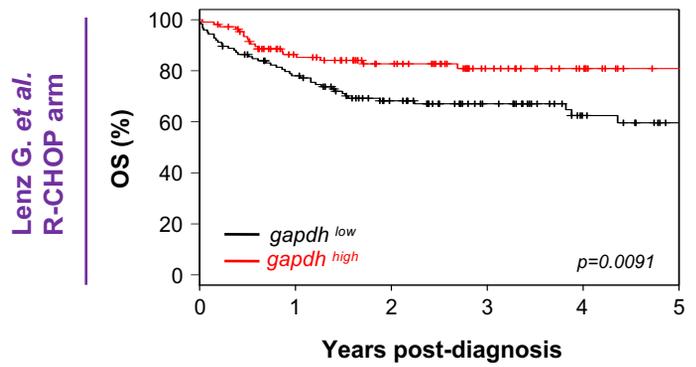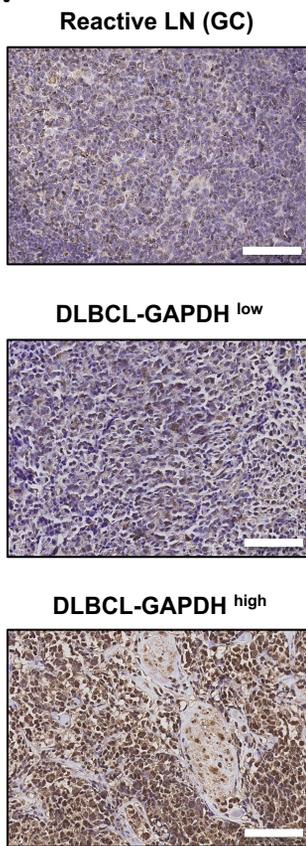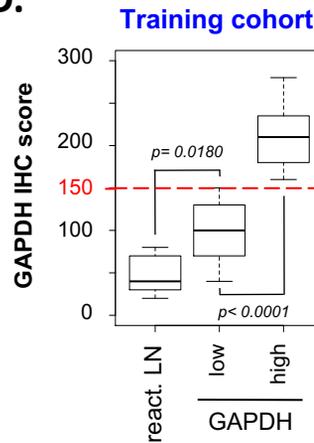
6 **Quantification and Statistical Analysis**

7 All survival analyses were performed on an intention-to-treat basis. Categorical variables were

8 compared using the chi-squared or Fisher exact tests. Progression-free survival (PFS) was defined as

9 the time from study entry until disease progression or death. Overall survival (OS) was defined as the

10 time from the start of treatment until death. Survival functions were estimated using the Kaplan-Meier

11 method and compared with the log-rank test. Comparative tests were considered significant if a 2-

12 sided $p < 0.05$. Because the LNH2003-6 trial was not stratified by biological data, we controlled for the

13 effects of prognostic factors on outcome due to sampling fluctuations in the treatment groups with a

14 multivariate analysis of survival in a Cox model. Statistical analyses of clinical data were performed

15 using R project software or SAS 9.2 (SAS Institute) and S-Plus 6.2 (MathSoft) software. Data

16 distributions are presented using boxplots when needed, to provide a graphical representation of the

17 variance. Statistical significance was assessed using the Wilcoxon rank sum test. For all other clinical

18 data, statistical analyses used for each of the figures is specified in the legends. For *in vitro*
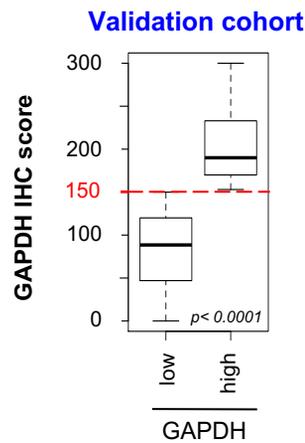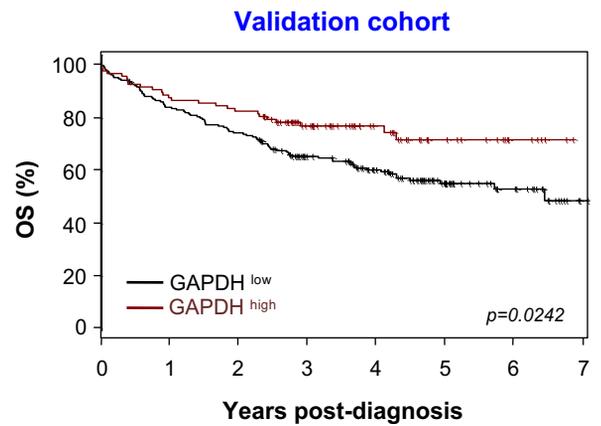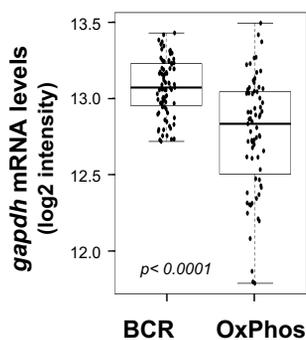
19 experiments, results are expressed as mean (-/+ s.d.) and statistical analyses were performed using

20 two-tailed Student's t-test. A p value of less than 0.05 was considered to indicate statistical

21 significance (*p <0.05, **p <0.01 and ***p <0.001). n for the different groups are provided in the

22 figure legends.

23

24 **Data analysis and Software availability**

25 *Code availability*

26 R code for microarray analysis of Lenz G. et al. data set is available at doi:10.17632/f4f3wbszmd.1

A. Lenz G. *et al.* R-CHOP arm

gapdh mRNA levels (log2 intensity)

p< *0.0001*

low   high

gapdh

B. Lenz G. *et al.* R-CHOP arm

OS (%)

gapdh low
gapdh high

p=*0.0091*

Years post-diagnosis

C.

Reactive LN (GC)

DLBCL-GAPDH low

DLBCL-GAPDH high

D. Training cohort

GAPDH IHC score

p= *0.0180*

150

p< *0.0001*

react. LN   low   high

GAPDH

E. Training cohort

OS (%)

GAPDH low
GAPDH high

p< *0.0001*

Years post-diagnosis

F. Validation cohort

GAPDH IHC score

150

p< *0.0001*

low   high

GAPDH

G. Validation cohort

OS (%)

GAPDH low
GAPDH high

p=*0.0242*

Years post-diagnosis

H.

gapdh mRNA levels (log2 intensity)

p< *0.0001*

BCR   OxPhos

I.

Glycolytic ATP (%)

R=0.792
p=*0.0036*

150

GAPDH score (IHC)

Figure 1 Chiche J *et al.*

Figure 2 Chiche J *et al.*

A.

GAPDH low   GAPDH high

G   H   J   F   D   C   B   E   : Eµ-*Myc* #

◄ P-p70S6K (T389)
◄ n.s.

p70S6K

Erk2

B.

GAPDH   P-p70S6K (T389)

DLBCL -GAPDH low

DLBCL -GAPDH high

DLBCL	#	P-p70S6K (T389)		
		Negative	Positive	*p*
GAPDH low	7	3	4	*0.04739*
GAPDH high	11	10	1	

C.

GAPDH low

Ctl   10   20   : nM Rapa

◄ P-p70S6K (T389)
◄ n.s.

p70S6K

Erk2

D. Baseline OCR (pmol/min)
***
Ctl / 10 / 20 — Rapa (nM)

E. Maximal respiration (pmol/min)
***
Ctl / 10 / 20 — Rapa (nM)

F. ATP-coupled OCR (pmol/min)
***
Ctl / 10 / 20 — Rapa (nM)

G. Glycolytic ATP (%)
***
Ctl / 10 / 20 — Rapa (nM)

H. % dead cells

Ctl / Rapa 10 nM / Rapa 20 nM

15h / 24h / 48h — GAPDH low   15h / 24h / 48h — GAPDH high

I. Survival (%)

Vehicle
Temsirolimus
***

Days post-cell injection

J.

Vehicle   Temsirolimus

#1 #2 #3 #4 #5 #6   #1 #2 #3 #4 #5 #6

◄ P-p70S6K (T389)
◄ n.s.

p70S6K

Erk2

Figure 3 Chiche J *et al.*

A. Gln consumption (mg/L/10⁶ cells) — GAPDH low vs high — **

B. ¹⁴C-Gln uptake (cpm × 10²) — GAPDH low vs high — ***

C. GAPDH low (F H G) / GAPDH high (D B C) : Eµ-Myc #

Glycolysis or non-oxidative PPP:
- Glucose-6-phosphate
- Glyceraldehyde-3-phosphate
- Lactate
- Sedoheptulose-7-phosphate

Glutaminolysis or TCA cycle:
- Glutamate
- α-Ketoglutarate
- Acetyl-CoA

D. GAPDH low (G H F) / GAPDH high (D C B) : Eµ-Myc #

GLS
Erk2
2.2 2.5 2.1 1.0 1.8 1.2 : GLS/Erk2

E. Gln (mM) and Asn (mM)

GAPDH low / GAPDH high

Ctl (T=0), Ctl, 0.3, 1 : L-ase (IU/ml), T=4h

F. % of dead cells

GAPDH low / GAPDH high

Ctl, 0.1, 0.3, 1 : L-ase (IU/ml)

G. Plasma Gln (µM)

GAPDH low / GAPDH high

Vehicle, L-ase, Vehicle, L-ase

p= 0.056, ***, ***

H. GAPDH low — Survival (%) vs Days post-cell injection

Vehicle, E-Coli L-ase, ***

I. GAPDH high — Survival (%) vs Days post-cell injection

Vehicle, E-Coli L-ase

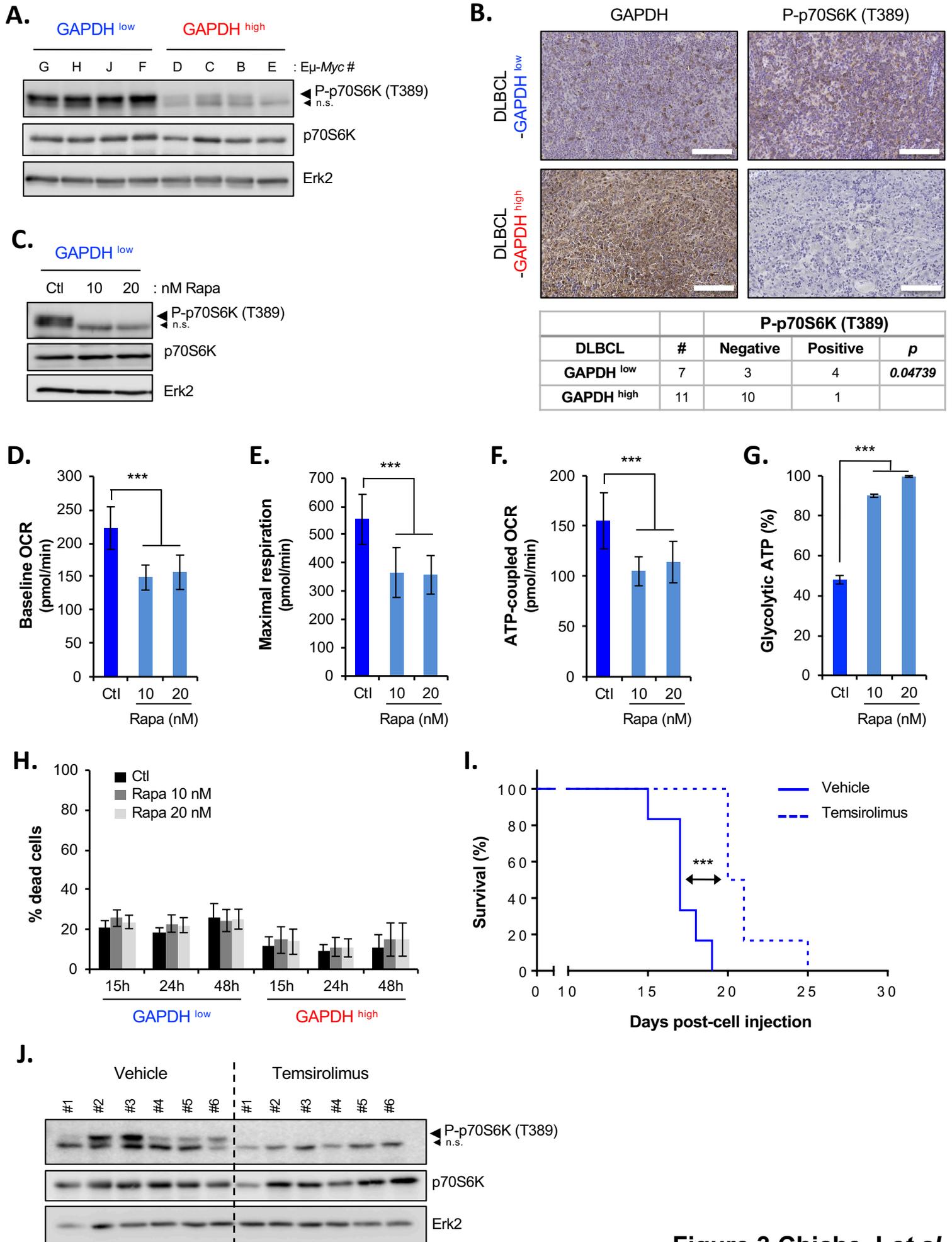**Figure 4 Chiche J _et al._**

Figure 5 Chiche J *et al.*

A.

DLBCL-GAPDH ^{low}

DLBCL → R-CHOP → ✓ 60%

R-CHOP → ✗ 40% → R-DHAP / R-ICE → ✓ 60%

R-DHAP / R-ICE → ✗ 40% → **KTM treatment**

B.

KTM cycle

| D1 | D3 | D5 | D7 | D9 | D11 | D13 | D14 | | D28 |

K K K K K K K → Metformin

T   T   T

C.

Cycle 1-D1   *Cycle 1-D7*   *Cycle 2-D1*

Patient #1- DLBCL-*Myc*$^+$/GAPDH ^{low}
Refractory to R-based therapies

D.

Day 0   Day 30

Patient #1

E.

Patient #1 — GAPDH score= 35

Patient #2 — GAPDH score= 40

Patient #3 — GAPDH score= 140

Patient #4 — GAPDH score= 30

F.

Legend:
- Duration of KTM treatment
- Duration post- KTM treatment
- Discontinued treatment due to AE
- CR
- PR
- PD
- * PAC

Patient #4, Patient #3, Patient #2, Patient #1

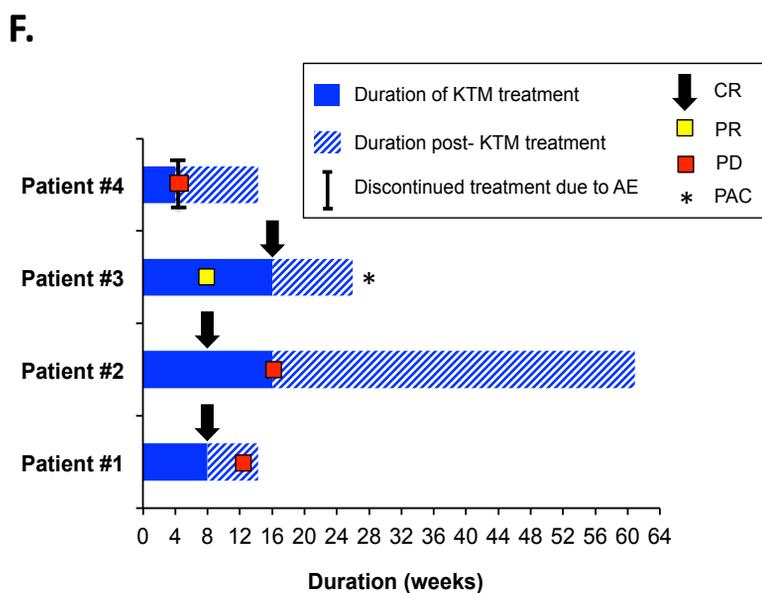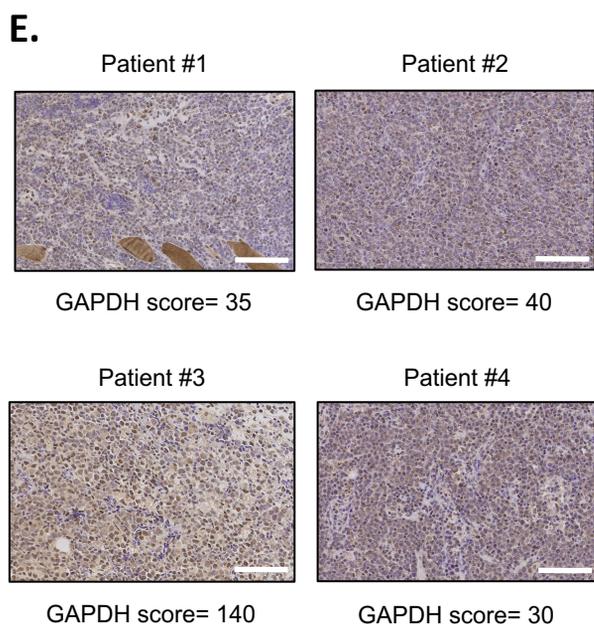Duration (weeks): 0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64

Figure 6 Chiche J *et al*.

Table 1. GAPDH expression levels are not associated with other biological prognostic factors.

DLBCL		TRAINING COHORT (n=43)					VALIDATION COHORT (n=294)				
		GAPDH low (n=15)		GAPDH high (n=28)			GAPDH low (n=204)		GAPDH high (n=90)		
		No.	%	No.	%	p	No.	%	No.	%	p
PARAMETERS											
Bcl2	< 70%	1	6.7	10	35.7	*0.0648*	66 (a)	37.7	35 (b)	40.2	*0.7876*
	≥ 70%	14	93.3	18	64.3		109 (a)	62.3	52 (b)	59.8	
Bcl6	≤ 25%	6	40	18	64.3	*0.1982*	63 (c)	36.2	33 (d)	39.3	*0.6808*
	> 25%	9	60	10	35.7		111 (c)	63.8	51 (d)	60.7	
Myc	< 40%	5	33.3	23 (e)	85.2	*0.0014*	98 (f)	62.4	48 (g)	72.7	*0.1657*
	≥ 40%	10	66.7	4 (e)	14.8		59 (f)	37.6	18 (g)	27.3	
DE (Myc-Bcl2)	Myc < 40% ; Bcl2 < 70%	6	40	26 (e)	85.2	*9.38 e-05*	115 (h)	73.3	51 (i)	78.5	*0.4990*
	Myc ≥ 40% ; Bcl2 ≥ 70%	9	60	1 (e)	14.8		41 (h)	26.3	14 (i)	21.5	
Double Hit *Myc/Bcl2*	No	4 (j)	100	11 (k)	91.67	*1.0000*	148 (l)	92.5	60 (m)	95.2	*0.5652*
	Yes	0 (j)	0	1 (k)	8.33		12 (l)	7.5	3 (m)	4.8	
Triple Hit *Myc/Bcl2/Bcl6*	No	4 (j)	100	12 (k)	100	*n.a*	158 (l)	98.7	62 (m)	98.4	*1.0000*
	Yes	0 (j)	0	0 (k)	0		2 (l)	1.3	1 (m)	1.6	

Abbreviations: DE, double expressors (Myc-Bcl2); GAPDH, glyceraldehyde-3-phosphate dehydrogenase; R-CHOP, Rituximab with cyclophosphamide, doxorubicin, oncovin and prednisone.
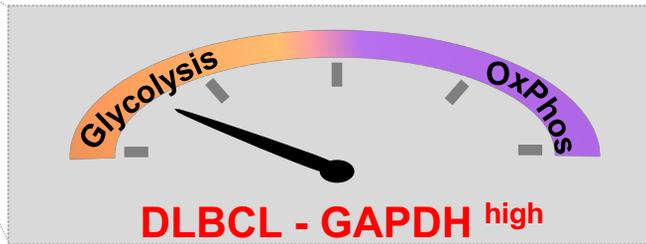
n.a, not applicable.

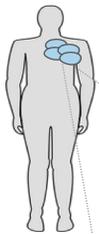Number of samples that could not be analyzed: (a) 29; (b) 3; (c) 30; (d) 6; (e) 1; (f) 47; (g) 24; (h) 48; (i) 25; (j) 11; (k) 16; (l) 44; (m) 27.