Dysregulation of autophagy in human follicular lymphoma is independent of overexpression of BCL-2

Supplementary Material

Suppl Table 1: Information on FL patients used for qRT-PCR microarray

| Vial ID | Gender | Age at diag | Date of Diagnosis | Date of transformation | Stage* | FLIPI score§ | Date of the 1st treatment | Response* | Date of death | Cause of death† |
|---------|--------|-------------|-------------------|------------------------|--------|--------------|---------------------------|-----------|---------------|-----------------|
| F9835   | M      | 56          | 20/06/1997        | 15/04/2008             | II     | 1            | 15/04/2008                | Progression | 03/12/2008    | NHL             |
| F9220   | M      | 57          | 01/04/2001        | 22/01/2008             | IV     | 3            | 26/04/2001                | CR(U) / GPR |               |                 |
| R8402   | F      | 67          | 22/08/2005        | 26/04/2011             | II     | 1            | 14/05/2010                | CR        |               |                 |
| T1979   | F      | 74          | 01/12/2005        | 18/05/2009             | IV     | U            | 08/05/2009                | Progression | 08/07/2009    | NHL             |
| T5728   | M      | 29          | 15/07/2011        | 13/02/2012             | IV     | 2            | 02/03/2012                | CR        |               |                 |
| R9130   | F      | 25          | 31/05/2007        |                        | II     | 1            | 04/07/2007                | CR(U) / GPR |               |                 |
| T6697   | M      | 48          | 24/03/2011        |                        | IV     | 2            | 21/03/2012                | PR        |               |                 |
| R0481   | F      | 54          | 12/04/2002        |                        | IV     | 2            | 11/01/2005                | CR(U) / GPR |               |                 |
| F5997   | F      | 63          | 01/01/1998        |                        | IV     | 3            | 27/08/1998                | Relapse    | 13/01/2005    | NHL             |
| F9713   | F      | 89          | 31/08/2001        |                        | IV     | 2            | 14/09/2001                | N/A       | 28/09/2002    | N/A             |
| T6713   | M      | 62          | 07/03/2012        |                        | III    | U            | N/A                       |           |               |                 |
| R2856   | M      | 76          | 06/10/2003        |                        | III    | 2            | N/A                       | 20/02/2006 | Other          |                 |
| T0167   | F      | 74          | 15/04/2006        |                        | IV     | U            | N/A                       |           |               |                 |

¶ Staging ranges from I to IV with a higher stage indicating more widespread disease. § The Follicular Lymphoma International Prognostic Index (FLIPI) score ranges from 1 to 4 where 1 = low risk (0-1 prognostic factor), 2 = intermediate risk (2 prognostic factors), 3 = high risk (3-5 prognostic factors) and 4 = not assessable; U = unknown. * CR= complete remission; CR(U)= undetermined complete remission; GPR= good partial remission; N/A= not applicable. † NHL= Non-Hodgkin lymphoma; N/A= not applicable.
### Suppl Table 2: Information on DLBCL patients used for qRT-PCR microarray

| Vial ID | Age at diagnosis | Gender | Date of diagnosis | Stage$^\dagger$ | IPI score$^\$ | Date of 1st treatment | Response* | Date of death | Cause of death$^\dagger$ |
|---------|------------------|--------|-------------------|----------------|----------------|----------------------|-----------|---------------|--------------------------|
| R1542   | 26               | F      | 23/12/2002        | VI             | 1              | 15/01/2003           | CR        |               |                           |
| R8374   | 20               | M      | 29/11/2006        | V              | 1              | 15/12/2006           | CR        |               |                           |
| T1486   | 33               | M      | 08/01/2009        | IV             | 1              | 27/01/2009           | CR        |               |                           |
| T1485   | 55               | M      | 13/01/2009        | II             | 0              | 20/01/2009           | CR        |               |                           |
| T2628   | 58               | M      | 23/07/2009        | I              | 0              | 20/08/2009           | CR        |               |                           |
| T3531   | 60               | M      | 21/12/2009        | III            | 1              | 03/02/2010           | CR        |               |                           |
| R1723   | 55               | F      | 28/01/2003        | IV             | 2              | 28/02/2003           | CR        | 02/01/2006    | NHL                      |
| R8878   | 79               | F      | 01/02/2007        | IV             | 4              | 17/04/2007           | Progression | 18/03/2008  | NHL                      |
| R9083   | 33               | M      | 09/05/2007        | III            | 3              | 15/05/2007           | CR(U) / GPR | 15/11/2007  | NHL                      |
| R9515   | 80               | M      | 16/08/2007        | IV             | 4              | 16/08/2007           | Stable disease | 19/10/2007 | Cardiac failure |
| T0082   | 18               | F      | 15/01/2008        | IV             | 1              | 01/02/2008           | Progression |               |                           |
| T0978   | 58               | M      | 12/08/2008        | IV             | 4              | 22/08/2008           | Progression |               |                           |

$^\dagger$ Staging ranges from I to VI with a higher stage indicating more widespread disease. $^\$ The International Prognostic Index (IPI) score ranges from 0 to 5, with 0 indicating absence of all prognostic factors and 5 indicating all prognostic factors are present. * CR= complete remission; CR(U)= undetermined complete remission; GPR= good partial remission. $^\dagger$ NHL= Non-Hodgkin lymphoma.
Suppl Table 3: Summary of clinical characteristics of FL patients’ samples used for the TMA

| No. of patients | Percentage |
|-----------------|------------|
| 128             | 100        |

| Age, years | No. of patients | Percentage |
|------------|-----------------|------------|
| <60        | 77              | 60.16      |
| ≥60        | 51              | 39.84      |

| Gender | No. of patients | Percentage |
|--------|-----------------|------------|
| Male   | 64              | 50.00      |
| Female | 64              | 50.00      |

| Stage at diagnosis | No. of patients | Percentage |
|-------------------|-----------------|------------|
| I                 | 14              | 10.94      |
| II                | 14              | 10.94      |
| III               | 20              | 15.53      |
| IV-VI             | 71              | 55.47      |
| Unknown           | 6               | 4.69       |

| FLIPI | No. of patients | Percentage |
|-------|-----------------|------------|
| 1 (Low) | 31              | 24.22      |
| 2 (Intermediate) | 28             | 21.88      |
| 3 (High)  | 34              | 26.56      |
| 4 (Not assessable) | 17             | 13.28      |
| Unknown   | 19              | 14.84      |

| Proceeded to transformation | No. of patients | Percentage |
|-----------------------------|-----------------|------------|
| 36                          | 28.13           |

| Time to transformation yr | No. of patients | Percentage |
|---------------------------|-----------------|------------|
| 0-5                       | 44              | 34.38\textsuperscript{5} |
| 6-10                      | 37              | 28.91\textsuperscript{5} |
| >11                       | 48              | 37.50\textsuperscript{5} |

| Cause of death | No. of patients | Percentage |
|----------------|-----------------|------------|
| Alive          | 51              | 39.84      |
| NHL-specific death | 64             | 50.00      |
| Unrelated/Other disease | 5             | 3.91       |
| Unknown        | 8               | 6.25       |

\textsuperscript{¶} Staging ranges from I to IV with a higher stage indicating more widespread disease. \textsuperscript{§} The Follicular Lymphoma International Prognostic Index (FLIPI) score ranges from 1 to 4 where 1 = low risk (0-1 prognostic factor), 2 = intermediate risk (2 prognostic factors), 3 = high risk (3-5 prognostic factors) and 4 = not assessable; U = unknown. \textsuperscript{Ʌ} Percentages of transformed.
Suppl Table 4: Summary of clinical characteristics of DLBCL patients’ samples used for the TMA

|                           | No. of patients | Percentage |
|---------------------------|-----------------|------------|
| **Age, years**            |                 |            |
| <60                       | 84              | 58.33      |
| ≥60                       | 60              | 41.67      |
| **Gender**                |                 |            |
| Male                      | 89              | 61.81      |
| Female                    | 55              | 38.19      |
| **Stage at diagnosis**    |                 |            |
| I                         | 13              | 9.02       |
| II                        | 22              | 15.28      |
| III                       | 28              | 19.44      |
| IV-VI                     | 79              | 54.86      |
| Unknown                   | 2               | 1.38       |
| **B Symptoms**            |                 |            |
| Yes                       | 44              | 30.56      |
| No                        | 69              | 47.92      |
| Unknown                   | 31              | 21.53      |
| **Serum LDH**             |                 |            |
| Normal                    | 54              | 37.50      |
| Elevated                  | 57              | 39.58      |
| Unknown                   | 33              | 22.92      |
| **IPI score**             |                 |            |
| 0 (Low)                   | 23              | 15.97      |
| 1 (Low)                   | 37              | 25.69      |
| 2 (Intermediate)          | 28              | 19.44      |
| 3 (High)                  | 27              | 18.75      |
| 4-5 (High)                | 9               | 6.25       |
| Unknown                   | 20              | 13.89      |
| **Cause of death**        |                 |            |
| Alive                     | 67              | 46.53      |
| NHL-specific death        | 49              | 34.03      |
| Unrelated/Other diseases  | 28              | 19.44      |

¶ Staging ranges from I to VI with a higher stage indicating more widespread disease; in some cases the stage was not clear at diagnosis and so none was assigned. ¥ B symptoms are weight low, fever and night sweats; yes indicates they were observed in patients; no indicates their absence. ⤵ The lactate dehydrogenase value was deemed elevated if it was greater than 480 U/L. § The International Prognostic Index (IPI) score ranges from 0 to 5, with 0 indicating absence of all prognostic factors and 5 indicating all prognostic factors are present; low, intermediate and high refer to risk groups based on IPI scores.
Suppl Table 5: List of primary antibodies and their dilutions

| Name of antibody | Type | Company      | Cat No   | Application | Dilution |
|------------------|------|--------------|----------|-------------|----------|
| BCL-2            | Mouse| Santa Cruz   | sc-509   | WB          | 1:1000   |
| BCL-2            | Mouse| DAKO         | M0887    | IH          | 1:200    |
| BCL-xL           | Rabbit| Santa Cruz | sc-634   | WB          | 1:1000   |
| Beclin-1         | Mouse| Santa Cruz   | sc-48341 | WB          | 1:1000   |
|                  |       |              |          | IH          | 1:250    |
| GAPDH            | Mouse| Cell Signaling| 2118L    | WB          | 1:2000   |
| LC3B             | Rabbit| Sigma       | L7543    | WB          | 1:1000   |
|                  |       |              |          | IH          | 1:5000   |
| SQSTM1/p62       | Mouse| Santa Cruz   | sc-28359 | WB          | 1:1000   |
|                  |       |              |          | IH          |          |
| Cathepsin D      | Mouse| Sigma        | C0715    | IH          | 1:1000   |
| CD68             | Mouse| DAKO         | M0814    | IH          | 1:8000   |
| TGM2             | Rabbit| Abcam      | Ab421    | IH          | 1:750    |
| APC-H7-CD20      | Mouse| BD           | 641396   | FC          | 2.5 µl/10^6 cells |
| FITC-CD3         | Mouse| Biolegend    | 344804   | FC          | 1 µl/10^6 cells |
| APC-H7-CD19      | Mouse| Biolegend    | 302218   | FC          | 2.5 µl/10^6 cells |
| PE-CD10          | Mouse| Biolegend    | 312204   | FC          | 2.5 µl/10^6 cells |
| APC-κ light chain| Mouse| Biolegend    | 31510    | FC          | 20 µl/10^6 cells |
| PerCpCy5.5-λ light chain | Mouse| Biolegend    | 316618   | FC          | 5 µl/10^6 cells |

FC= flow cytometry; IH= immuno-histochemistry; WB= Western blotting
### Suppl Table 6: Genes of autophagy machinery components

| Gene code | Full name | Function |
|-----------|-----------|----------|
| AMBRA1    | Autophagy/Beclin-1 regulator 1 | Autophagic vacuole formation |
| ATG12     | Autophagy related gene 12 | Autophagic vacuole formation/co-regulator for autophagy and apoptosis |
| ATG16L1   | Autophagy related gene 16-1 | Autophagic vacuole formation/protein transport |
| ATG4 A, B, C, D | Autophagy related gene 4 A, B, C, D | Autophagic vacuole formation/protein targeting to vacuole/protein transport/with protease activity |
| ATG5      | Autophagy related gene 5 | Autophagic vacuole formation |
| ATG9A     | Autophagy related gene 9A | Autophagic vacuole formation/protein transport |
| ATG9B     | Autophagy related gene 9B | Autophagic vacuole formation |
| BECN1     | Beclin-1 | Autophagic vacuole formation/co-regulator of autophagy and apoptosis |
| GABARAP   | GABA(A) receptor-associated protein | Autophagic vacuole formation/protein targeting to vacuole/protein transport/linking to lysosome |
| GABARAPL1 | GABA(A) receptor-associated protein-like 1 | Autophagic vacuole formation |
| GABARAPL2 | GABA(A) receptor-associated protein-like 2 | Autophagic vacuole formation/protein transport |
| IRGM      | Immunity-related GTPase family, M | Autophagic vacuole formation |
| MAP1LC3A  | Microtubule-associated protein 1 light chain 3-α (LC3A) | Autophagic vacuole formation |
| MAP1LC3B  | Microtubule-associated protein 1 light chain 3-β (LC3B) | Autophagic vacuole formation |
| RGS19     | Regulator of G-protein signaling 19 | Autophagic vacuole formation |
| ULK1      | Serine/threonine-protein kinase ULK1 (ATG1) | Autophagic vacuole formation |
| WIPI1     | WD repeat domain, phosphoinositide interacting 1 | Autophagic vacuole formation |
| ATG10     | Autophagy related protein 10 | Protein transport |
| ATG16L2   | Autophagy related protein 16-2 | Protein transport |
| ATG3      | Autophagy related protein 3 | Protein transport/ubiquitination |
| ATG7      | Autophagy related protein 7 | Protein transport/ubiquitination |
| RAB24     | RAB24 | Protein transport |
| DRAM1     | DNA-damage regulated autophagy modulator 1 | Linking to lysosome/co-regulator of autophagy and apoptosis |
| LAMP1     | Lysosomal-associated membrane protein 1 | Linking to lysosome and autophagy induction |
| NPC1      | Niemann-Pick disease, type C1 | Linking to lysosome |
| CTS B, D, S | Cathepsin B, D, S | Linking to lysosome |
| HDAC6     | Histone deacetylase 6 | Protein ubiquitination |
### Suppl Table 7: Genes for autophagy regulation

| Gene code | Full name                                                                 | Function                                                                                      |
|-----------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| AKT1      | v-akt murine thymoma viral oncogene homolog 1                              | Co-regulator of autophagy and apoptosis                                                       |
| APP       | Amyloid beta (A4) precursor protein                                        | Co-regulator of autophagy and apoptosis                                                       |
| ATG5      | Autophagy related protein 5                                                 | Co-regulator of autophagy and apoptosis                                                       |
| BAD       | BCL2-associated agonist of cell death (Bad)                                | Co-regulator of autophagy and apoptosis                                                       |
| BAK1      | BCL2 antagonist/killer 1                                                   | Co-regulator of autophagy and apoptosis                                                       |
| BAX       | Bcl-2 associated X protein                                                 | Co-regulator of autophagy and apoptosis                                                       |
| BCL2      | B cell CLL/lymphoma 2                                                      | Co-regulator of autophagy and apoptosis                                                       |
| BCL2L1    | BCL2 like 1                                                                | Co-regulator of autophagy and apoptosis                                                       |
| BID       | BH3 interacting domain death agonist                                       | Co-regulator of autophagy and apoptosis                                                       |
| BNIP3     | BCL2/adenovirus E1B 19kDa interacting protein 3                            | Co-regulator of autophagy and apoptosis                                                       |
| CASP3, 8  | Caspase-3, 8                                                               | Co-regulator of autophagy and apoptosis                                                       |
| CDKN1B    | Cyclin-dependent kinase inhibitor 1B (p27)                                 | Co-regulator of autophagy and apoptosis and cell cycle                                         |
| CDKN2A    | Cyclin-dependent kinase inhibitor 2A (p16)                                 | Co-regulator of autophagy and apoptosis and cell cycle                                         |
| CLN3      | Battenin                                                                  | Co-regulator of autophagy and apoptosis                                                       |
| CXCR4     | Chemokine (C-X-C motif) receptor 4                                         | Co-regulator of autophagy and apoptosis                                                       |
| DAPK1     | Death-associated protein kinase 1                                          | Co-regulator of autophagy and apoptosis                                                       |
| EIF2AK3   | Eukaryotic translation initiation factor 2 alpha kinase 3                  | Co-regulator of autophagy and apoptosis and autophagy induction                               |
| FADD      | Fas (TNFRSF6)-associated via death domain                                  | Co-regulator of autophagy and apoptosis                                                       |
| FAS       | Fas cell surface death receptor                                             | Co-regulator of autophagy and apoptosis                                                       |
| HDAC1     | Histone deacetylase 1                                                     | Co-regulator of autophagy and apoptosis                                                       |
| HTT       | Huntingtin                                                                | Co-regulator of autophagy and apoptosis                                                       |
| IFNG      | Interferon γ                                                              | Co-regulator of autophagy and apoptosis and cell cycle and autophagy induction                |
| INS       | Insulin                                                                   | Co-regulator of autophagy and apoptosis                                                       |
| MAPK8     | Mitogen-activated protein kinase 8                                         | Co-regulator of autophagy and apoptosis                                                       |
| MTO1      | Mechanistic target of rapamycin                                           | Co-regulator of autophagy and apoptosis                                                       |
| NFKB1     | Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1       | Co-regulator of autophagy and apoptosis                                                       |
| PIK3CG    | Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit γ        | Co-regulator of autophagy and apoptosis                                                       |
| PRKAA1    | Protein Kinase, AMP-Activated, Alpha 1                                     | Co-regulator of autophagy and apoptosis                                                       |
| PTEN      | Phosphatase and tensin homolog                                             | Co-regulator of autophagy and apoptosis and cell cycle                                         |
| SNCA      | Synuclein, alpha                                                          | Co-regulator of autophagy and apoptosis                                                       |
| SQSTM1    | Sequestosome 1 (p62)                                                      | Co-regulator of autophagy and apoptosis                                                       |
| TGFBI     | Transforming growth factor beta 1                                           | Co-regulator of autophagy and apoptosis and cell cycle                                         |
| TGM2      | Transglutaminase 2                                                         | Co-regulator of autophagy and apoptosis                                                       |
| TNF       | Tumour necrosis factor                                                     | Co-regulator of autophagy and apoptosis                                                       |
| TNFSF10   | Tumour necrosis factor (ligand) superfamily, member 10                    | Co-regulator of autophagy and apoptosis                                                       |
| TP53      | Tumour p53                                                                 | Co-regulator of autophagy and apoptosis and cell cycle                                         |
| RB1       | Retinoblastoma 1                                                          | Co-regulator of autophagy and cell cycle                                                      |
| EIF4G1    | Eukaryotic translation initiation factor 4 gamma, 1                        | Autophagy in response to other intracellular signals                                           |
| ESR1      | Estrogen receptor 1                                                        | Autophagy in response to other intracellular signals                                           |
| GAA       | Glucosidase, α; acid                                                      | Autophagy in response to other intracellular signals                                           |
| Gene          | Description                                                                 | Function                          |
|--------------|------------------------------------------------------------------------------|-----------------------------------|
| HGS          | Hepatocyte growth factor-regulated tyrosine kinase substrate                 | Autophagy in response to other intracellular signals |
| MAPK14       | Mitogen-activated protein kinase 14                                           | Autophagy in response to other intracellular signals |
| PIK3C3       | Phosphatidylinositol 3-kinase, catalytic subunit type 3                       | Autophagy in response to other intracellular signals |
| PIK3R4       | Phosphatidylinositol 3-kinase, regulatory subunit 4                          | Autophagy in response to other intracellular signals |
| RPS6KB1      | Ribosomal protein S6 kinase, 70kDa, polypeptide 1                            | Autophagy in response to other intracellular signals |
| TMEM74       | Transmembrane protein 74                                                     | Autophagy in response to other intracellular signals |
| ULRK2        | Unc-51 like autophagy activating kinase 2                                     | Autophagy in response to other intracellular signals |
| UVRAG        | UV radiation resistance associated gene                                       | Autophagy in response to other intracellular signals |
| HSP90AA1     | Heat shock protein 90 alpha class A 1                                         | Chaperone-mediated autophagy       |
| HSPA8        | Heat shock protein 8                                                          | Chaperone-mediated autophagy       |
Suppl Table 8: Validation of differentially expressed autophagy-related genes.

| Gene code | FL   | DLBCL |
|-----------|------|-------|
|           | F.C. (Array) | P value | F.C. (V) | P value | F.C. (Array) | P value |
| BECN1     | 2.07 | <0.005 | 1.46     | 0.06    | 1.23       | 0.46    |
| MAP1LC3A  | 2.60 | <0.005 | 2.08     | <0.05   | 1.59       | 0.32    |
| ATG4B     | 4.66 | <0.005 | 2.72     | <0.05   | 1.78       | 0.13    |
| DRAM1     | 2.38 | <0.05  | 1.05     | 0.18    | 4.24       | <0.05   |
| CTSD      | 2.41 | <0.005 | 2.27     | <0.05   | 4.14       | <0.05   |

Samples used for validation were those previously analyzed in the unpurified PCR array; RA n=5, FL n=4, and DLBCL n=5. Primers used for validation were the same as for PCR array. RPLPO was used as the housekeeping gene. (Array) indicates the initial PCR array and (V) means validation. Validation samples were analyzed in triplicate and the average CT value of these triplicates used to calculate the RQ value provided standard deviation (SD) was <0.5. In cases where SD was >0.5, data was visualized and if a clear outlier was identified this value was excluded and the average of the remaining duplicates used. Data were normalized to averaged RPLPO, RQ values calculated using the average CT and the 2-ΔΔCT method as before and FCs calculated as previously described.