**Additional file 2 for**

### Immunoglobulin somatic hypermutation has clinical impact in DLBCL and potential implications for immune checkpoint blockade and neoantigen-based immunotherapies

**Table S1.** Clinical features of 378 patients in the training and validation cohort whose DLBCL biopsies were sequenced and 290 patients whose sequencing results showed sufficient sequence reads.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Overall study cohort** | **Training set** | **Validation set** | **Cases with results** |
| **Characteristic** | **(n = 378)** | **(n = 192)** | **(n = 186)** | **(n = 290)** |
| **Age** |  |  |  |  |
| < 60 years | 158 | 75 | 82 | 118 |
| ≥ 60 years | 215 | 117 | 98 | 167 |
| Sex |  |  |  |  |
| Male | 217 | 111 | 106 | 163 |
| Female | 155 | 81 | 74 | 122 |
| **Stage** |  |  |  |  |
| I - II | 166 | 84 | 82 | 124 |
| III - IV | 192 | 100 | 92 | 151 |
| **B symptoms** |  |  |  |  |
| No | 230 | 129 | 101 | 177 |
| Yes | 126 | 52 | 74 | 93 |
| **Serum LDH levels** |  |  |  |  |
| Normal | 137 | 85 | 52 | 109 |
| Elevated | 193 | 83 | 110 | 146 |
| **No. of extranodal sites** | |  |  |  |
| 0 - 1 | 274 | 140 | 134 | 211 |
| ≥ 2 | 79 | 41 | 38 | 59 |
| **ECOG performance status** | |  |  |  |
| 0 - 1 | 264 | 143 | 121 | 201 |
| ≥ 2 | 60 | 22 | 38 | 43 |
| **Largest tumor size** |  |  |  |  |
| < 5cm | 176 | 88 | 88 | 129 |
| ≥ 5cm | 123 | 67 | 56 | 100 |
| **IPI score** |  |  |  |  |
| 0 - 2 | 219 | 115 | 104 | 167 |
| 3 - 5 | 137 | 69 | 68 | 105 |
| **Therapy response** |  |  |  |  |
| CR | 276 | 145 | 131 | 211 |
| PR | 53 | 28 | 25 | 40 |
| SD | 15 | 4 | 11 | 11 |
| PD | 28 | 15 | 13 | 22 |
| **GCB/ABC subtype** |  |  |  |  |
| GCB | 202 | 97 | 105 | 146 |
| ABC | 171 | 95 | 76 | 141 |

**Abbreviations:** IGH, immunoglobulin heavy chain gene; IGK/L,immunoglobulin kappa or lambdalight chain gene; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

**Table S2.** Comparisons of clinicopathologic and molecular characteristics between patients with germinal-center B-cell*–*like (GCB) diffuse large B-cell lymphoma (DLBCL) with a low or high degree of SHM in immunoglobulin variable region genes

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **GCB-DLBCL** |  |  |  |
| **Characteristic** | **IGHV SHMlow** | **IGHV SHMhigh** |  | **IGK/LV SHMlow** | **IGK/LV SHMhigh** |  |
|  | n (%) | n (%) | *P* | n (%) | n (%) | *P* |
| **Age** |  |  |  |  |  |  |
| < 60 years | 12 (44%) | 17 (46%) | 1.0 | 36 (48%) | 9 (45%) | 1.0 |
| ≥ 60 years | 15 (56%) | 20 (54%) |  | 39 (52%) | 11 (55%) |  |
| **Sex** |  |  |  |  |  |  |
| Male | 16 (59%) | 19 (51%) | .62 | 43 (57%) | 12 (60%) | 1.0 |
| Female | 11 (41%) | 18 (49%) |  | 32 (43%) | 8 (40%) |  |
| **Stage** |  |  |  |  |  |  |
| I - II | 12 (48%) | 20 (56%) | .61 | 38 (53%) | 8 (40%) | .45 |
| III - IV | 13 (52%) | 16 (44%) |  | 34 (47%) | 12 (60%) |  |
| **B symptoms** |  |  |  |  |  |  |
| No | 15 (60%) | 24 (65%) | .79 | 52 (74%) | 15 (75%) | 1.0 |
| Yes | 10 (40%) | 13 (35%) |  | 18 (26%) | 5 (25%) |  |
| **Serum LDH level** | |  |  |  |  |  |
| Normal | 10 (43%) | 17 (47%) | .80 | 36 (55%) | 10 (56%) | 1.0 |
| Elevated | 13 (57%) | 19 (53%) |  | 30 (45%) | 8 (44%) |  |
| **No. of extranodal sites** | |  |  |  |  |  |
| 0 or 1 | 20 (83%) | 28 (80%) | 1.0 | 59 (83%) | 13 (68%) | .20 |
| ≥ 2 | 4 (17%) | 7 (20%) |  | 12 (17%) | 6 (32%) |  |
| **ECOG performance status** | | |  |  |  |  |
| 0 or 1 | 18 (90%) | 24 (77%) | .45 | 53 (83%) | 12 (71%) | .31 |
| ≥ 2 | 2 (10%) | 7 (23%) |  | 11 (17%) | 5 (29%) |  |
| **Largest tumor size** | |  |  |  |  |  |
| < 5 cm | 13 (65%) | 16 (55%) | .56 | 35 (57%) | 5 (33%) | .15 |
| ≥ 5 cm | 7 (35%) | 13 (45%) |  | 26 (43%) | 10 (67%) |  |
| **IPI score** | |  |  |  |  |  |
| 0 - 2 | 16 (64%) | 26 (72%) | .58 | 49 (68%) | 11 (55%) | .30 |
| 3 - 5 | 9 (36%) | 10 (28%) |  | 23 (32%) | 9 (45%) |  |
| **Therapy response** | |  |  |  |  |  |
| CR | 19 (70%) | 28 (76%) | .78\* | 57 (76%) | 11 (55) | .093\* |
| PR | 4 | 4 |  | 9 | 31 |  |
| SD | 1 | 2 |  | 3 | 1 |  |
| PD | 3 | 3 |  | 6 | 6 |  |
| ***BCL2* translocation** | |  |  |  |  |  |
| No | 18 (75%) | 15 (45%) | **.032** | 47 (66%) | 8 (42%) | .068 |
| Yes | 6 (25%) | 18 (55) |  | 24 (34%) | 11 (58%) |  |
| **MDM2 expression** | |  |  |  |  |  |
| < 40% | 19 (70%) | 34 (97%) | **.0075** | 53 (74%) | 13 (65%) | .57 |
| ≥ 40% | 8 (30%) | 1 (3%) |  | 19 (26%) | 7 (35%) |  |
| **p53 expression** | |  |  |  |  |  |
| < 20% | 12 (50%) | 25 (78%) | **.045** | 36 (53%) | 9 (50%) | 1.0 |
| ≥ 20% | 12 (50%) | 7 (22%) |  | 32 (47%) | 9 (50%) |  |
| **No. of IGK/LV sequences with ongoing SHM** | | | |  |  |  |
| 0 to 16 | 2 (29%) | 8 (100%) | **.007** | 24 (75%) | 21 (100%) | **.016** |
| ≥ 17 | 5 (71%) | 0 (0%) |  | 8 (25%) | 0 (0%) |  |

**Abbreviations:** IGHV, immunoglobulin heavy chain variable region gene; IGK/LV,immunoglobulin kappa or lambdalight chain variable region gene; SHMlow, low degree of SHM; SHMhigh, high degree of SHM; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

**Note:** Not all patients had data available. Significant *P* values (Fisher’s exact test) are in bold. \*For therapy response, *P* values were for comparisons between CR and non-CR cases.

**Table S3.** Comparisons of clinicopathologic and molecular characteristics between patients with activated B-cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL) with a low or high degree of somatic hypermutation (SHM) in immunoglobulin variable region genes

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  |  | **ABC-DLBCL** |  |  |  |
| **Characteristic** | **IGHV SHMlow** | **IGHV SHMhigh** |  | **IGK/LV SHMlow** | **IGK/LV SHMhigh** |  |
|  | n (%) | n (%) | *P* | n (%) | n (%) | *P* |
| **Age** |  |  |  |  |  |  |
| < 60 years | 18 (42%) | 8 (24%) | .14 | 29 (33%) | 4 (25%) | .58 |
| ≥ 60 years | 25 (58%) | 25 (76%) |  | 58 (67%) | 12 (75%) |  |
| **Sex** |  |  |  |  |  |  |
| Male | 19 (44%) | 22 (67%) | .065 | 51 (59%) | 13 (81%) | .10 |
| Female | 24 (56%) | 11 (33%) |  | 36 (41%) | 3 (19%) |  |
| **Stage** |  |  |  |  |  |  |
| I - II | 16 (38%) | 14 (47%) | .48 | 34 (40%) | 5 (33%) | .78 |
| III - IV | 26 (62%) | 16 (53%) |  | 51 (60%) | 10 (67%) |  |
| **B symptoms** |  |  |  |  |  |  |
| No | 22 (52%) | 19 (61%) | .48 | 50 (59%) | 12 (80%) | .15 |
| Yes | 20 (48%) | 12 (39%) |  | 35 (41%) | 3 (20%) |  |
| **Serum LDH level** | |  |  |  |  |  |
| Normal | 11 (28%) | 12 (43%) | .30 | 31 (40%) | 7 (50%) | .56 |
| Elevated | 28 (72%) | 16 (57%) |  | 47 (60%) | 7 (50%) |  |
| **No. of extranodal sites** | |  |  |  |  |  |
| 0 or 1 | 30 (71%) | 25 (83%) | .27 | 66 (79%) | 11 (69%) | .52 |
| ≥ 2 | 12 (29%) | 5 (17%) |  | 18 (21%) | 5 (31%) |  |
| **ECOG performance status** | | |  |  |  |  |
| 0 or 1 | 28 (74%) | 20 (74%) | 1.0 | 59 (76%) | 12 (92%) | .28 |
| ≥ 2 | 10 (26%) | 7 (26%) |  | 19 (24%) | 1 (8%) |  |
| **Largest tumor size** | |  |  |  |  |  |
| < 5 cm | 23 (66%) | 11 (50%) | .28 | 38 (57%) | 6 (46%) | .55 |
| ≥ 5 cm | 12 (34%) | 11 (50%) |  | 29 (43%) | 7 (54%) |  |
| **IPI score** | |  |  |  |  |  |
| 0 - 2 | 23 (56%) | 16 (53%) | 1.0 | 47 (56%) | 8 (53%) | 1.0 |
| 3 - 5 | 18 (44%) | 14 (47%) |  | 37 (44%) | 7 (47%) |  |
| **Therapy response** | |  |  |  |  |  |
| CR | 30 (70%) | 26 (79%) | .44\* | 68 (78%) | 15 (94%) | .19\* |
| PR | 6 | 4 |  | 12 | 1 |  |
| SD | 1 | 1 |  | 1 | 0 |  |
| PD | 6 | 2 |  | 6 | 0 |  |
| ***MYC* translocation** | |  |  |  |  |  |
| No | 33 (87%) | 30 (100%) | .062 | 75 (90%) | 14 (93%) | 1.0 |
| Yes | 5 (13%) | 0 (0%) |  | 8 (10%) | 1 (7%) |  |
| **MYCmutation** | |  |  |  |  |  |
| No | 36 (90%) | 36 (95%) | .68 | 77 (92%) | 11 (73%) | **.04** |
| Yes | 4 (10%) | 2 (5%) |  | 7 (8%) | 4 (27%) |  |
| **PI3K expression** | |  |  |  |  |  |
| < 70% | 27 (69%) | 25 (78%) | .43 | 61 (73%) | 5 (31%) | **.0025** |
| ≥ 70% | 12 (31%) | 7 (22%) |  | 22 (27%) | 11 (69%) |  |
| **p63 expression** | |  |  |  |  |  |
| < 10% | 28 (65%) | 17 (52%) | .25 | 50 (58%) | 4 (25%) | **.027** |
| ≥ 10% | 15 (35%) | 16 (48%) |  | 36 (42%) | 12 (75%) |  |
| **PD-L1+CD20+ cells to PD-1+CD8+ T cells** | | |  |  |  |  |
| Far | 4 (13%) | 7 (32%) | .17 | 7 (11%) | 5 (42%) | **.018** |
| Close | 27 (87%) | 15 (68%) |  | 57 (89%) | 7 (58%) |  |

**Abbreviations:** IGHV, immunoglobulin heavy chain variable region gene; IGK/LV, **i**mmunoglobulin kappa or lambdalight chain variable region gene; SHMlow, low degree of SHM; SHMhigh, high degree of SHM; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

**Note:** Not all patients had data available. Significant *P* values (Fisher’s exact test) are in bold. \*For therapy response, *P* values were for comparisons between CR and non-CR cases.

**Table S4.** Significant prognostic effects of immunoglobulin molecular characteristics in diffuse large B-cell lymphoma (DLBCL) patients treated with R-CHOP (rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone) by multivariate survival analysis

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | |  | | **OS** | |  |  | **PFS** |  |
| **Variable** | | **HR** | **95% CI** | | ***P*** | | **HR** | **95% CI** | ***P*** |
| **ABC-DLBCL** | | | | |  | |  |  |  |
| IGHV SHMhigh | 0.45 | | 0.17-1.24 | | 0.12 | | 0.35 | 0.13-0.94 | **0.036** |
| IPI score >2 | 4.53 | | 1.61-12.7 | | **0.004** | | 2.83 | 1.07-7.46 | **0.036** |
| Female sex | 1.45 | | 0.55-3.79 | | 0.45 | | 1.41 | 0.55-3.59 | 0.47 |
| Tumor size ≥5 cm | 2.29 | | 0.91-5.75 | | 0.079 | | 1.74 | 0.72-4.18 | 0.22 |
| B symptoms | 1.00 | | 0.40-2.51 | | 0.99 | | 1.50 | 0.62-3.63 | 0.37 |
| *MYC* rearrangement | 0.97 | | 0.18-5.27 | | 0.97 | | 1.01 | 0.19-5.22 | 0.99 |
| **DLBCL** |  | |  | |  | |  |  |  |
| IGHV ongoing SHM+ | 2.56 | | 1.32-4.97 | | **0.006** | | 1.96 | 1.04-3.69 | **0.036** |
| IPI score >2 | 2.49 | | 1.26-4.92 | | **0.009** | | 2.16 | 1.12-4.16 | **0.021** |
| Female sex | 1.46 | | 0.67-3.20 | | 0.34 | | 1.10 | 0.52-2.32 | 0.81 |
| Tumor size ≥5 cm | 2.41 | | 1.11-5.22 | | **0.026** | | 1.96 | 0.94-4.11 | 0.073 |
| B symptoms | 0.95 | | 0.48-1.90 | | 0.89 | | 1.01 | 0.52-1.95 | 0.98 |
| ABC subtype | 1.13 | | 0.59-2.17 | | 0.72 | | 1.06 | 0.56-1.98 | 0.84 |
| **GCB-DLBCL** |  | |  | |  | |  |  |  |
| IGK/LV SHMhigh | 3.55 | | 1.44-8.77 | | **0.006** | | 2.64 | 1.11-6.30 | **0.028** |
| IPI score >2 | 2.50 | | 1.06-5.87 | | **0.036** | | 2.63 | 1.17-5.91 | **0.019** |
| Female sex | 0.22 | | 0.074-0.65 | | **0.006** | | 0.37 | 0.14-0.95 | 0.038 |
| Tumor size ≥5 cm | 4.50 | | 1.72-11.7 | | **0.002** | | 3.62 | 1.49-8.79 | **0.004** |
| B symptoms | 2.28 | | 0.89-5.84 | | 0.085 | | 2.02 | 0.82-5.00 | 0.13 |
| **GCB-DLBCL** |  | |  | |  | |  |  |  |
| Long HCDR3 | 3.55 | | 1.44-8.77 | | **0.026** | | 2.92 | 1.03-8.26 | **0.043** |
| IPI score >2 | 4.18 | | 1.58-11.0 | | **0.004** | | 2.95 | 1.22-7.11 | **0.016** |
| Female sex | 1.80 | | 0.52-6.25 | | 0.36 | | 1.17 | 0.39-3.50 | 0.78 |
| Tumor size ≥5 cm | 1.40 | | 0.35-5.59 | | 0.63 | | 1.32 | 0.41-4.30 | 0.64 |
| B symptoms | 2.42 | | 0.92-6.33 | | 0.073 | | 1.85 | 0.76-4.53 | 0.18 |
| **DLBCL** |  | |  | |  | |  |  |  |
| Long K/LCDR3 | 3.96 | | 1.34-11.7 | | **0.013** | | 2.83 | 0.97-8.20 | 0.056 |
| IPI score >2 | 2.84 | | 1.60-5.06 | | **<0.001** | | 2.22 | 1.30-3.80 | **0.004** |
| Female sex | 0.66 | | 0.38-1.15 | | 0.14 | | 0.80 | 0.48-1.34 | 0.40 |
| Tumor size ≥5 cm | 3.06 | | 1.75-5.34 | | **<0.001** | | 2.45 | 1.47-4.11 | **0.001** |
| B symptoms | 2.11 | | 1.21-3.67 | | **0.008** | | 1.95 | 1.15-3.30 | **0.013** |

**Abbreviations**: OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; ABC, activated B-cell–like; IGHV, immunoglobulin heavy chain variable region gene; SHM, somatic hypermutation; IPI, International Prognostic Index; GCB, germinal center B-cell–like; IGK/LV,immunoglobulin kappa or lambda light chain variable region gene; CDR3, complementarity determining region 3.

**Note:** Significant *P* values (Cox regression model) are in bold.

**Table S5.** Clinicopathologic and molecular characteristics of patients with diffuse large B-cell lymphoma (DLBCL) with a short or long immunoglobulin heavy/light chain complementarity determining region 3 (CDR3) length

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **GCB-DLBCL** | |  | **DLBCL** | |  |
|  | **HCDR3short** | **HCDR3long** |  | **K/LCDR3short** | **K/LCDR3long** |  |
| **Characteristic** | n (%) | n (%) | *P* | n (%) | n (%) | *P* |
| **Age** |  |  |  |  |  |  |
| < 60 years | 12 (41%) | 14 (44%) | 1.0 | 61 (39%) | 6 (46%) | .77 |
| ≥ 60 years | 17 (59%) | 18 (56%) |  | 96 (61%) | 7 (54%) |  |
| **Sex** |  |  |  |  |  |  |
| Male | 16 (55%) | 17 (53%) | 1.0 | 94 (60%) | 7 (54%) | .77 |
| Female | 13 (45%) | 15 (47%) |  | 63 (40%) | 6 (46%) |  |
| **Stage** |  |  |  |  |  |  |
| I - II | 8 (28%) | 19 (66%) | **.0079** | 66 (43%) | 6 (50%) | .77 |
| III - IV | 21 (72%) | 10 (34%) |  | 86 (57%) | 6 (50%) |  |
| **B symptoms** |  |  |  |  |  |  |
| No | 17 (59%) | 20 (67%) | .60 | 99 (66%) | 8 (73%) | .75 |
| Yes | 12 (41%) | 10 (33%) |  | 52 (34%) | 3 (27%) |  |
| **Serum LDH level** | |  |  |  |  |  |
| Normal | 17 (61%) | 6 (21%) | **.006** | 64 (45%) | 5 (50%) | .76 |
| Elevated | 11 (39%) | 22 (79%) |  | 79 (55%) | 5 (50%) |  |
| **No. of extranodal sites** | | |  |  |  |  |
| 0 or 1 | 22 (81%) | 24 (83%) | 1.0 | 117 (77%) | 10 (83%) | 1.0 |
| ≥ 2 | 5 (19%) | 5 (17%) |  | 35 (23%) | 2 (17%) |  |
| **ECOG performance status** | | |  |  |  |  |
| 0 or 1 | 18 (75%) | 22 (92%) | .24 | 109 (79%) | 7 (70%) | .45 |
| ≥ 2 | 6 (25%) | 2 (8%) |  | 29 (21%) | 3 (30%) |  |
| **Largest tumor size** | |  |  |  |  |  |
| <5 cm | 17 (74%) | 9 (38%) | **.019** | 74 (57%) | 4 (57%) | 1.0 |
| ≥5 cm | 6 (26%) | 15 (63%) |  | 56 (43%) | 3 (43%) |  |
| **IPI score** | |  |  |  |  |  |
| 0 - 2 | 19 (68%) | 19 (63%) | .79 | 90 (59%) | 9 (82%) | .20 |
| 3 - 5 | 9 (32%) | 11 (37%) |  | 62 (41%) | 2 (18%) |  |
| **Therapy response** | |  |  |  |  |  |
| CR | 24 (83%) | 18 (56%) | **.03\*** | 114 (73%) | 7 (54%) | .20\* |
| PR | 1 | 7 |  | 21 | 4 |  |
| SD | 1 | 2 |  | 6 | 0 |  |
| PD | 3 | 5 |  | 16 | 2 |  |
| **CD37 expression** | |  |  |  |  |  |
| No | 15 (56%) | 26 (87%) | **.017** | 97 (63%) | 7 (58%) | .76 |
| Yes | 12 (44%) | 4 (13%) |  | 56 (37%) | 5 (42%) |  |
| ***MYC* gene mutations** | |  |  |  |  |  |
| No | 16 (55%) | 23 (82%) | **.045** | 110 (72%) | 9 (69%) | .76 |
| Yes | 13 (45%) | 5 (18%) |  | 42 (28%) | 4 (31%) |  |
| **Kappa/Lambda** |  |  |  |  |  |  |
| Lambda | 4 (22%) | 6 (38%) | .46 | 39 (27%) | 9 (69%) | **.0029** |
| Kappa | 14 (78%) | 10 (63%) |  | 107 (73%) | 4 (31%) |  |

**Abbreviations**: GCB, germinal center B-cell-like; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; HCDR3, immunoglobulin heavy chain complementarity determining region 3; K/LCDR3, immunoglobulin light chain (kappa or lambda type) complementarity determining region 3.

**Note:** Not all patients had data available. Significant *P* values (Fisher’s exact test) are in bold. \*For therapy response, *P* values were for comparisons between CR and non-CR cases.

**Table S6.** Clinicopathologic and molecular characteristics of patients with diffuse large B-cell lymphoma (DLBCL) with ongoing somatic hypermutation (SHM) in immunoglobulin variable region genes

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **IGHV SHMongoing+** | **IGHV SHMongoing−** |  | **IGK/LV SHMongoinghigh** | **IGK/LV SHMongoinglow** |  |
| **Characteristic** | n (%) | n (%) | *P* | n (%) | n (%) | *P* |
| **Age** |  |  |  |  |  |  |
| < 60 years | 15 (39%) | 31 (38%) | 1.0 | 2 (22%) | 34 (37%) | .48 |
| ≥ 60 years | 23 (61%) | 51 (62%) |  | 7 (78%) | 58 (63%) |  |
| **Sex** |  |  |  |  |  |  |
| Male | 21 (55%) | 44 (54%) | 1.0 | 5 (56%) | 62 (67%) | .48 |
| Female | 17 (45%) | 38 (46%) |  | 4 (44%) | 30 (33%) |  |
| **Stage** |  |  |  |  |  |  |
| I - II | 13 (37%) | 41 (53%) | .16 | 3 (38%) | 38 (42%) | 1.0 |
| III - IV | 22 (63%) | 37 (47%) |  | 5 (63%) | 52 (58%) |  |
| **B symptoms** |  |  |  |  |  |  |
| No | 19 (54%) | 53 (66%) | .30 | 3 (38%) | 64 (73%) | .052 |
| Yes | 16 (46%) | 27 (34%) |  | 5 (63%) | 24 (27%) |  |
| **Serum LDH level** | |  |  |  |  |  |
| Normal | 18 (50%) | 26 (36%) | .21 | 0 (0%) | 47 (57%) | **.0021** |
| Elevated | 18 (50%) | 47 (64%) |  | 8 (100%) | 36 (43%) |  |
| **No. of extranodal sites** | | |  |  |  |  |
| 0 or 1 | 25 (74%) | 64 (82%) | .32 | 6 (67%) | 69 (78%) | .43 |
| ≥ 2 | 9 (26%) | 14 (18%) |  | 3 (33%) | 20 (22%) |  |
| **ECOG performance status** | | |  |  |  |  |
| 0 or 1 | 23 (70%) | 56 (82%) | .20 | 6 (75%) | 62 (77%) | 1.0 |
| ≥ 2 | 10 (30%) | 12 (18%) |  | 2 (25%) | 19 (23%) |  |
| **Largest tumor size** | |  |  |  |  |  |
| < 5 cm | 19 (63%) | 38 (61%) | 1.0 | 3 (43%) | 40 (53%) | .70 |
| ≥ 5 cm | 11 (37%) | 24 (39%) |  | 4 (57%) | 35 (47%) |  |
| **IPI score** | |  |  |  |  |  |
| 0 - 2 | 20 (56%) | 52 (68%) | .29 | 1 (13%) | 53 (60%) | **.020** |
| 3 - 5 | 16 (44%) | 25 (32%) |  | 7 (81%) | 35 (40%) |  |
| **Therapy response** | |  |  |  |  |  |
| CR | 28 (74%) | 58 (58%) | .83\* | 3 (33%) | 71 (77%) | **.010\*** |
| PR | 4 | 12 |  | 4 | 9 |  |
| SD | 0 | 5 |  | 0 | 4 |  |
| PD | 6 | 7 |  | 2 | 8 |  |
| **GCB/ABC subtype** | |  |  |  |  |  |
| GCB | 16 (43%) | 42 (49%) | .56 | 8 (89%) | 45 (48%) | **.032** |
| ABC | 21 (57%) | 43 (51%) |  | 1 (11%) | 48 (52%) |  |
| **BAGS classification** | |  |  |  |  |  |
| Centroblast | 12 (63%) | 10 (25%) | **.0086**§ | 4 (67%) | 19 (39%) | .22§ |
| Centrocyte | 7 (37%) | 30 (75%) |  | 2 (33%) | 30 (61%) |  |
| Others | 9 | 21 |  | 1 | 18 |  |
| **CD5 expression** | |  |  |  |  |  |
| No | 82 (96%) | 29 (78%) | **.003** | 8 (89%) | 86 (93%) | .49 |
| Yes | 3 (4%) | 8 (22%) |  | 1 (11%) | 6 (7%) |  |
| **IgM expression** | |  |  |  |  |  |
| < 20% | 19 (50%) | 59 (73%) | **.022** | 8 (89%) | 64 (73%) | .44 |
| ≥ 20% | 19 (50%) | 22 (27%) |  | 1 (11%) | 24 (27%) |  |
| **PD-L2+% of CD68+ cells** | |  |  |  |  |  |
| < 14.2% | 25 (83%) | 61 (97%) | **.034** | 8 (100%) | 64 (97%) | 1.0 |
| ≥ 14.2% | 5 (17%) | 2 (3%) |  | 0 (0%) | 2 (3%) |  |
| **9p24 locus by a *PDL1* probe** | |  |  |  |  |  |
| Normal/gain | 66 (97%) | 22 (85%) | **.048** | 72 (94%) | 4 (50%) | **.0036** |
| Amplification/polyploid | 2 (3%) | 4 (15%) |  | 5 (6%) | 4 (50%) |  |
| **9p24 locus by a *PDL2* probe** | |  |  |  |  |  |
| Normal/gain | 64 (97%) | 25 (83%) | **.029** | 71 (91%) | 4 (50%) | **.0082** |
| Amplification/polyploid | 2 (3%) | 5 (17%) |  | 7 (9%) | 4 (50%) |  |
| **SHM degree** |  |  |  |  |  |  |
| SHMlow | 32 (40%) | 19 (50%) | .19 | 56 (60%) | 9 (100%) | **.025** |
| SHMhigh | 49 (60%) | 19 (50%) |  | 38 (40%) | 0 (0%) |  |

**Abbreviations**: IGHV, immunoglobulin heavy chain variable region gene; IGK/LV, **i**mmunoglobulin kappa or lambda light chain variable region gene; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; GCB, germinal center B-cell-like; ABC, activated B-cell–like; BAGS, B-cell–associated gene signature.

**Note:** Not all patients had data available. Significant *P* values (Fisher’s exact test) are in bold. \*For therapy response, *P* values were for comparisons between CR and non-CR cases. §Combined centroblast and centrocyte case numbers vs others.

**Table S7.** Gene signaturesassociated with SHM in immunoglobulin sequences of diffuse large B-cell lymphoma (DLBCL) samples

|  |  |  |
| --- | --- | --- |
| **Comparison** | **Upregulated genes** | **Downregulated genes** |
| High vs. low degree of IGHV SHM (FDR 0.35) | *BTG1, ATP2B1, C1orf71, ADRBK2, SYPL1, EEA1, USP34, APPL2, JMJD1C, ATXN3, BDH2, GARNL1, CHIC2, KIAA1715, RAB39B* | *ZNF667,* ***CD6****, CETP, ICAM5, SAP30, SYDE1, MFI2, ALDH1B1, C1QTNF6, GJB1,* ***WNT6****, LOC100134017, SLC1A7,* ***AKNA****, CHRNA4, SNX20, ST6GALNAC4, LOC390595****, IL27****, HR, LOC100291944, LOC100293662, DGCR14, ZNF564/ZNF709, PRRT1, B3GNT7, CNTN2, CABP5, KIAA1522, C1orf62* |
| Intraclonal sequences with ongoing IGHV SHM ≥2% the repertoire in ABC-DLBCL (FDR 0.3) | *SLC17A7, OR2H1, SLC14A2, NCRNA00105, GSG1, SPAG8,* ***EBF3****, HOXC10, DYRK1B, MAN1A2, LOC729652,* ***PCNA****, HYDIN/HYDIN2, ADCY8, C14orf56, IRX4, ZFYVE28, FAM38B, SCARNA17, INVS, C6orf105* | *COL4A1, SPARC, H3F3B, CMTM6, SLC35A4, VTA1, STK16, LMBR1* |
| High vs. low numbers of sequences with ongoing IGK/LV SHM (FDR 0.01) | *CYP2U1, C11orf41, PDZRN4, CSTF1, MORN1, C14orf23, C9orf93, LOC100268168, IGF2BP2, RGNEF, LOC727924, METTL6, SLITRK5, LOC100130958* | *BMI1* |
| High vs. low numbers of sequences with ongoing IGK/LV SHM in GCB-DLBCL (FDR 0.05) | *C14orf23, FOXG1, CCDC78, IGF2BP2, LOC728543, C11orf57, PDZRN4, C9orf24, MORN1, LOC100132077, LOC100130958, C16orf55, ETV1, RGNEF, C9orf93, LOC100268168, LOC727924, URB1, SCEL, LOC157931, MYT1L, CNBD1, IL1RAPL1* | *BMI1* |

**Abbreviations**: IGH, immunoglobulin heavy chain; ABC, activated B-cell–like; FDR, false discovery rate; IGHV*,* IGH variable region gene; SHM, somatic hypermutation; IGK/LV,immunoglobulin kappa or lambda light chain variable region gene; GCB, germinal center B-cell–like. Abbreviations for highlighted genes (in bold): *CD6*, CD6 molecule or T-cell differentiation antigen CD6; *WNT6,* Wnt family member 6; AKNA, AT-hook transcription factor; *IL27*, interleukin 27; *EBF3*, transcription factor 3; *PCNA*, proliferating cell nuclear antigen.

**Table S8.** Multiple testing corrections for prognostic effects found in the overall cohort of diffuse large B-cell lymphoma treated with R-CHOP (rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone) by the Benjamini-Hochberg method with a false discovery rate of 0.10

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | |  | | **OS** | |  |  | **PFS** |  |
| **Factor** | | *P*-value by Log-rank test | Benjamini-Hochberg q-value | | Benjamini-Hochberg significance | | *P*-value by Log-rank test | Benjamini-Hochberg q-value | Benjamini-Hochberg significance |
| IGHV SHMhigh | **0.011** | | 0.0231 | | **Significant** | | 0.1 | 0.12353 | Insignificant |
| IGHV SHMhigh in *BCL2*-R− | **0.006** | | 0.016275 | | **Significant** | | **0.012** | 0.036 | **Significant** |
| IGHV SHMhigh in *MYC*-R− | **0.0012** | | 0.0084 | | **Significant** | | **0.0047** | 0.0252 | **Significant** |
| IGHV SHMhigh in MYC− | **0.0058** | | 0.016275 | | **Significant** | | 0.059 | 0.09 | **Significant** |
| IGHV SHMhigh in *BCL2*-R+ | 0.44 | | 0.4863 | | Insignificant | | 0.64 | 0.67 | Insignificant |
| IGHV SHMhigh in *MYC*-R+ | 0.48 | | 0.504 | | Insignificant | | 0.34 | 0.3967 | Insignificant |
| IGHV SHMhigh in MYC+ | 0.57 | | 0.57 | | Insignificant | | 0.67 | 0.67 | Insignificant |
| IGK/LV SHMhigh in GCB | **<0.0001** | | 0.001 | | **Significant** | | **0.0016** | 0.021 | **Significant** |
| IGK/LV SHMhigh in ABC | 0.24 | | 0.28 | | Insignificant | | 0.65 | 0.67 | Insignificant |
| IGHV ongoing SHM+ | **0.003** | | 0.01575 | | **Significant** | | 0.1 | 0.12353 | Insignificant |
| IGHV ongoing SHM+ in *BCL2*-R− | **0.0007** | | 0.00735 | | **Significant** | | **0.01** | 0.035 | **Significant** |
| IGK/LV ongoing SHMhigh | **0.0047** | | 0.016275 | | **Significant** | | **0.0048** | 0.0252 | **Significant** |
| IGK/LV ongoing SHMhigh in GCB | **0.042** | | 0.06785 | | **Significant** | | **0.033** | 0.077 | **Significant** |
| HCDR3 short length | **0.0077** | | 0.01797 | | **Significant** | | **0.002** | 0.021 | **Significant** |
| HCDR3 short length in GCB | **0.0062** | | 0.16275 | | **Significant** | | **0.0091** | 0.035 | **Significant** |
| HCDR3 short length in ABC | 0.16 | | 0.19764 | | Insignificant | | 0.054 | 0.09 | **Significant** |
| K/LCDR3 short length | **0.026** | | 0.0496 | | **Significant** | | 0.094 | 0.12353 | Insignificant |
| K/LCDR3 short length in ABC | **0.031** | | 0.05425 | | **Significant** | | 0.06 | 0.09 | **Significant** |
| IGH only-D-J-resolved or unproductive V-D-J clones in GCB | 0.13 | | 0.17063 | | Insignificant | | **0.047** | 0.08973 | **Significant** |
| IGK/L unproductive VJ | 0.11 | | 0.154 | | Insignificant | | **0.024** | 0.063 | **Significant** |
| IGK/L unproductive VJ in GCB | 0.11 | | 0.154 | | Insignificant | | **0.045** | 0.08973 | **Significant** |

**Abbreviations**: OS, overall survival; PFS, progression-free survival; IGHV, immunoglobulin heavy chain variable region gene; SHM, somatic hypermutation; GCB, germinal center B-cell–like; ABC, activated B-cell–like; *BCL2*-R, *BCL2* rearrangement ; *MYC*-R, *MYC* rearrangement; IGK/LV,immunoglobulin kappa or lambda light chain variable region gene.CDR3, complementarity determining region 3.

**Note:** The order of factors listed are according to heavy chain and light chain factors but not the significance by Benjamini-Hochberg procedure. Light chain factors are in shading. Significant values are in bold.