Defining signatures of peripheral T-cell lymphoma with a targeted 20-marker gene expression profiling assay

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**Supplemental Material**

**Patients and tumor samples**

As shown in Figure S1, the cases were divided into two cohorts. The first cohort (classification cohort, n = 230) used to build and train a predictive classification model, was composed of 230 cases, including 30 angioimmunoblastic T-cell lymphoma (AITL), 33 T<sub>FH</sub>-PTCL, 21 ALK-positive anaplastic large cell lymphomas (ALCL), 34 ALK-negative ALCL, 16 extranodal NK/T-cell lymphomas, nasal-type (NKTCL), 6 hepatosplenic T-cell lymphomas (HSTL), and 13 adult T-cell leukemia/lymphomas (ATLL), and 77 PTCL-NOS cases, according to the WHO 2017 classification. The second cohort (diagnostic cohort) of 40 FFPE PTCL samples (6 ALK-positive ALCL, 4 ALK-negative ALCL, 13 AITL, 9 NKTCL, 6 ATLL, and 2 PTCL-NOS) was used to validate the robustness of the assay and its reproducibility between three independent centers.

**Immunohistochemistry and EBV in situ hybridization**

Deparaffinized tissue sections were stained for a panel of T-cell (CD3, CD2, CD7, CD5, CD8, CD4), cytotoxic (TIA-1, granzyme B, perforin), T<sub>FH</sub> (PD1, CXCL13, BCL6, ICOS, CD10), follicular dendritic-cell (CD21, CD23), B-cell (CD20, CD79a, PAX5), and other (CD30, CD56, ALK) antigens. Immunostains for GATA3 (clone HG3-31, Santa Cruz Biotechnology, Santa Cruz, CA) and TBX21 (clone 4B10, BD Biosciences, San Jose, CA) were performed on a subset of cases. The scoring system was evaluated as follows: score 0: negative (below threshold of 10% positive tumor cells), score 1: 10-50% positive tumor cells, score 2: >50% positive tumor cells as previously described (Dobay, Haematologica, 2017;102(4): e148-e151). The detection of EBV was performed by in situ hybridization using EBER probes and was scored as previously described (de Leval, Haemalogica 2015;100(9):e361-364): score 0: absence of large EBV-positive cells, score 1: up to 5 large EBV-positive cells per high power fields (hpf), score 2 : 5 to 50 per hpf and score 3 : > 50 per hpf , or sheets or aggregates of large EBV-positive cells.

**FISH for **<i>DUSP22/IRF4</i>** rearrangement**

Laboratory-developed fluorescence in situ hybridization FISH-probes using bacterial artificial chromosomes (BACs) were used to explore <i>DUSP22/IRF4</i> rearrangements by interphase FISH in FFPE tissue sections of ALK-negative ALCL. Break-apart probes consisted of telomeric
RP3-416J7 labelled with SpectrumOrange™ and centromeric RP11-615C17 and CTD-3139L20 labelled with SpectrumGreen™ (Institute of Pathology, Lausanne).

**Allele-specific PCR and targeted deep sequencing**

*RHOA* G17V and *IDH2* R172K/T mutational status were also determined by allele-specific qPCR (AS-qPCR) or next generation sequencing (NGS) on a MiSeq instrument with a mean coverage depth of 1200X. (Dupuy et al. J Mol Diag)

**RNA extraction and microarray procedure**

Total RNA was extracted from frozen and FFPE tissue samples with Trizol reagent and the Maxwell 16 LEV RNA kit (Promega), respectively. High-throughput gene-expression data (HG-U133 plus 2.0 chips, Affymetrix, Santa Clara, CA) from 72 cases (23 AITL and 49 PTCL-NOS) were previously reported (de Leval et al, Blood. 2007;109(11):4952–4963).

**RT-MLPA procedure**

All probes consisted of a gene-specific region complementary to the cDNA target linked to a tail. The 5’ and 3’ tails correspond to the primers U1 (TCCAACCCTTAGGGAACCC) and U2 (GTGCCAGCAAGATCCAATCTAGA) used for the final PCR amplification step. Spacers were added between these tails and the gene-specific regions to allow the separation and identification of the PCR products based on size. The 3’ probes were phosphorylated at the 5’ ends. For four genes, specific probes without the PCR tails were used as competitors to normalize the amplification signal. The competitors were added to the corresponding probes at a ratio of 1 (MLPA) to 4 (competitor) for *GZB, CXCL13, TCRα*, and 1 (MLPA) to 3 (competitor) for *PRF*. For the identification of *RHOAG17V* and *IDH2R172K/T* hotspot mutations, 5’ probes with the last nucleotide corresponding to the mutated nucleotide were designed.

**Bioinformatic analysis**

Starting from raw (.fsa) files generated by the genetic analyzer, the web interface provides a graphical representation of the gene expression profile and a table of normalized gene expression calculated as a function of the FAM fluorescence intensity normalized to the
median intensity of the 20 genes of the signature. For each sample, this interface also returns a class prediction deduced from a support vector machine classifier (SVM), established using the e1071 R package with default settings. To minimize the risks of misclassification, this algorithm was coupled with a second nearest centroid classifier to minimize the risks of misclassification. For each class $C$, the coordinates of the centroid $\mu_c$ was calculated so that

$$\mu_c = \frac{1}{N} \sum_{i=1}^{N} S_i$$

where $N$ represents the number of cases belonging to the class $C$ defined by the SVM classifier in the training series and $S_i$, a sample defined by the expression of the 20 RT-MLPA markers. For each sample $S_i$, the distance $d(S_i, C)$ to the centroid $\mu_c$ of the class $C$ is given by

$$d(S_i, C) = \sqrt{\sum_{i=1}^{N} (S_i - \mu_c)^2}$$

For each sample, the class prediction returned by the web interface consists of the results of the SVM algorithm completed by the arithmetic distance to the centroids of each class.

A stratified cross validation was performed to assess the accuracy of our assay. A bootstrap resampling process was first used to build 100 independent training series, randomly selecting two-thirds of the samples within the six categories of the unsupervised hierarchical analysis. For each iteration, a SVM predictor was trained and tested against all remaining samples. A definitive SVM predictor was thus trained using the 184 cases classified by hierarchical analysis.

Post-tests were then built to distinguish ALCL from cytotoxic/Th1 cases. A specific threshold was determined using ROC curves, based on the expression of the CD30 gene values by RT-MLPA (CD30 threshold = 0.8). A second post-test was performed to distinguish ALK-positive from ALK-negative ALCLs, based on the expression of the ALK gene (threshold = 0.2).

Another CD30 post-test was designed among the Th2 category to catch misclassified CD30-positive Th2 cases (threshold = 1.4) due to overlapping FOXP3 and ICOS expression in both the CD30-positive and CD30-negative Th2 groups.
An algorithm was finally performed to calculate the distance of the sample to the centroid of the predicted class and compare it to the other intraclass distances. The algorithm constructs a boxplot based on these intraclass distances and calculates the first and third quartile values, deducting the IQR (InterQuartile Range) value, which is equal to Q3-Q1.

Finally, if the distance of the tested sample was higher than Q3 + 1.5 IQR, the sample was considered to be a mild outlier, which defined the NOS category.
# Tables

## Table S1 - Sequences of the oligonucleotides used for the RT-MLPA assay

Genes are listed according to the probes size. Specific sequences are represented in pink (5' probe) and red (3' probe). The blue sequence corresponds to the common 5' and 3' tails. Nucleotide spacers are in green.

| Gene     | Oligo | probe | Sequence                                                                 |
|----------|-------|-------|--------------------------------------------------------------------------|
| CD8      | CD8E5L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TGCTGCCGGTCTTCCTGCCAG                          |
|          | CD8E6R| 3'prob| CAAGGCCATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC                                 |
| IDH2R172K| IDH2R172KL| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| IDH2R172T| IDH2R172TL| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| EBER1    | EBER1L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| GATA3    | GATA3E2L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|           | GATA3E4R| 3'prob| TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC                                 |
| ALK      | ALKE23F| 5'prob| GTGCCAGCAAGATCCAATCTAGA AAGCCAGCTCCCTCGCGGGTGGTGACCATCTCTCCCTTAGGGGAACCC |
|          | ALKE24R| 3'prob| GCACGCCCATGGCGACCAGT TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC          |
| FOXP3    | FOXP3E3L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TAC GTAGCCACCCGTCCCGGGTA                       |
|          | FOXP3E4R| 3'prob| TAC GTAGCCACCCGTCCCGGGTA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| CD4      | CD4E4L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| CD30     | CD30E5L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| PFR      | PFRE2L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | PFRE3R| 3'prob| AAGCCAGCTCCCTCGCGGGTGGTGACCATCTCTCCCTTAGGGGAACCC                      |
| BCL6     | BCL6E1L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | BCL6E1R| 3'prob| TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC                                 |
| RHOA mut | RHOAMutE2L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| RHOA     | RHOAR| 3'prob| GAAGCCAGCTCCCTCGCGGGTGGTGACCATCTCTCCCTTAGGGGAACCC                      |
| GNB2     | GNB2E5L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TAC GTAGCCACCCGTCCCGGGTA                       |
|          | GNB2E6R| 3'prob| TAC GTAGCCACCCGTCCCGGGTA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| TET      | TETE2L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | TETE6R| 3'prob| CATGACACACATCTCTCTCGACCTAATCCCGACCAGT TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC |
| CD56     | CD56E1L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | CD56E2R| 3'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| CXCL13   | CXCL13E2L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | CXCL13E3R| 3'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| ICOS     | ICOSEL| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | ICOSER| 3'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| TRAC     | TRACE2L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | TRACE3R| 3'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| CXCR5    | CXCR5E2L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | CXCR5E3R| 3'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| INFg     | INFgE3L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | INFgE4R| 3'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
| CCR4     | CCR4E1L| 5'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
|          | CCR4E2R| 3'prob| GTGCCAGCAAGATCCAATCTAGA TCCGAACATCCACGCGGCGTTCCAAACCCTTAGGGGAACCC        |
Table S2. Comparison of RhoAG17V (n = 33) and IDH2R172K/T (n = 8) mutations detected by RT-MLPA and next generation sequencing (NGS) or allele-specific-qPCR (AS-qPCR) analysis. Concordant results are represented in green, while discordant result in red.

| Id    | Pathology | RhoAG17V status by RT-MLPA | RhoA status by AS-qPCR/NGS (allele frequency) | IDH2 R172K/T status by RT-MLPA | IDH2 status with AS-qPCR/NGS (allele frequency) |
|-------|-----------|----------------------------|-----------------------------------------------|-------------------------------|-----------------------------------------------|
| UPN001 | AITL      | +                          | +/NA                                          | WT                            | WT/NA                                         |
| UPN002 | AITL      | +                          | /+ (6.12%)                                    | WT                            | NA/NA                                         |
| UPN003 | AITL      | +                          | +/NA                                          | +                             | +/NA                                          |
| UPN004 | AITL      | +                          | +/NA                                          | WT                            | WT/NA                                         |
| UPN005 | AITL      | +                          | +/NA                                          | +                             | +/NA                                          |
| UPN006 | AITL      | +                          | +/ (19.27%)                                   | WT                            | NA/NA                                         |
| UPN007 | AITL      | +                          | +/ (12.93%)                                   | +                             | +/ (5.08%)                                    |
| UPN010 | AITL      | +                          | +/ (8.53%)                                    | WT                            | NA/NA                                         |
| UPN012 | AITL      | +                          | +/ (23.3%)                                    | WT                            | NA/NA                                         |
| UPN016 | AITL      | +                          | +/ (22.38%)                                   | +                             | +/ (14.9%)                                    |
| UPN018 | AITL      | +                          | +/ (9.13%)                                    | WT                            | NA/NA                                         |
| UPN020 | AITL      | +                          | +/ (12.24%)                                   | +                             | +/ (10.23%)                                   |
| UPN024 | AITL      | +                          | +/ (18.2%)                                    | WT                            | NA/NA                                         |
| UPN025 | AITL      | +                          | +/ (7.44%)                                    | WT                            | WT/ + (2.83%)                                 |
| UPN026 | AITL      | +                          | +/ (17.52%)                                   | +                             | +/NA                                          |
| UPN028 | AITL      | +                          | +/NA                                          | WT                            | NA/NA                                         |
| UPN029 | AITL      | +                          | +/ (18.31%)                                   | WT                            | NA/NA                                         |
| UPN017 | AITL      | +                          | +/NA                                          | +                             | +/NA                                          |
| UPN117 | PTCL TFH  | +                          | +/ (38.93%)                                   | WT                            | NA/NA                                         |
| UPN136 | PTCL TFH  | +                          | +/NA                                          | WT                            | NA/NA                                         |
| UPN113 | PTCL TFH  | +                          | +/NA                                          | WT                            | NA/NA                                         |
| UPN120 | PTCL TFH  | +                          | +/ (23.54%)                                   | WT                            | NA/NA                                         |
| UPN118 | PTCL TFH  | +                          | +/NA                                          | +                             | +/ (2.22%)                                    |
| UPN115 | PTCL TFH  | +                          | +/ (23.15%)                                   | WT                            | NA/NA                                         |
| UPN114 | PTCL TFH  | +                          | +/NA                                          | WT                            | NA/NA                                         |
| UPN138 | PTCL TFH  | +                          | +/ (21.87%)                                   | WT                            | NA/NA                                         |
| UPN134 | PTCL TFH  | +                          | +/NA                                          | WT                            | NA/NA                                         |
| UPN165 | PTCL TFH  | +                          | +/NA                                          | WT                            | NA/NA                                         |
| UPN116 | PTCLnos   | +                          | +/ (50%)                                      | WT                            | NA/NA                                         |
| UPN125 | PTCLnos   | +                          | +/NA                                          | WT                            | NA/NA                                         |
| UPN137 | PTCLnos   | +                          | +/NA                                          | WT                            | NA/NA                                         |
| UPN091 | AITL      | +                          | +/NA                                          | WT                            | NA/NA                                         |
Table S3. Clinical parameters of ALK-negative ALCL and comparison of the non-cytotoxic ALCL ALK-negative subgroup according to DUSP22 status

|                  | ALCL ALK- n=13 | Non-cytotoxic ALCL ALK-negative n=24 | DUSP22R (n=8) | DUSP22NR (n=8) | p     |
|------------------|----------------|--------------------------------------|---------------|----------------|-------|
| age median (range) | 53.8 (40.8-67.2) | 55 (52-70) | 55 (43-72) | 56.45 (53.1-70.3) | 0.63  |
| <= 60 years      | 63.6% (7/11) | 54.2% (13/24) | 62.5% (5/8) | 62.5% (5/8) | 1     |
| gender male      | 53.8% (7/13) | 79.2% (19/24) | 87.5% (7/8) | 75% (6/8) | 1     |
| IPI>=3           | 52.5% (5/8) | 33.3% (7/21) | 50% (4/8) | 16.6% (1/6) | 0.3   |
| PHT>=2           | 85.7% (6/7) | 42.8% (9/21) | 50% (4/8) | 16.6% (1/6) | 0.3   |
| extranodal site >=2 | 85.7% (6/7) | 23.7% (5/23) | 25% (2/8) | 28.6% (2/7) | 1     |
| stage >=3        | 77.8% (7/9) | 73.9% (17/23) | 75% (6/8) | 85.7% (6/7) | 1     |
| PS>=2            | 50% (4/8) | 21% (4/19) | 28.6% (2/7) | 0 (0/5) | 0.47  |
| LDH>=1           | 38.5% (5/13) | 50% (11/22) | 50% (4/8) | 60% (3/5) | 1     |
| B symptoms       | 50% (4/8) | 35% (7/20) | 57.1% (4/7) | 20% (1/5) | 0.33  |
Table S4. Correlation scores of the RT-MLPA values (n = 20) of 40 FFPE samples between three independent centers (very strong correlation (VSC): rho > 0.9, strong correlation (SC) rho > 0.7)

| Gene       | corr-C2-C3 | corr-C1-C3 | corr-C1-C2 |   |
|------------|------------|------------|------------|---|
| CD8        | 0.9777741  | 0.9553372  | 0.9683209  | VSC |
| IDH2       | -0.2502684 | 0.4048631  | 0.3518407  | NA  |
| EBER       | 0.9951752  | 0.9954745  | 0.9877439  | VSC |
| GATA3      | 0.9771558  | 0.939488   | 0.9531494  | VSC |
| ALK        | 0.9894248  | 0.9789414  | 0.9823311  | VSC |
| FOXP3      | 0.9509514  | 0.927661   | 0.9438251  | VSC |
| CD4        | 0.967501   | 0.8858032  | 0.9321464  | VSC |
| CD30       | 0.9897987  | 0.9903066  | 0.9864354  | VSC |
| PRF        | 0.971159   | 0.987516   | 0.9687311  | VSC |
| BCL6       | 0.9412178  | 0.8655903  | 0.8919673  | SC  |
| RHOA       | 0.9324257  | 0.7869005  | 0.7019153  | SC  |
| GRB        | 0.9740104  | 0.9800914  | 0.9456018  | VSC |
| TBX21      | 0.9366044  | 0.9629732  | 0.9254932  | VSC |
| CD56       | 0.9362774  | 0.9749429  | 0.9381184  | VSC |
| CXCL13     | 0.9633031  | 0.9472914  | 0.9743159  | VSC |
| ICOS       | 0.904137   | 0.8439778  | 0.8613445  | SC  |
| TRAC       | 0.9728271  | 0.9564053  | 0.9828094  | VSC |
| CXCR5      | 0.9810425  | 0.9339171  | 0.9412344  | VSC |
| IFNg       | 0.8904373  | 0.8534687  | 0.8465664  | SC  |
| CCR4       | 0.9921214  | 0.9822178  | 0.9819727  | VSC |

* There was no correlation for IDH2 R172m in the absence of IDH2 mutation detected in these 40 FFPE samples
Figures

Figure S1. Study design. 270 cases were divided into two cohorts. The classification cohort (n=230 including 30 AITL, 33 PTCL-TFH, 55 ALCL, 13 ATLL, 6 HSTL, 16 NKTC and 77 PTCL-NOS and consisting mostly of fresh-frozen (FF) samples) was used to train a SVM classifier. The diagnostic cohort (n=40 including 13 AITL, 10 ALCL, 6 ATLL, 9 NKTC and 2 PTCL-NOS) was used for the independent validation on FFPE samples and for the inter-laboratory reproducibility study.

Unsupervised hierarchical analysis
Specified entities n=153 (Figure 2)
Whole cohort n=230 (Figure 4)

Exclusion of 46 PTCLs (heterogenous cluster)

SVM Training (Figure 4B) n=184

SVM classification
*16 FFPE **13 FFPE ***9 FFPE $ all FFPE

Reproducibility study (Table 2)
Figure S2. Representation of the RT-MLPA profiles of each PTCL molecular category

A) AITL/T_{FH} profile, showing the expression of CXCL13, CXCR5, ICOS, and BCL6, and in this case the presence of RHOA and IDH2 mutations. B) NKTCL signature, characterized by high EBER expression, and that of CD56 and cytotoxic markers. C and D) Cytotoxic ALCL profile, defined by the expression of CD30 and cytotoxic markers with (C) or without (D) ALK. E) Non-cytotoxic ALK-negative ALCL signature, characterized by the expression of CD30 and TH2 markers (GATA3 and CCR4). F) Cytotoxic/Th1 signature, defined by the expression of cytotoxic markers with inconsistent expression of Th1 markers. G) ATLL/TH2 signature, characterized by the expression of GATA3, CCR4, and ICOS, with inconsistent FOXP3 expression. H) HSTL profile, showing the expression of CD56, TBX21, GATA3, and BCL6.
Figure S3. ALCL case reclassified from ALK-negative to ALK-positive based on RT-MLPA assay (UPN051) a) Histopathology of the misdiagnosed ALK- ALCL case, based on the negative immunohistochemistry with ALK1 (performed twice). Strong cytoplasmic staining with the D5F3 clone was obtained, retrospectively. b) RT-MLPA profile of the case showing ALK expression (red arrow). c) Sequencing of the specific RT-PCR products confirmed an ATIC-ALK fusion transcript. No mutation in the region coding for the epitope of ALK1 was found (data not shown).
Figure S4. Scatterplot representation of the correlation between RT-MLPA and Affymetrix gene expression values (n = 71 cases, 23 AITL, and 49 PTCL NOS). There were significant correlations (Spearman test, p < 0.05) for each gene, with rho > 0.9 for CXCL13 and CCR4, rho > 0.7 for PRF, GZMB, GATA3, ICOS, CXCR5, BCL6, TNFRSF8/CD30, CD8, and TBX21, and rho > 0.5 for CD4 and FOXP3 (x = Affymetrix values log, y = RT-MLPA values). The correlation for ALK and CD56 was not determined because these genes were not evaluated in the Affymetrix series in the absence of ALCL, HSTL, and NKTCL. RHOAm, IDH2m, TCR, and EBER expression were not studied by the Affymetrix chip.
Figure S5. Comparison of individual RT-MLPA gene expression values and immunohistochemical results in 224 PTCLs, including 20 ALK+ ALCLs, 34 ALK- ALCLs, 29 AITLs, 36 PTCL-TFH, 15 NKTCLs, 13 ATLLs, 6 HSTLs, and 70 PTCL-NOS (6 cases with no IHC data were not considered). There was a significant correlation by Wilcoxon’s rank-sum test between the RT-MLPA expression level of each gene and negative (-) or positive (+) staining by immunohistochemistry (CD30, TBX21, PRF, GZMB, GATA3, ALK, CXCL13, CD56, ICOS, CD8, CD4, and BCL6) or in situ hybridization (EBER).
Figure S6. Examples of RT-MLPA profiles for paired FFPE and frozen samples. A) Superimposed profiles showed similar peaks for each 3 paired cases (NKTCL, AITL and non-cytotoxic ALK-negative ALCL). B) There was a strong correlation (rho>0.7) of RTMLPA normalized data between frozen (blue) and FFPE (orange) samples.
Figure S7. Molecular prediction of PTCL-NOS (n = 77) by the SVM model and correlation with immunohistochemical data. The SVM classification is presented in the top line, the pathological diagnosis in the second line, and immunohistochemical markers in the map. The SVM proposed a molecular class for 92% (69/75) of PTCL-NOS: 17 with a TFH signature, 28 with a cytotoxic/Th1 signature (5 ALK-negative ALCL, 19 cytotoxic/Th1, 5 NKTCL) and 24 with a Th2 signature. Among the 29 cases with a cytotoxic molecular signature, 23 were characterized as cytotoxic by immunohistochemistry and 6 were undetermined. Among the 24 molecular Th2 PTCL-NOS, 14/18 tested cases had a positive immunostaining for GATA3. Only four discrepancies (5%) were noted: 4 cases with a cytotoxic phenotype were classified in the T<sub>FH</sub> /AITL group.