



UTRECHT
THE NETHERLANDS

27-30
SEPTEMBER 2023

ESCCA 2023: *Bridging the (cytometry) flows*

B-LPD, diagnosis beyond typical immunophenotypic fingerprint entities

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All that is gold does not glitter
J.R.R. Tolkien

ESCCA 2023 Utrecht

Disclosure commercial conflict of interest

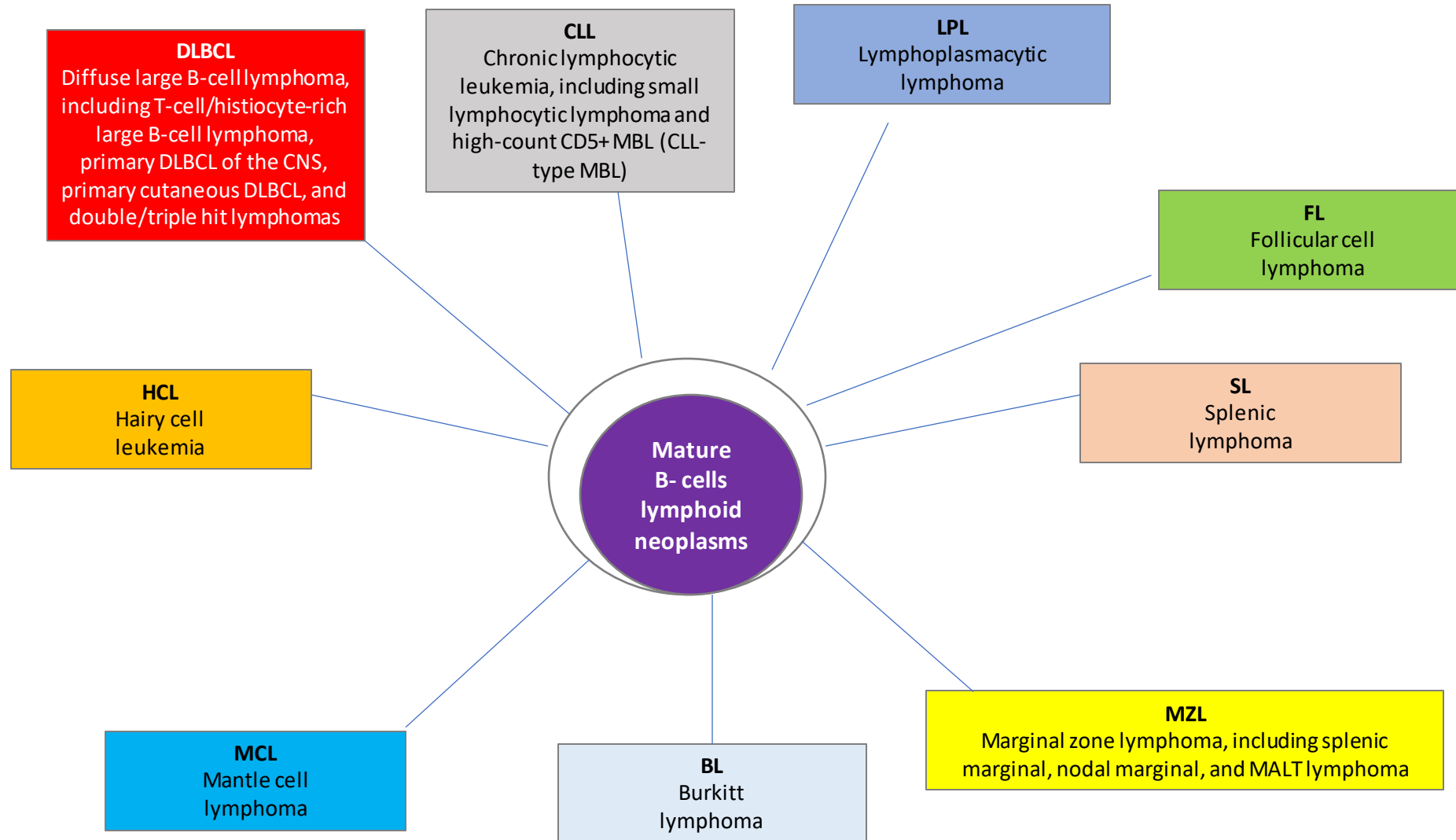
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|---|--------------------------|
| X | No, nothing to disclose |
| | Yes, as specified below: |

| Company Name | Specification |
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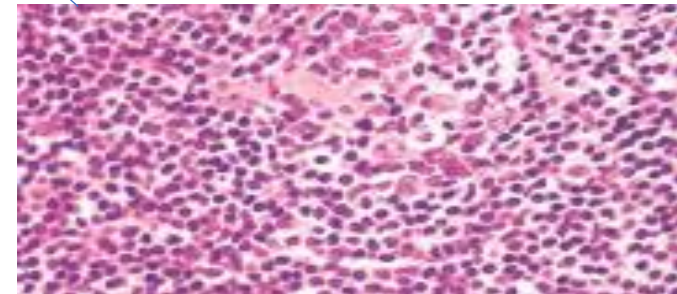
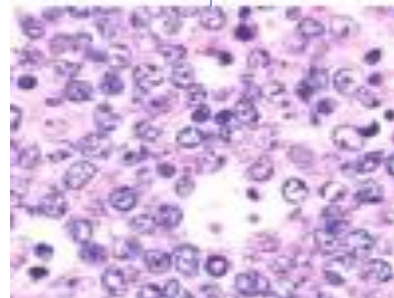
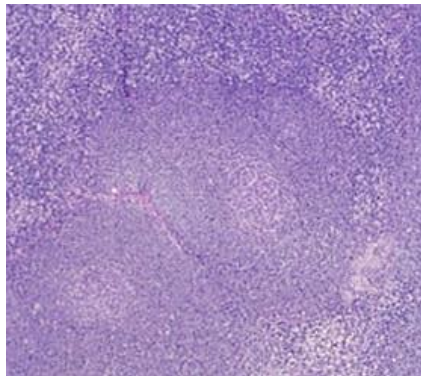
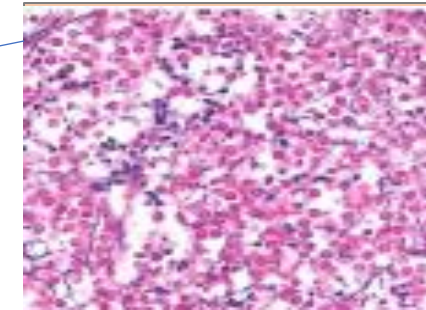
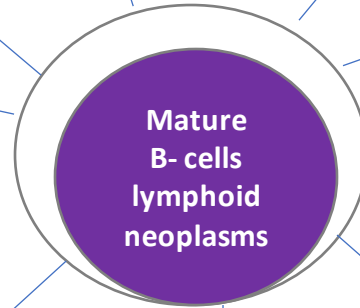
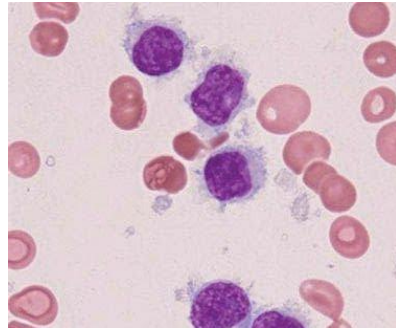
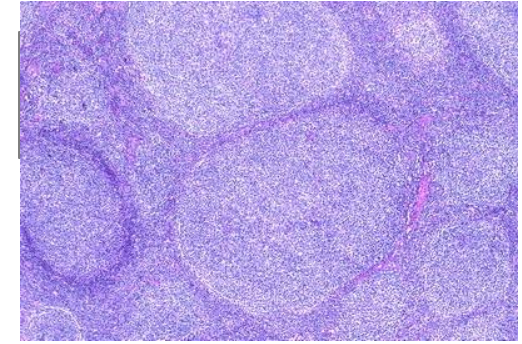
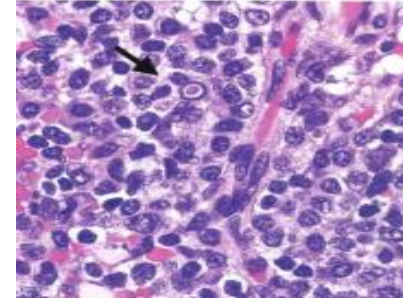
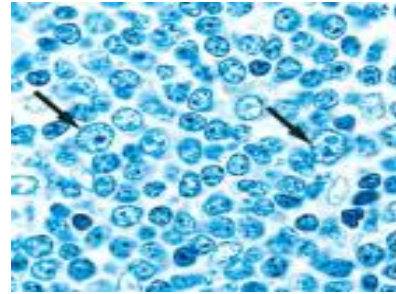
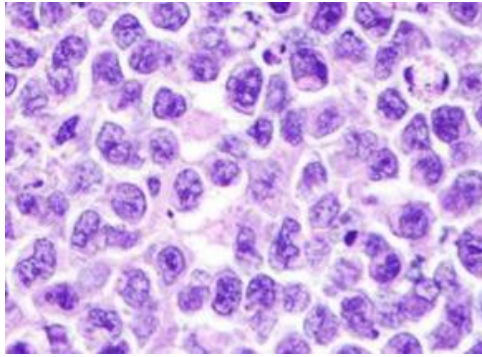
Background

- Despite the well known advantages that the use of Flow Cytometry (FC) to the study (and diagnosis) of mature B cell neoplasms presents, it is largely limited to the characterization of leukemias (mainly B cell Chronic Lymphocytic Leukemia) and non-Hodgkin lymphomas with peripheral blood or bone marrow involvement.
- Only few laboratories routinely use FC for tissue biopsies, except for samples obtained by fine needle aspiration, and even fewer perform a wide characterization with extended panels of monoclonal antibodies. The reasons for this underuse could lie both at the practical and at the theoretical level: difficulties in sample preparations, the belief that morphology and IHC are generally enough and, above all, the lack of a long-standing practice and of a vast literature in support.
- The WHO-HAEM4 (2017), the WHO-HAEM5 (2022) and the ICC (2022) deserve a very little role to the FC in diagnosis of BNHL, being the immunophenotype of different lymphoma entities mainly based on immunohistochemistry.

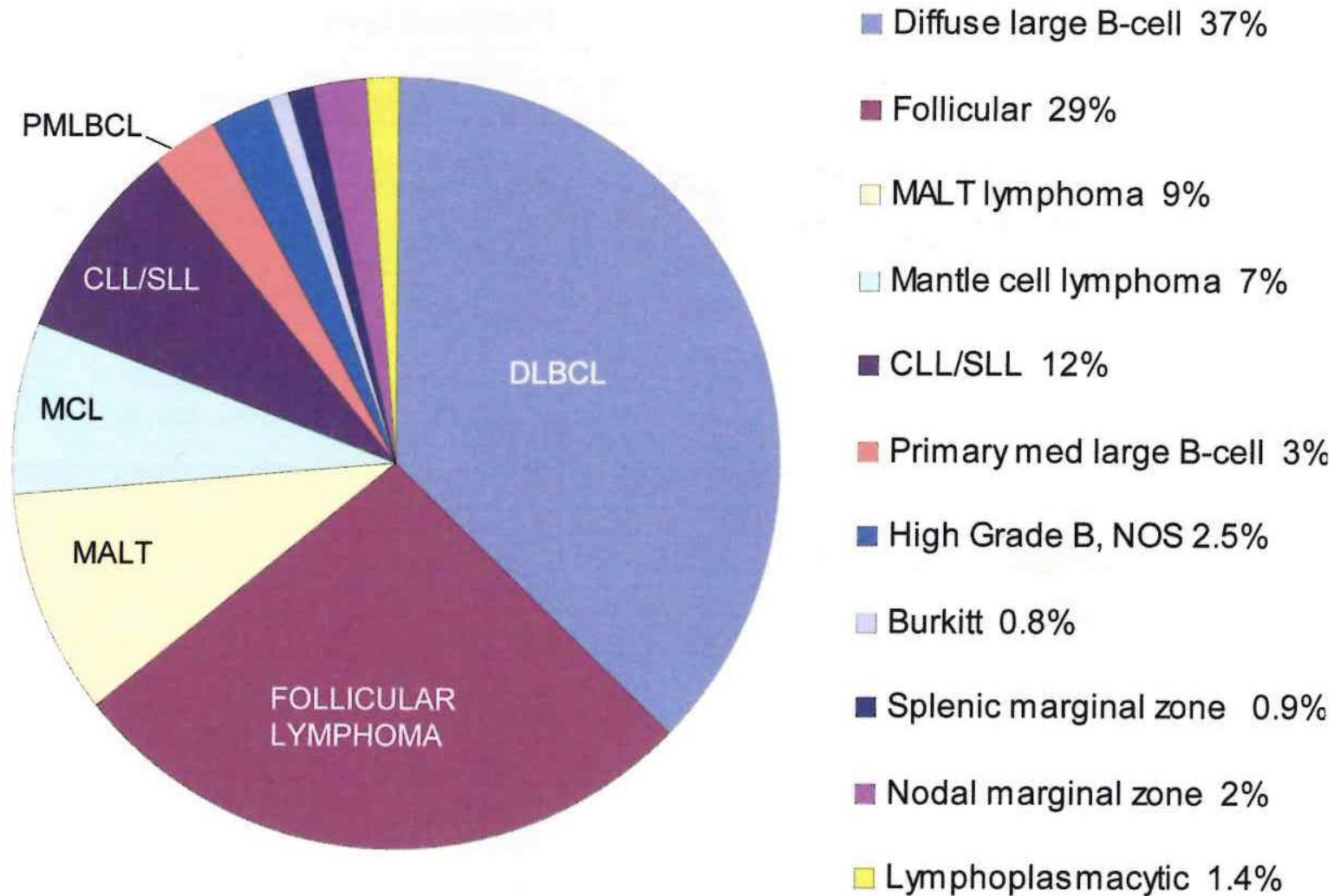
B-NHL heterogeneity



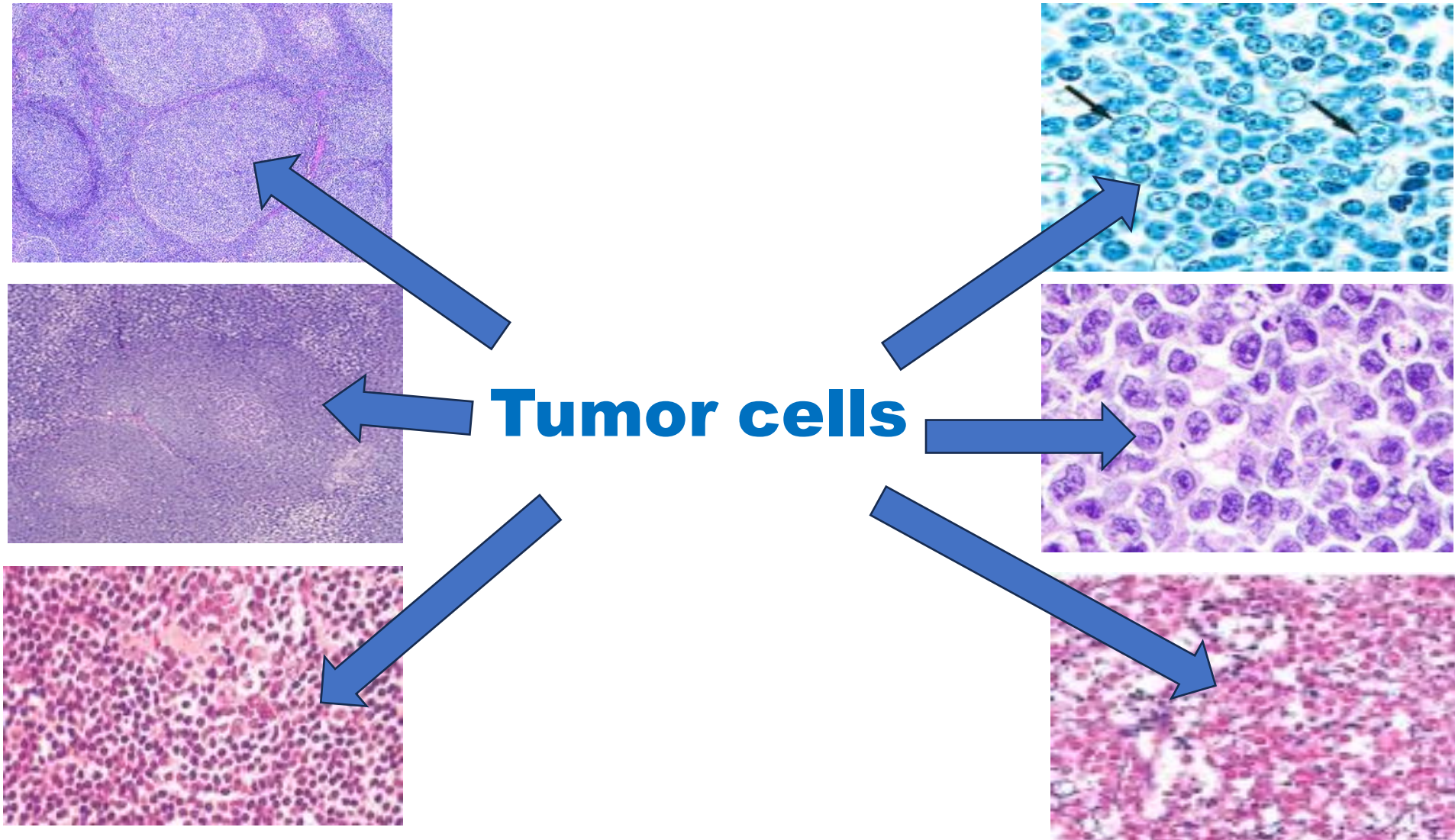
B-NHL heterogeneity



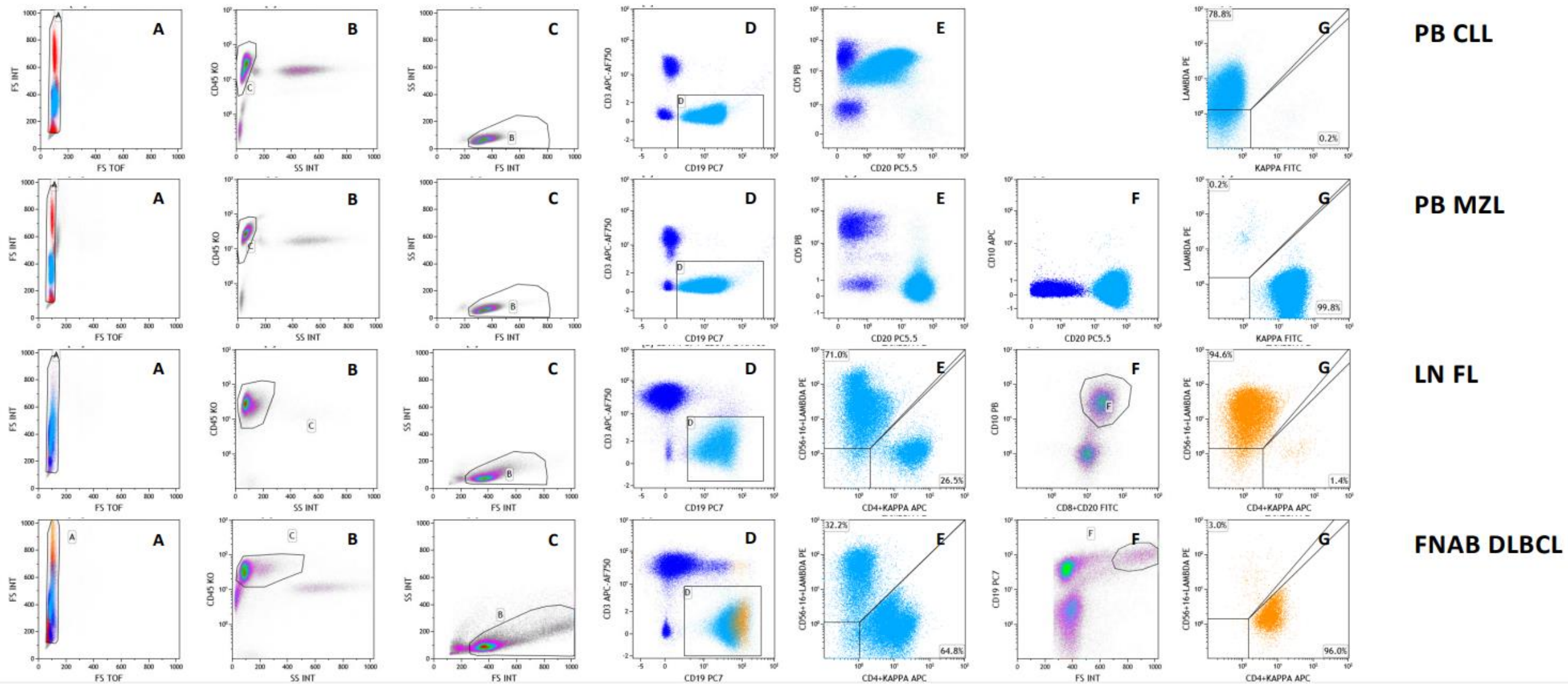
Relative frequency of B cell NHL subtypes (WHO 2017)



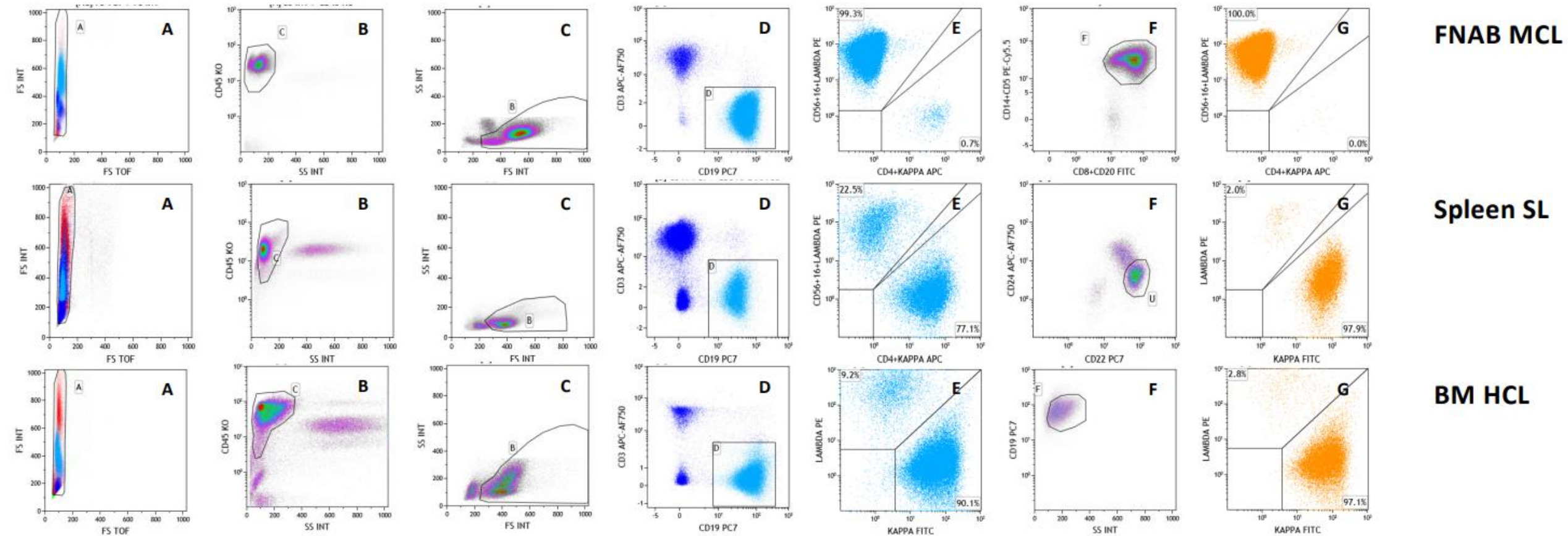
B-NHL heterogeneity



Strategy for detection of clonality



Strategy for detection of clonality



The identification of the clonal population with a purity greater than 95% of monoclonal cells is a fundamental prerequisite to a correct phenotyping of pathological cells and is the strategy used routinely in our laboratory

Complete immunophenotyping

From 2003 to 2019, cases with evidence of monoclonality were characterized with a large panel of antibodies, including the following:

Surface Markers:

CD19, CD20, CD5, CD6, CD9, CD10, CD11c, CD21, CD22, CD23, CD24, CD25, CD31, CD38, CD43, CD44, CD49d, CD52, CD72, CD74, CD79b, CD81, CD103, CD123, CD138, CD180, CD183 (CXCR3), CD196 (CCR6), CD197 (CCR7), CD200, CD220, CD305, CD307d, FMC7

Intracellular Markers (cytoplasmic and nuclear):

cyCD79a, cyBCL-2, cyZAP70, nuMIB-1(Ki-67) and more recently nuIRF4/MUM1, nuBCL-6 and nuMNDA

Analysis method

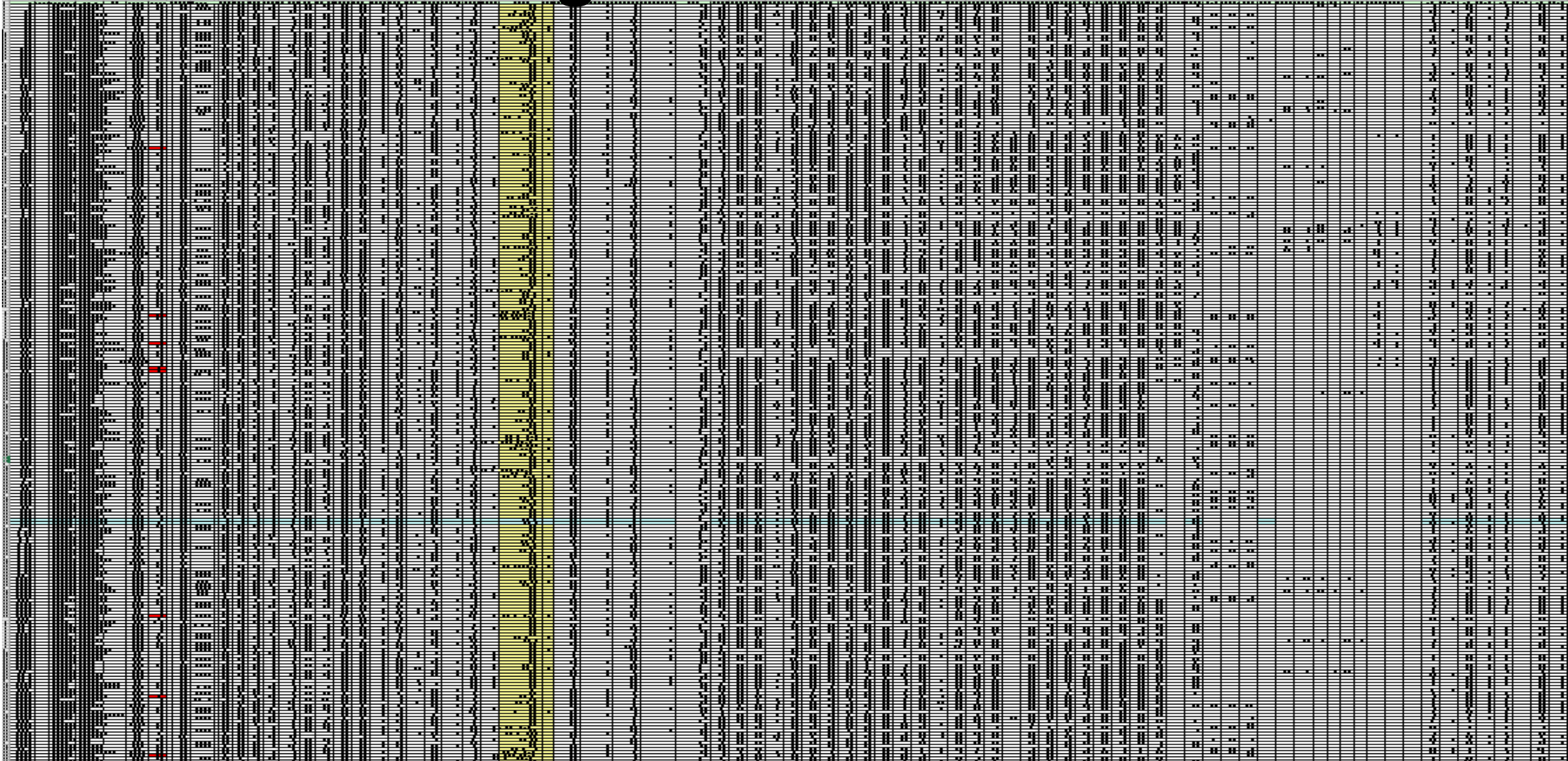
Multicolor (6-8 colors) flow cytometry on “pure” clonal population previously identified by mean of “backbone markers” (CD19, CD20, or any combination giving a clonality on gated cells greater than 95%)

Distribution of 1465 samples in B-NHL categories

| B-NHL Category | Total Samples | Blood Samples | Non-Blood Samples |
|----------------|---------------|---------------|-------------------|
| CLL | 670 | 602 | 68 |
| FCL | 199 | 43 | 156 |
| SL | 19 | 17 | 2 |
| MZL | 174 | 94 | 80 |
| DLBCL | 220 | 25 | 195 |
| LPL | 60 | 53 | 7 |
| MCL | 83 | 51 | 32 |
| HCL | 26 | 26 | 0 |
| BL | 14 | 4 | 10 |

1465 cases, ≥ 50 markers. ~ 90.000 data

A huge amount of data



Cluster analysis

Immunology Letters 134 (2011) 137–144

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immllet



Cluster analysis of immunophenotypic data: The example of chronic lymphocytic leukemia

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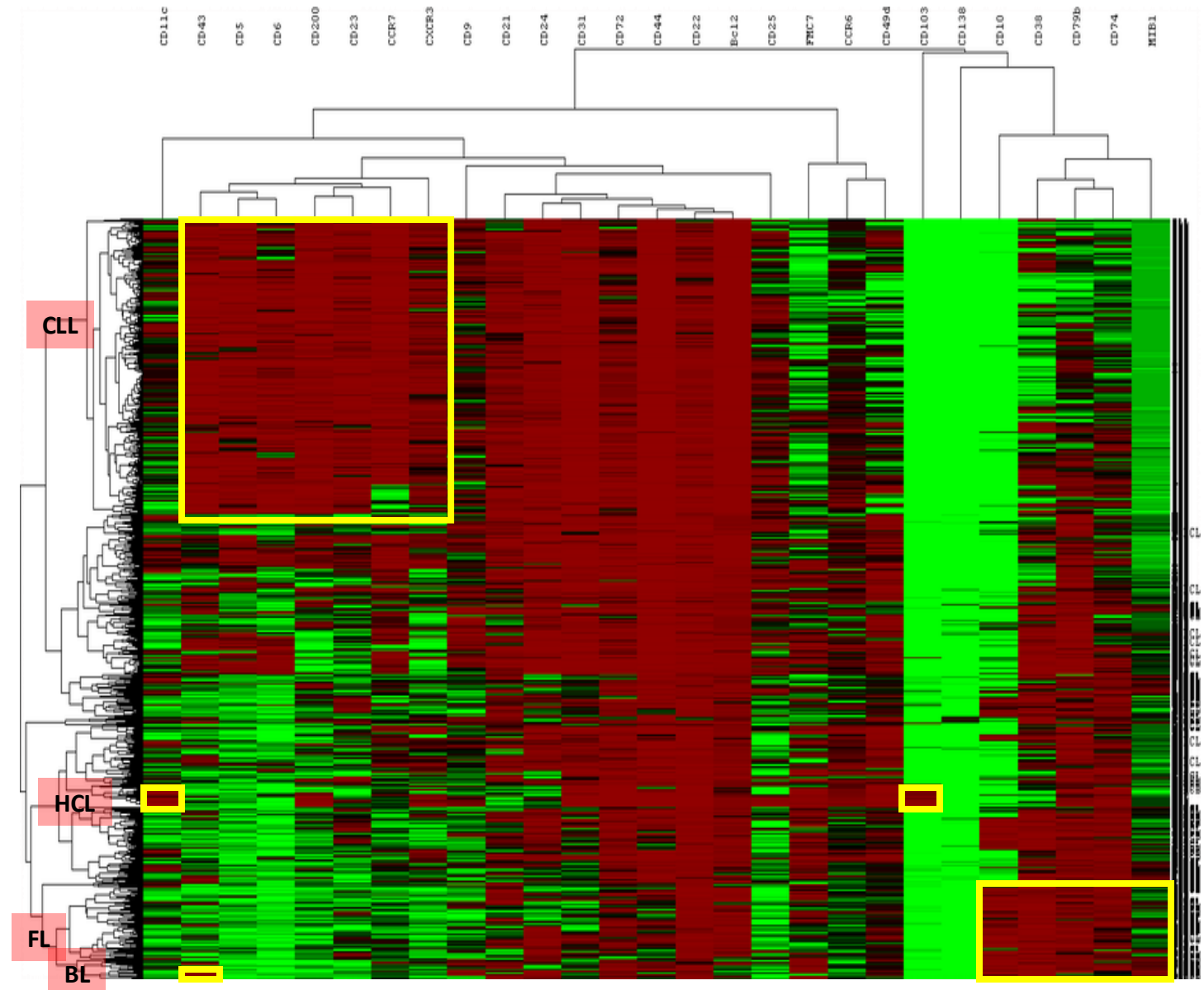
^a Clinical and Experimental Hematology Research Unit, Centro di Riferimento Oncologico, I.R.C.C.S., Via Franco Gallini 2, Aviano (PN), Italy

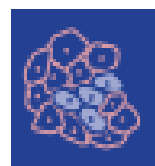
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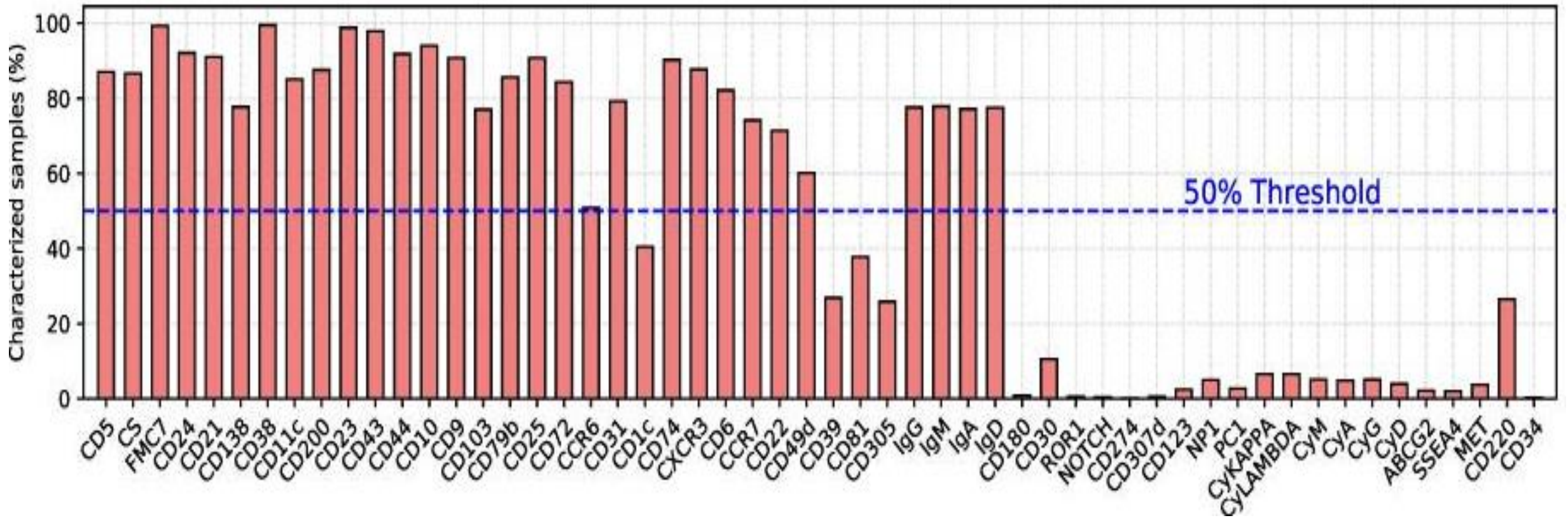
Article

A Clinically Applicable Approach to the Classification of B-Cell Non-Hodgkin Lymphomas with Flow Cytometry and Machine Learning

Valentina Gaidano ^{1,2,†} , Valerio Tenace ^{3,*,†} , Nathalie Santoro ⁴, Silvia Varvello ⁵,
Alessandro Cignetti ⁵ , Giuseppina Prato ⁶, Giuseppe Saglio ^{1,5} , Giovanni De Rosa ⁴
and Massimo Geuna ^{4,*}

Cancers **2020**, *12*(6), 1684; <https://doi.org/10.3390/cancers12061684>

Machine Learning: model preprocessing

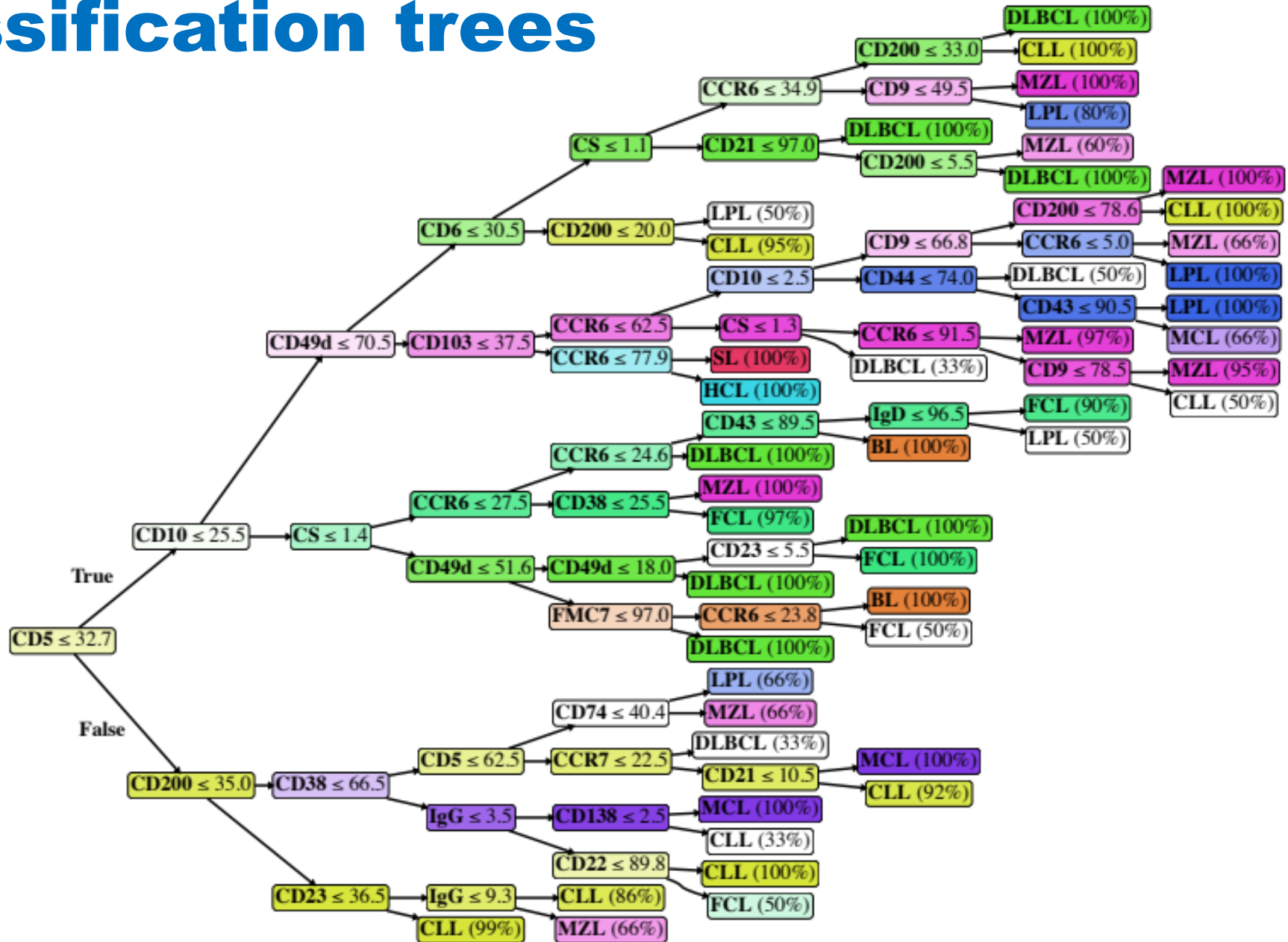


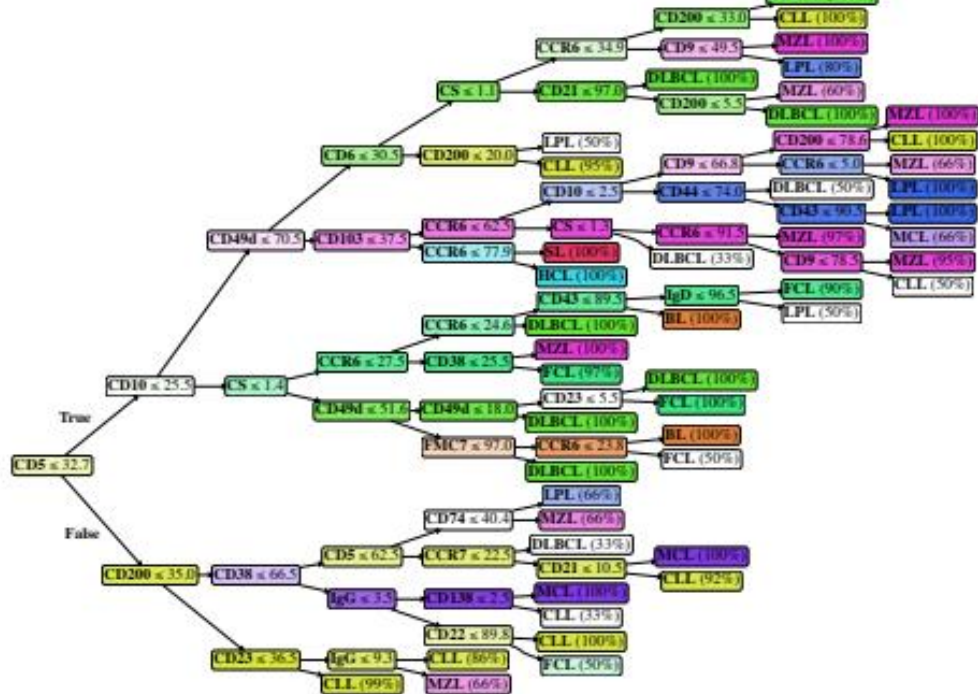
Machine Learning: model generation

| Model | Samples | Classes | Samples/Class | Samples/TS | Samples/VS | Markers |
|-------|---------|---------|---------------|------------|------------|-------------------|
| I | 1465 | BL | 14 | 11 | 3 | SM |
| | | CLL | 670 | 502 | 168 | |
| | | DLBCL | 220 | 165 | 55 | |
| | | FCL | 199 | 149 | 50 | |
| | | HCL | 26 | 20 | 6 | |
| | | LPL | 60 | 45 | 15 | |
| | | MCL | 83 | 62 | 21 | |
| | | MZL | 174 | 130 | 44 | |
| | | SL | 19 | 14 | 5 | |
| II | 1420 | BL | 14 | 10 | 4 | SM, Bcl2, MIB1 |
| | | CLL | 670 | 503 | 167 | |
| | | DLBCL | 220 | 165 | 55 | |
| | | FCL | 199 | 149 | 50 | |
| | | LPL | 60 | 45 | 15 | |
| | | MCL | 83 | 62 | 21 | |
| | | MZL | 174 | 131 | 43 | |

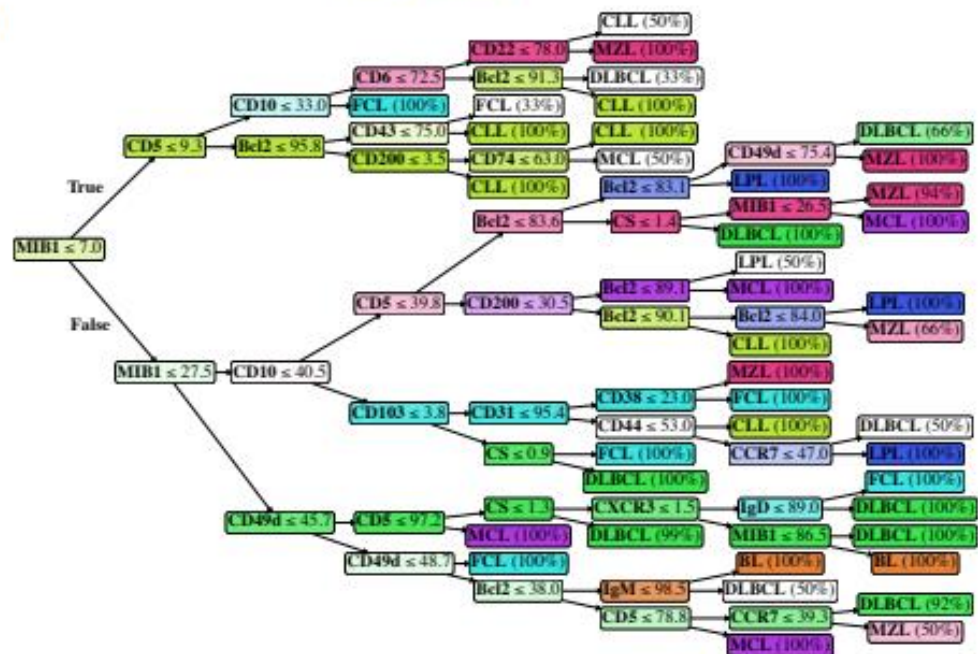
| Model | Samples | Classes | Samples/Class | Samples/TS | Samples/VS | Markers |
|-------|---------|---------|---------------|------------|------------|---------|
| III | 1420 | BL | 14 | 10 | 4 | SM |
| | | CLL | 670 | 503 | 167 | |
| | | DLBCL | 220 | 165 | 55 | |
| | | FCL | 199 | 149 | 50 | |
| | | LPL | 60 | 45 | 15 | |
| | | MCL | 83 | 62 | 21 | |
| | | MZL | 174 | 131 | 43 | |
| | | IV | 548 | BL | 10 | |
| CLL | 68 | | | 51 | 17 | |
| DLBCL | 195 | | | 146 | 49 | |
| FCL | 156 | | | 117 | 39 | |
| LPL | 7 | | | 5 | 2 | |
| MCL | 32 | | | 24 | 8 | |
| MZL | 80 | | | 60 | 20 | |

Machine Learning: training set classification trees

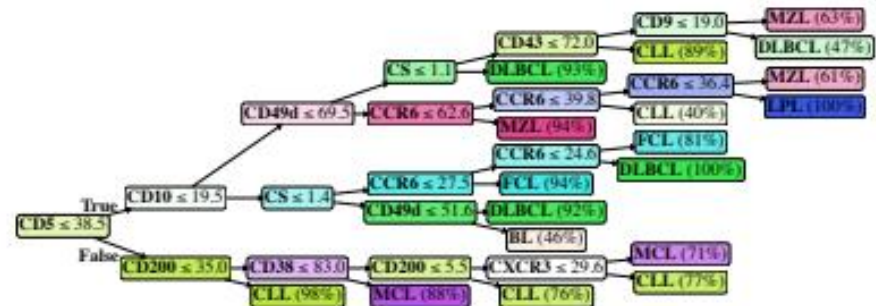




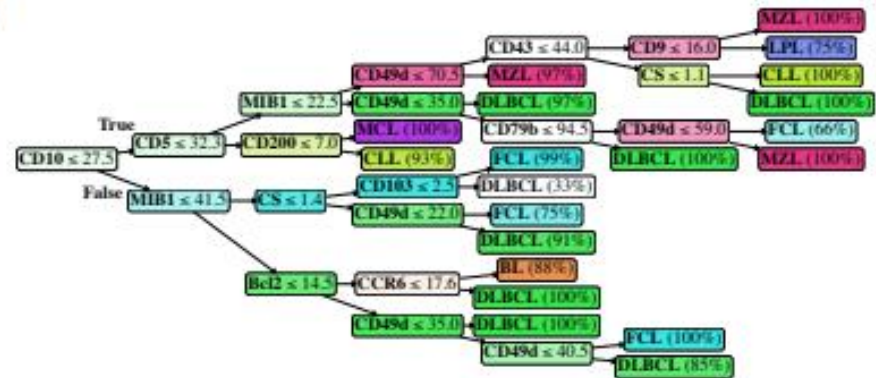
(b)



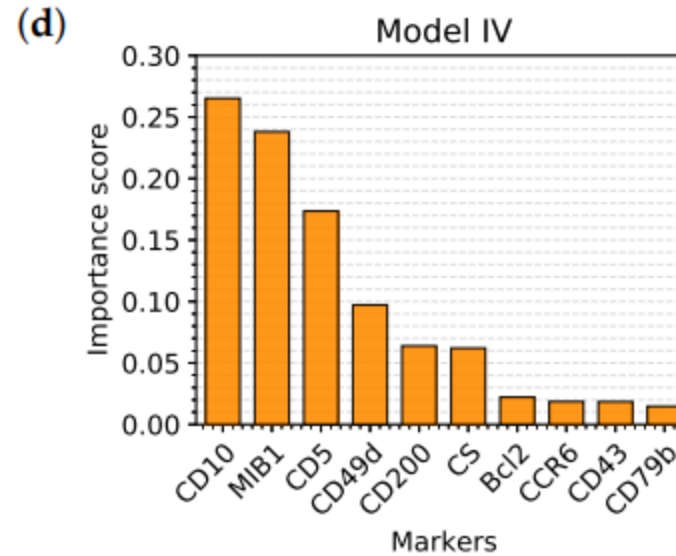
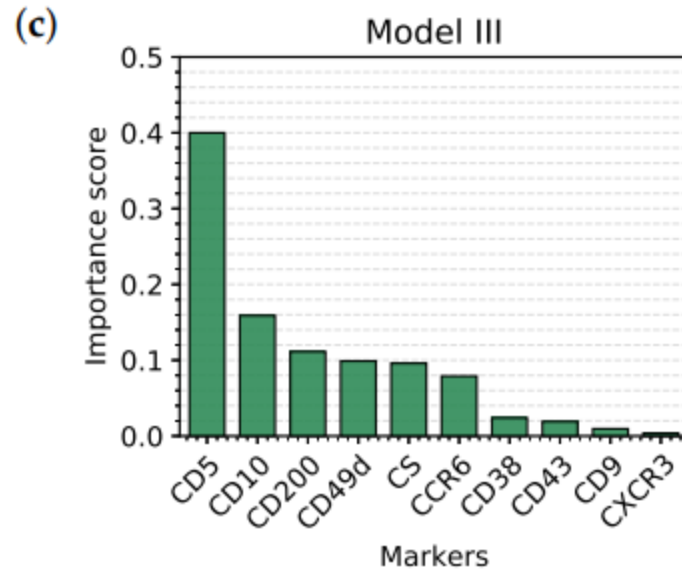
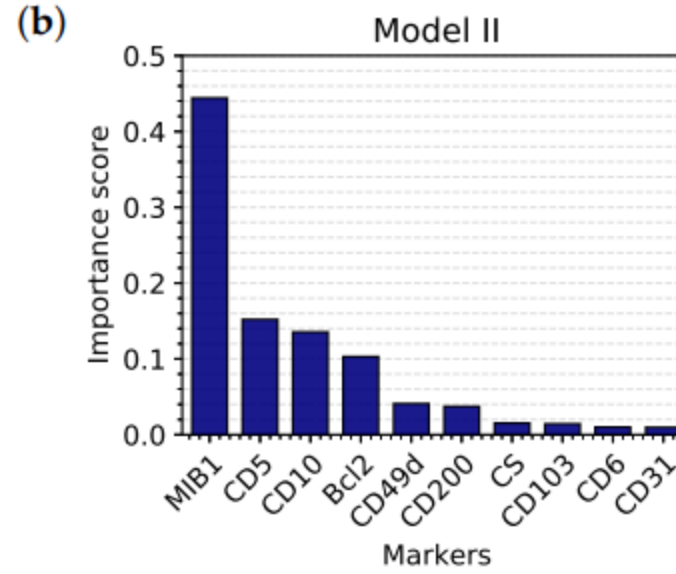
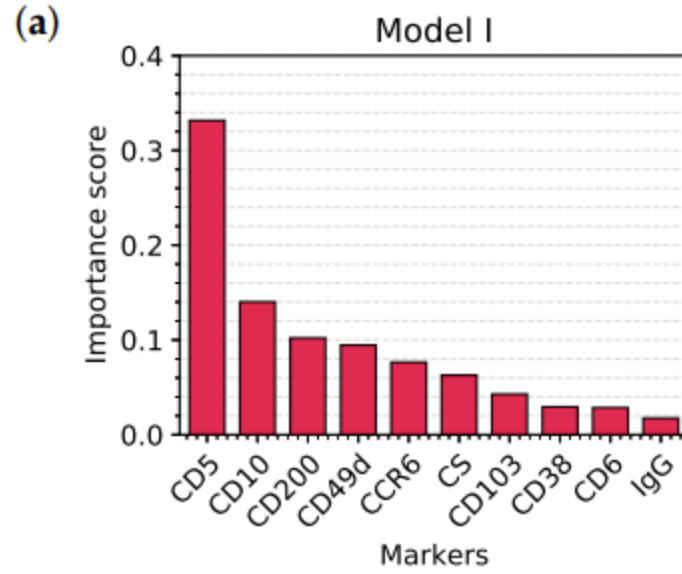
(c)



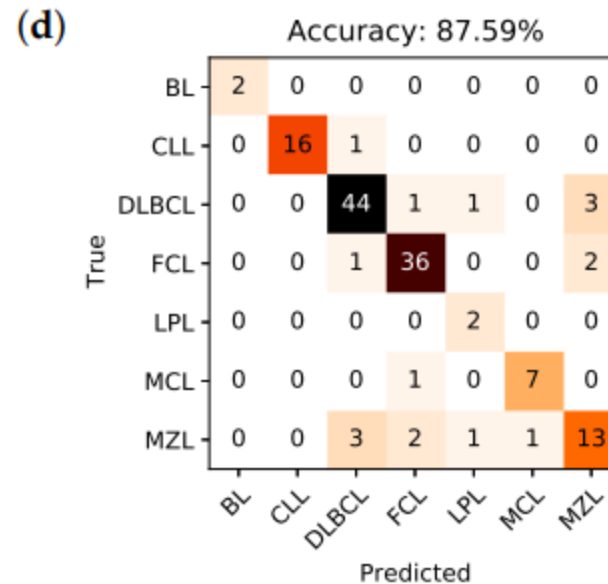
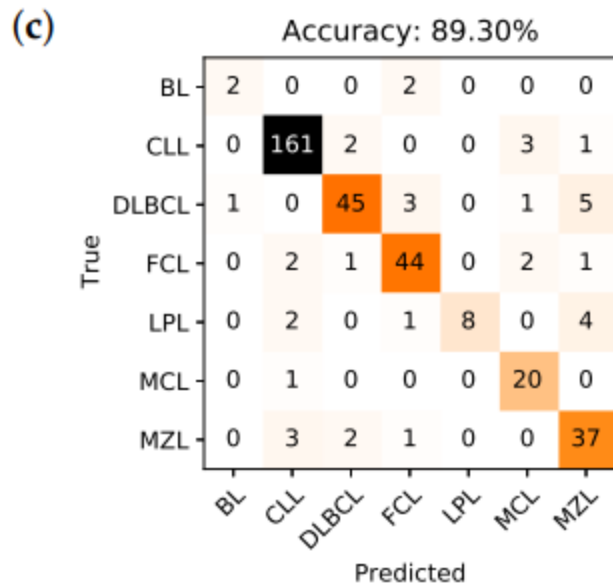
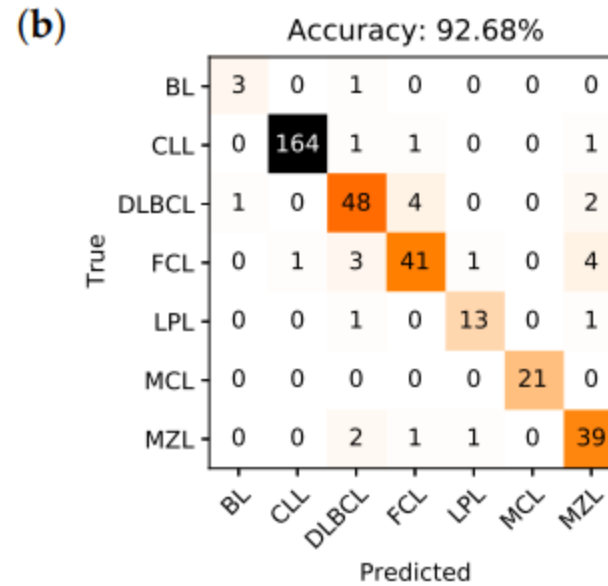
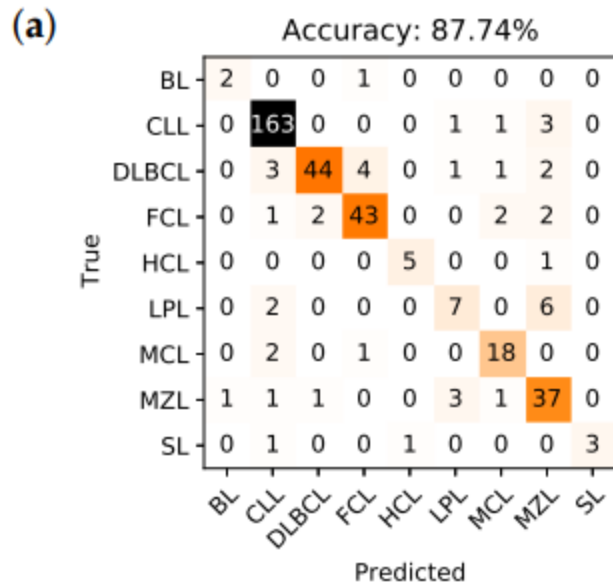
(d)



Machine Learning: training set top ten markers



Machine Learning: Validation set confusion matrix and predictive accuracy



Training set accuracy

Model I: 85.97%

Model II: 91.27%

Model III: 86.67%

Model IV: 87.35%

No overfitting:

the predictive models are not too rigid nor calibrated on the training set, but they are rather capable of generalizing classification rules on new data quite effectively

The on-line interface

Deep Marple Home Dynamic Analysis Complete Analysis Static Tube Analysis Logout

Logged in as test_user_3245

Dynamic Analysis

A guided cytoflow analysis procedure

Two predictive models are available with slightly different performance are available.

Try it

Complete Analysis

Predict samples described through 23 markers.

A single model is available. All marker values must be provided.

Try it

Static Tube Analysis

A Tube-wise cytoflow analysis tool

Four different *hand-crafted* tubes can be combined together. The best model is generated each time depending on tubes arrangement.

Try it

The on-line interface

Dynamic analysis

Evaluate Sample

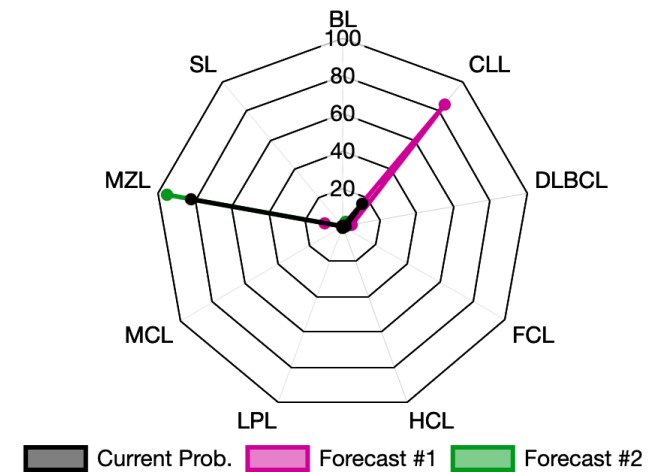
Select which model you would like to use. **Model1** includes all markers, whereas **Model2** does not take into account Intracellular and Immunoglobulin markers.

Adopted model: Model1

Once a model is selected, provide a value for each marker.

| | |
|-------|----|
| CD5 | 1 |
| MIB1 | 12 |
| CD10 | 1 |
| Bcl2 | 99 |
| CD103 | 0 |
| CD6 | |

[Evaluate](#) [New sample](#)



The on-line interface

Dynamic analysis

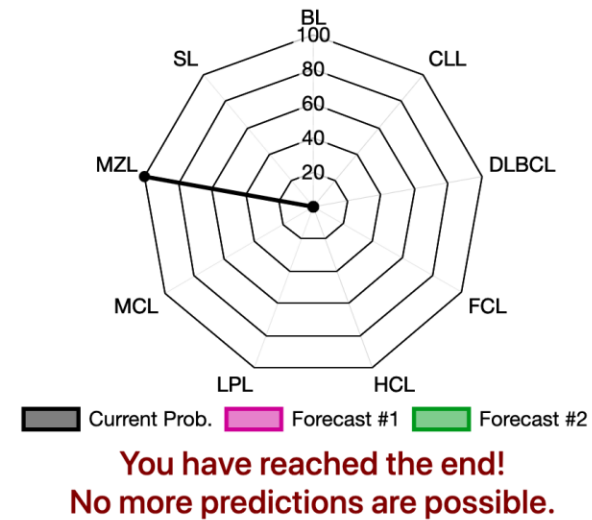
Evaluate Sample

Select which model you would like to use. **Model1** includes all markers, whereas **Model2** does not take into account Intracellular and Immunoglobulin markers.

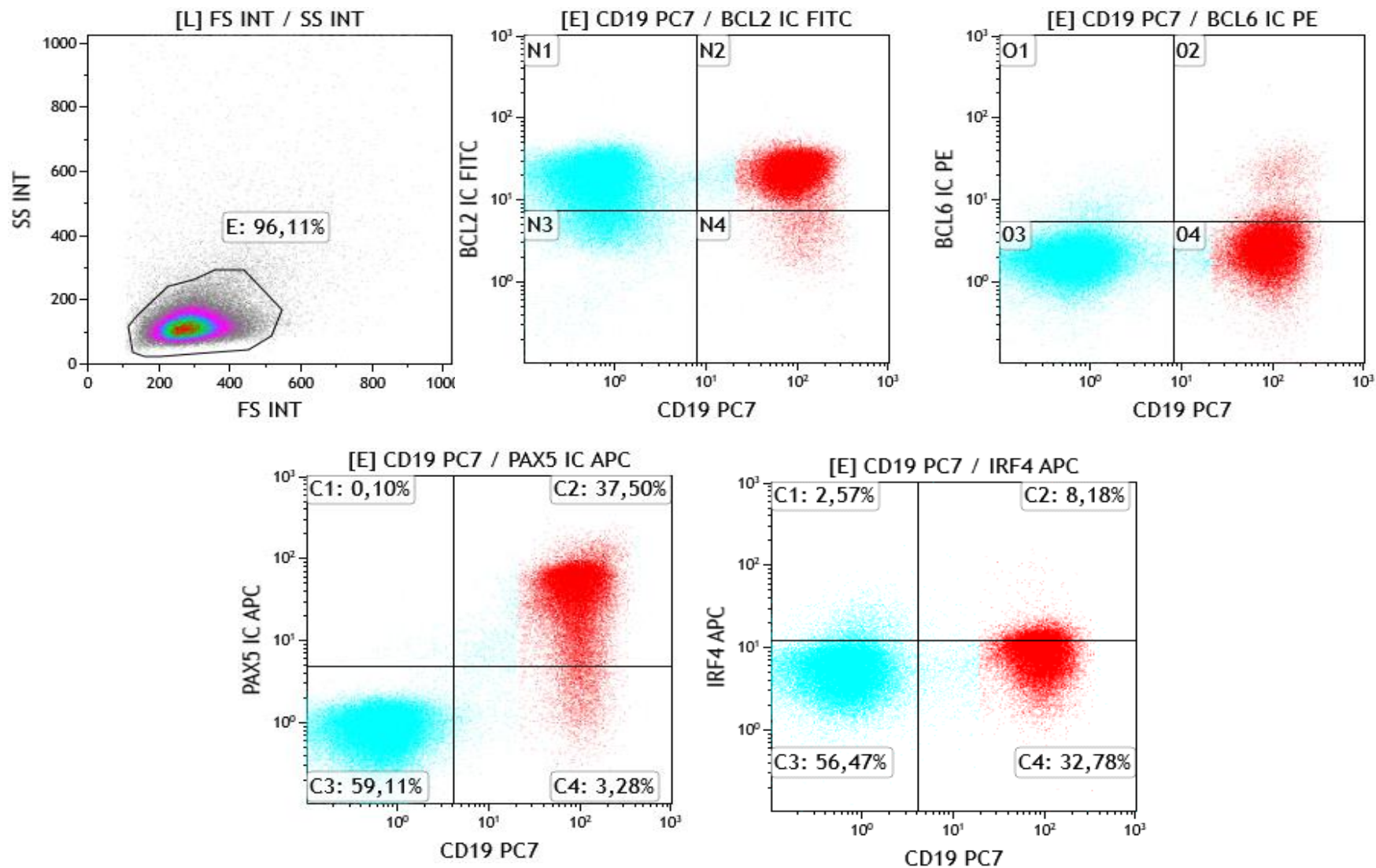
Adopted model: Model1

Once a model is selected, provide a value for each marker.

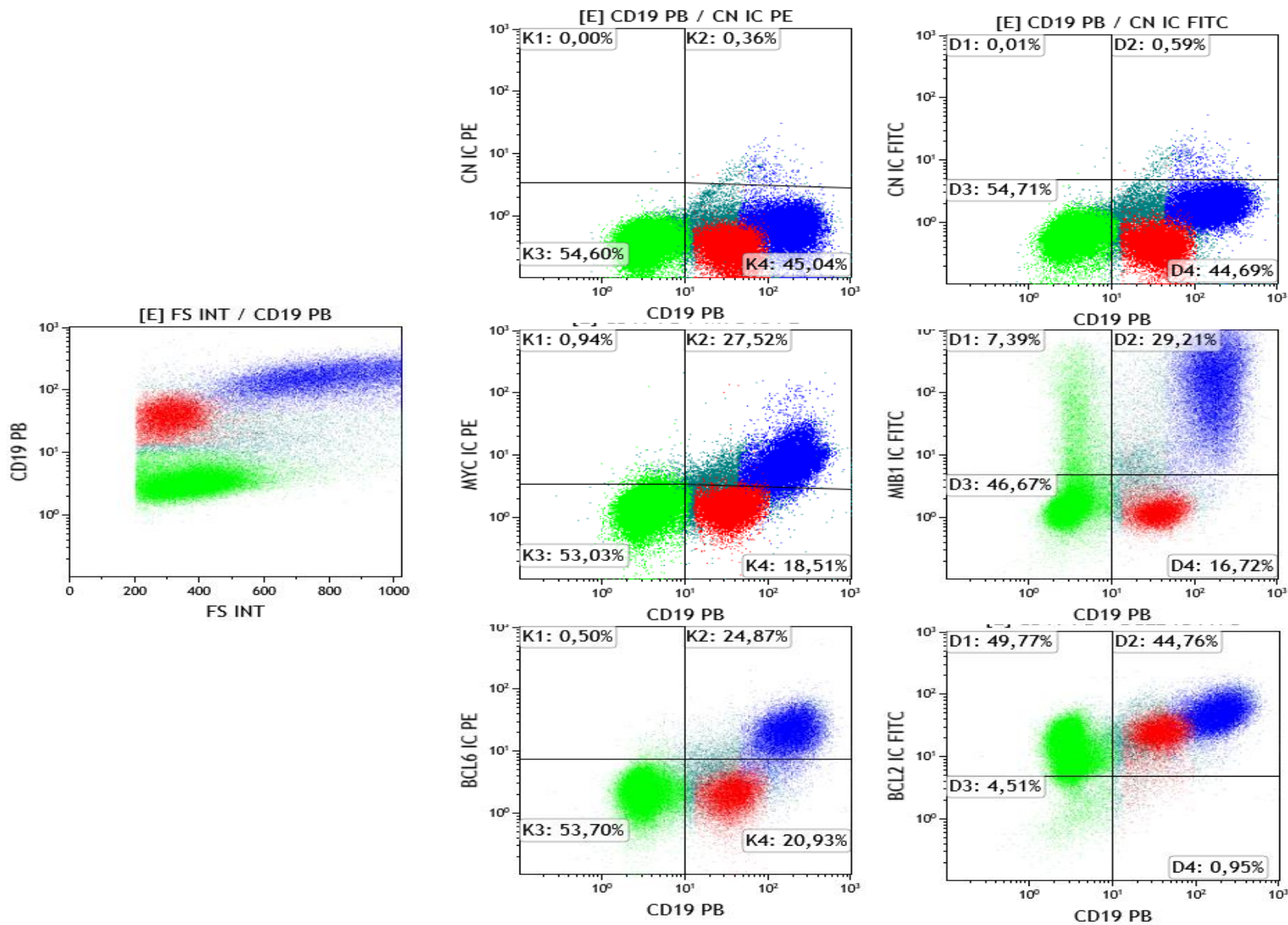
| | |
|-------|----|
| CD5 | 1 |
| MIB1 | 12 |
| CD10 | 1 |
| Bcl2 | 99 |
| CD103 | 0 |
| CD6 | 3 |
| FMC7 | 67 |



BCL2, BCL6, PAX5, IRF4



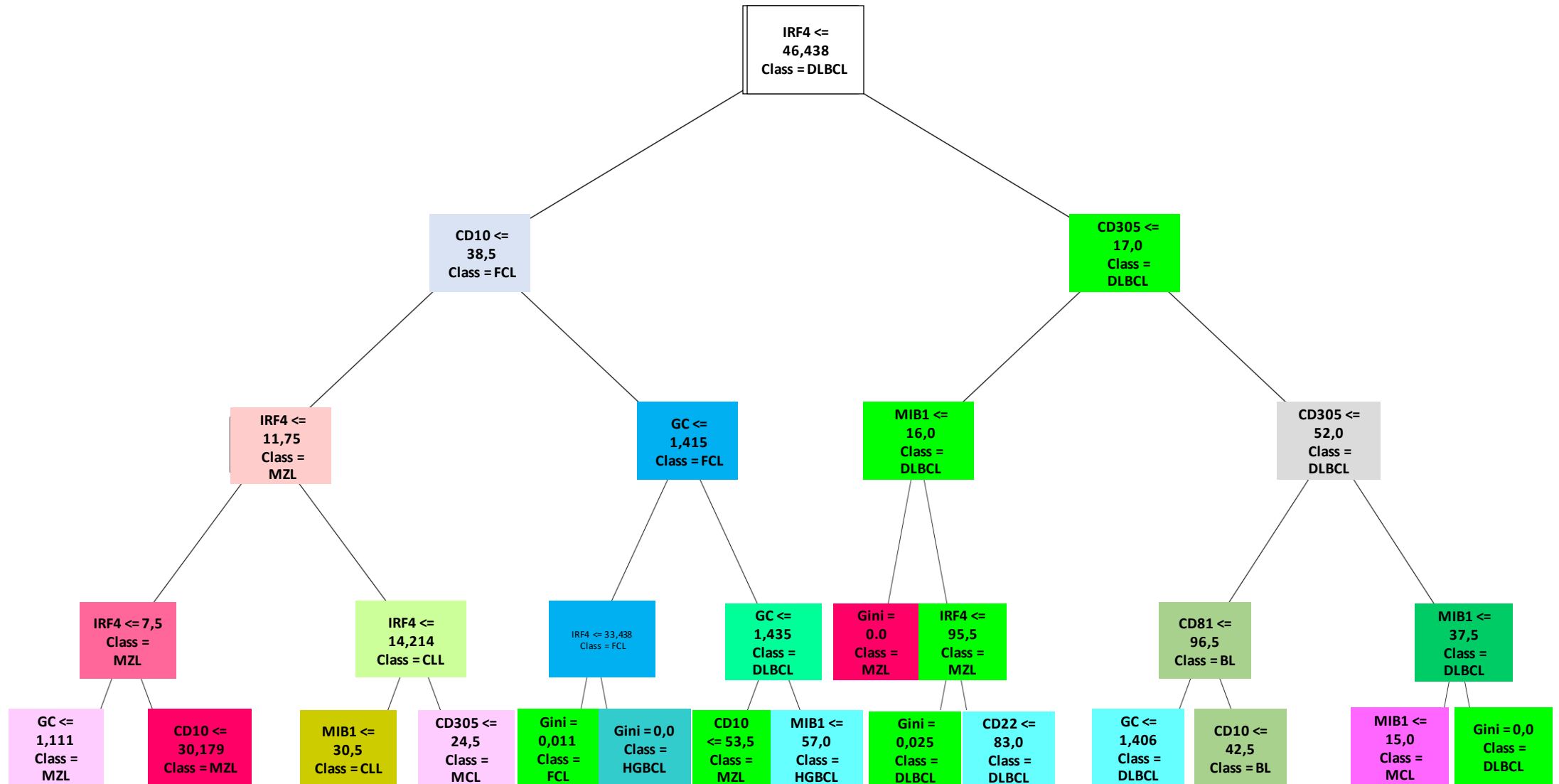
MYC, BCL6, MIB1, BCL2



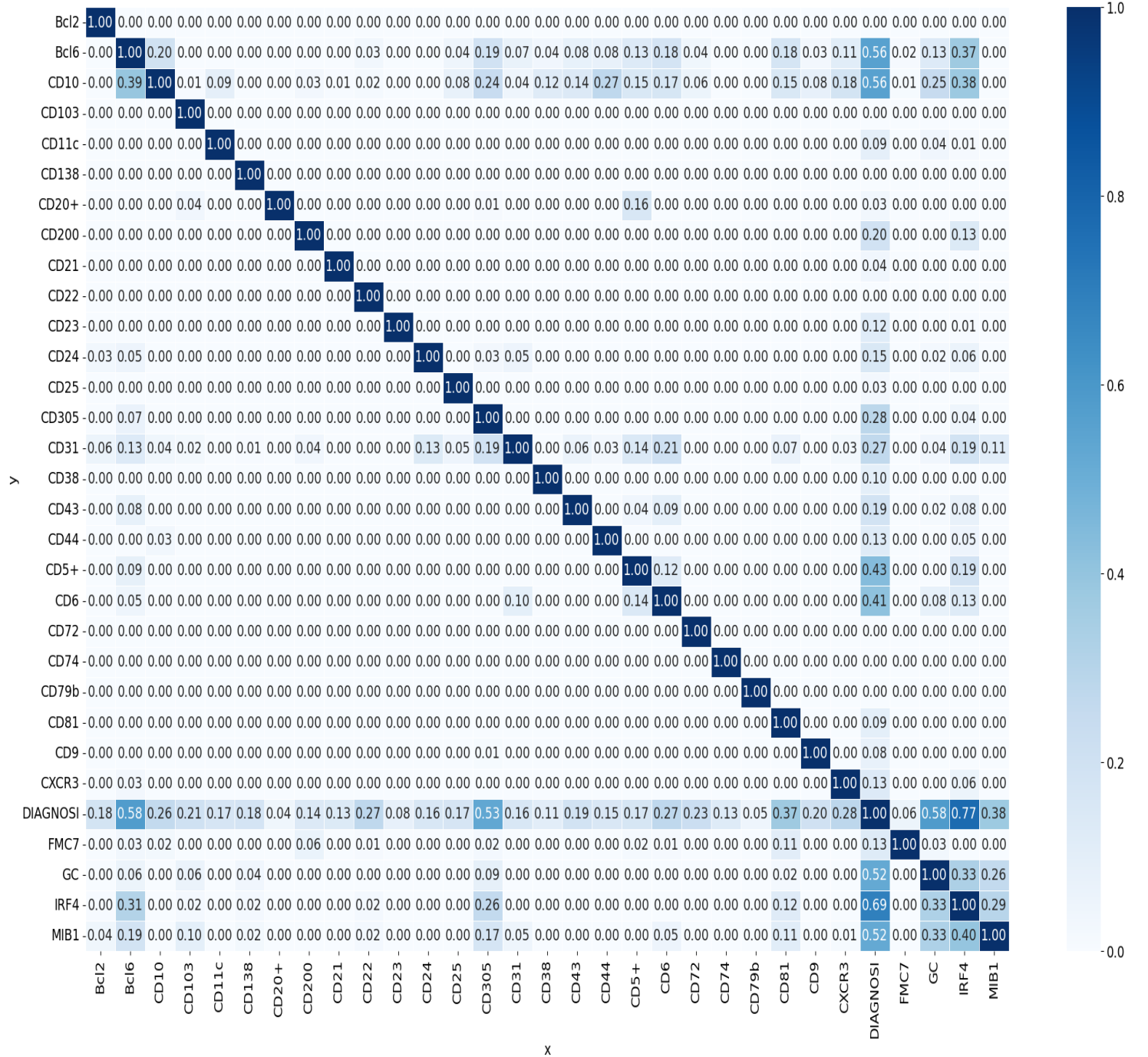
Distribution of 618 tissue samples in B-NHL categories according to WHO-HAEM4R

| diagnosis | tot cases | average age at diagnosis | cases F | average age F | cases M | average age M |
|-------------|-----------|--------------------------|---------|---------------|---------|---------------|
| DLBCL | 193 | 66 | 96 | 68 | 97 | 63 |
| FL | 193 | 64 | 102 | 63 | 91 | 65 |
| MZL | 92 | 67 | 41 | 70 | 51 | 68 |
| CLL | 67 | 71 | 31 | 72 | 36 | 69 |
| MCL | 27 | 64 | 13 | 65 | 14 | 63 |
| LPL | 16 | 69 | 7 | 71 | 9 | 68 |
| HGBL | 20 | 67 | 9 | 73 | 11 | 62 |
| BL | 10 | 48 | 1 | 70 | 9 | 46 |
| Total cases | 618 | | 300 | | 318 | |

Classification tree identifies as root node the intracellular marker IRF4/MUM1



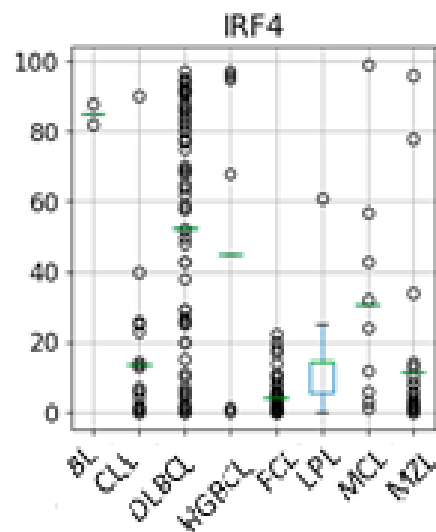
PPScore to assess the impact of each marker in defining each lymphoma category



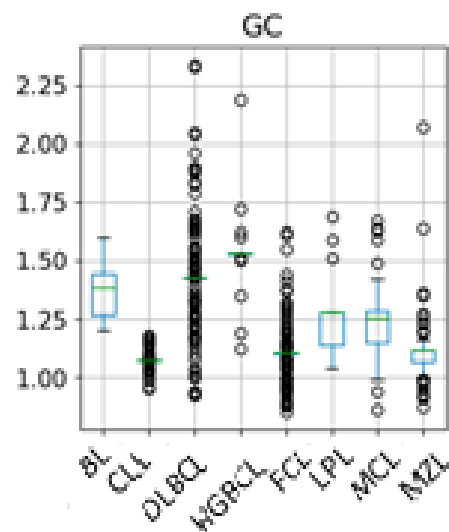
PPScore: top ten markers

| x | y | ppscore | case | is_valid_score | metric | baseline_score | model_score | model |
|-------|----------|----------|----------------|----------------|-------------|----------------|-------------|--------------------------|
| IRF4 | DIAGNOSI | 0.772383 | classification | True | weighted F1 | 0.234146 | 0.825679 | DecisionTreeClassifier() |
| GC | DIAGNOSI | 0.582396 | classification | True | weighted F1 | 0.234146 | 0.680176 | DecisionTreeClassifier() |
| Bcl6 | DIAGNOSI | 0.580251 | classification | True | weighted F1 | 0.234146 | 0.678534 | DecisionTreeClassifier() |
| CD305 | DIAGNOSI | 0.528243 | classification | True | weighted F1 | 0.234146 | 0.638703 | DecisionTreeClassifier() |
| MIB1 | DIAGNOSI | 0.376755 | classification | True | weighted F1 | 0.234146 | 0.522685 | DecisionTreeClassifier() |
| CD81 | DIAGNOSI | 0.374881 | classification | True | weighted F1 | 0.234146 | 0.521250 | DecisionTreeClassifier() |
| CXCR3 | DIAGNOSI | 0.279717 | classification | True | weighted F1 | 0.234146 | 0.448369 | DecisionTreeClassifier() |
| CD6 | DIAGNOSI | 0.272696 | classification | True | weighted F1 | 0.234146 | 0.442992 | DecisionTreeClassifier() |
| CD22 | DIAGNOSI | 0.271921 | classification | True | weighted F1 | 0.234146 | 0.442398 | DecisionTreeClassifier() |
| CD10 | DIAGNOSI | 0.260594 | classification | True | weighted F1 | 0.234146 | 0.433723 | DecisionTreeClassifier() |

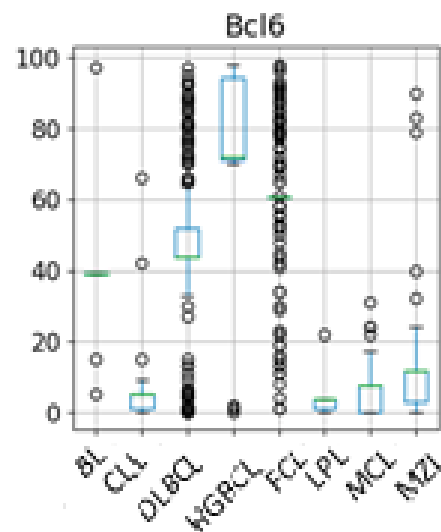
PPScore: top ten markers distribution



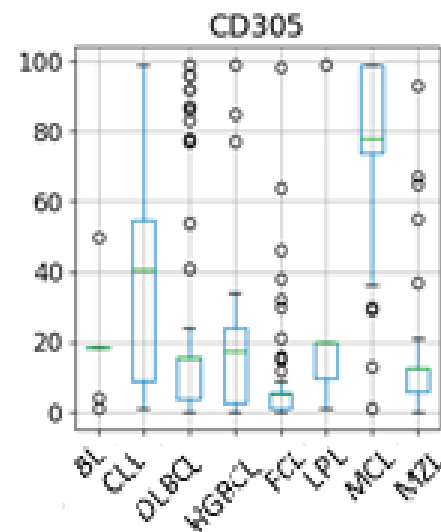
DIAGNOSIS



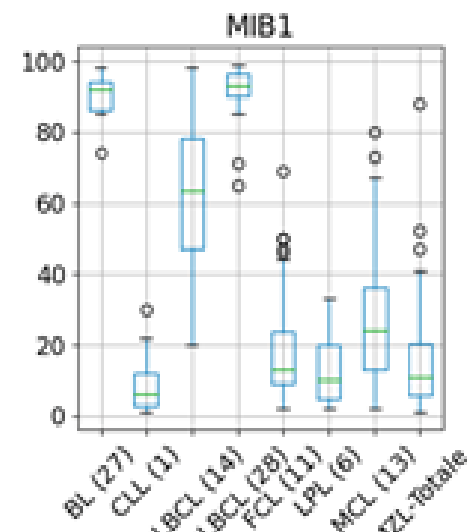
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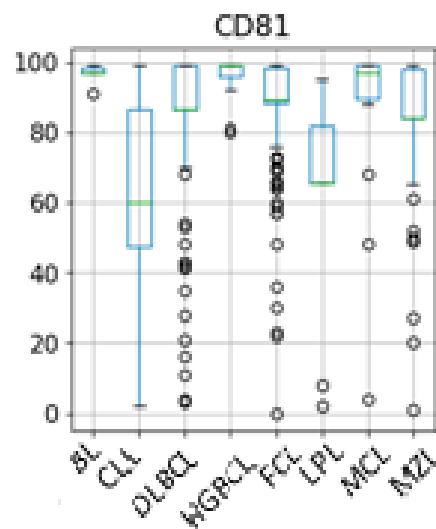
DIAGNOSIS



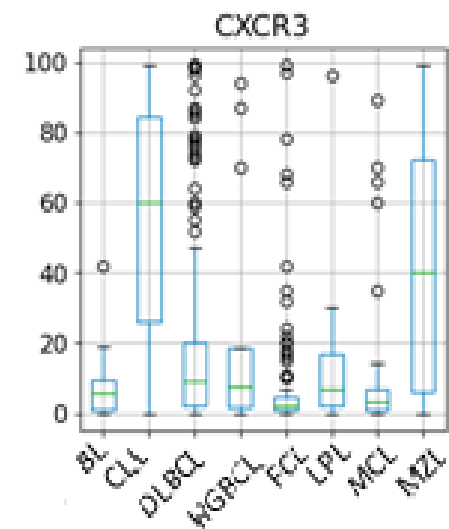
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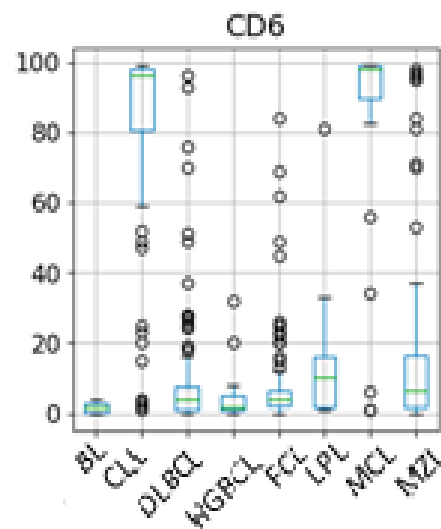
DIAGNOSIS



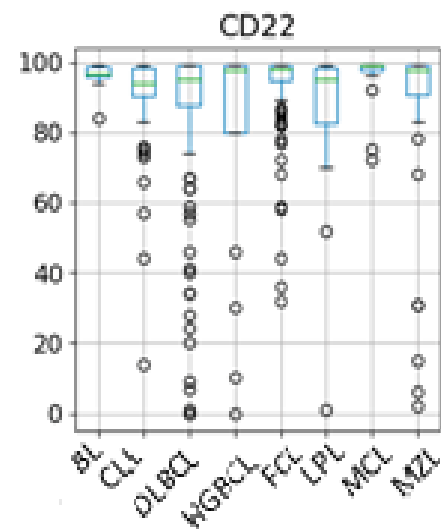
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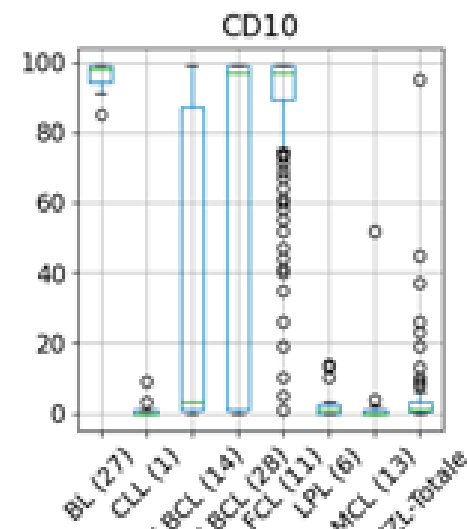
DIAGNOSIS



DIAGNOSIS



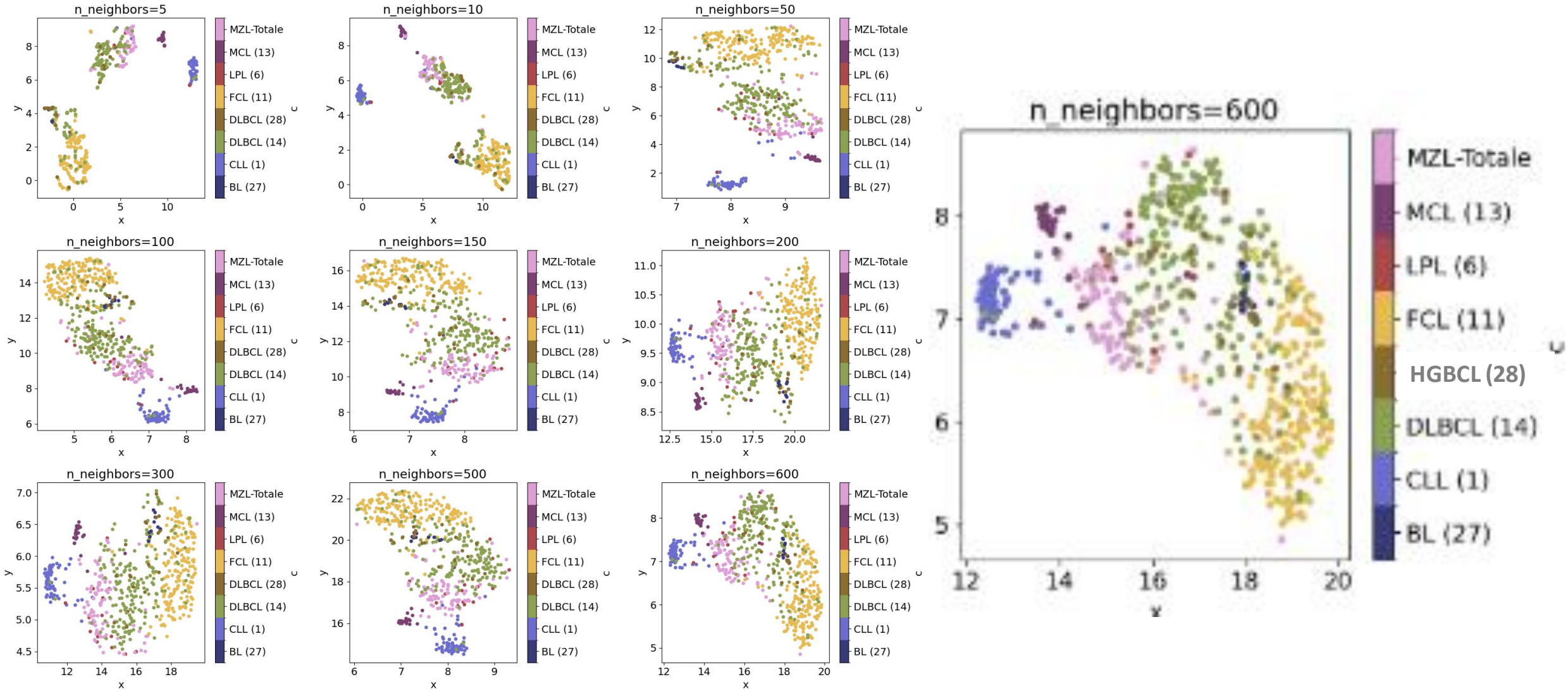
DIAGNOSIS



DIAGNOSIS

UMAP dimensionality reduction analysis

UMAP Exploration (all markers)



Conclusions and perspectives

- **The FC phenotype of mature B-cell lymphomas is effective in identifying the major groups of B-NHLs.**
- **The use of a larger number of markers increases the discriminatory power of the immunophenotype**
- **The use of intracellular markers, although requiring more technical effort, is very useful for the classification of B-NHLs, and increases the predictive power of immunophenotype**
- **Many of the current artificial intelligence algorithms are able to correctly use information derived from immunophenotypic analysis and create predictive models useful for guiding diagnosis**
- **It is desirable that the efforts of researchers and companies will be focused on achieving an appropriate level of standardization of analytical procedures, data analysis and processing of results**

Acknowledgments

Elena Casanova
Nathalie Santoro
Margherita Vizzini
Giada Cocchiara

Giovanni De Rosa

Valentina Gaidano
Valerio Tenace



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