EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes

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Introduction

Laboratory diagnostics of hematological malignancies has three major applications: 1, establishing the diagnosis; 2, prognostic (sub)classification; and 3, evaluation of treatment effectiveness via detection of "minimal residual disease" (MRD). Over the past decade, several molecular techniques have brought new insights into classification and monitoring of treatment effectiveness. However, they have several major disadvantages: they frequently are time consuming (1-3 days or more), not applicable in all categories of patients, and cannot focus on cellular subpopulations without preceding purification steps. Flow cytometric immunophenotyping is the sole technique that fulfils the requirements of high speed, broad applicability at diagnosis and during follow-up, and accurate focusing on the malignant cell population using membrane-bound and intracellular proteins as targets.

However, innovations are needed in flow cytometry, such as development of novel antibodies, 8-color immunostaining protocols, and novel flow cytometry software for fast and easy interpretation of complex data and for automated pattern recognition, which are all key objectives of the EuroFlow consortium (EU-FP6 project LSHB-CT-2006-018708: "*Flow cytometry for fast and sensitive diagnosis and follow-up of haematological malignancies*"). These innovations need a multidisciplinary translational research approach using cutting edge technologies and biological data arising from genomic research, which can be addressed best via close collaboration between industry and academia. The EuroFlow consortium consists of two SME's and twelve diagnostic research groups, which are regarded as experts in the fields of flow cytometric and molecular diagnostics.

Consequently the EuroFlow members are working on: 1, development of new software for fast and easy handling of large data sets and for integration of 8-color stainings into a single multicolor data file (INFINICYT™); 2, development of standardized 8-color antibody panels for fast and easy flow cytometric diagnosis and classification of hematological malignancies as well as for sensitive monitoring of patients for evaluation of treatment effectiveness; 3, development of multiplex immunobead assays for detection of fusion proteins and oncoproteins per disease category (particularly ALL and AML) (see Weerkamp et al. Leukemia 2009; 23, in press); 4, development of software for automated pattern recognition of normal, reactive, and aberrant (malignant) leukocyte populations in blood and bone marrow; and 5, creation of a large data base with hundreds of well-defined normal, reactive and malignant cell samples, which can be used as ready-to-use template for fully automated comparison with newly analyzed patient samples.

Here we present the 8-color EuroFlow antibody panels, which have been developed over the last three years. The 8-color EuroFlow antibody panels and the EuroFlow standard operating procedure (SOP) have been designed for usage in combination with the INFINICYT software for data calculation and efficient data interpretation. It should be emphasized that the here provided panels are not random lists of antibodies per panel. On the contrary, the provided antibody combinations have been carefully attuned with special combinations of backbone markers and characterization markers for selecting and characterizing the cell

populations of interest. The INFINICYT software can automatically combine the immunophenotypic information of the selected cell populations from multiple tubes according to the so-called nearest neighbor calculations in which individual cells are matched with corresponding individual cells according to their backbone markers and scatter profile. This INFINICYT procedure transforms the here presented 8-color EuroFlow panels into 12, 16, or ≥ 20-color immunostainings, dependent on the number of tubes per panel and the number of backbone markers per tube.

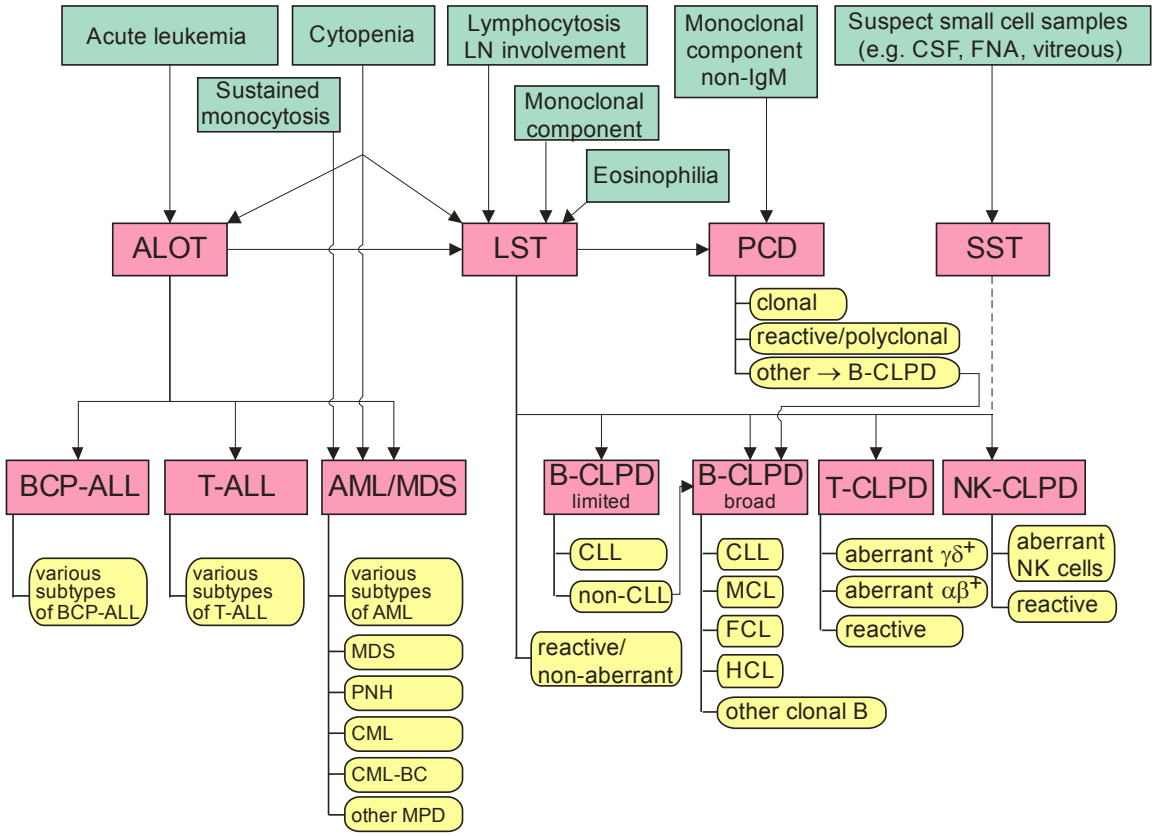

Figure 1. Diagnostic flow diagram showing the potential usage of the EuroFlow antibody panels. Multiple entries are possible, which are dependent on the clinical-diagnostic question and on the availability of cell material. For example, analysis with the "small sample tube" (SST) can be followed by analysis with additional antibody panels (see dotted line), if sufficient cell material is left over.

The 8 fluorochromes have been selected in several testing rounds for brightness, limited spectral overlap and limited need for compensation, stability, etc. After multiple testing rounds the EuroFlow Consortium has choosen for the following fluorochromes: Pacific Blue, Pacific Orange, fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyl protein/cyanine 5.5 (PerCp-Cy5.5), PE-Cy7, allophycocyanine (APC), and APC-H7.

The EuroFlow antibody panels, the EuroFlow SOP, and the INFINICYT software can be used in combination with all currently available flow cytometers that allow 8-color immunostainings, such as FACSCanto™ II, FACSAria, LSRII, DAKO CyAn™, etc. New flow cytometers should be checked for comparability with the proposed filter combinations (see EuroFlow SOP) and data acquisition.

Section 1: Acute leukemia orientation tube (ALOT)

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Background

The EuroFlow acute leukemia orientation tube (ALOT) was designed and approved for assessment of the nature of immature blast cell populations in acute leukemia samples (B, T versus non-lymphoid or mixed phenotype) and consequent orientation towards the most appropriate complementary antibody panel(s): BCP-ALL, T-ALL, and/or AML/MDS. The precise composition of the ALOT is provided in Table 1. The markers CD45, CD34 and CD19 serve as backbone markers when the information of the ALOT is combined with the BCP-ALL panel (using the INFINICYT software), whereas the markers cyCD3, CD45, and smCD3 serve as backbone markers when the information of the ALOT is combined with the T-ALL panel (using the INFINICYT software). CD45 and CD34 are also used as part of the backbone marker set in the AML/MDS protocol (see Section 7).

The ALOT should not be used for exclusion of an hematological malignancy, because the ALOT antibody combination is not sufficient for that purpose. However, when the ALOT is combined with the LST and 4 tubes of the AML/MDS protocol (tubes 1, 2, 3, and 4), virtually all types of hematological malignancies can be detected (not classified) or excluded.

TABLE 1. Single tube EuroFlow screening combinations for acute leukemias (ALOT)*

Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
cyCD3	**CD45**	cyMPO	cyCD79a	**CD34**	**CD19**	CD7	**smCD3**

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; cy= cytoplasmic; sm= surface membrane.

Section 2: Lymphoid screening tube (LST)

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Background

The EuroFlow LST was designed and approved for evaluation of several suspected clinical conditions, such as lymphocytosis, lymph node enlargement, splenomegaly, monoclonal serum components, unexplained neurological symptoms, unexplained cytopenias, etc. The precise composition of the LST is provided in Table 2. This tube detects aberrant mature lymphocyte populations of B, T and NK lineage. However, this 8-color tube does not allow the precise diagnosis and classification of the detected aberrant lymphocyte populations. This needs further characterization with the PCD, B-CLPD, T-CLPD or NK-CLPD panels (see Sections 4, 8, 9 and 10, respectively).

TABLE 2. Single tube EuroFlow screening combination for mature lymphoid cells.*

Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
CD20 and CD4	**CD45**	CD8 and smIgλ	CD56 and smIgκ	CD5	**CD19** and TCRγδ	smCD3	CD38

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; sm= surface membrane.

Section 3: Small sample tube (SST)

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Background

The EuroFlow small sample tube (SST) is a modified version of the EuroFlow LST, specially designed for evaluation of suspect small samples and samples with (very) low cell counts, such as fine needle aspirates (FNA), cerebrospinal fluid (CSF), vitreous humor, etc. For this special aim, the tube allows the unequivocal recognition of normal leukocytes present in these samples, e.g. B, T, NK cells and monocytes as well as any coexisting aberrant cell population. The precise composition of the SST is provided in Table 3.

TABLE 3. Single tube EuroFlow combination for mature lymphoid cells for small cell samples.*

Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
CD20	**CD45**	CD8 and smIgλ	CD56 and smIgκ	CD4	**CD19**	smCD3 and CD14	CD38

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; sm= surface membrane.

Section 4: Plasma cell dyscrasia (PCD) tubes

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Background

The EuroFlow antibody panel for plasma cell dyscrasias (PCD) consists of two tubes with four backbone markers: CD45, CD138, CD38 and CD19 (Table 4). The PCD panel aims at the identification and enumeration of plasma cells as well as at the discrimination between normal polyclonal plasma cells such as in reactive plasmacytosis versus aberrant monoclonal plasma cells such as in monoclonal gammapathies of undetermined significance (MGUS), smoldering and symptomatic multiple myeloma (MM), plasma cell leukemias (PCL), and extramedullary plasmacytoma. In combination with the EuroFlow LST and B-CLPD panels, this multi-tube antibody panel will also contribute to the diagnosis of other plasma cell dyscrasias such as Waldenström's macroglobulinemia and lymphoplasmacytic lymphoma (LPL).

TABLE 4. Two-tube EuroFlow classification panel for plasma cell dyscrasias (PCD).*

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	**CD45**	**CD138**	**CD38**	CD56	β2micro	**CD19**	cylgκ	cylgλ	Detection of aberrant and clonal plasma cells
2	**CD45**	**CD138**	**CD38**	CD28	CD27	**CD19**	CD117	CD81	Complementary phenotypic characterization and evaluation of markers with potential prognostic impact

***** Further information about the markers and the availability of hybridoma clones is summarized in Appendix A Backbone markers are indicated in bold; cy= cytoplasmic.

****** The described marker combinations can also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Section 5: Antibody panel for B-cell precursor ALL (BCP-ALL)

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Background

The EuroFlow BCP-ALL panel aims at the recognition and classification of all classically defined BCP-ALL (pro-B-ALL, common-ALL, pre-B-ALL) or alternative BCP-ALL classifications, including immunophenotypic classifications associated with well-defined molecular aberrations, such as specific fusion genes. The information obtained with the BCP-ALL tube set needs to be combined with the ALOT (Table 1), based on the backbone markers CD45, CD34 and CD19 and using the INFINICYT software (Table 5).

TABLE 5. Multi-tube EuroFlow classification panel for BCP-ALL*

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	CD20	**CD45**	CD58	CD66c	**CD34**	**CD19**	CD10	CD38	Diagnosis and classification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations
2	smIgκ	**CD45**	cyIgμ	CD33	**CD34**	**CD19**	smIgμ and CD117	smIgλ	Diagnosis and classification of BCP-ALL
3	CD9	**CD45**	nuTdT	CD13	**CD34**	**CD19**	CD22	CD24	Diagnosis and classification of BCP-ALL; Detection of phenotypes associated with molecular aberrations; Detection of LAP markers
4	CD21	**CD45**	CD15 and CDw65	NG2	**CD34**	**CD19**	CD123	CD81	Subclassification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; cy= cytoplasmic; sm= surface membrane; nu= nuclear; LAP= leukemia associated phenotype.
** The described marker combinations can also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Section 6: Antibody panel for T-cell ALL (T-ALL)

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Background

The EuroFlow T-ALL panel consists of four tubes and uses cyCD3, CD45, and smCD3 as backbone markers (see Table 6). The T-ALL panel aims at the recognition and classification of all classically defined T-ALL (immature T-ALL, common thymocytic T-ALL, mature T-ALL) or alternative T-ALL classification, e.g. based on TCR protein expression (cyTCRβ, TCRαβ, TCRγδ) or based on association with well-defined molecular aberrations. The information obtained with the T-ALL tube set needs to be combined with the ALOT (Table 1), based on the backbone markers CD45, CD3 and cyCD3 and using the INFINICYT software (Table 5).

TABLE 6 Multi-tube EuroFlow classification combinations for T-ALL.*

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	**cyCD3**	**CD45**	nuTdT	CD99	CD5	CD10	CD1a	**smCD3**	Diagnosis of T-ALL, classification of T-ALL, and determine the maturation stage of arrest; identification of LAP markers
2	**cyCD3**	**CD45**	CD2	CD117	CD4	CD8	CD7	**smCD3**	Diagnosis of T-ALL, classification of T-ALL, and determine the maturation stage of arrest; identification of LAP markers
3	**cyCD3**	**CD45**	TCRγδ	TCRαβ	CD33	CD56	cyTCRβ	**smCD3**	Diagnosis of T-ALL and determine the maturation stage of arrest; identification of LAP markers
4	**cyCD3**	**CD45**	CD44	CD13	HLADR	CD45RA	CD123	**smCD3**	Subclassification of T-ALL

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; cy= cytoplasmic; sm= surface membrane; nu= nuclear; LAP= leukemia associated phenotype.
** The described marker combinations can also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Section 7: Antibody panel for AML and MDS

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Background

The EuroFlow antibody panel for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) consists of three complementary marker combinations (tube 1-4, tube 5-6, and tube 7 in Table 7), all of them containing HLA-DR, CD45, CD34 and CD117 as backbone markers (Table 7). The first set of tubes (tubes 1-4) aims at the detection and classification (lineage assignment and maturation) of myeloid malignancies, such as in AML and MDS, with a major focus on immature neutrophilic lineage (tube 1), monocytic lineage (tube 2), erythroid lineage (tube 3), and aberrant expression of lymphoid-associated markers and abnormal lymphoid maturation (tube 4). These four tubes also contribute to the detection of paroxysmal nocturnal hemoglobinuria (PNH) (tube 1 and 2) and other aberrant myeloid phenotypes. In case of AML suspicion, these tubes should always be used in combination with EuroFlow ALOT (see Table 1).

The second set of tubes (tubes 5-6) should be used in addition to tube 1-4 and the ALOT in AML cases and provides information about megakaryocytic, basophilic, and plasmacytoid dendritic lineages (tube 5), as well as additional information about mono/myeloid development and aberrant myeloid phenotypes.

The third set (tube 7) should be used (together with tube 1-6 and ALOT) if a megakaryocytic leukemia is suspected. This tube may also be used to detect mastocytosis in association (or not) with AML/MDS.

TABLE 7. Multi-tube EuroFlow classification combinations for AML/MDS. *

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerC-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
AML/MDS									
1	**HLADR**	**CD45**	CD16	CD13	**CD34**	**CD117**	CD11b	CD10	Diagnosis and subclassification of AML and PNH especially focused on neutrophilic lineage
2	**HLADR**	**CD45**	CD35	CD64	**CD34**	**CD117**	IREM2	CD14	Diagnosis and subclassification of AML and PNH especially focussed on monocytic lineage
3	**HLADR**	**CD45**	CD36	CD105	**CD34**	**CD117**	CD33	CD71	Diagnosis and subclassification of AML especially focused on erythroid lineage
4	**HLADR**	**CD45**	nuTdT	CD56	**CD34**	**CD117**	CD7	CD19	Aberrant expression of lymphoid-associated markers and abnormal lymphoid maturation
AML									
5	**HLADR**	**CD45**	CD15	NG2	**CD34**	**CD117**	CD22	CD38	Aberrant expression of markers; detection of stem cells
6	**HLADR**	**CD45**	CD42a and CD61	CD203c	**CD34**	**CD117**	CD123	CD4	Diagnosis and subclassification of AML especially focused on megakaryocytic, basophilic, and plasmacytoid dendritic lineages
AML-M7									
7	**HLADR**	**CD45**	CD41	CD25	**CD34**	**CD117**	CD42b	CD9	Characterization of AML-M7, mastocytosis

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; nu= nuclear.
** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Section 8: Antibody panel for B-cell chronic lymphoproliferative diseases (B-CLPD)

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Background

The B-CLPD panel is designed to diagnose mature B-cell malignancies according to WHO entities using flow cytometric data only (see Table 8). The B-CLPD panel was designed to work when gating on the backbone markers CD20, CD19 and CD45 results in at least 90% purity of the malignant B-cell population. The integration of LST information (e.g. via INFINICYT software) is a prerequisite for the diagnosis of B-NHL entities in all cases. Tubes 2 and 3 contain the most informative markers for B-NHL classification. Those markers on their own allow the classification of most mature B-cell malignancies with typical immunophenotypes (e.g. CLL, HCL). The addition of tubes 4 and 5 is useful to distinguish immunophenotypically similar diseases from each other (e.g. DLBCL, LPL, MZL) or for further clarification in atypical cases.

The modular design of the panel avoids the necessity to stain the whole panel if the pretest probability for a particular B-cell malignancy is high. In those instances the panel will allow to diagnose a particular entity using a reduced number of tubes. For example, the LST plus tube 2 are sufficient to indentify CLL with a very high positive predictive value (PPV).

TABLE 8. Multi-tube EuroFlow classification combinations for B-CLPD *

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim **
1	**CD20** and CD4	**CD45**	CD8 and smIgλ	CD56 and smIgκ	CD5	**CD19** and TCRγδ	smCD3	CD38	LST tube; detection of B-CLPD
2	**CD20**	**CD45**	CD23	CD10	CD79b	**CD19**	CD200	CD43	Identification of CLL vs other B-CLPD cases, when combined with LST
3	**CD20**	**CD45**	CD31	LAIR1	CD11c	**CD19**	smIgμ	CD81	
4	**CD20**	**CD45**	CD103	CD95	CD22	**CD19**	CXCR5	CD49d	Further subclassification of non-CLL B-CLPD, e.g. HCL, MCL, FL, MZL, LPL, DLBCL and other B-CLPD
5	**CD20**	**CD45**	CD62L	CD39	HLADR	**CD19**	CD27		

*Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; sm= surface membrane. ** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Section 9: Antibody panel for T-cell chronic lymphoproliferative diseases (T-CLPD)

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11, Cytognos SL, Salamanca, ES.

Background

The EuroFlow T-CLPD aims for diagnosis and classification of mature T-cell malignancies (Table 9). The panel is designed to work in cases in which the malignant T cell population can be purified to > 90% using the backbone-markers smCD3, CD4, CD8, and CD45, regardless of the cell material analyzed. The combination of LST and T-CLPD tubes detects T-cell malignancies of both TCR$\alpha\beta$ and TCR$\gamma\delta$ lineages.

TABLE 9. Multi-tube EuroFlow classification combinations for T-CLPD.*

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	**CD4**	**CD45**	CD7	CD26	**smCD3**	CD2	CD28	**CD8**	Phenotypic characterization; Identification of Sézary syndrome
2	**CD4**	**CD45**	CD27	CCR7	**smCD3**	CD45RO	CD45RA	**CD8**	Phenotypic characterization; Assessment of maturation stage
3	**CD4**	**CD45**	CD5	CD25	**smCD3**	HLADR	cyTCL1	**CD8**	Phenotypic characterization; Identification of T-PLL
4	**CD4**	**CD45**	CD57	CD30	**smCD3**		CD11c	**CD8**	Phenotypic characterization; Cytotoxic phenotype and identification of anaplastic T-cell lymphoma
5	**CD4**	**CD45**	cyPerforin	cyGranzyme	**smCD3**	CD16	CD94	**CD8**	Phenotypic characterization; Assessment of cytotoxic-associated phenotypes; Identification of T-LGL
6	**CD4**	**CD45**		CD279	**smCD3**			**CD8**	Identification of lymphomas derived from follicular helper T cells (angioimmunoblastic T-cell lymphomas)

*Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; sm= surface membrane; cy= cytoplasmic.

** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Section 10: Antibody panel for NK-cell chronic lymphoproliferative diseases (NK-CLPD)

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11, Cytognos SL, Salamanca, ES.

Background

The EuroFlow NK-CLPD tubes aim at the discrimination between aberrant and normal/reactive NK-cells. The NK-CLPD panel uses four backbone markers: CD45, smCD3, CD56 and CD19 (Table 10).

TABLE 10. Multi-tube EuroFlow classification combinations for NK-CLPD.*

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	CD2	**CD45**	CD7	CD26	**smCD3**	**CD56**	CD5	**CD19**	Detection of aberrant NK cell phenotype
2	CD16	**CD45**	CD57	CD25	**smCD3**	**CD56**	CD11c	**CD19**	Detection of aberrant NK cell phenotype
3	HLADR	**CD45**	cyPerforin	cyGranzyme	**smCD3**	**CD56**	CD94	**CD19**	Detection of aberrant NK cell phenotype; Assessment of cytotoxic effector phenotype

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; sm= surface membrane; cy= cytoplasmic.

** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Conclusion

In conclusion, the presented EuroFlow antibody panels are composed of subsets of one or multiple combinations of antibodies (tubes) conjugated with eight different fluorochromes, each of said combinations of reagents having specific aims. These panels teach how to combine, fluorochrome-conjugated antibodies composed of 1, a set of markers required to be stained in common for appropriate and reproducible identification of the cell populations of interest in all stained aliquots of a sample, and 2, further characterization markers. In addition, information about the aim of each combination is also given as indication about when and how to apply it.

The EuroFlow antibody panels have been designed, for the immunophenotypic characterization of normal, reactive, regenerating vs. neoplastic cells in a wide variety of types of samples (i.e.: peripheral blood, bone marrow, pleural effusions, ascitis, spinal fluid, vitreous humor, synovial fluid, bronchoalveolar lavage, urine, spleen, liver, lymph node samples, among other samples). After careful selection of the relevant markers, design of appropriate combinations of antibodies in multi-color tubes, and the selection of suited fluorochromes (based on need for brightness, compensation, stability, etc.), a set of antibody reagents was developed. The studies were complemented with extensive multicentric evaluation of the consensus panels in order to reshape and achieve an optimal efficiency.

APPENDIX A. Markers applied in the EuroFlow panels and availability of antibodies

Marker*	Fluorochrome	Clone name	Company	Catalogue number	Application in EuroFlow panel
CD1a	APC	HI149	BD Biosciences	559775	T-ALL
CD2	FITC	RPA-2.10	BD Biosciences	555326	T-ALL
CD2	Pacific Blue	TS1/8	Biolegend	309216	NK-CLPD
CD2	PE-Cy7	S5.2	BD Biosciences	335821	T-CLPD
cyCD3	Pacific Blue	UCHT1	BD Biosciences	558117	ALOT, T-ALL
smCD3	APC	SK7	BD Biosciences	345767	LST, SST, B-CLPD
smCD3	APC-H7	SK7	BD Biosciences	641397	ALOT
smCD3	PerCP-Cy5.5	SK7	BD Biosciences	332771	T-CLPD, NK-CLPD
CD4	APC-H7	SK3	BD Biosciences	641398	AML
CD4	Pacific Blue	RPA-T4	BD Biosciences	558116	LST, B-CLPD, T-CLPD
CD4	PerCP-Cy5.5	SK3	BD Biosciences	332772	SST, T-ALL
CD5	APC	L17F12	BD Biosciences	345783	NK-CLPD
CD5	FITC	L17F12	BD Biosciences	345781	T-CLPD
CD5	PerCP-Cy5.5	L17F12	BD Biosciences	341109	LST, T-ALL, B-CLPD
CD7	APC	124-1D1	eBioscience	17-0079	ALOT, T-ALL, AML
CD7	FITC	4H9	BD Biosciences	347483	T-CLPD, NK-CLPD
CD8	APC-H7	SK1	BD Biosciences	641400	T-CLPD
CD8	FITC	UCH-T4	Cytognos	CYT-8F8	LST, SST, B-CLPD
CD8	PE-Cy7	SFCI21Thy2D3	Beckman Coulter	737661	T-ALL
CD9	APC-H7	M-L13	BD Biosciences	custom-conjugate	AML
CD9	Pacific Blue	MEM-61	Exbio	PB-208-T100	BCP-ALL
CD10	APC	HI10A	BD Biosciences	332777	BCP-ALL
CD10	APC-H7	HI10A	BD Biosciences	custom-conjugate	AML
CD10	PE	ALB1	Beckman Coulter	A07760	B-CLPD
CD10	PE-Cy7	HI10A	BD Biosciences	341112	T-ALL
CD11b	APC	D12	BD Biosciences	333143	AML
CD11c	APC	S-HCL-3	BD Biosciences	333144	T-CLPD, NK-CLPD
CD11c	PerCP-Cy5.5	B-Ly6	BD Biosciences	custom-conjugate	B-CLPD
CD13	PE	L138	BD Biosciences	347406	BCP-ALL, T-ALL, AML
CD14	APC	MϕP9	BD Biosciences	345787	SST
CD14	APC-H7	MϕP9	BD Biosciences	641394	AML
CD15	FITC	MMA	BD Biosciences	332778	BCP-ALL, AML
CD16	FITC	CLB-Fc-gran/15D2	Sanquin	M1604	AML
CD16	Pacific Blue	3G8	BD Biosciences	558122	NK-CLPD
CD16	PE-Cy7	3G8	BD Biosciences	557744	T-CLPD
CD19	APC-H7	SJ25C1	BD Biosciences	641395	AML, NK-CLPD
CD19	PE-Cy7	J3-119	Beckman Coulter	IM3628	ALOT, LST, SST, BCP-ALL, PCD
CD20	Pacific Blue	2H7	eBioscience	57-0209	LST, SST, BCP-ALL, B-CLPD
CD21	Pacific Blue	LT21	Exbio	PB-306-T100	BCP-ALL
CD22	APC	S-HCL-1	BD Biosciences	333145	BCP-ALL, AML
CD22	PerCP-Cy5.5	S-HCL-1	BD Biosciences	custom-conjugate	B-CLPD
CD23	FITC	MHM6	Dako	F7062	B-CLPD
CD24	APC-H7	ML5	BD Biosciences	custom-conjugate	BCP-ALL
CD25	PE	2A3	BD Biosciences	341011	AML, T-CLPD, NK-CLPD
CD26	PE	L272	BD Biosciences	340423	T-CLPD, NK-CLPD
CD27	APC	L128	BD Biosciences	337169	B-CLPD
CD27	FITC	L128	BD Biosciences	340424	T-CLPD
CD27	PerCP-Cy5.5	L128	BD Biosciences	custom-conjugate	B-CLPD, PCD
CD28	APC	CD28.2	BD Biosciences	559770	T-CLPD
CD28	PE	L293	BD Biosciences	348047	PCD

Marker*	Fluorochrome	Clone name	Company	Catalogue number	Application in EuroFlow panel
CD30	PE	BerH8	BD Biosciences	550041	T-CLPD
CD31	FITC	WM59	BD Biosciences	555445	B-CLPD
CD33	APC	P67,6	BD Biosciences	345800	AML
CD33	PE	P67,6	BD Biosciences	345799	BCP-ALL
CD33	PerCP-Cy5.5	P67,6	BD Biosciences	333146	T-ALL
CD34	PerCP-Cy5.5	8G12	BD Biosciences	347222	ALOT, BCP-ALL, AML
CD35	FITC	E11	BD Biosciences	555452	AML
CD36	FITC	CLB-IVC7	Sanquin	M1613	AML
CD38	APC-H7	HB7	BD Biosciences	Custom-conjugate	LST, SST, BCP-ALL, AML, B-CLPD
CD38	FITC	LD38	Cytognos	CYT-38F	PCD
CD39	PE	TU66	BD Biosciences	555464	B-CLPD
CD41	FITC	CLB-tromb/7, 6C9	Sanquin	M1674	AML
CD42a	FITC	GRP-P	Serotec	MCA1227F	AML
CD42b	APC	HIP1	BD Biosciences	551061	AML
CD43	APC-H7	IG10	BD Biosciences	custom-conjugate	B-CLPD
CD44	FITC	L178	BD Biosciences	347943	T-ALL
CD45	Pacific Blue	T29/33	Dako	PB986	PCD
CD45	Pacific Orange	HI30	Invitrogen	MHCD4530	ALOT, LST, SST, BCP-ALL, T-ALL, AML, B-CLPD, T-CLPD, NK-CLPD
CD45RA	APC	HI100	BD Biosciences	550855	T-CLPD
CD45RA	PE-Cy7	L48	BD Biosciences	337186	T-ALL
CD45RO	PE-Cy7	UCHL1	BD Biosciences	337168	T-CLPD
CD49d	APC-H7	9F10	BD Biosciences	custom-conjugate	B-CLPD
CD56	PE	C5.9	Cytognos	CYT-56PE	LST, SST, B-CLPD, PCD, AML
CD56	PE-Cy7	N901/NKH1	Beckman Coulter	A21692	T-ALL, NK-CLPD
CD57	FITC	HNK-1	BD Biosciences	333169	T-CLPD, NK-CLPD
CD58	FITC	1C3	BD Biosciences	555920	BCP-ALL
CD61	FITC	RUU-PL7F12	BD Biosciences	347407	AML
CD62L	FITC	SK11	BD Biosciences	347443	B-CLPD
CD64	PE	10.1	Serotec	MCA756PE	AML
CDw65	FITC	88H7	Beckman Coulter	IM1654U	BCP-ALL
CD66c	PE	KOR-SA3544	Beckman Coulter	IM2357U	BCP-ALL
CD71	APC-H7	M-A712	BD Biosciences	custom-conjugate	AML
cyCD79a	PE	HM57	Dako	R7159	ALOT
CD79b	PerCP-Cy5.5	SN8	BD Biosciences	custom-conjugate	B-CLPD
CD81	APC-H7	JS-81	BD Biosciences	custom-conjugate	BCP-ALL, PCD, B-CLPD
CD94	APC	HP-3D9	BD Biosciences	559876	T-CLPD, NK-CLPD
CD95	PE	DX2	BD Biosciences	555674	B-CLPD
CD99	PE	Tü12	BD Biosciences	555689	T-ALL
CD103	FITC	Ber-ACT8	BD Biosciences	550259	B-CLPD
CD105	PE	1G2	Beckman Coulter	A07414	AML
CD117	APC	104D2	BD Biosciences	333233	PCD, BCP-ALL
CD117	PE	104D2	BD Biosciences	332785	T-ALL
CD117	PE-Cy7	104D2D1	Beckman Coulter	IM3698	AML
CD123	APC	AC145	Miltenyi Biotec	130-090-901	BCP-ALL, T-ALL, AML
CD138	Pacific Orange	B-A38	Molecular Probes	custom-conjugation via outsourcing e.g. Exbio	PCD
CD200	APC	OX104	eBioscience	17-9200	B-CLPD
CD203c	PE	97A6	Beckman Coulter	IM3575	AML
CD279	PE	MIH4	BD Biosciences	557946	T-CLPD
β2micro	PerCP-Cy5.5	Tü99	BD Biosciences	custom-conjugate	PCD

Marker*	Fluorochrome	Clone name	Company	Catalogue number	Application in EuroFlow panel
CCR7	PE	150503	R&D Systems	FAB197P	T-CLPD
cyGranzyme	PE	CLB-GB11	Sanquin	M2289	T-CLPD, NK-CLPD
cyIgκ	APC	polyclonal rabbit serum	Dako	C0222	PCD
cyIgλ**	APC-H7	1-155-2	BD Biosciences	custom-conjugate	PCD
cyIgμ	FITC	polyclonal rabbit serum	Dako	F0058	BCP-ALL
cyMPO	FITC	MPO-7	Dako	F0714	ALOT
cyPerforin	FITC	δG9	BD Biosciences	556577	T-CLPD, NK-CLPD
cyTCL1	APC	eBio1-21	eBioscience	17-6699	T-CLPD
CXCR5	APC	51505	R&D Systems	FAB190A	B-CLPD
HLADR	Pacific Blue	L243	Biolegend	307624	AML, NK-CLPD
HLADR	PE-Cy7	L243	BD Biosciences	335830	T-CLPD
HLADR	PerCP-Cy5.5	L243	BD Biosciences	552764	T-ALL, B-CLPD
IREM-2	APC	UP-H2	Immunostep	IREM2A-T100	AML
LAIR1	PE	DX26	BD Biosciences	550811	B-CLPD
NG2	PE	7.1	Beckman Coulter	IM3454U	BCP-ALL, AML
smIgκ	Pacific Blue	TB28-2	Leeds team	custom-conjugation via outsourcing e.g. Exbio	BCP-ALL
smIgκ	PE	polyclonal	Cytognos	CYT-KF2-LPE	LST, SST, B-CLPD
smIgλ**	APC-H7	1-155-2	BD Biosciences	custom-conjugate	BCP-ALL
smIgλ	FITC	polyclonal	Cytognos	CYT-KF2-LPE	LST, SST, B-CLPD
smIgμ	APC	G20-127	BD Biosciences	551042	BCP-ALL, B-CLPD
TCRαβ	PE	IP26A	Beckman Coulter	A39499	T-ALL
cyTCRβ	APC	8A3 (βF1)	Cytognos	custom-conjugate	T-ALL
TCRγδ	FITC	IMMU510	Beckman Coulter	IM1571U	T-ALL
TCRγδ	PE-Cy7	11F2	BD Biosciences	custom-conjugate	LST, B-CLPD
nuTdT	FITC	HT6	Dako	F7139	BCP-ALL, T-ALL, AML

* cy= cytoplasmic; sm= surface membrane; nu= nuclear

**The same APC-H7 conjugated anti-Igλ antibody is used for both cytoplasmic Igλ (cyIgλ) as well as surface membrane Igλ (smIgλ) staining.