

# Multiparameter Flow Cytometry in the Diagnosis of Hematologic Malignancies



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**Preface** 

Together with cytological examination, flow cytometry is often the first exploration step in patients with clinical symptoms suggesting haematological malignancy or with fortuitously discovered anomalies in a whole blood cell count. Depending on the healthcare organisation, flow cytometry results will stand alone and be discussed later during a diagnostic conference, or be integrated in a comprehensive set of investigations including bone marrow biopsy morphology, cytogenetics and sophisticated molecular studies.

Over the years, knowledge and skills have developed so that in many cases the subtleties of the sets of markers, as well as their expression of absence, have become familiar to clinicians expecting a diagnosis. Yet, the thousands of references in the literature, dealing with this specific part of laboratory haematology, provide a good idea of the puzzlement that may overwhelm any novice in the field.

Thinking about the outlines of this book, we placed ourselves in the position of a young laboratory haematologist or haematopathologist and wondered which questions would need an answer likely to be found in a single document. We then asked Expert Friends to work with us with this aim in mind. Moreover, we wanted to focus on the new 8- and 10-colour methodologies.

We decided to start with basic characteristics of the structure and functions of flow cytometers, trying to provide a clear explanation of what sometimes seems to be very complex. We also depicted the analysis tools available in current software to make the most of acquired data (Chapter 1).

We then collected pertinent information about the structure, function and expression of a large number of the antigens investigated in flow cytometry, all mentioned somewhere in this book, together with a brief history of the way they were characterised or discovered (Chapter 2).

Before tackling pathological issues, we thought that readers would appreciate some information about what to expect when flow cytometry is applied to normal samples of blood, bone marrow or lymphatic tissue (Chapter 3). We also listed a series of non-malignant conditions where the hypothesis of malignancy is plausible and must be ruled out (Chapter 4).

In Chapter 5, we present a collection of typical flow cytometry graphs characteristic for various categories of acute leukaemia.

From Chapters 6 to 13, the authors considered specific sets of diseases and their idiosyncratic flow cytometry features.

Finally, Chapter 14 provides a glimpse at what lays ahead, in the already foreseeable developments of the versatile and powerful technology of cell analysis.

We built this book, not only as a manual that may be read through while starting to work with flow cytometry diagnostics, but also as a reference document to consult when interested in any aspect of flow cytometry diagnostics of haematological malignancies.

We hope that, together with our co-authors, we have reached that goal.

Anna Porwit and Marie-Christine Béné



### **Abbreviations**

7-AAD	7-aminoactinomycin D	ECD	Energy coupled dye
AIHA	Auto immune haemolytic anaemia		(phycoerythrine-Texas red
AITL	Angio-immunoblastic T-cell		conjugate)
	lymphoma	EDTA	Ethylene diamine tetraacetic acid
ALL	Acute lymphoblastic leukaemia	ETP-ALL	Early T-cell precursor acute
AML	Acute myeloid (or myeloblastic)		lymphoblastic leukaemia
	leukaemia	FCM	Flow cytometry
APC	Allophycocyanin	FITC	Fluorescein isothiocyanate
APL	Acute promyelocytic leukemia	FNA	Fine-needle aspirate
ATLL	Adult T-cell leukemia/lymphoma	FL	Follicular lymphoma
BCP ALL	B-cell progenitor acute	FLAER	Fluorescein-labelled proaerolysin
	lymphoblastic leukaemia	FLT3-ITD	FMS-like tyrosine kinase-3 inversion
BCR	B-cell receptor		tandem duplication mutation
BCR-ABL	Breakpoint cluster region-abelson	FSC	Forward scatter
	[t(9;22) also called Philadelphia	HCL	Hairy cell leukaemia
	chromosome]	GFP	Green fluorescent protein
BDCA	Blood-derived dendritic cell antigens	GvL	Graft versus leukaemia
BF	Body fluid	HIV	Human immunodeficiency virus
BM	Bone marrow	HL	Hodgkin lymphoma
BPDCN	Blastic plasmacytoid dendritic cell	HLA-DR	Human leukocyte antigen – antigen
	neoplasm		D related
BR	Blast region	HSL	Hepatosplenic lymphoma
CALLA	Common acute lymphoblastic	HSCT	Haematopoeitic stem cell
	leukemia antigen		transplantation
CAR T-cell	Chimeric antigen receptor T-cell	HTLV-1	Human T-cell lymphotropic virus-1
CBF	Core binding factor	ICOS	Inducible costimulatory
CCR	Chemokine receptor	Ig	Immunoglobulin
CD	Cluster of differentiation	IL	Interleukin
ChIP	Chromatin immunoprecipitation	JAK	Janus kinase
CLL	Chronic lymphocytic leukemia	KIR	Killer immunoglobulin-like
CLPD	Chronic lymphoproliferative		receptors
	disorders	KrO	Krome orange
CML	Chronic myeloid leukaemia	LAIP	Leukemia associated
CMML	Chronic myelomonocytic leukaemia		immunophenotype
CRLF2	Cytokine receptor-like factor	LCA	Leucocyte common antigen
Су	Cyanin	LGL	Large granular lymphocyte
DAPI	4',6-diamidino-2-phenylindole	LSC	Leukemic stem cell
DC	Dendritic cell	Lin	Lineage
DLBCL	Diffuse large B-cell lymphoma	LPD	Lymphoproliferative disorder
DNA	Deoxyribonucleic acid	MAPK	Mitogen activated protein kinase
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More Information

#### **Abbreviations**

MBL	Monoclonal B-cell lymphocytosis	PCR	Polymerase chain reaction
MCL	Mantle cell lymphoma	PD-1	Programmed death-1
MDS	Myelodysplastic syndrome	Percp	Peridinin chlorophyll-A protein
MF	Mycosis fungoïdes	PE R-	Phycoerythrin
MFI	Mean fluorescence intensity	PI	Propidium iodide
MHC	Major histocompatibility	PI3K	Phosphoinositide 3 kinase
	complex	PML-RARA	Promyelocytic leukemia/
MLL/KMT2A	Mixed lineage leukaemia/lysine		retinoic acid receptor A [t(15;17)
	methyl transferase 2A		translocation]
MRD	Minimal residual disease	PMT	Photomultiplier
m-TOR	mammalian transporter of	RNA	Ribonucleic acid
	rapamycin	RBC	Red blood cell
MZL	Marginal zone lymphoma	SC	Sézary cell
MPAL	Mixed phenotype acute leukemia	SLL	Small lymphocytic lymphoma
MRD	Minimal residual disease	SS	Sézary syndrome
NF	Nuclear factor-kappa B	SSC	Side scatter
NGS	Next generation sequencing	STAT	Signal transducer and activator of
NK	Natural killer		transcription
NHL	Non-hodgkin lymphoma	TCR	T-cell receptor
NPM	Nucleophosmin	TdT	Terminal deoxynucleotidyl
PB	Peripheral blood		transferase
Pbl	Pacific blue	TK	Tyrosine kinase
PC	Plasma cell	Tregs	Regulatory T-cells
PCA	Principal component analysis	WBC	White blood cell
PCM	Plasma cell myeloma	WHO	World Health Organization