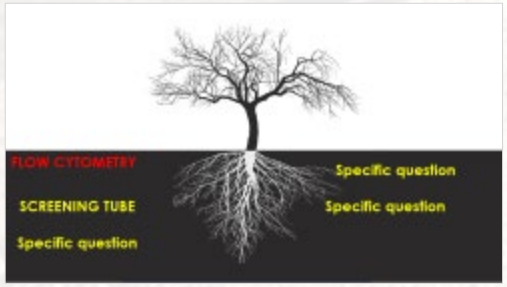
Screening tube. Much more tha a single tube



In conclussion


FAILURE
SUCCESS


SUCCESS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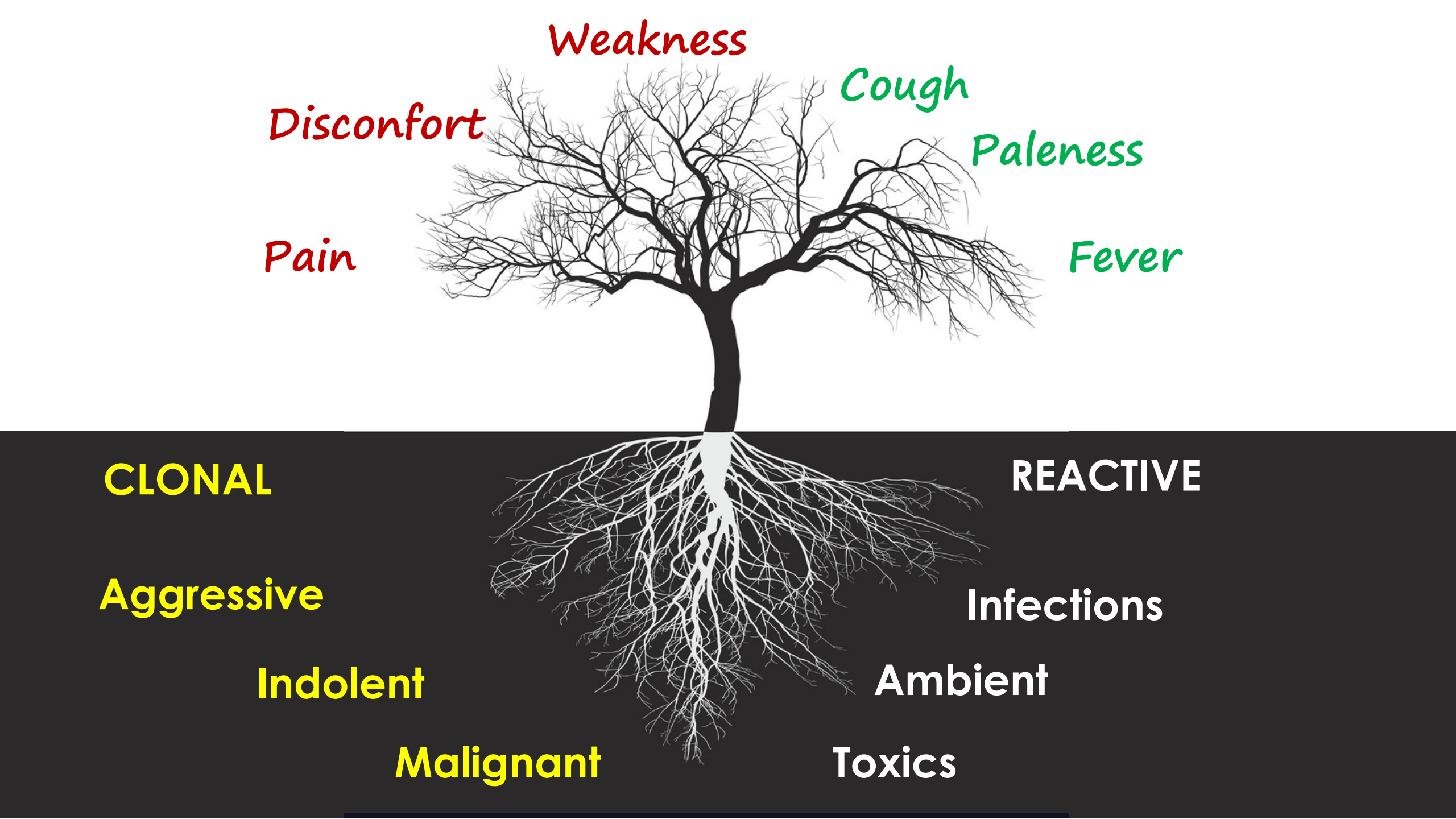

Weakness
Cough
Disconfort
Paleness
Pain
Fever
CLONAL
REACTIVE
Aggressive
Infections
Indolent
Ambient
Malignant
Toxics

Weakness

Cough

Disconfort

Paleness

Pain

Fever

IMMATURE

MATURE

Immature Lympho

Lympho

Immature myeloid

Myeloid

Other cells

Other cells

Weakness

Cough

Disconfort

Paleness

Pain

Fever

IMMATURE

MATURE

Immature Lympho

Lympho

Immature myeloid

Myeloid

Other cells

Other cells

Euroflow

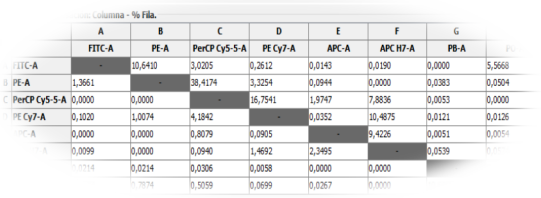
J.Van Dongen

Alberto Orfao

MCF must serve CLINICIANS and patients

Beginning

Techniques and FC



	A	B	C	D	E	F	G
	FITC-A	PE-A	PerCP Cy5-5-A	PE Cy7-A	APC-A	APC H7-A	PB-A
FITC-A	-	10,6410	3,0205	0,2612	0,0143	0,0190	0,0000
PE-A	1,3661	-	38,4174	3,3254	0,0944	0,0000	0,0383
PerCP Cy5-5-A	0,0000	0,0000	-	16,7541	1,9747	7,8836	0,0053
PE Cy7-A	0,1020	1,0074	4,1842	-	0,0352	10,4875	0,0121
	0,0000	0,0000	0,8079	0,0905	-	9,4226	0,0051
	0,0099	0,0000	0,0940	1,4692	2,3495	-	0,0539
		0,0214	0,0306	0,0058	0,0000	0,0000	

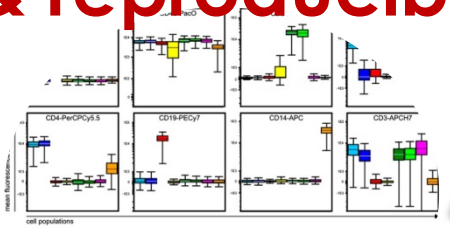
Panels

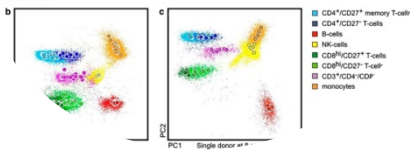
	PacB	AmCyan	FITC	PE	PerCPCy5.5	PECy7	
1	CD20	CD45	CD58	CD66c	CD34	CD19	
2	SmIgκ	CD45	SmIgM	CyIgμ	CD34	CD19	CD123
3	CD21	CD45	NuTdT	CD13	CD34	CD19	CD22
4	CD81	CD45	CD15 and CD65	NG2	CD34	CD19	CD33
1	CD20	CD45	CD58	CD66c	CD34	CD19	CD10
2	SmIgκ	CD45	CyIgμ	CD123	CD34	CD19	SmIgM
3	CD21	CD45	NuTdT	CD13	CD34	CD19	CD81
4	CD9	CD45	CD15 and CD65	NG2	CD34	CD19	CD33

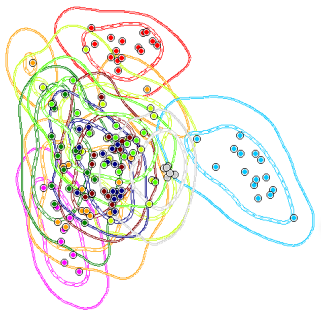
	PacB	PacO	FITC	PE	PerCPCy5.5	PECy7	APC	APCH7
1	CD20	CD45	CD58	CD66c	CD34	CD19	CD10	
2	SmIgκ	CD45	CyIgμ	CD33	CD34	CD19	SmIgM and CD117	
3	CD21	CD45	NuTdT	CD13	CD34	CD19	CD22	
4	CD9	CD45	CD15 and CD65	NG2	CD34	CD19	CD123	
1	CD20	CD45	CD58	CD66c	CD34	CD19	CD10	
2	SmIgκ	CD45	CyIgμ	CD33	CD34	CD19	SmIgM and CD117	
3	CD9	CD45	NuTdT	CD13	CD34	CD19	CD22	
4	CD21	CD45	CD15 and CD65	NG2	CD34	CD19	CD123	

alexa fluor 700; ALL, acute lymphoblastic leukemia; AmCyan, *Anemonia Majano* cyan fluorescent protein; APC
marrow; Cy, cytoplasmic; Cy7, cyanin7; FITC, fluorescein isothiocyanate; H7, hilite7; Nu, nuclear; PacB,
PE, phycoerythrin; PerCPCy5.5, peridinin–chlorophyll–protein–cyanin5.5; Sm, surface
in the Appendix. [b]A total of 215 samples (172 BM, 36 PB and 7 other types of
of 4 BM and PB from healthy donors, respectively. [c]Testing of
compared to previous version.

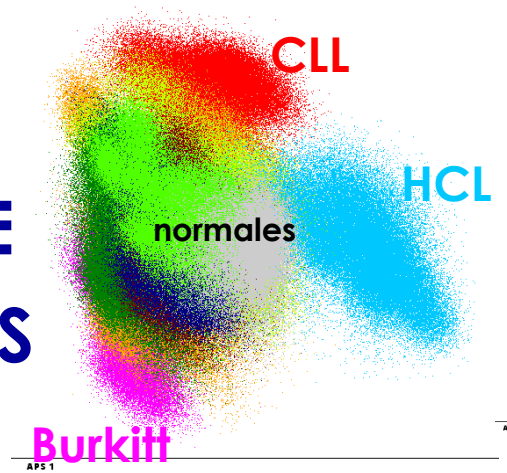
Efficiency & reproducibility



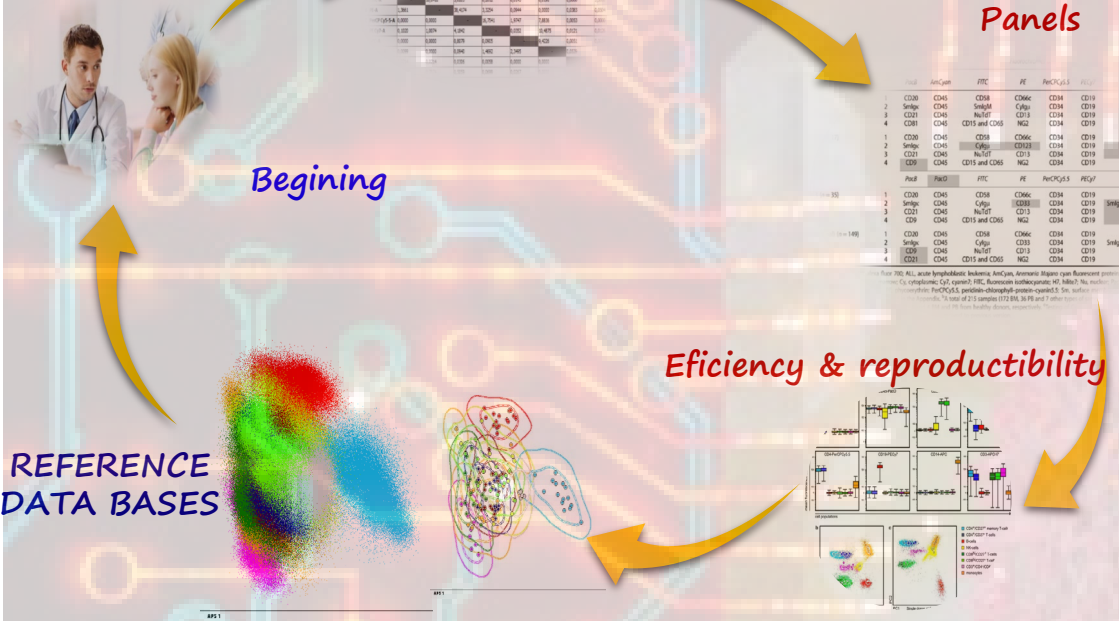




REFERENCE DATA BASES



NEW GENERATION FLOW



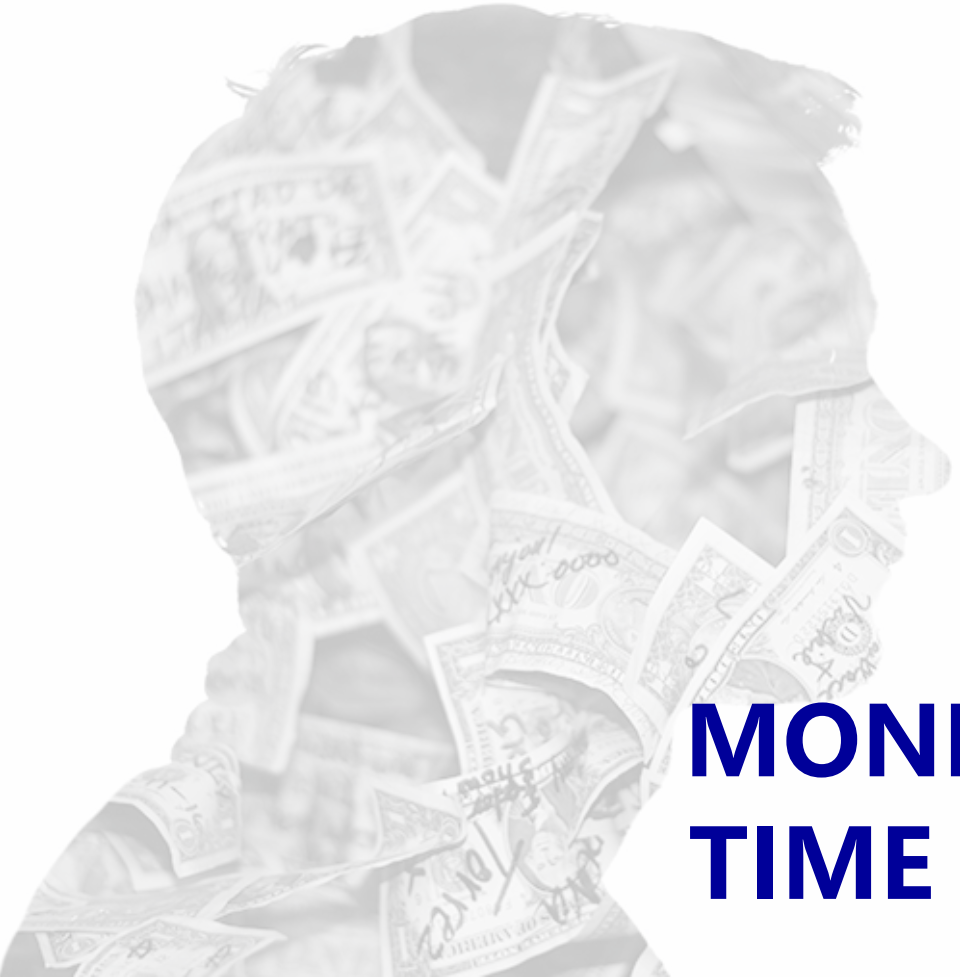
Best **objective** Criteria
(standardization)
Based on **evidence**

Better technology & Bioinformatics
More info in less time (Big Data)

NEW GENERATION FLOW

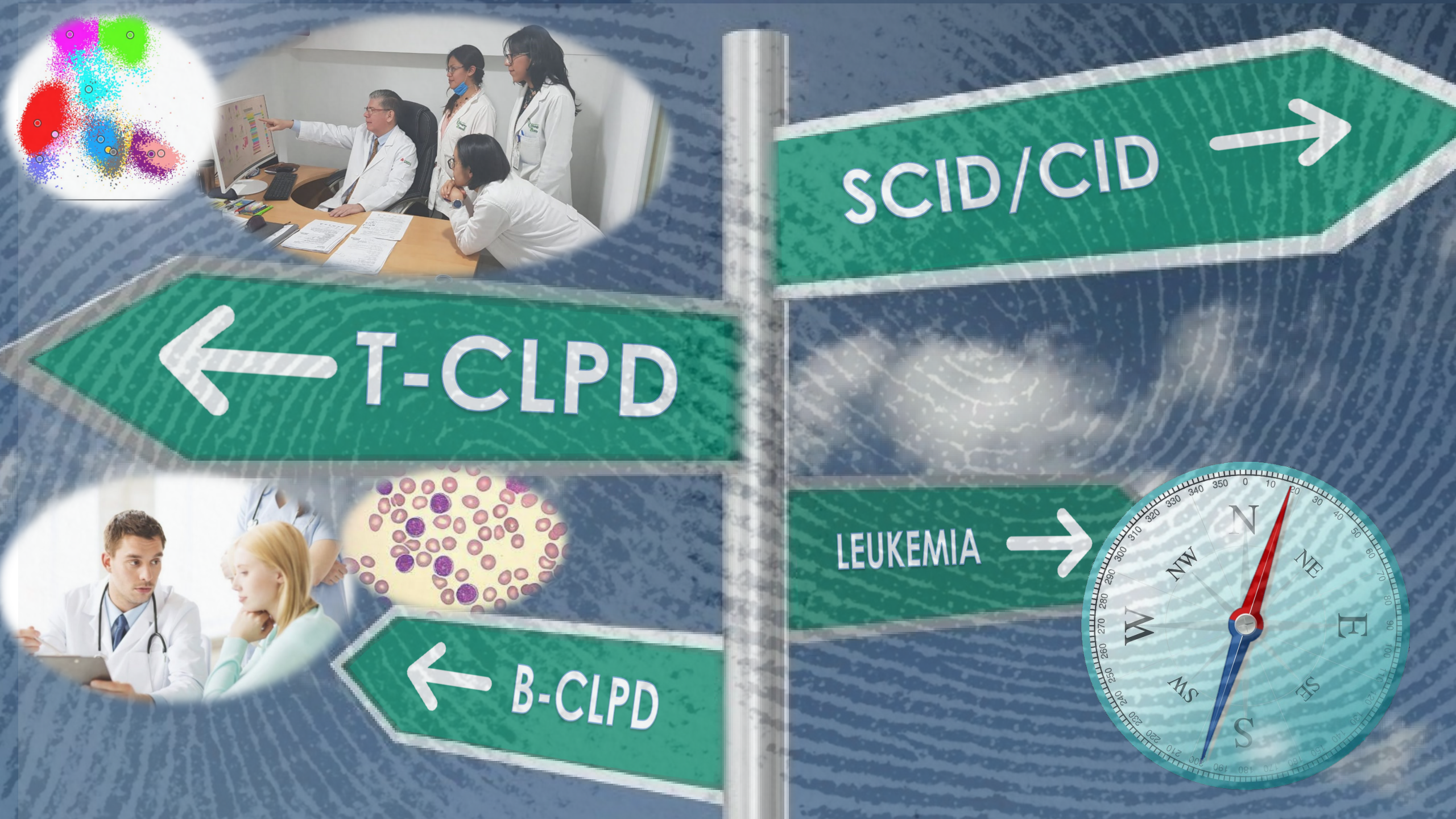
Standardization Process

Standardization Process



MONEY Investment : > 9,5 milllons €

TIME Investment : > 12 years


SCID/CID
T-CLPD
B-CLPD
LEUKEMIA
N
NE
E
SE
S
SW
W
NW


ALOT

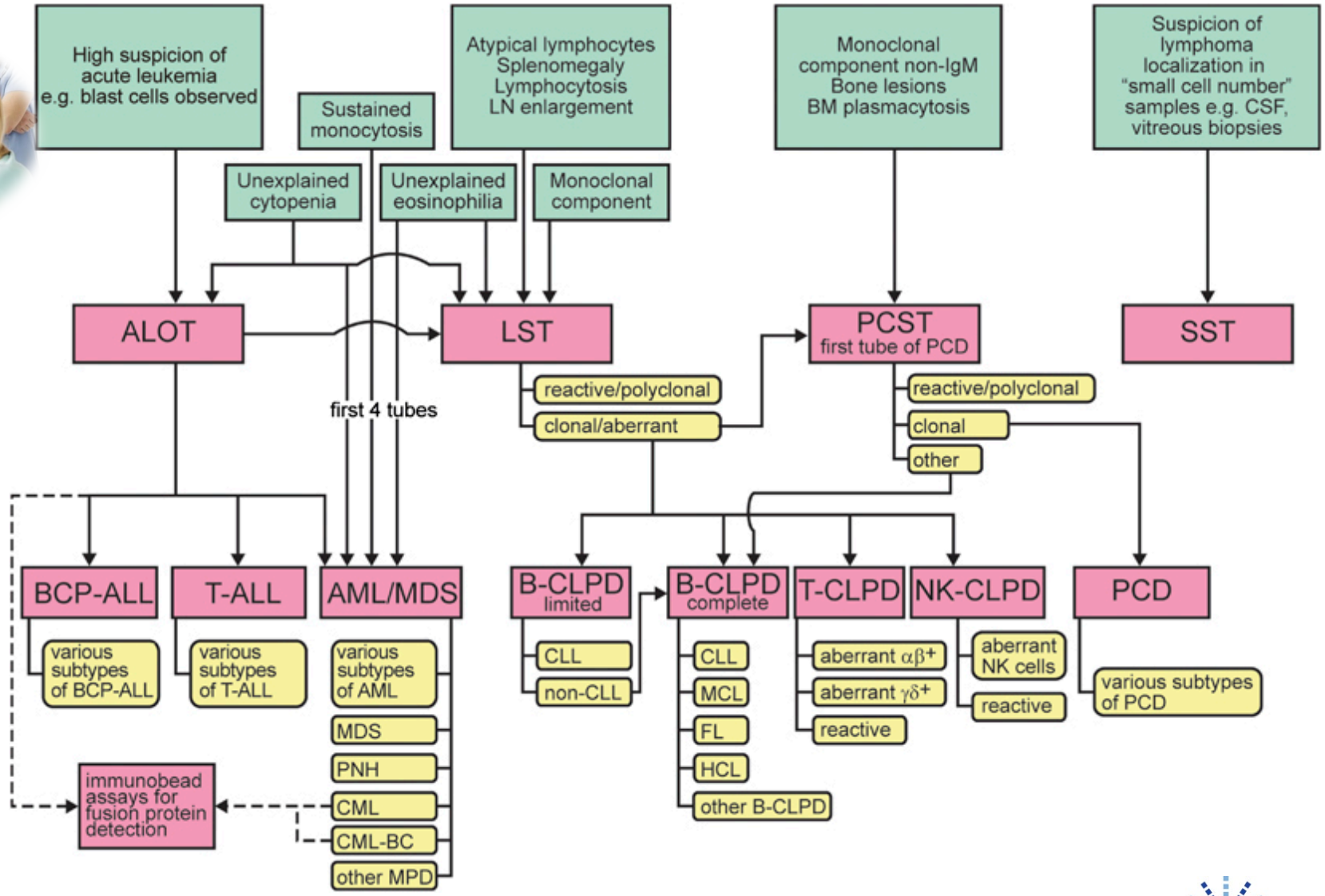
HIERARCHY

EUROFLOW FLOWCHART WORK

Begining

1. ORIENTATION

2. CHARACTERIZATION

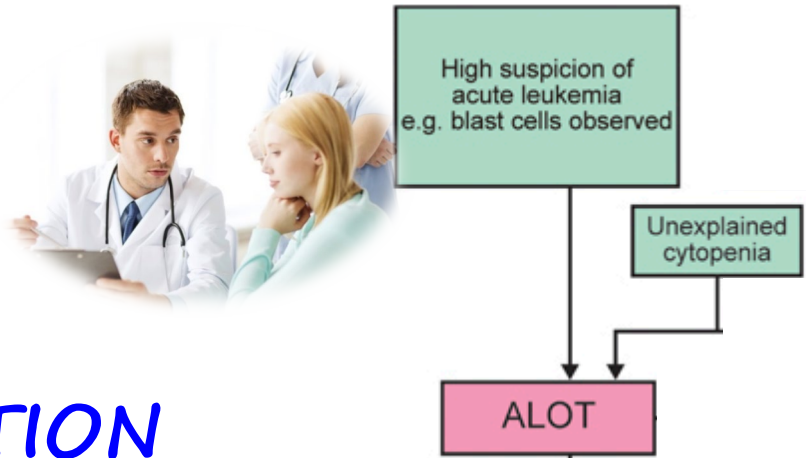


Leukemia 2012; 26, 1908–1975 (van Dongen et al on behalf of EuroFlow)

EuroFlow
a division of ESLHO

HIERARCHY EUROFLOW FLOWCHART WORK

Begining



1. ORIENTATION

Leukemia (2018) 32, 874–881
www.nature.com/leu

Accuracy: 99,3%

2. CHARACTER

ORIGINAL ARTICLE

Automated database-guided expert-supervised orientation for immunophenotypic diagnosis and classification of acute leukemia

L Lhermitte[1,2,18], E Mejstrikova[3,18], AJ van der Sluijs-Gelling[4,5,18], GE Grigore[6], L Sedek[7], AE Bras[8], G Gaipa[9], E Sobral da Costa[10], M Novakova[3], E Sonneveld[4], C Buracchi[8], T de Sá Bacelar[10], JG te Marvelde[8], A Trinquand[1], V Asnafi[1], T Szczepanski[11], S Matarraz[12], A Lopez[12], B Vidriales[13], J Bulsa[11], O Hrusak[3], T Kalina[3], Q Lecrevisse[12], M Martin Ayuso[6], M Brüggemann[14], J Verde[6], P Fernandez[15], L Burgos[16], B Paiva[16], CE Pedreira[17], JJM van Dongen[5], A Orfao[12,19] and VHJ van der Velden[8,19] on behalf of the EuroFlow Consortium

Leukemia 2012; 26, 1908–1975 (van Dongen et al on behalf of EuroFlow)

EuroFlow
a division of ESLHO

DIAGNOSIS APPROACHING

ALGORITHM for ACUTE LEUKEMIA at diagnosis

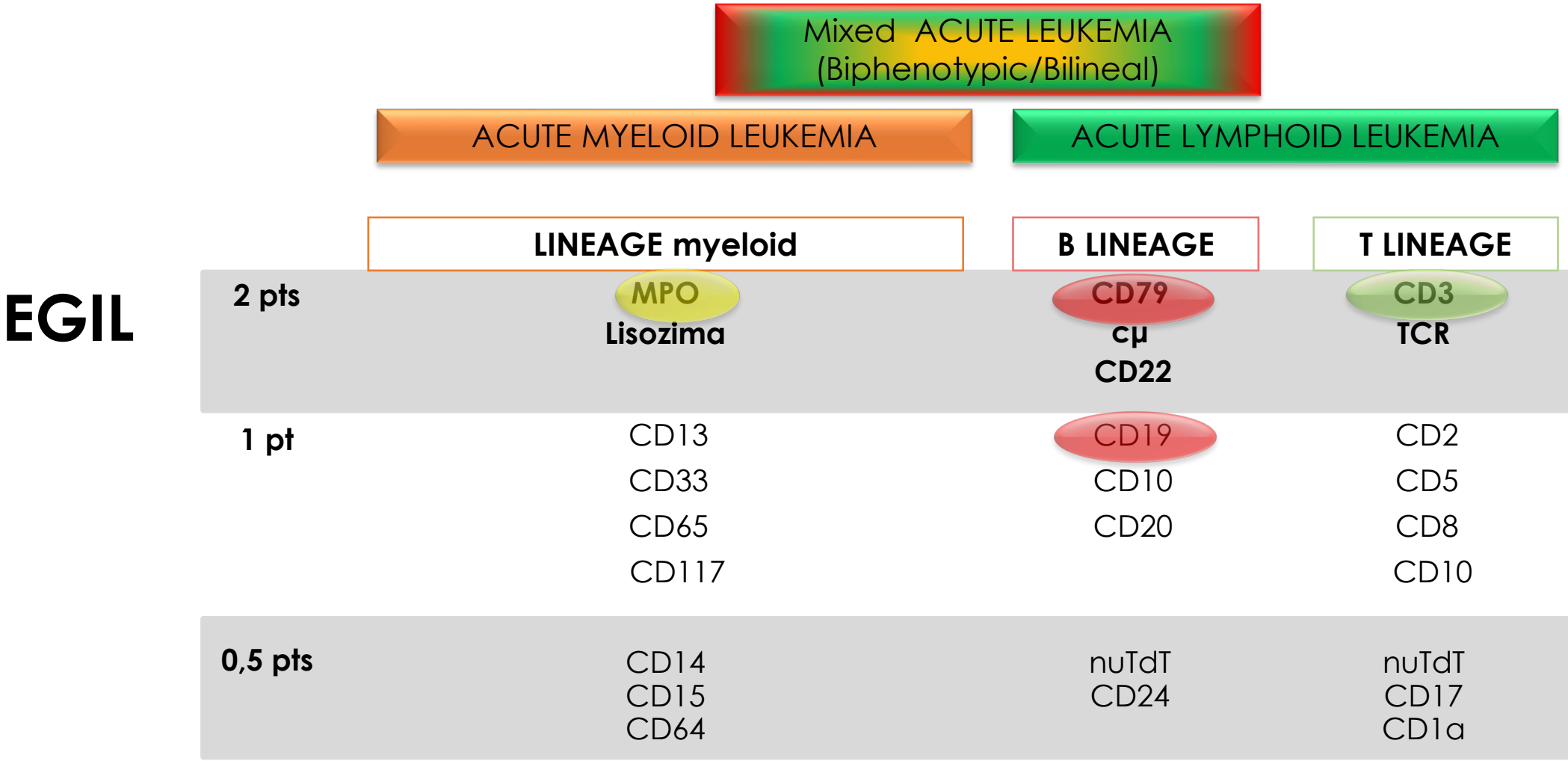
LINEAGE

myeloid = NO B NO T

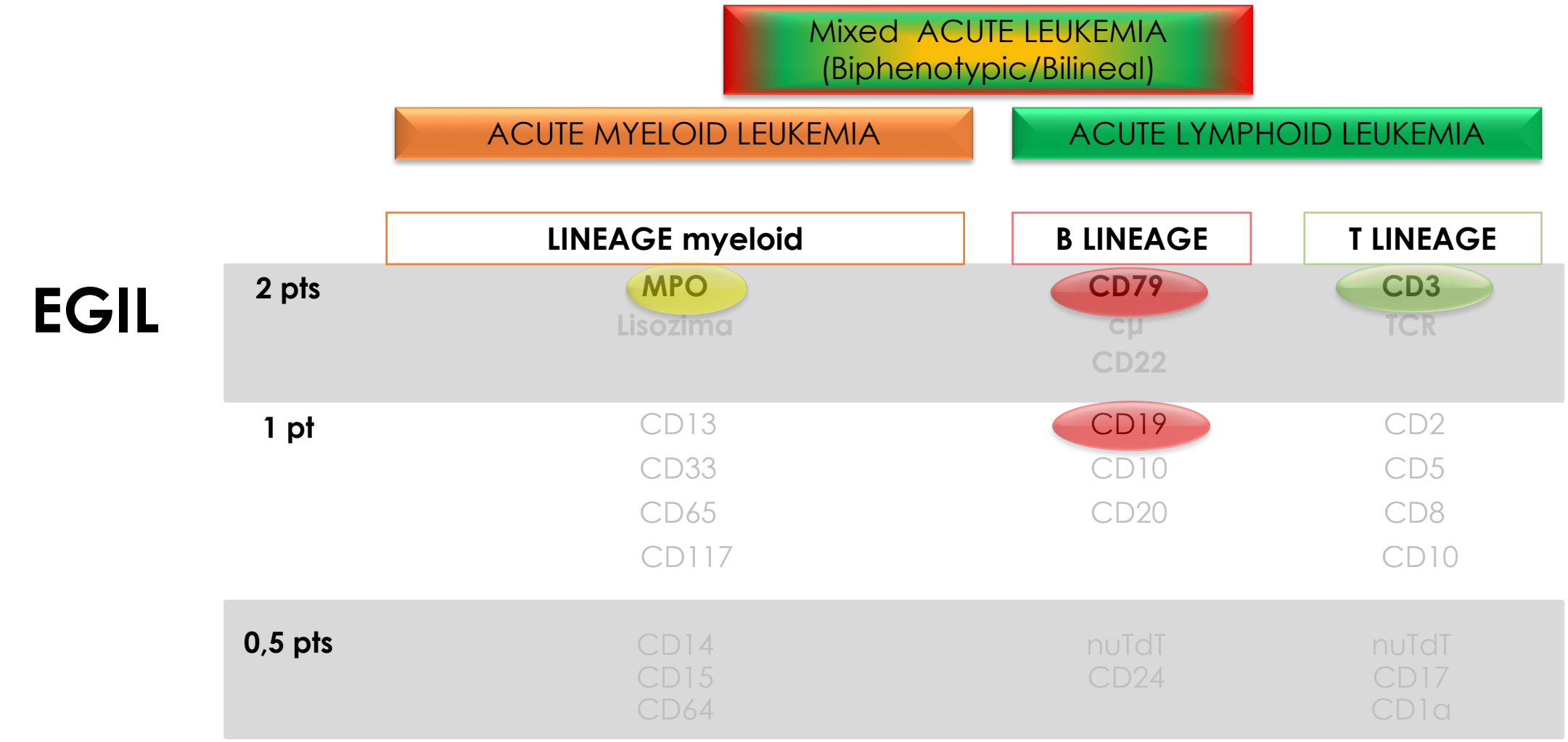
B Lymphoid = NO myeloid NO T

T Lymphoid = NO myeloid NO B

ALGORITHM for ACUTE LEUKEMIA at diagnosis



ALGORITHM for ACUTE LEUKEMIA at diagnosis



EUROFLOW (ALOT)

PB	PO	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
cyCD3	CD45	cyMPO	cyCD79a	CD34	CD19	CD7	CD3

ALGORITHM for ACUTE LEUKEMIA at diagnosis

Mixed ACUTE LEUKEMIA (Biphenotypic/Bilineal)

ACUTE MYELOID LEUKEMIA | ACUTE LYMPHOID LEUKEMIA

EGIL

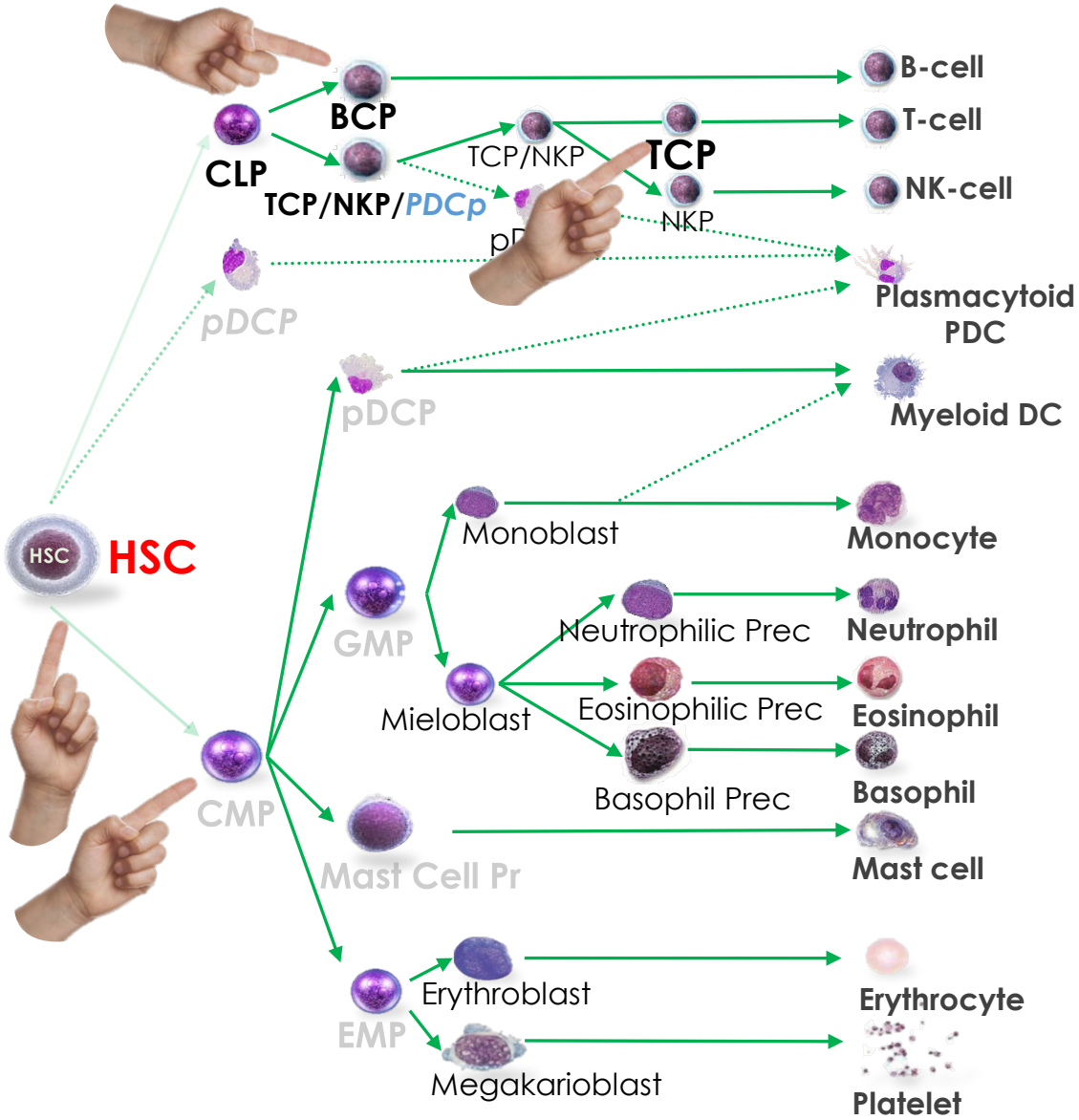
	LINEAGE myeloid	B LINEAGE	T LINEAGE
2 pts	MPO Lisozima	CD79 cμ CD22	CD3 TCR
1 pt	CD13 CD33 CD65 CD117	CD19 CD10 CD20	CD2 CD5 CD8 CD10
0,5 pts	CD14 CD15 CD64	nuTdT CD24	nuTdT CD17 CD1a

EUROFLOW (ALOT)

PB	PO	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
cyCD3	CD45	cyMPO	cyCD79a	CD34	CD19	CD7	CD3

Origin of Hematopoiesis: BLAST Characterization

HEMATOPOIESIS



ORIGIN of CLONAL Hematopoiesis

Acute Lymphoid Leukemia

BCP-ALL **TCP-ALL** NKCP-ALL

Acute Myeloid Leukemia

AML

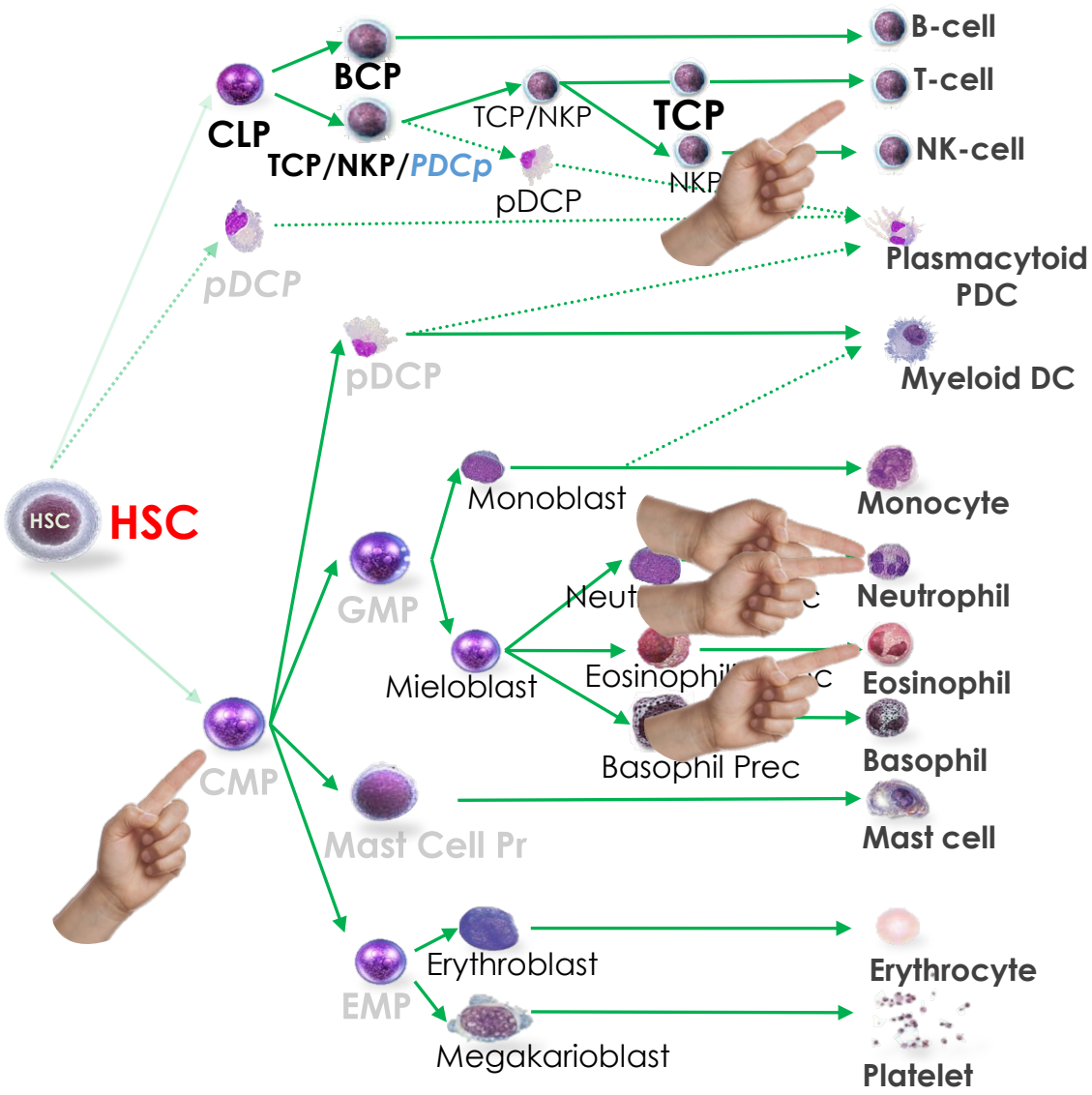
Acute leukemias of ambiguous lineage

Undifferentiated AL

Mixed phenotype

RESIDUAL Hematopoiesis: microenvironment

HEMATOPOIESIS



Mature/maturing Hematopoiesis

Normal distribution of lymphocytes

T/NK/B ratio Neturo/lymph ratio

Dysplastic features

CD34+: MPO pattern, CD19&CD79a; CD7

Neutrophils: MPO pattern; CD45; SSC/FSC+

Genetic patterns related with AML

Blasts: CD19+ het RUNX1
CD7 het CEBPA;
Dendritic cell involvement
Megakariocytic AL.
Eosinophils: MPO -/++ inv (16)

Summary

Summary

Why/when to use ALOT

BONE MARROW *& Peripheral Blood*

Diagnosis: ***Lineage assessment in AL***
MPAL identification
Dysplasia, CLPD infiltration, MM infiltration?

Prognosis: ***Correlation with cytogenetics***

Saving TIME & money. ***Orientates more specific questions***

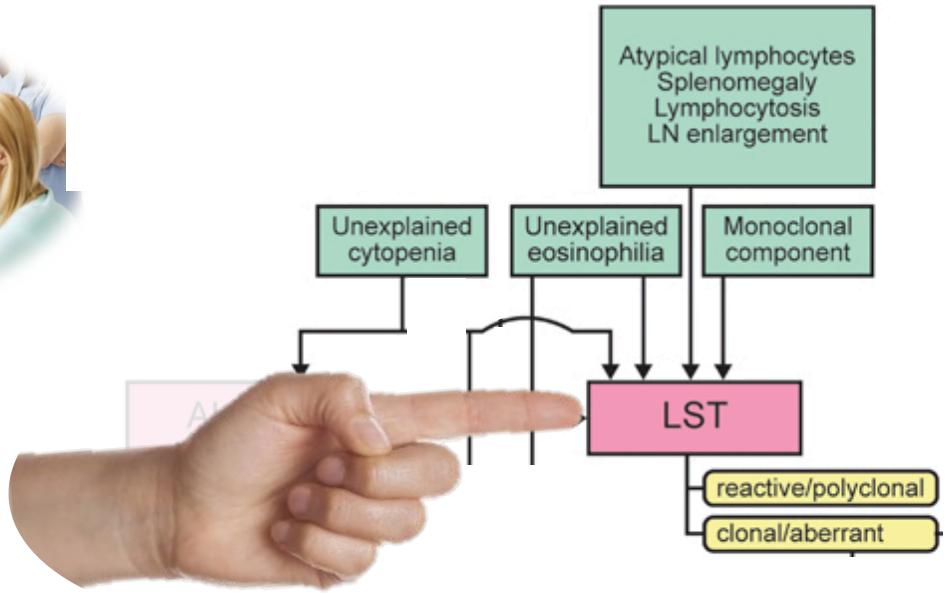

S

HIERARCHY

EUROFLOW FLOWCHART WORK

Begining

1. ORIENTATION



Leukemia 2012; 26, 1908–1975 (van Dongen et al on behalf of EuroFlow)

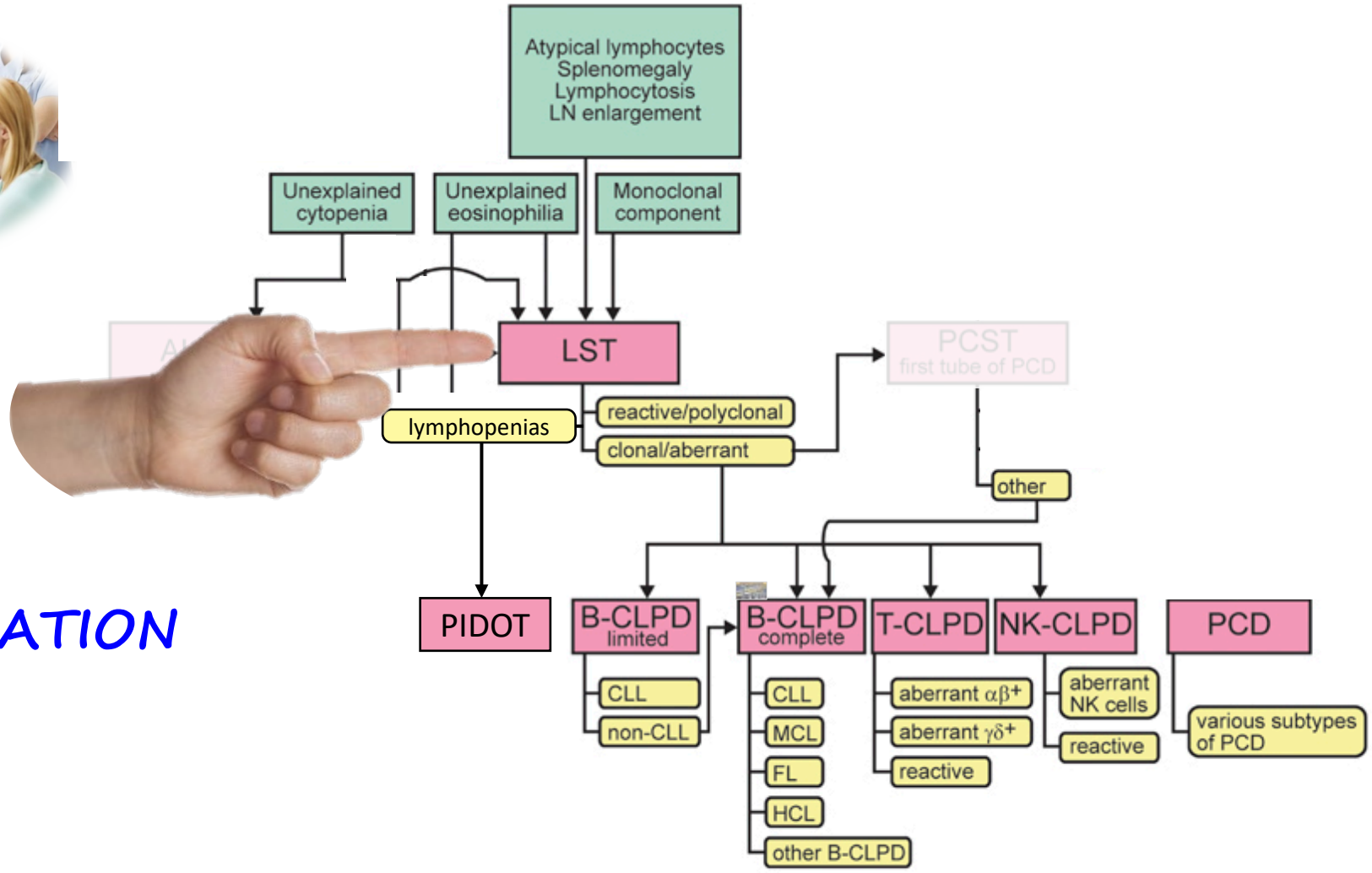
EuroFlow
a division of ESLHO

HIERARCHY EUROFLOW FLOWCHART WORK

Begining

1. ORIENTATION

2. CLPD CHARACTERIZATION



MODIFICATION of Leukemia 2012; 26, 1908–1975 (van Dongen et al on behalf of EuroFlow)

EuroFlow
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CLINICAL NEEDS

Knowledge of PATHOPHYSIOLOGY

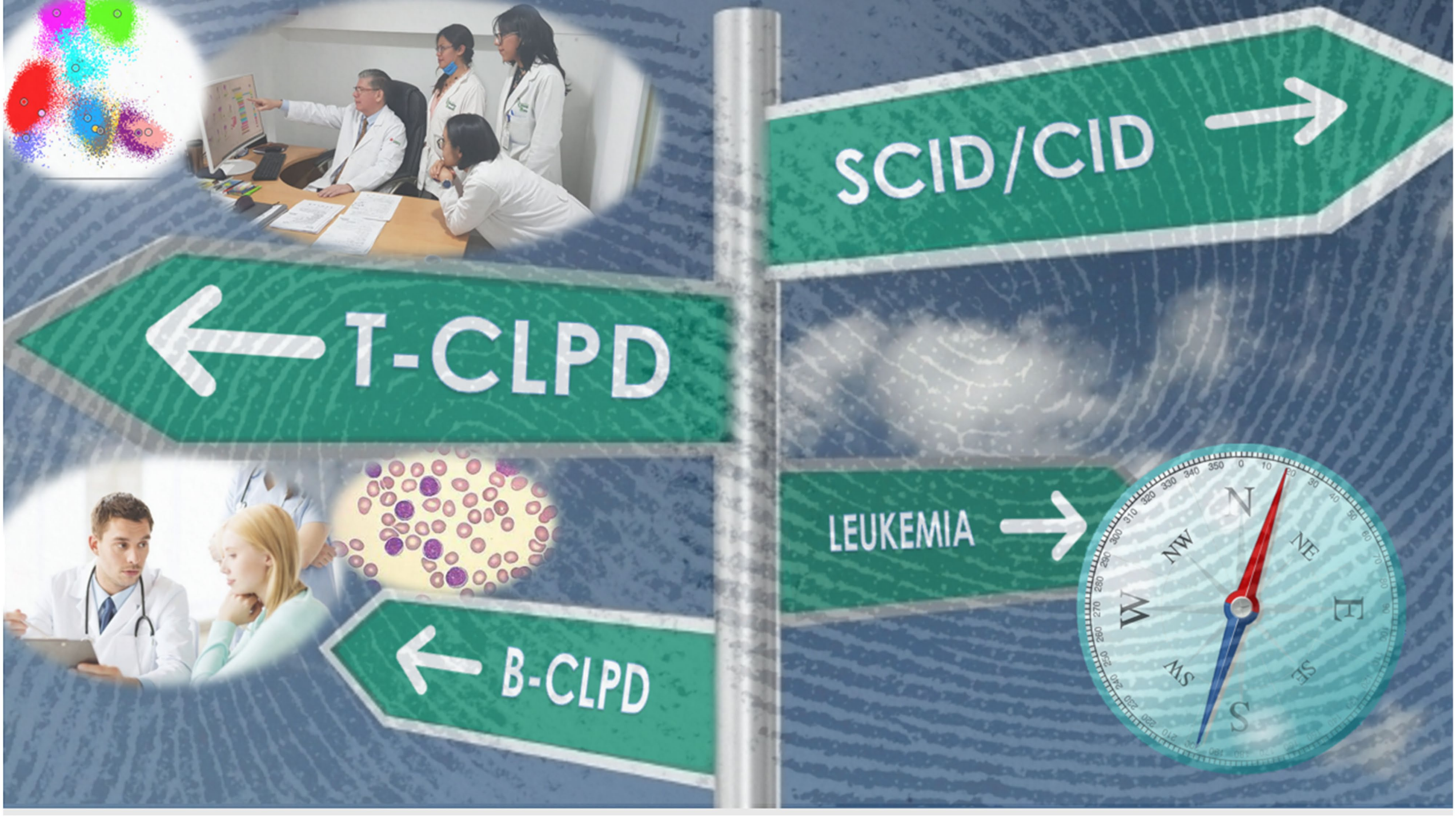
Diagnostic orientation

Differential Diagnosis

Biological heterogenity

New questions

Prognostic/predictive value


SCID/CID
T-CLPD
LEUKEMIA
B-CLPD

STANDARDIZED MFC PANEL allows **TO ASK SPECIFIC QUESTIONS**
& DESIGN STRATEGIES in order to **SAVE TIME, resources and MONEY.**

CLONALITY

B Cell: aberrant Phenotypes & Kappa or Lambda restriction

CD19/CD20/CD5/CD38/CD45 **WHY NOT CD10?**

T Cell: abnormal Phenotypes & CD4/CD8 ratio* & FSC/SSC* (TRBC1)

CD3/CD5/CD4/CD8/CD56

NK Cell: abnormal Phenotypes & >10% NK within whole nucleated cells.

CD56/CD8/CD5/CD4/CD38

Diagnostic algorithm (LST) >90% Accuracy

SIZE

SMALL CELL (FSC < normal T-cell or normal B FSC)

CD19+ Hom
CD20+ Hom

NO CLL — CD20+d

NO FL — CD19+d

CD5

\- MALT / LLP/ MZL

\+ MALT / LLP/ MZL VS MCL

BIG CELL (FSC > normal T-cell or normal B FSC)

CD19+ Hom
CD20+ Hom

NO HCL — CD19+++ & CD20+++

CD10

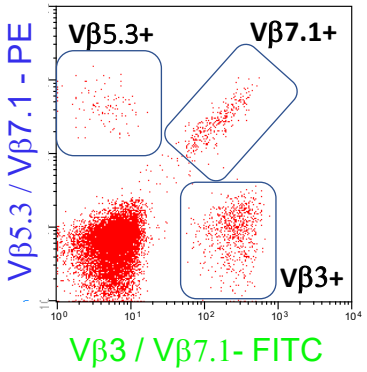
BCLPD Tube 1

\- LBCL CD10- → MALT / LLP/ MZL; MCL /CLL/FL

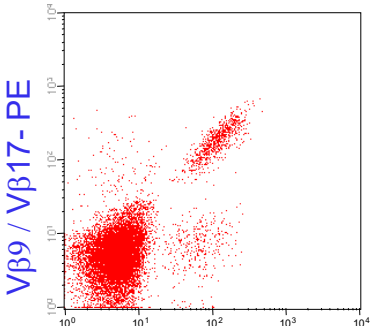
\+ LBCL CD10+ VS BURKITT

NORMAL PB CD4+ T-CELLS: TCRVβ REPERTOIRE

A



B



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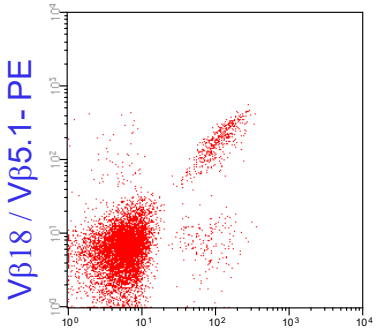
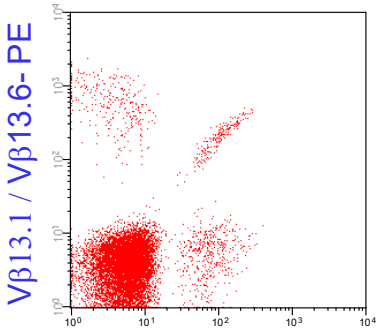


D



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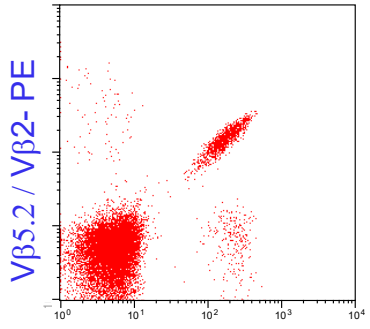
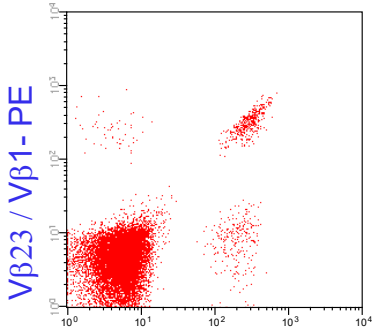
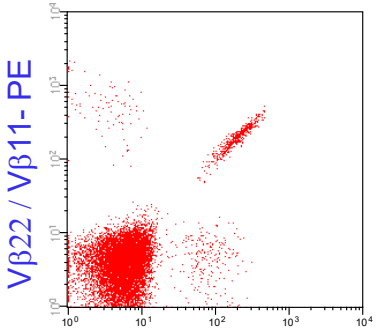


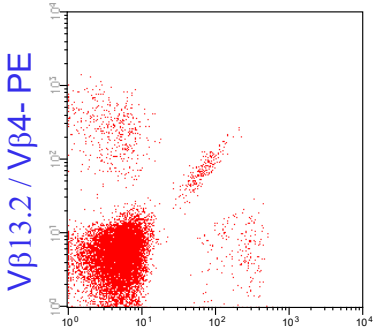
F



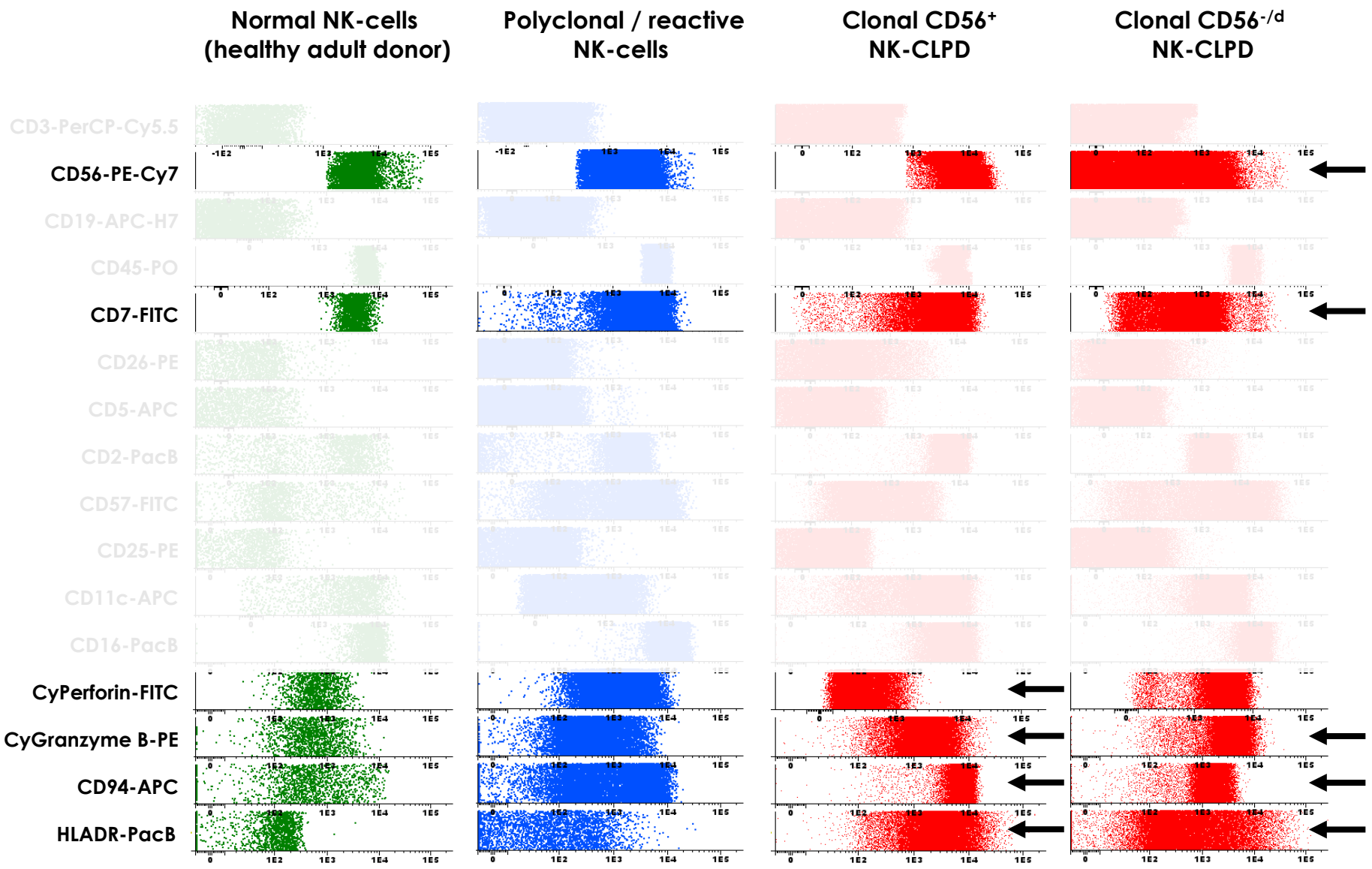
G



H



Phenotypic patterns of polyclonal and clonal NK-cells



Leukemia 2012; 26, 1908–1975 (van Dongen et al on behalf of EuroFlow)

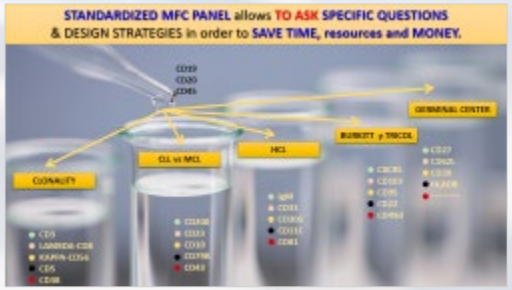
STANDARDIZED MFC PANEL allows **TO ASK SPECIFIC QUESTIONS**
& DESIGN STRATEGIES in order to **SAVE TIME, resources and MONEY.**

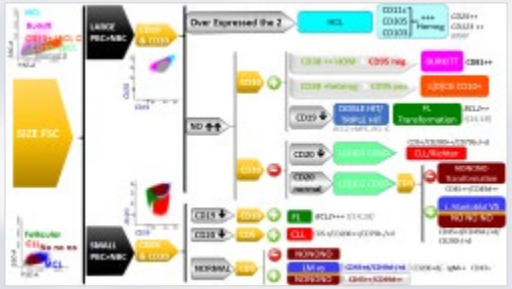
CLONALITY

B Cell: aberrant Phenotypes & Kappa or Lambda restriction

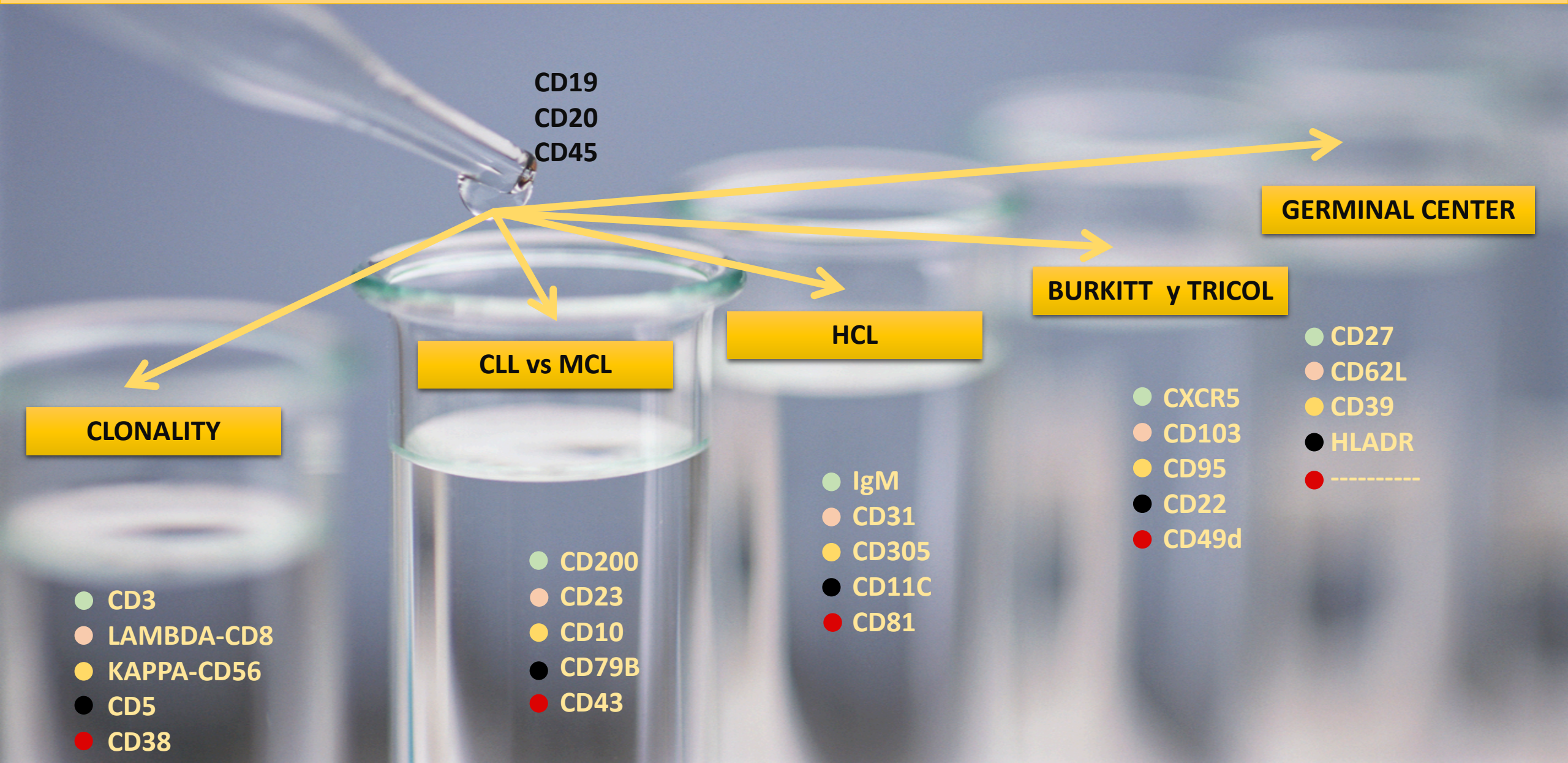
CD19/CD20/CD5/CD38/CD45

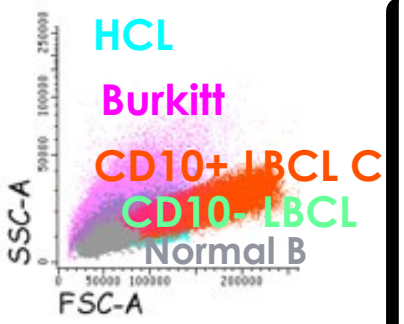
More Accuracy





STANDARDIZED MFC PANEL allows TO ASK SPECIFIC QUESTIONS & DESIGN STRATEGIES in order to SAVE TIME, resources and MONEY.

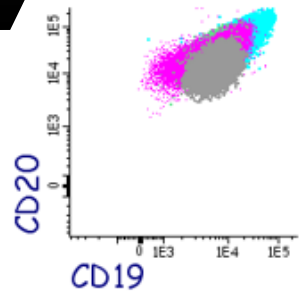




SIZE FSC

LARGE PBC>NBC

CD19 & CD20



Over Expressed the 2

HCL

CD11c
CD305
CD103
+++ Homog

CD25++
CD123 ++
BRAF

NO ↑↑

CD10 ⊕

CD38 ++ HOM & CD95 neg → BURKITT **CD81++**

CD38 +heterog & CD95 pos → L(D)CG CD10+

CD19 ↓ → DOBLE HIT/ TRIPLE HIT → FL Transformation *BCL2++ t(14;18)*

BCL2; cMYC, BCL-6

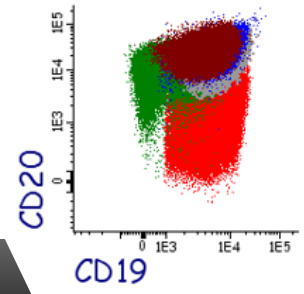
CD10 ⊖

CD20 ↓ → L(D)CG CD10- → CD5+/CD200++/CD79b-/+d CLL/Richter

CD20 normal → L(D)CG CD10- → CD5

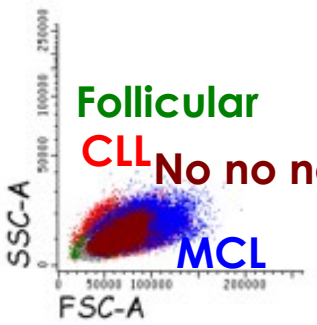
⊖ NONONO Transformation CD45++/CD49d++

⊕ L. Mantoblat VS NO NO NO CD45+d/CD49d-/+d/ CD200-/+d



SMALL PBC>NBC

CD19 & CD20



CD19 ↓ → CD10 ⊕ → FL *BCL2+++ t(14;18)*

CD20 ↓ → CD5 ⊕ → CLL CD5+/CD200++/CD79b-/+d

NORMAL → CD5

⊖ NONONO

⊕ LM vs NONONO → CD45+d/CD49d-/+d CD45++/CD49d++ CD200+d/- IgM++ CD43+

STANDARDIZED MFC PANEL allows **TO ASK SPECIFIC QUESTIONS**
& DESIGN STRATEGIES in order to **SAVE TIME, resources and MONEY.**

B Cell

CLONALITY

T Cell

NK Cell

MICROENVIRONMENT

CD38: Plasma cells (MM/ WM) + Precursors + Basophils + Monocytes + TIMAS

CD45: GLOBAL hematopoietic distribution and maturing cells

CD56: Dysplasia in monocytes and neutrophils

CD56+CD4+CD45+dim: aberrant vs normal PDC

Summary

Summary

Why/when to use LST

BONE MARROW *& Peripheral Blood*

Diagnosis: ***CLONALITY & categorization in B-CLPD***

Suspiction of clonality T&NK

Dysplasia, MM infiltration?,MPN?

Suspiction of PID (SCID or CID).

Identification of reactive patterns (lymphoid)

Saving TIME & money. ***Orientates more specific questions***

FLOW CYTOMETRY

SCREENING TUBE

Specific question

Specific question

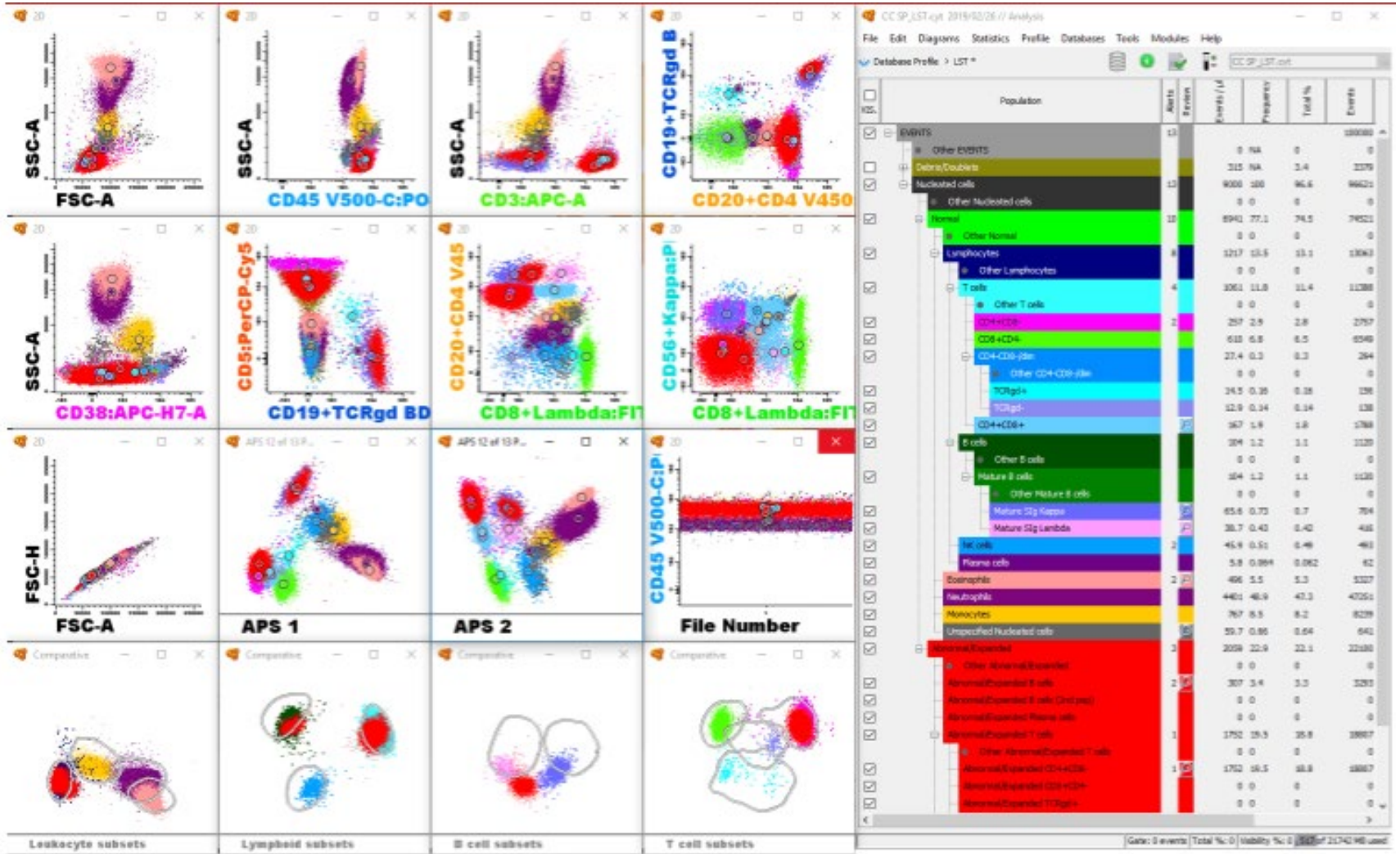
Specific question

FLOW CYTOMETRY

SCREENING TUBE

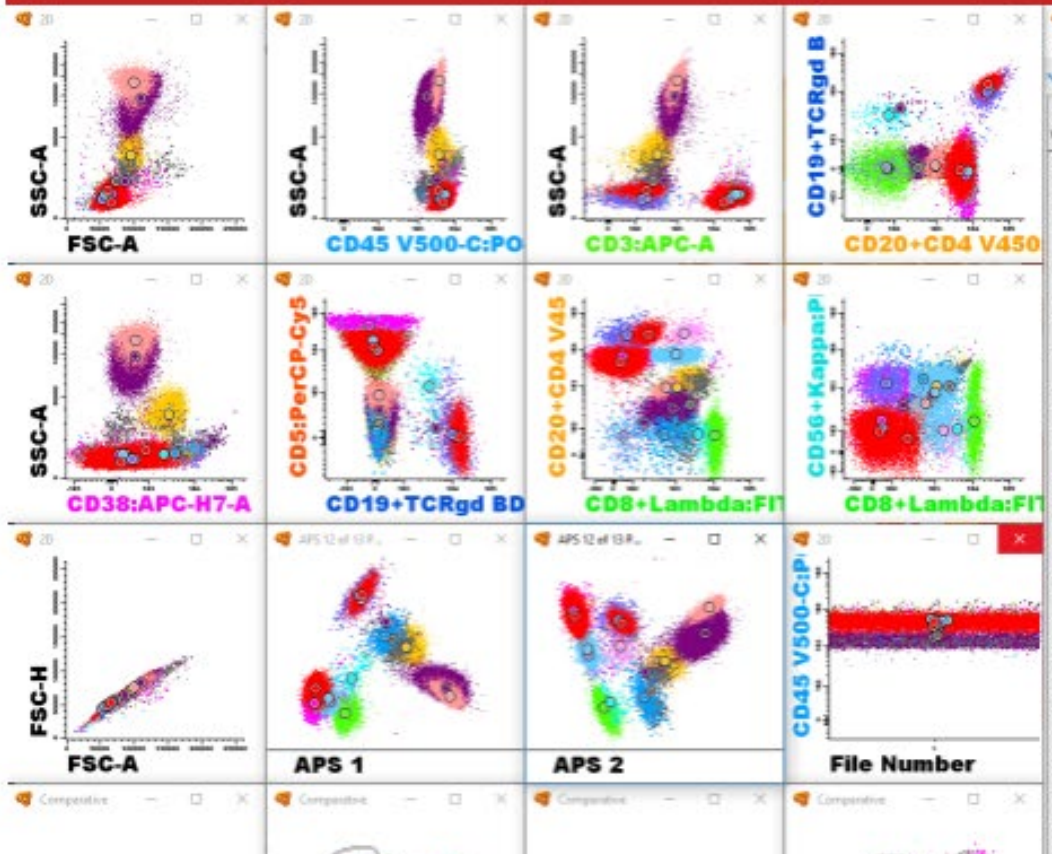
Specific tube

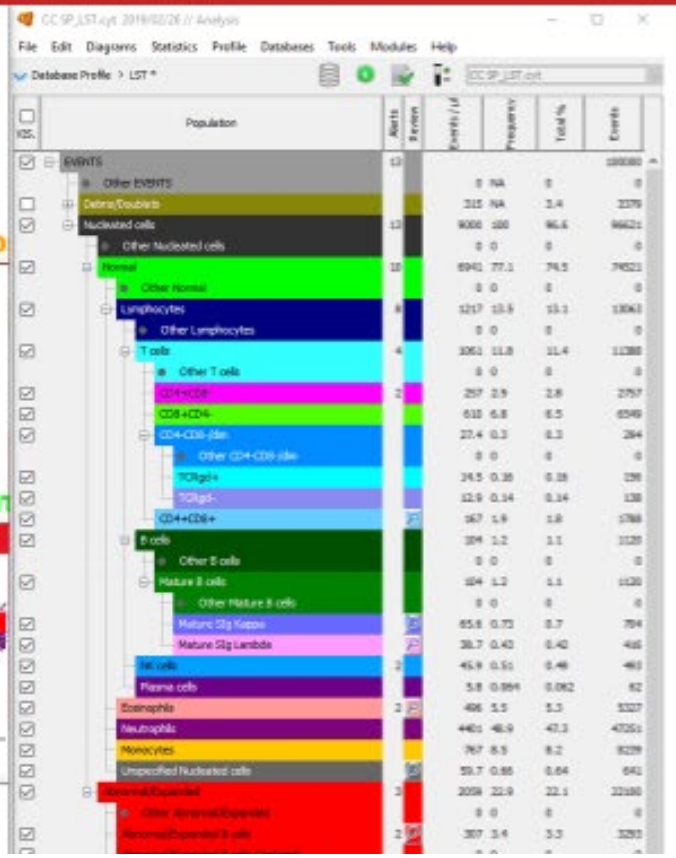
Comparing with normal IS THE KEY



Webinar – Why changing from manual to automated analysis of the LST tube? (2020/11/26) – 7:00 PM CET

November 26, 2020 @ 19:00 - 20:00 UTC+1





Invited speaker:
Maria Arroz,
MD and Clinical Pathologist

Director of the Flow Cytometry Laboratory CHLO, Hospital S. Francisco Xavier Lisbon, Portugal

Addressing all the questions!!!!


What
Would
Happen If...


What
Would
Happen If...

In conclussion

In conclussion

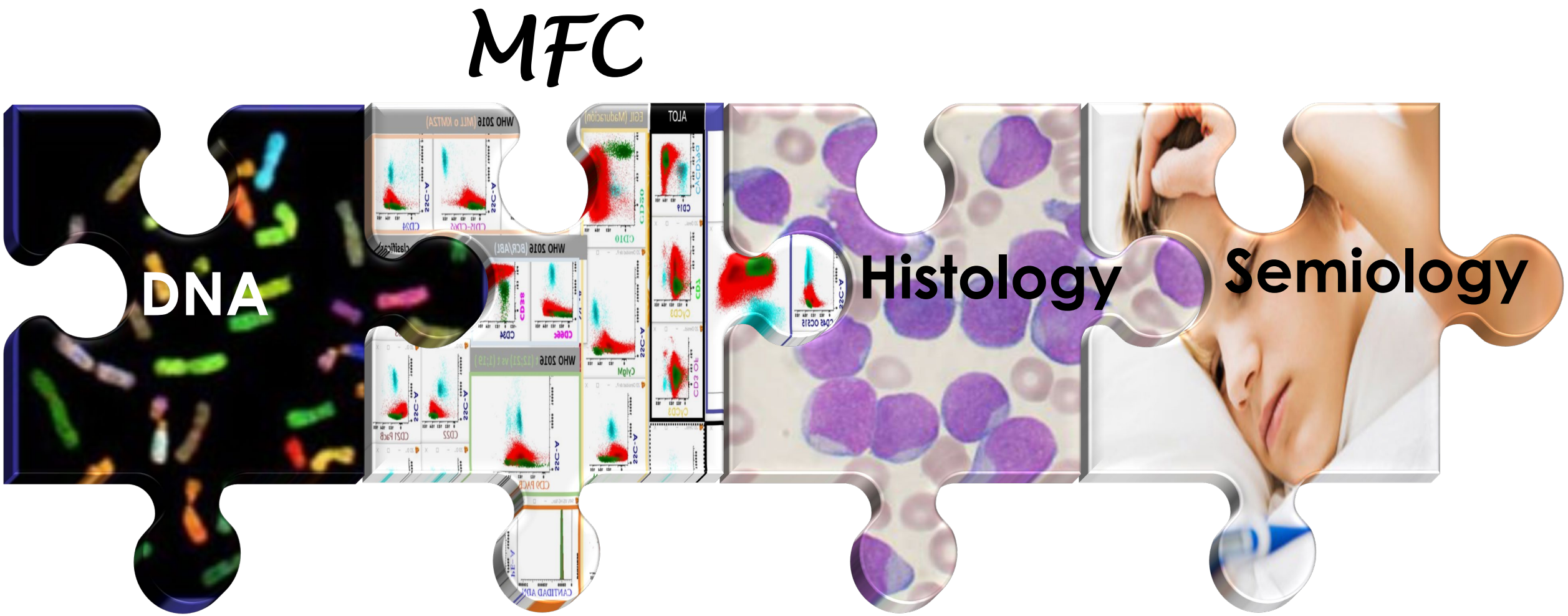
Screening tubes improve our approaching to DIAGNOSIS, PROGNOSIS (orientation).

Help us to choose the best option/s to follow the diagnosis proccess (optimization).

Provide huge information (BIG DATA) where the use of automatic analysis improve the diagnosis accuracy, arising new questions…being a source of knowledge.

Screening tubes optimize time, money and resources.

Diagnosis MUST BE INTEGRATED




What
Would
Happen If...