LABORATOIRE HOSPITALIER UNIVERSITAIRE DE BRUXELLES
LHUB-ULB
UNIVERSITAIR LABORATORIUM BRUSSEL

Cytométrie en flux et masse suspecte en hématologie

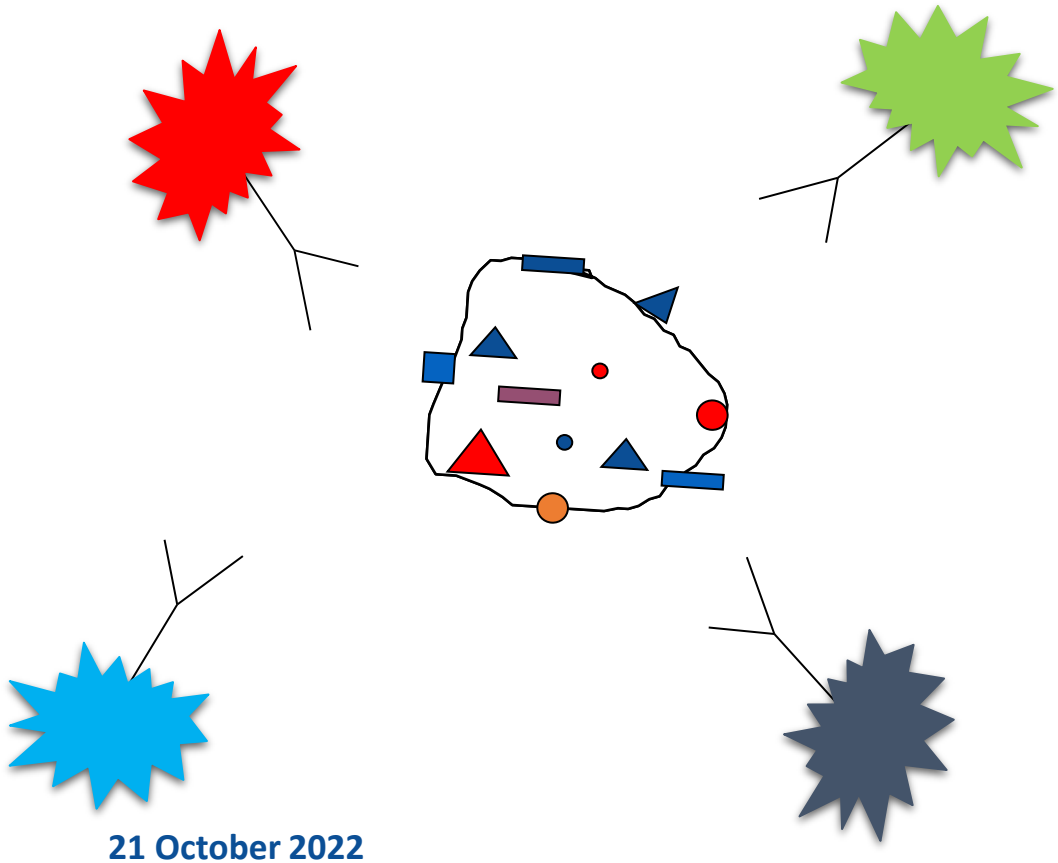
Valéry Daubie

21 October 2022

La cytométrie

- Cellules en suspension < sang, moelle osseuse, liquides (LCR, LBA, …), ganglion
- Marquage anticorps anti-CD + fluorochrome

Fluorochrome	Excitation	Emission
FITC	494-517	518
PE	470-570	576
PerCP	470-570	670
APC	650	660-680



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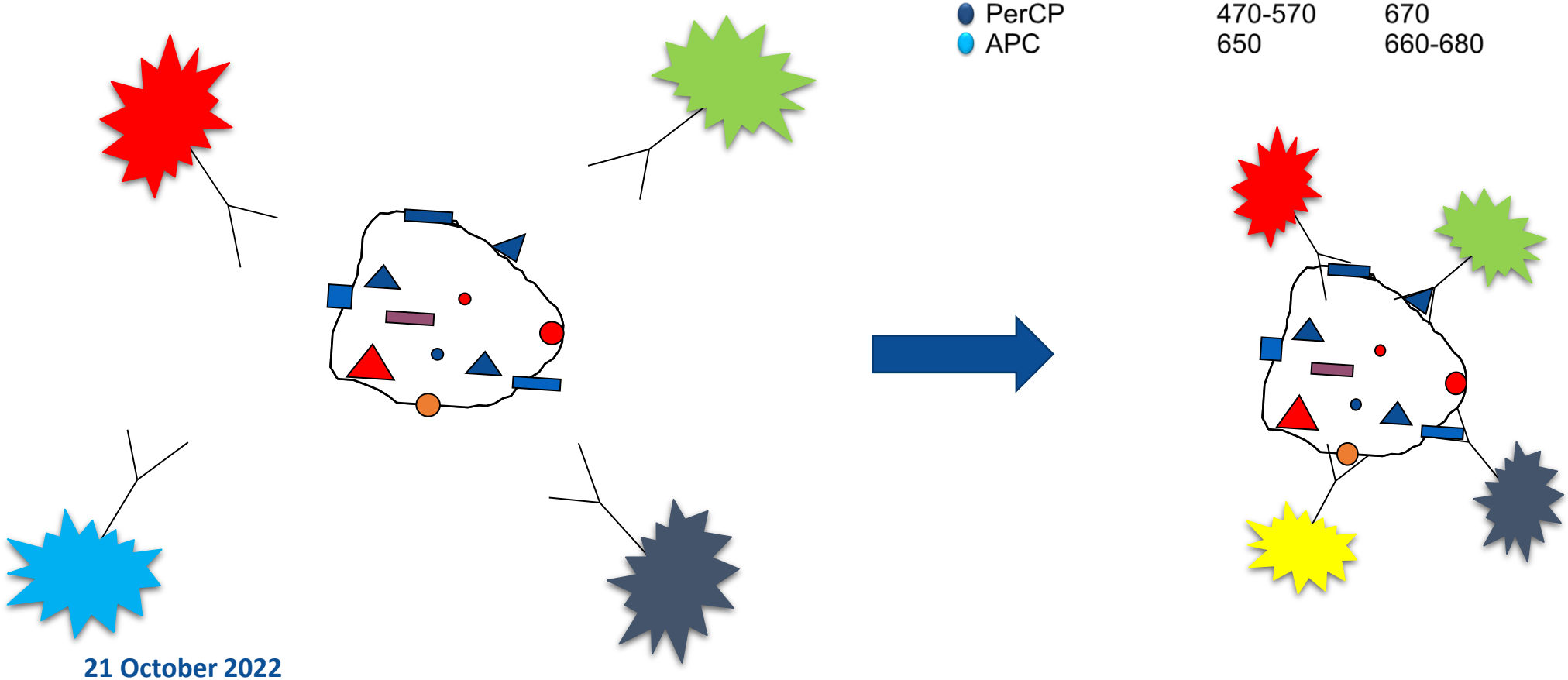
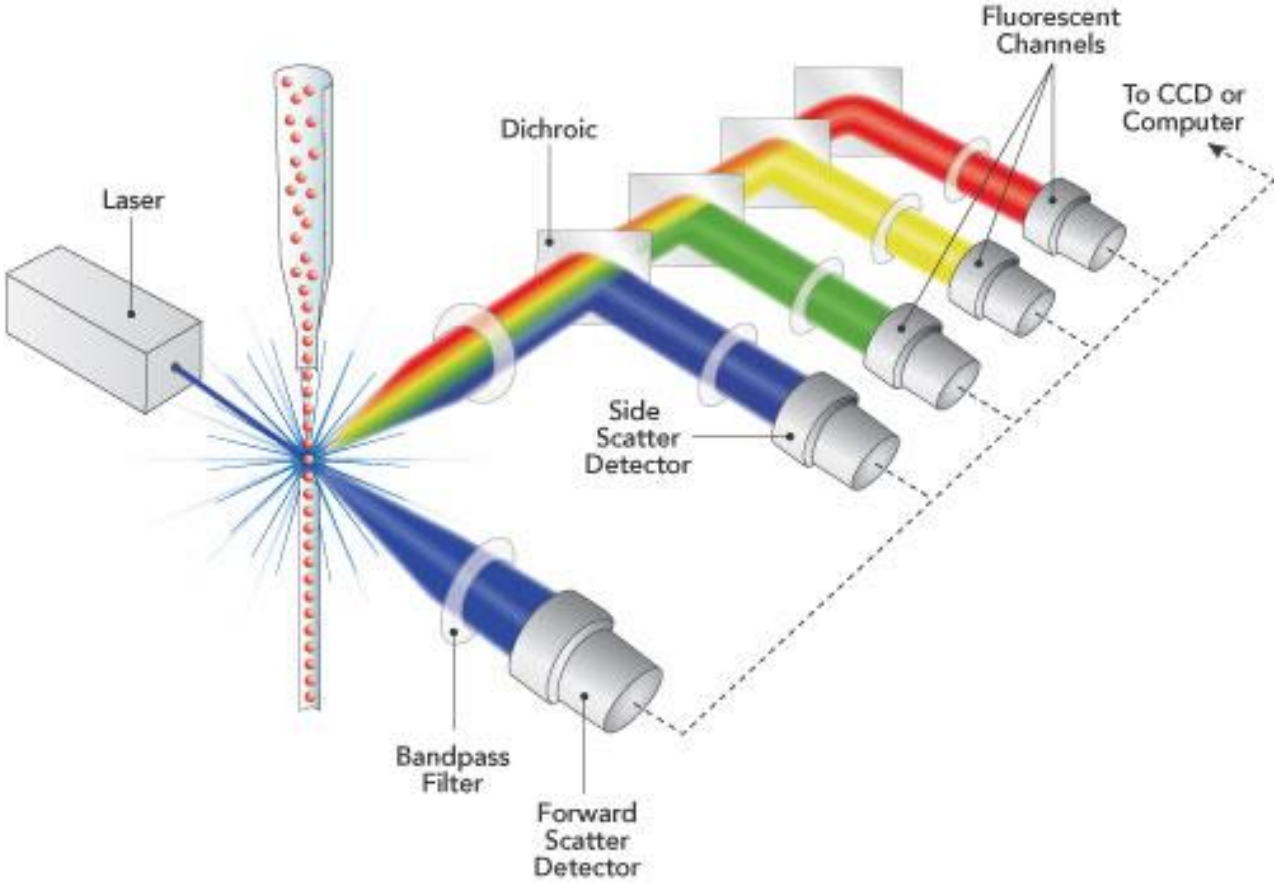
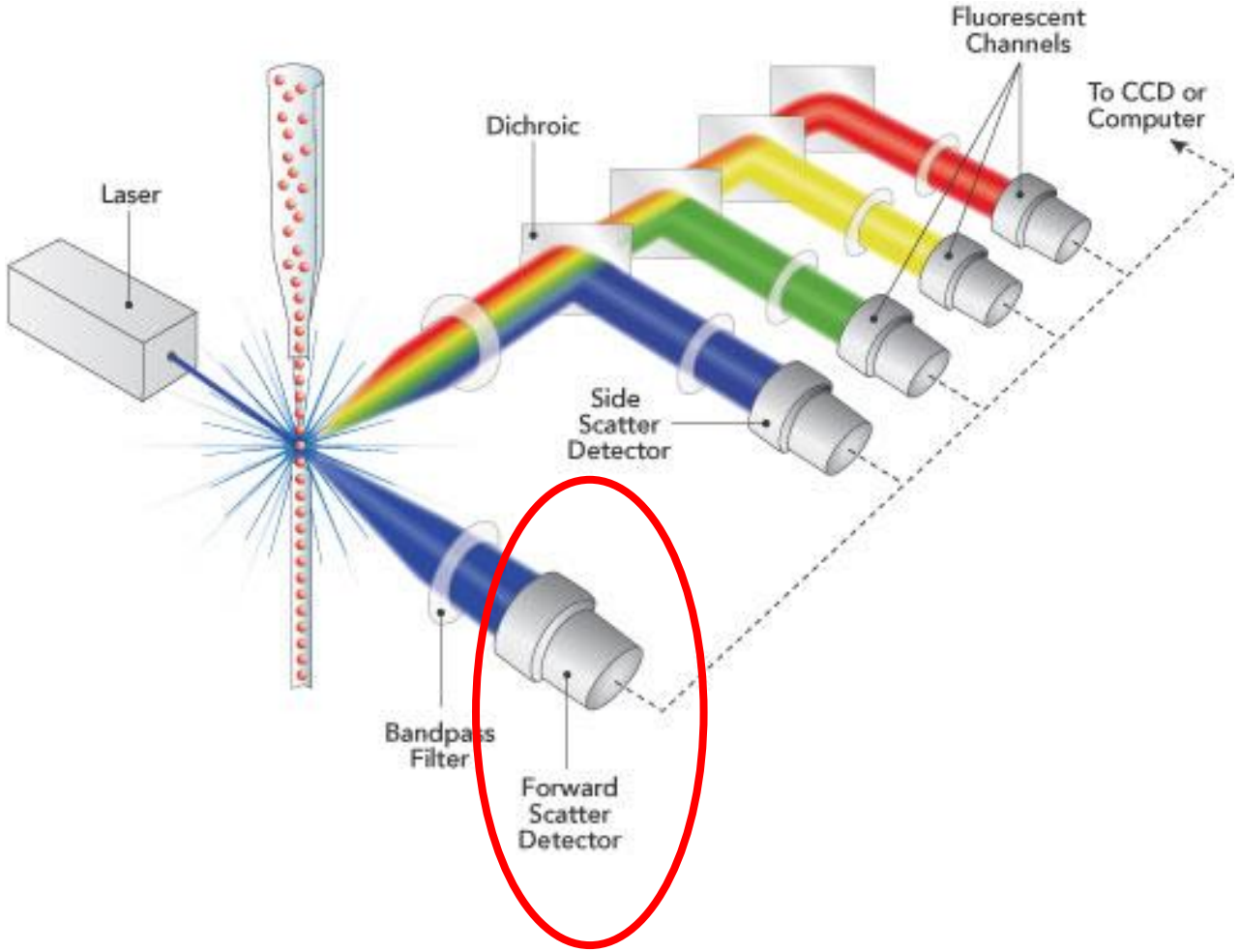


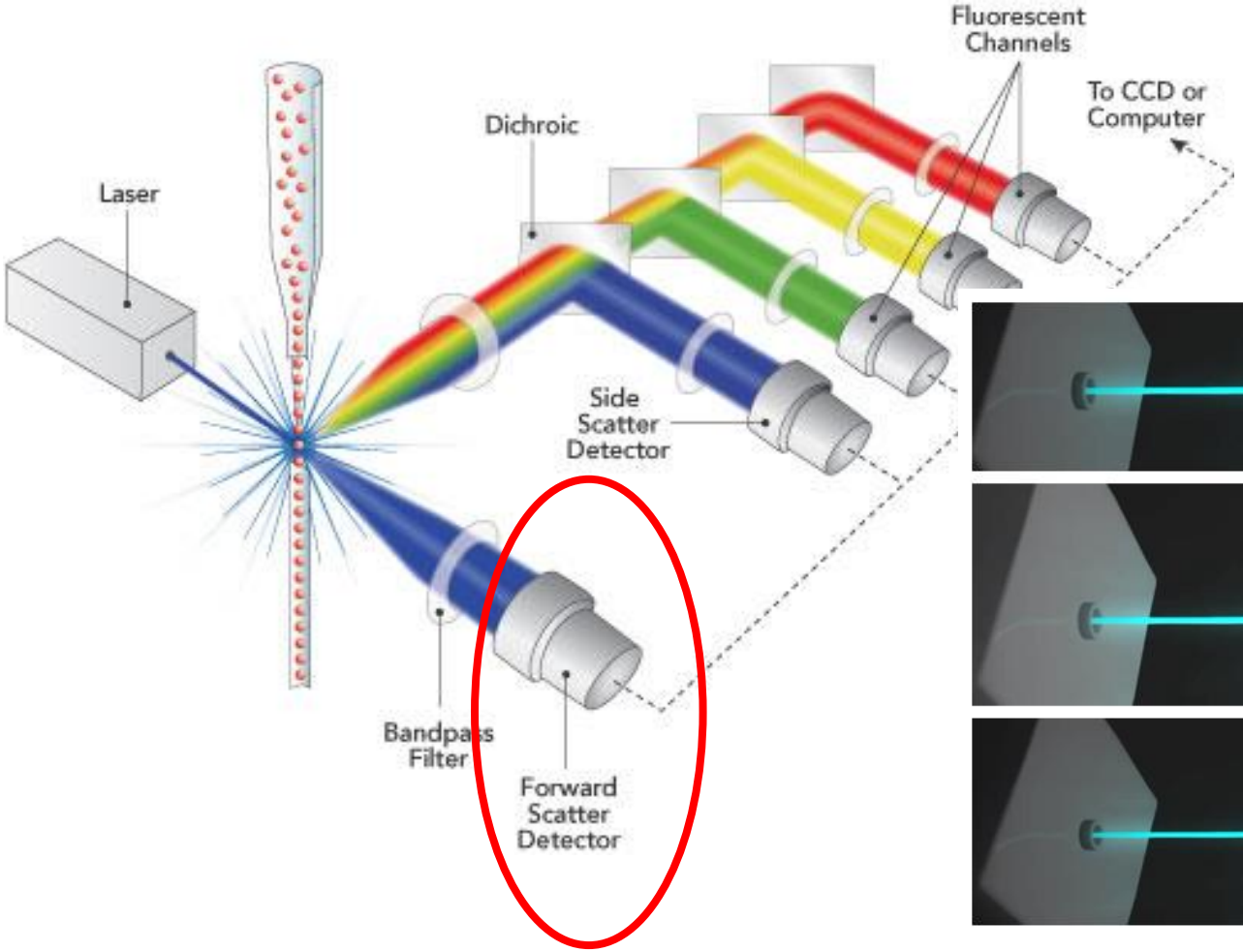
La cytométrie

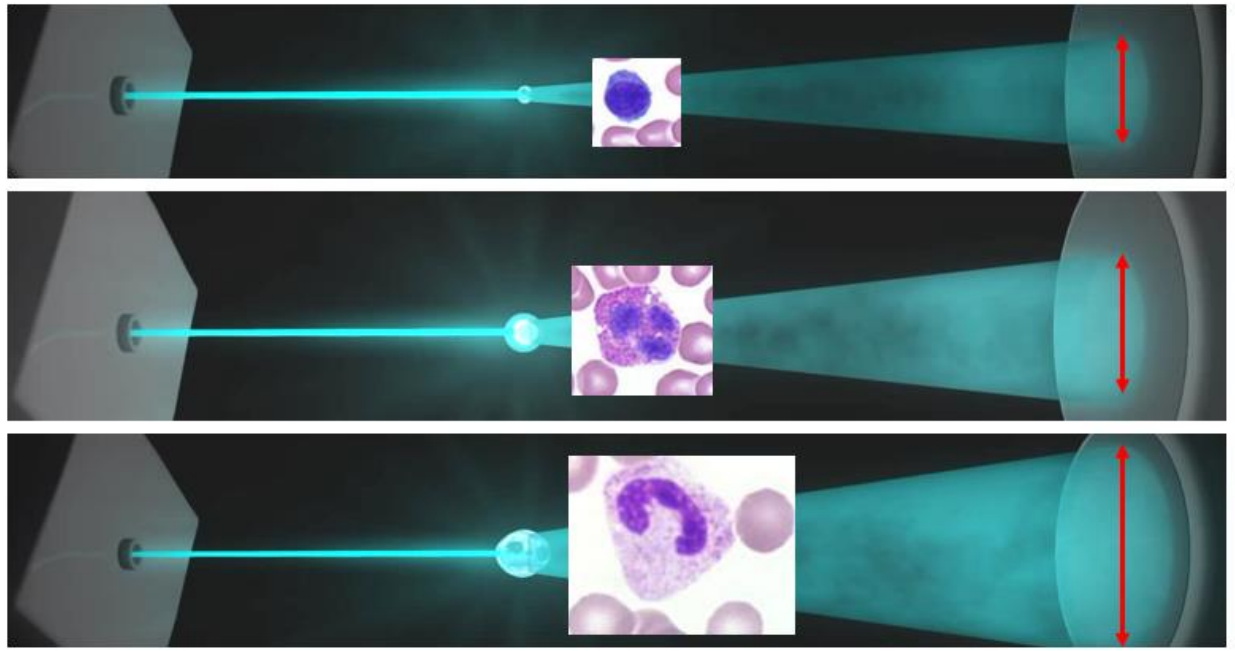


La cytométrie

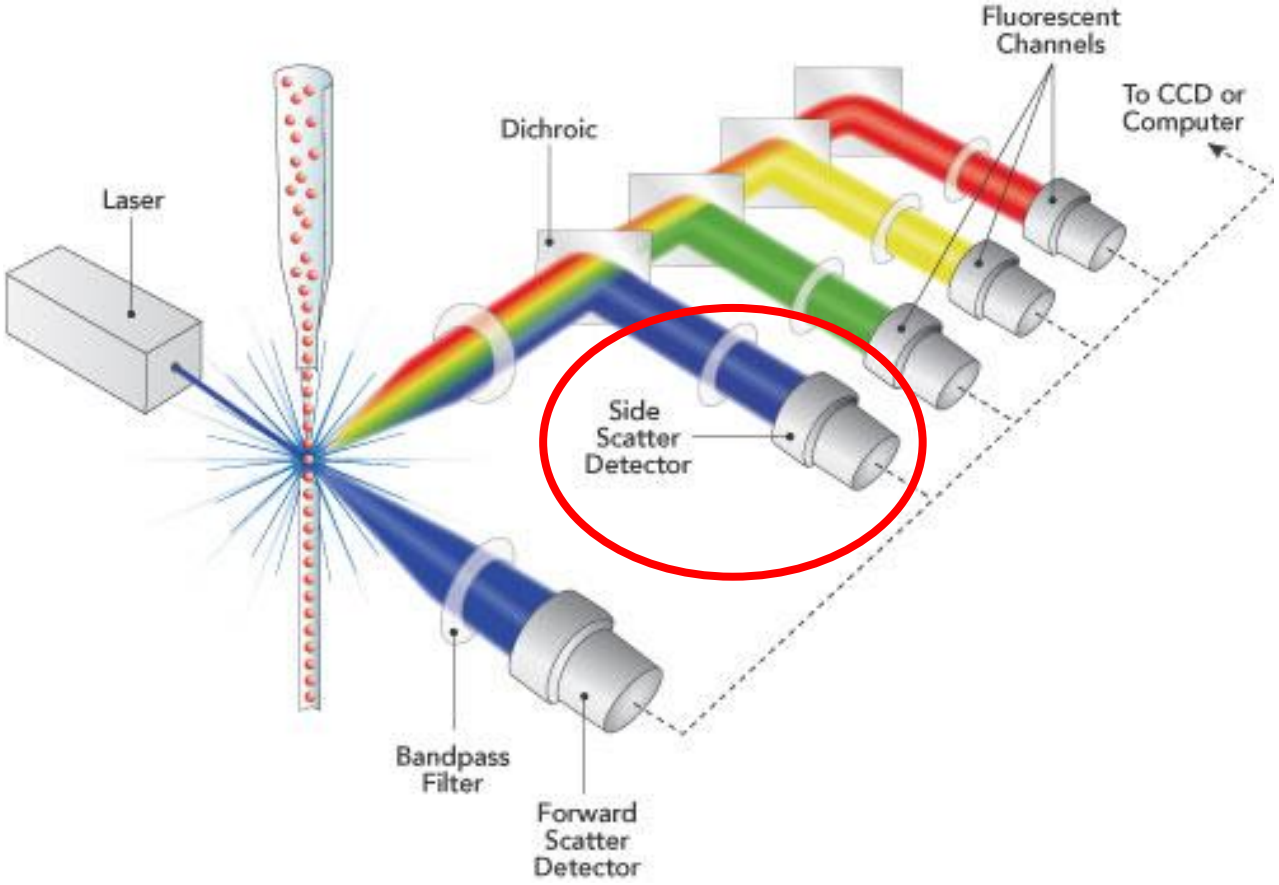


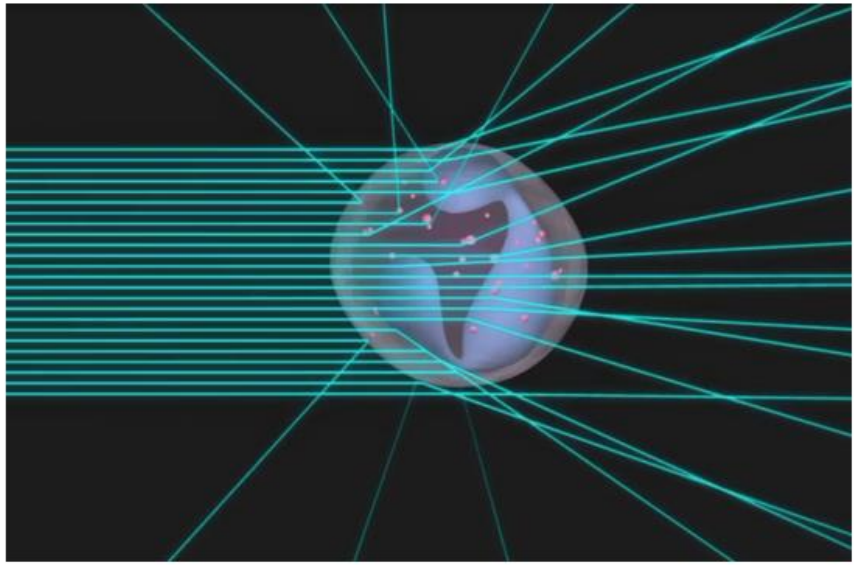
La cytométrie



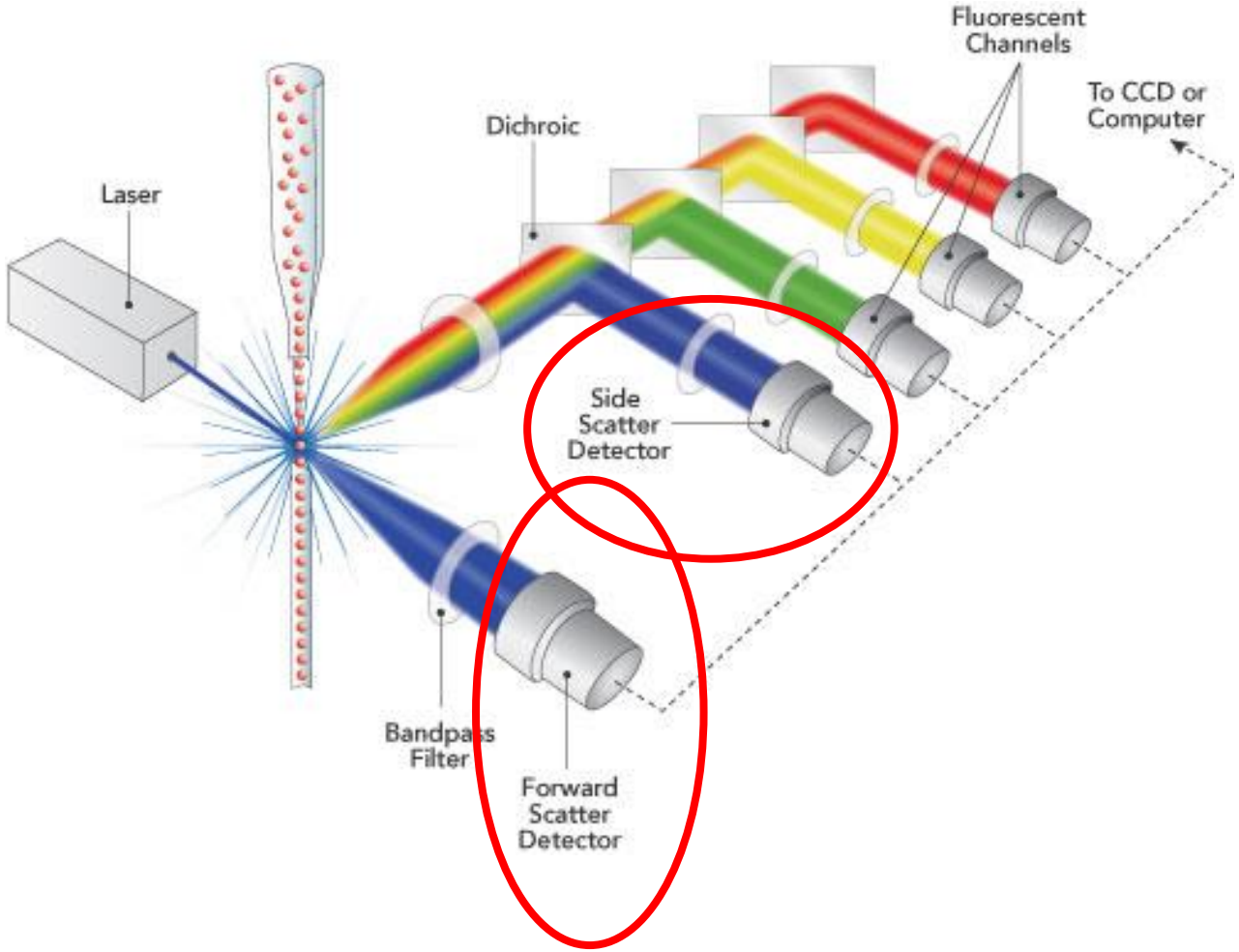


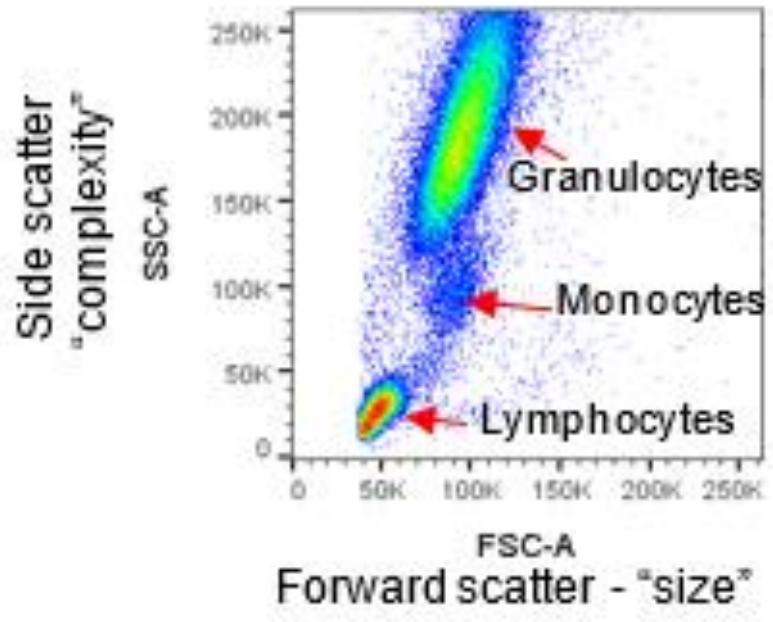
La cytométrie





La cytométrie





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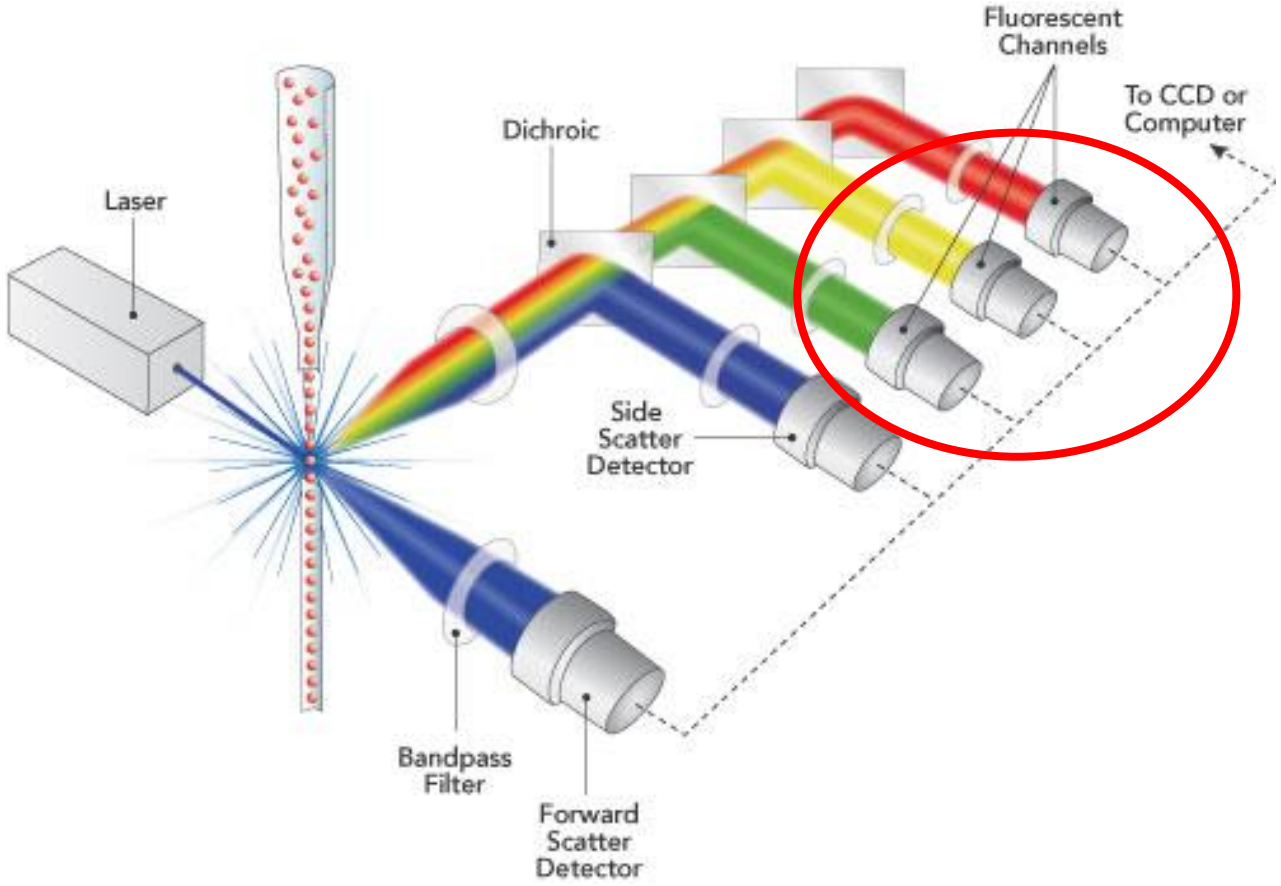

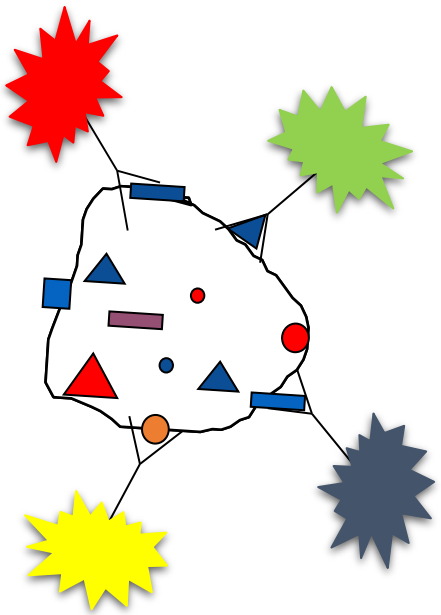

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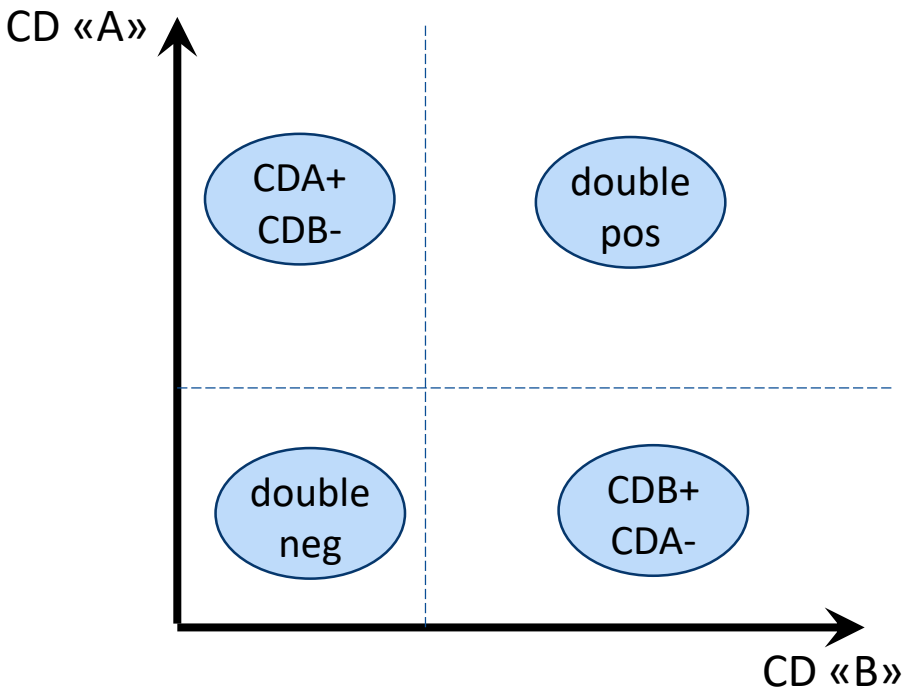
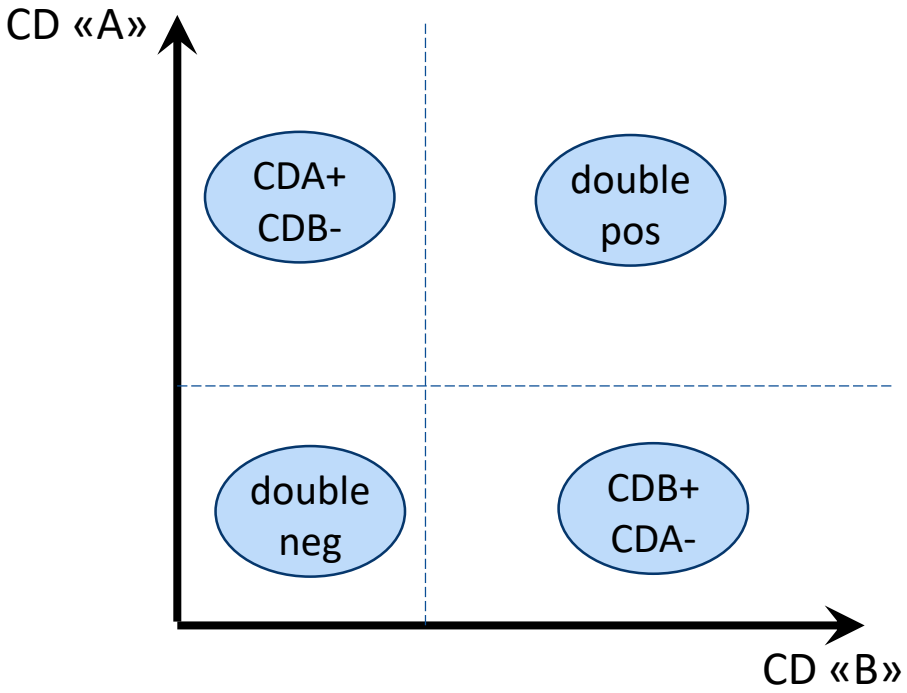
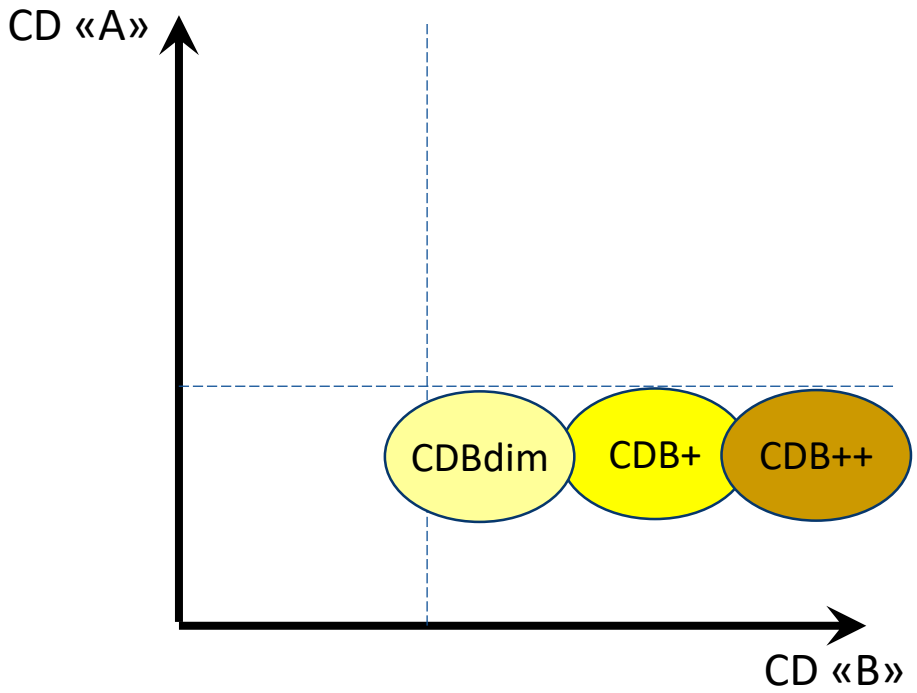
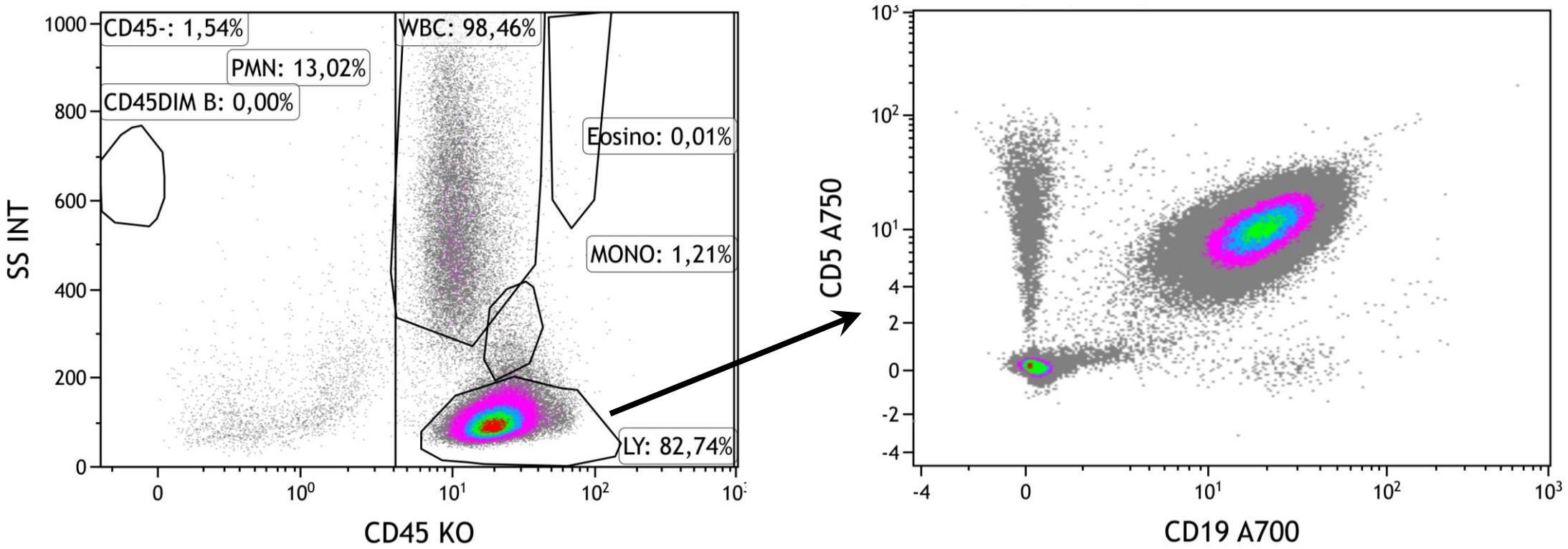


La cytométrie

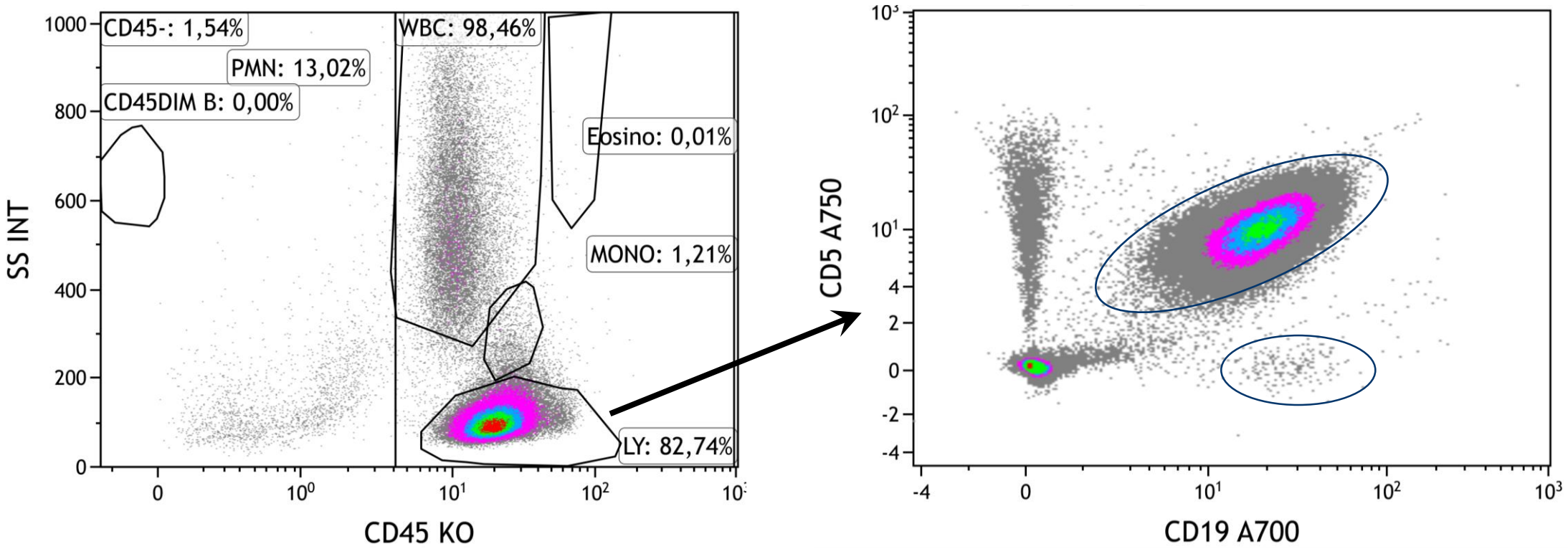




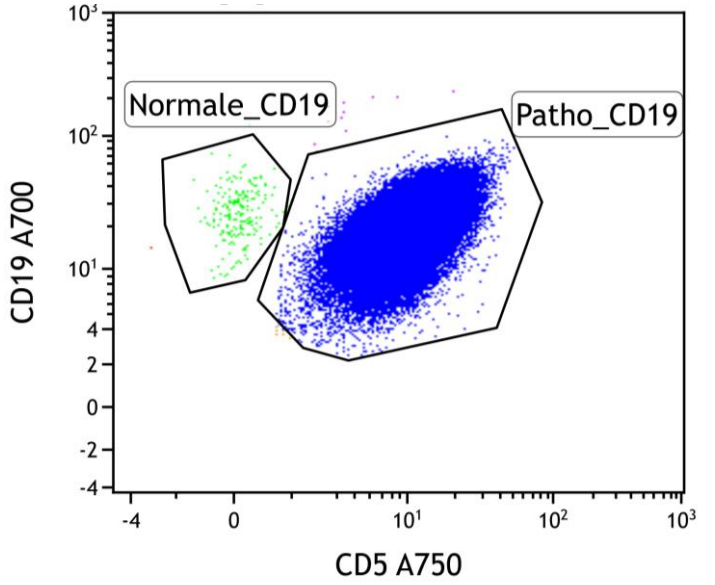
Application: la LLC-B



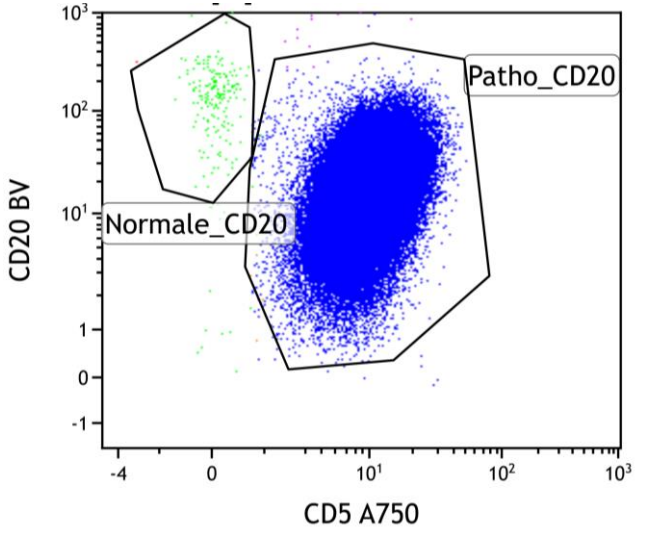
Application: la LLC-B



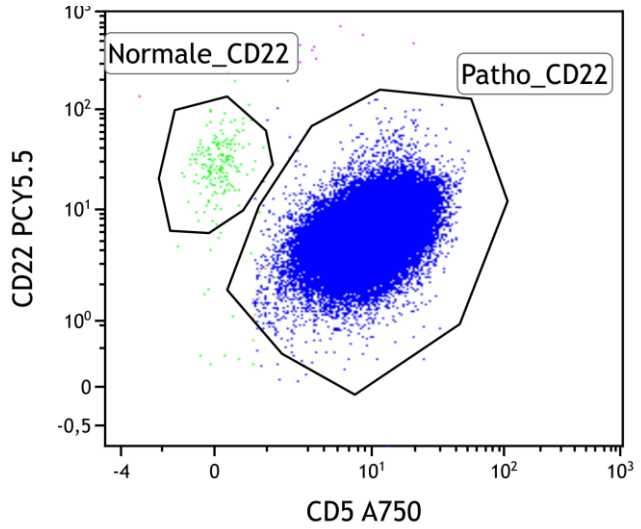
Application: la LLC-B



Gate	Y-Med	Y-AMean
Normale_CD19	24,74	27,11
Patho_CD19	18,38	19,91

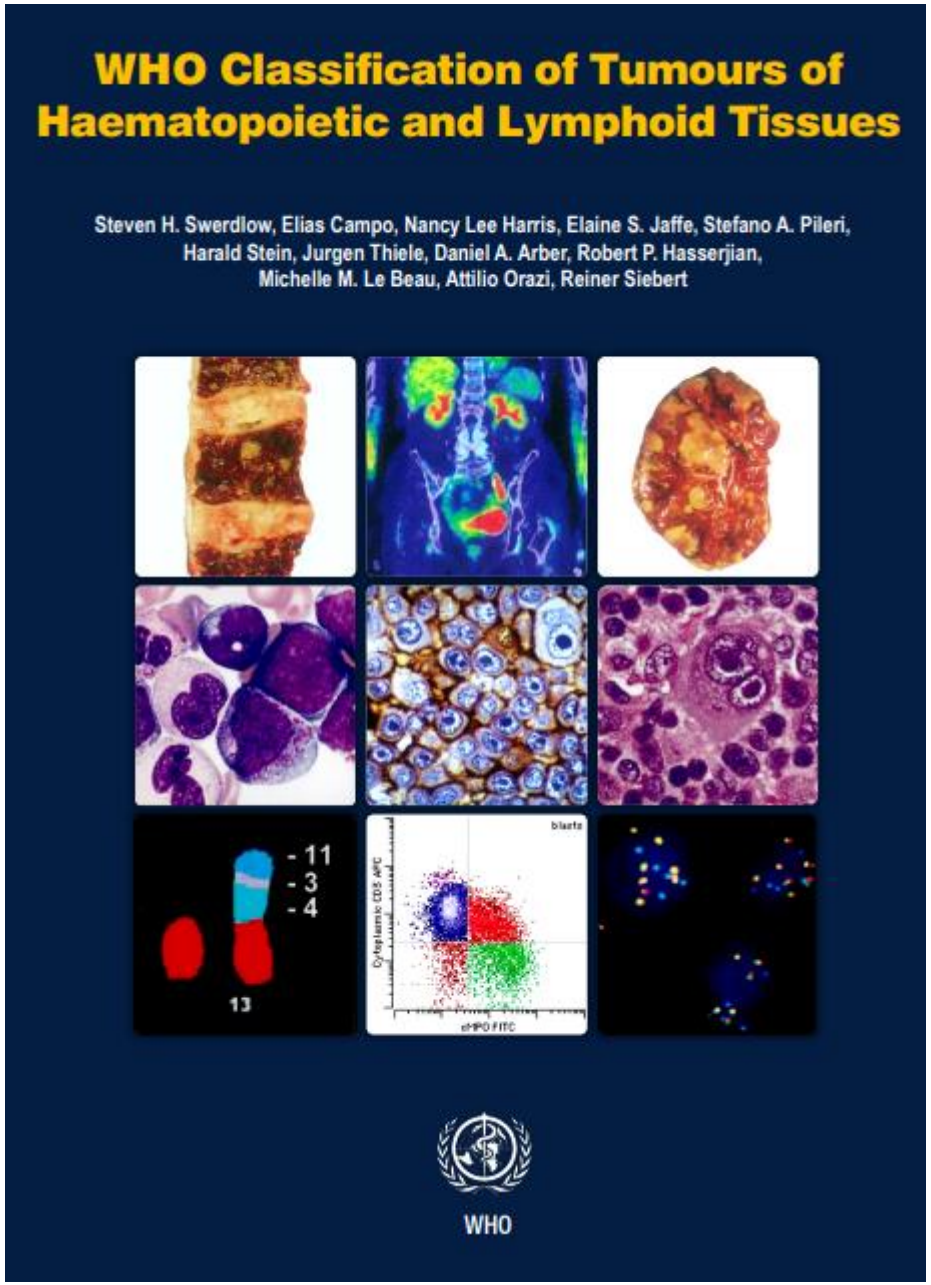


Gate	Y-Med	Y-AMean
Normale_CD20	144,94	157,42
Patho_CD20	14,51	19,70



Gate	Y-Med	Y-AMean
Normale_CD22	26,95	31,49
Patho_CD22	6,05	6,65

WHO



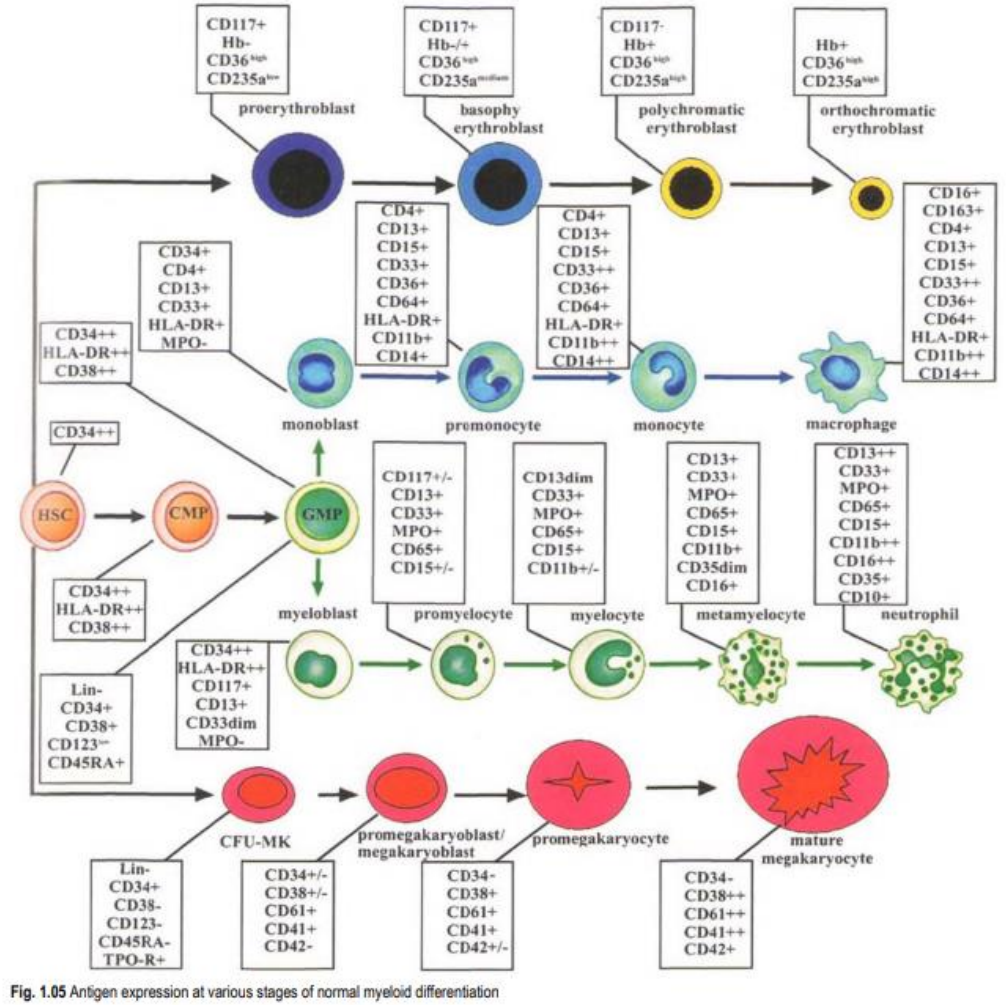


Fig. 1.05 Antigen expression at various stages of normal myeloid differentiation

Cas clinique n°I

De l’intérêt du « multi-canaux »

Septembre 2019

Depuis de nombreuses années, au moins depuis le début des années 2000, le patient est connu avec une leucocytose augmentée aux dépens des lignées neutrophiliques et lymphocytaires.

Le bilan biologique effectué en date du 9/10/2019 montre une hyperleucocytose avec 21380 él/mm³ dont 9490 lymphocytes. Parmi ceux-ci, il existe 6631 cellules dont l'immunophénotypage est compatible avec une leucémie lymphoïde chronique B. Il n'y a pas d'anémie, pas de thrombopénie, pas de stigmate d'hémolyse, pas de syndrome inflammatoire, pas d'élévation enzymatique suspecte, pas de pic à l'électrophorèse des protéines.

En conclusion :
Le patient présente une leucémie lymphoïde chronique B au stade A selon la classification de Binet.

Présence d'une population de lymphocytes B CD19+ CD20+ CD22faible+ CD5+ CD10- CD23+ CD38- CD43++ CD79b- CD81faible+ CD200+ et monoclonale kappa à 96% parmi les lymphocytes B soit 6631 cellules / µL compatible avec une LLC-B.

Pour l'instant, une simple surveillance est préconisée.

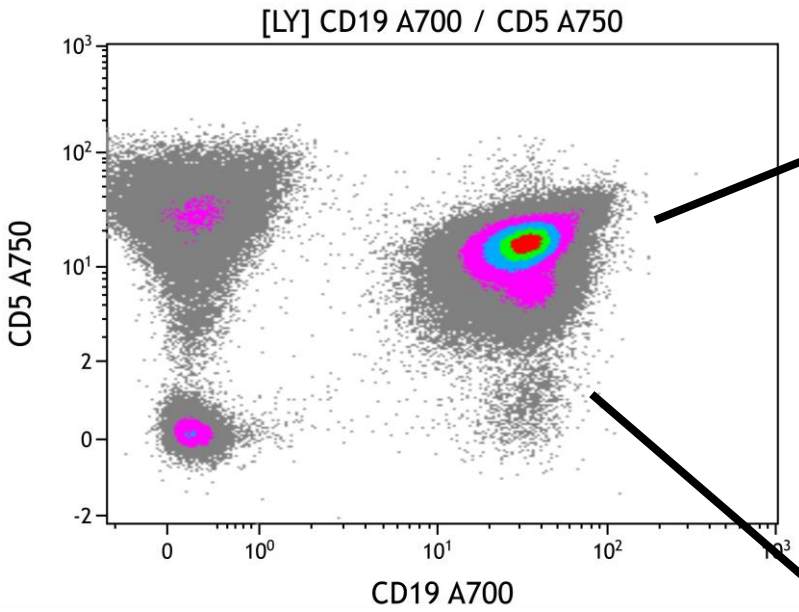
Un traitement pourrait être indiqué :

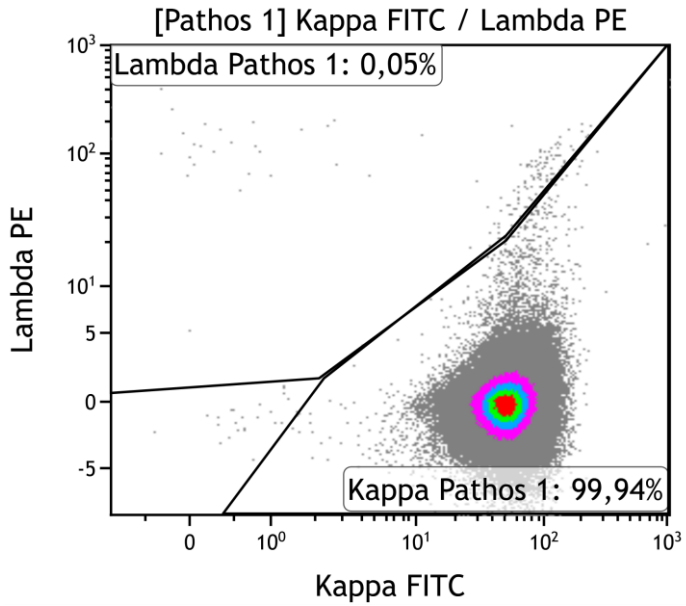
- Si les lymphocytes doublent en moins de 6 mois.
- Si le patient développe une anémie à moins de 10 g/dL ou une thrombopénie à moins de 100000 él/mm³.
- Si le patient développe des symptômes systémiques (amaigrissement, sudation…).
- S'il développe des adénopathies gênantes et/ou une splénomégalie.

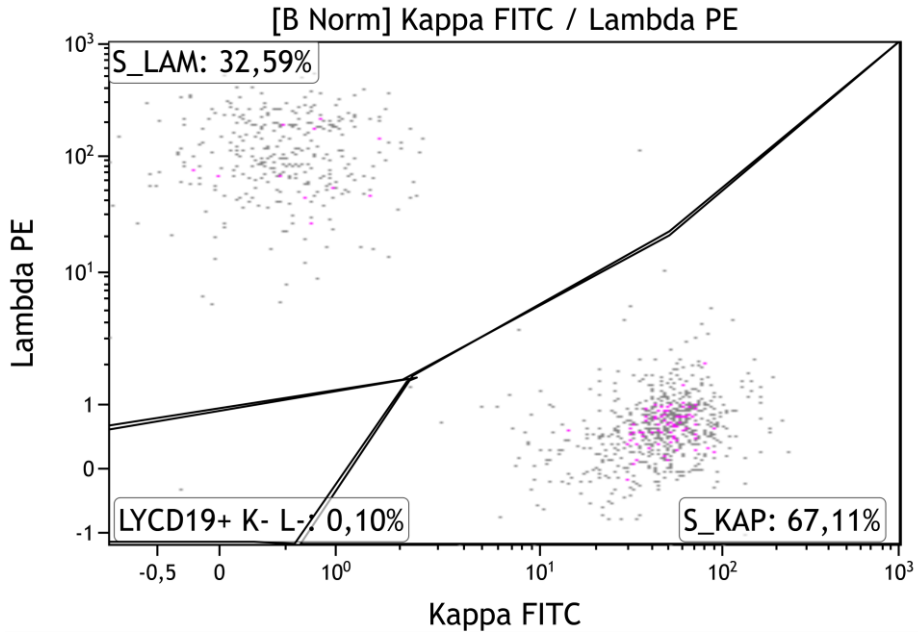
Août 2022

GR	4.48 x10^6/mm³
HB	13.3 g/dL
HCT	39.2 %
MCV	87.5 µm³
MCH	29.7 pg
MCHC	33.9 g/dL
RDW	14.3 %
PLT	218 x10^3/mm³
MPV	10.6 µm³
P-MFV	8.5 fL
RETIC %	1.55 %
RET VA	69.4 x10^3/mm³
GB	77.66 x10^3/mm³
GB_CONF	Formule réalisée au m
PMN	*7.1 %
PMN VA	*5.38 x10^3/mm³
PMN MANU	7.0 %
PMN MANU VA	5.44 x10^3/mm³
LYM Ctrl F	90.5 %
LYM VA	*70.28 x10^3/mm³
LYM VA	70.28 x10^3/mm³
MONO	1.0 %
MONO VA	0.78 x10^3/mm³
EOS	1.5 %
EOS VA	1.16 x10^3/mm³
BASO	0.0 %
BASO VA	0.00 x10^3/mm³

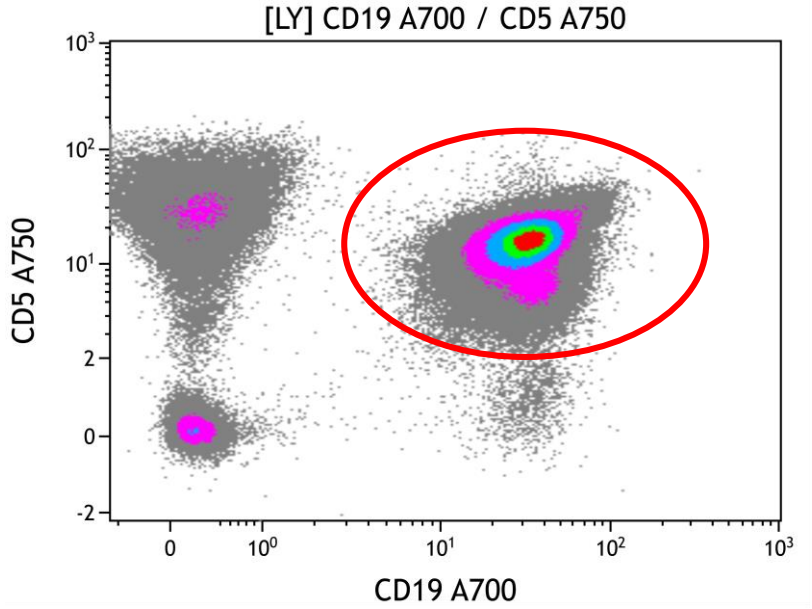
Août 2022

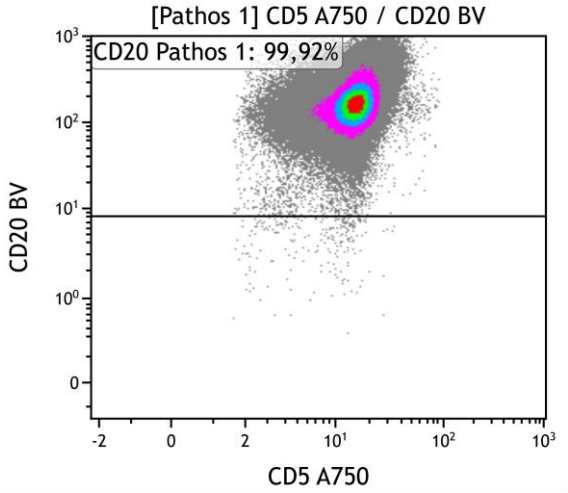

[LY] CD19 A700 / CD5 A750
CD5 A750
CD19 A700

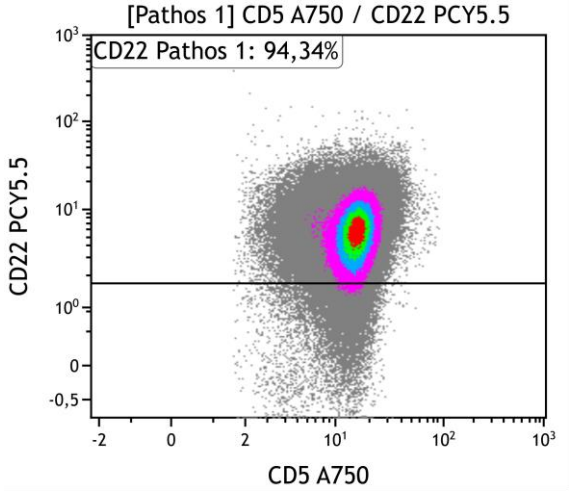

[Pathos 1] Kappa FITC / Lambda PE
Lambda Pathos 1: 0,05%
Kappa Pathos 1: 99,94%
Lambda PE
Kappa FITC

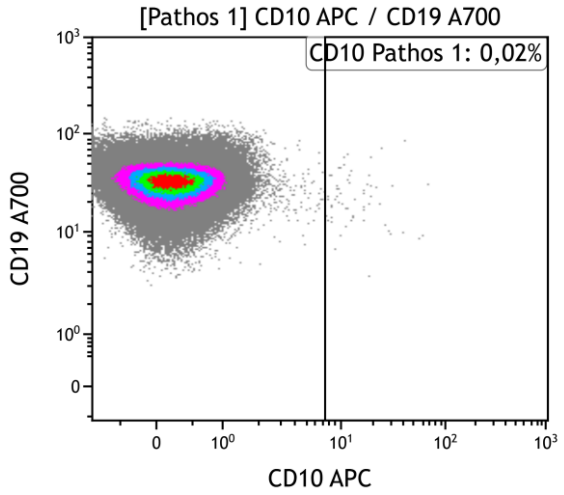

[B Norm] Kappa FITC / Lambda PE
S_LAM: 32,59%
LYCD19+ K- L-: 0,10%
S_KAP: 67,11%
Lambda PE
Kappa FITC

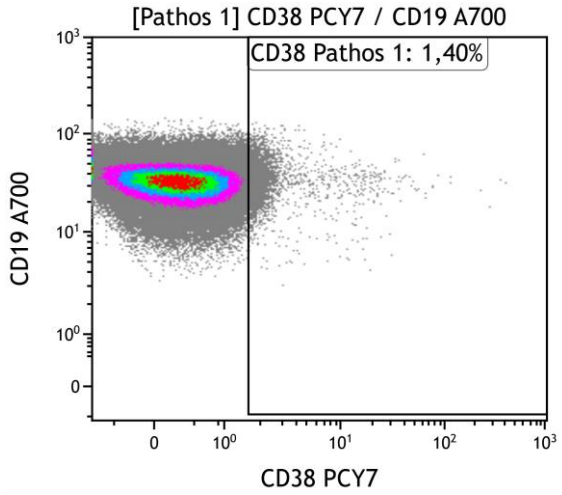
Août 2022





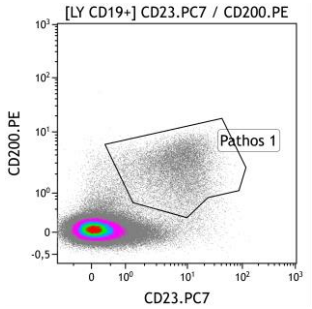


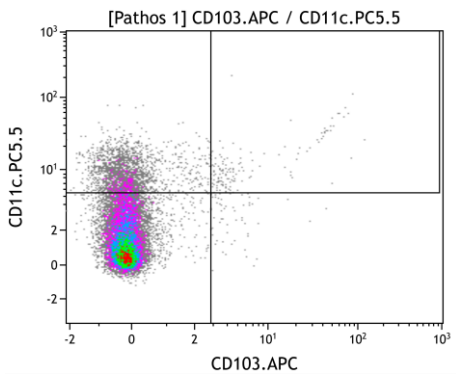


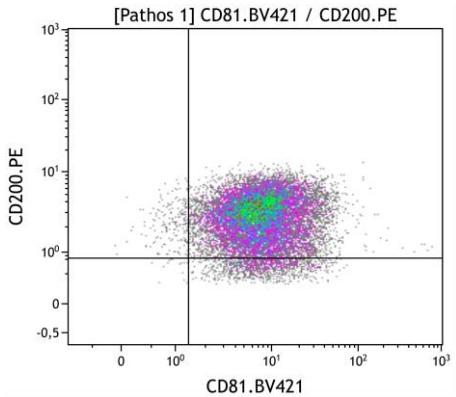


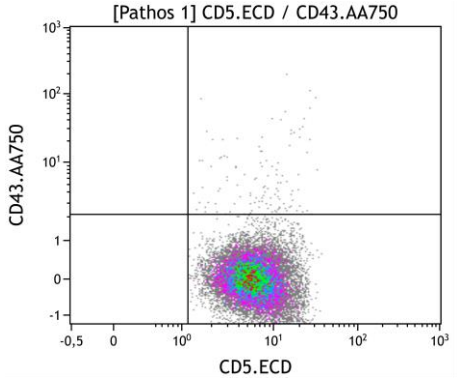
Présence d'une population de lymphocytes B CD19+ CD20+ CD22faible+ CD5+ CD10- CD38-

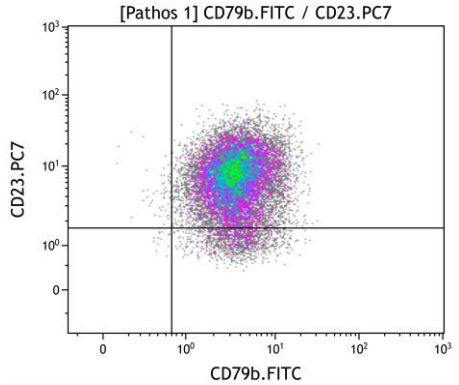
Août 2022

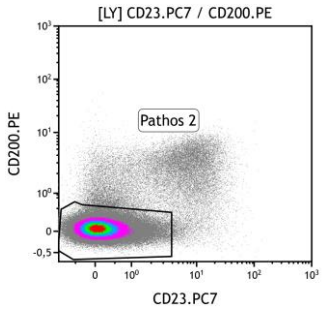

[LY CD19+] CD23.PC7 / CD200.PE
Pathos 1
CD200.PE
CD23.PC7

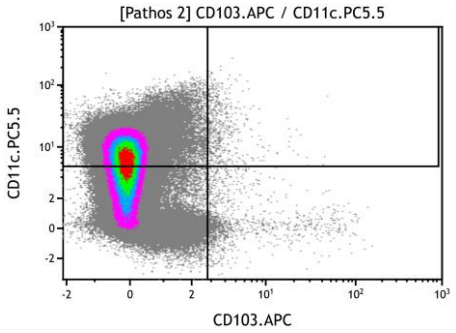

[Pathos 1] CD103.APC / CD11c.PC5.5
CD11c.PC5.5
CD103.APC

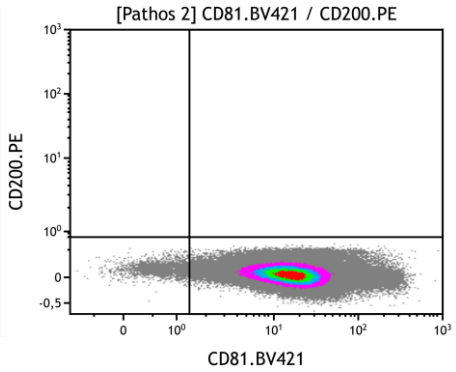

[Pathos 1] CD81.BV421 / CD200.PE
CD200.PE
CD81.BV421

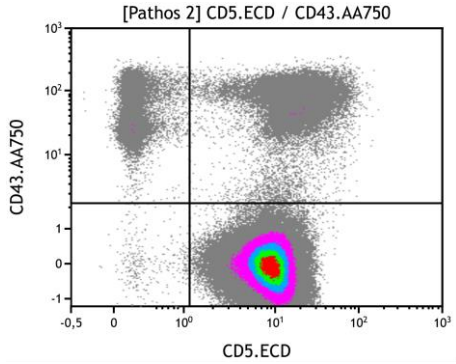

[Pathos 1] CD5.ECD / CD43.AA750
CD43.AA750
CD5.ECD

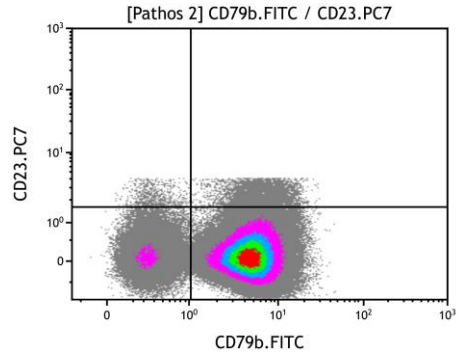

[Pathos 1] CD79b.FITC / CD23.PC7
CD23.PC7
CD79b.FITC


[LY] CD23.PC7 / CD200.PE
Pathos 2
CD200.PE
CD23.PC7


[Pathos 2] CD103.APC / CD11c.PC5.5
CD11c.PC5.5
CD103.APC


[Pathos 2] CD81.BV421 / CD200.PE
CD200.PE
CD81.BV421


[Pathos 2] CD5.ECD / CD43.AA750
CD43.AA750
CD5.ECD


[Pathos 2] CD79b.FITC / CD23.PC7
CD23.PC7
CD79b.FITC

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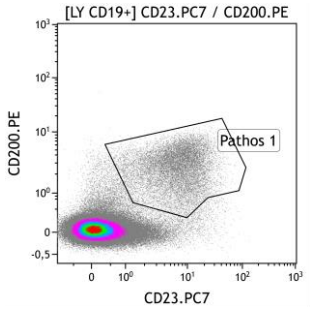
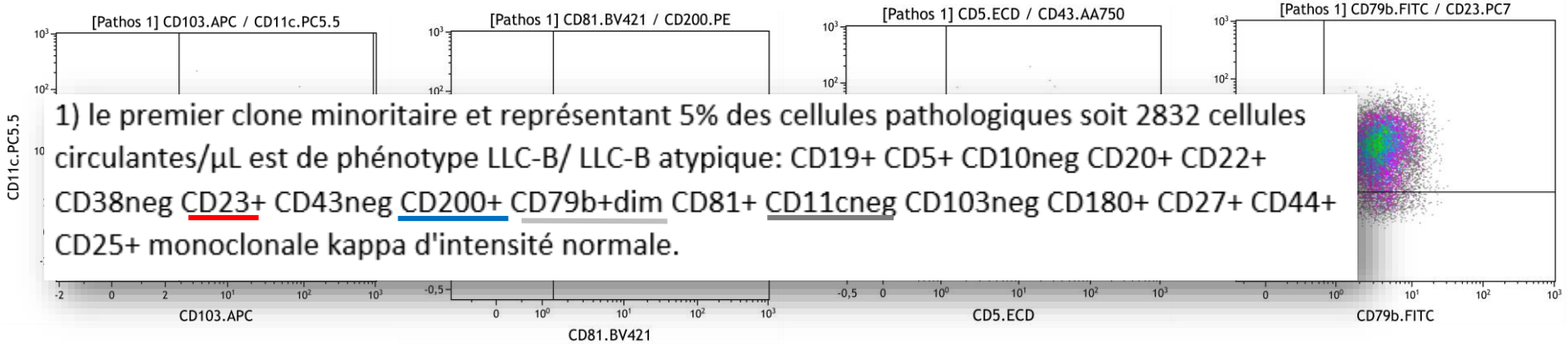
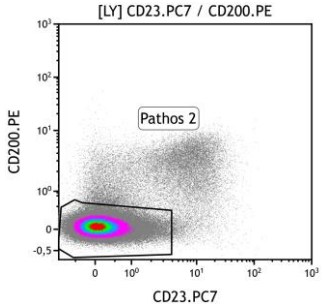
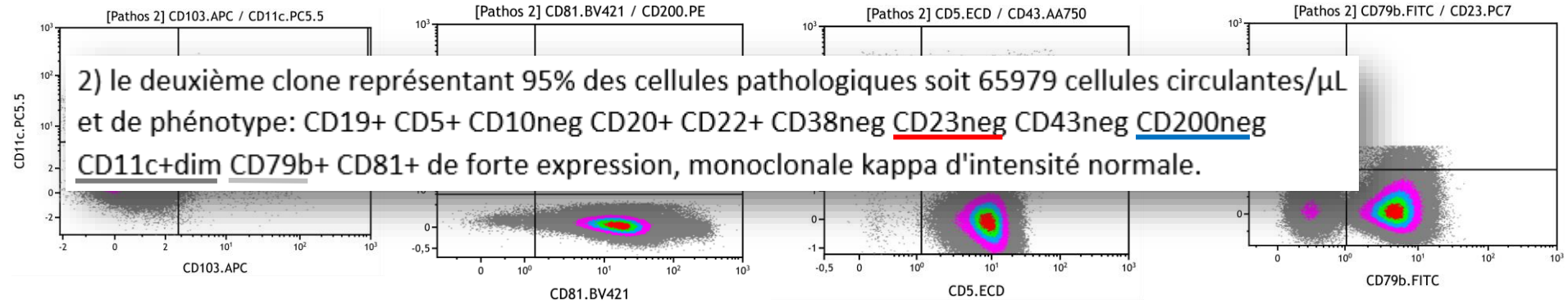




1) le premier clone minoritaire et représentant 5% des cellules pathologiques soit 2832 cellules circulantes/µL est de phénotype LLC-B/ LLC-B atypique: CD19+ CD5+ CD10neg CD20+ CD22+ CD38neg CD23+ CD43neg CD200+ CD79b+dim CD81+ CD11cneg CD103neg CD180+ CD27+ CD44+ CD25+ monoclonale kappa d'intensité normale.



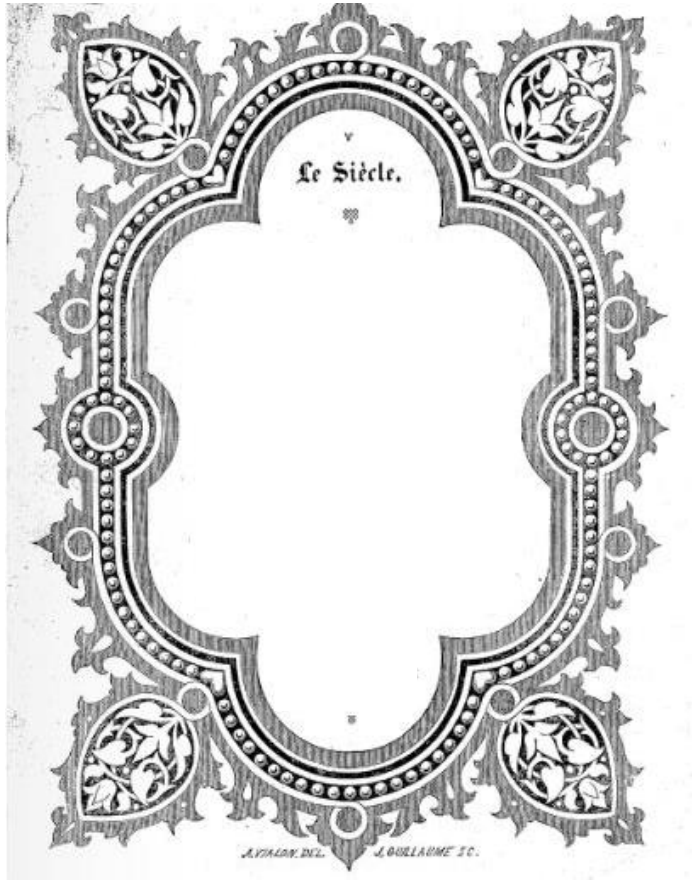


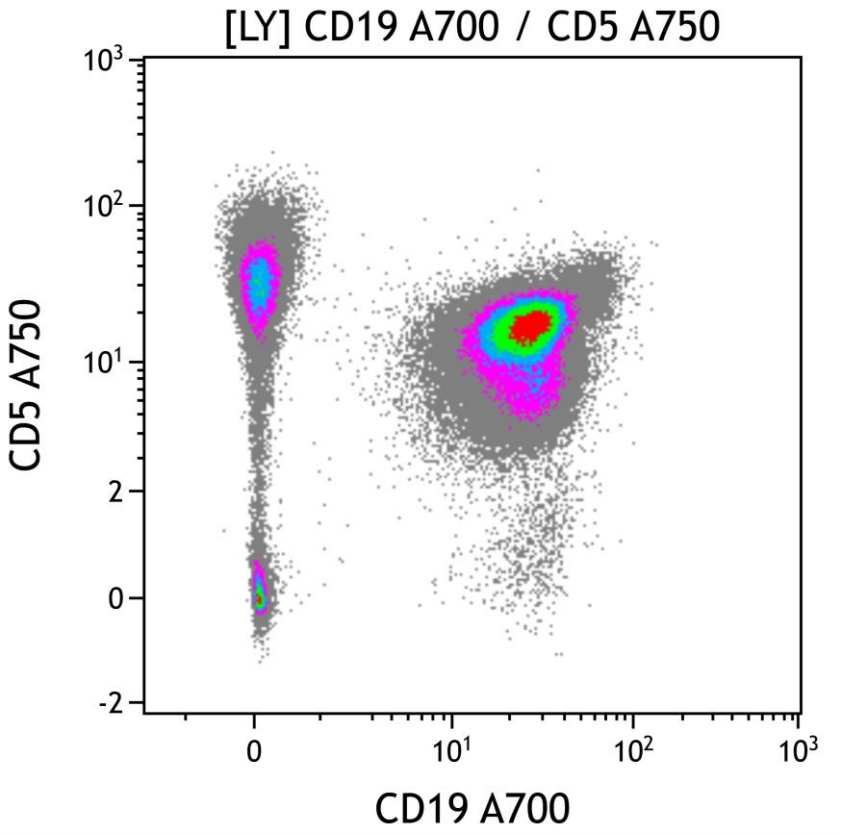
2) le deuxième clone représentant 95% des cellules pathologiques soit 65979 cellules circulantes/µL et de phénotype: CD19+ CD5+ CD10neg CD20+ CD22+ CD38neg CD23neg CD43neg CD200neg CD11c+dim CD79b+ CD81+ de forte expression, monoclonale kappa d'intensité normale.

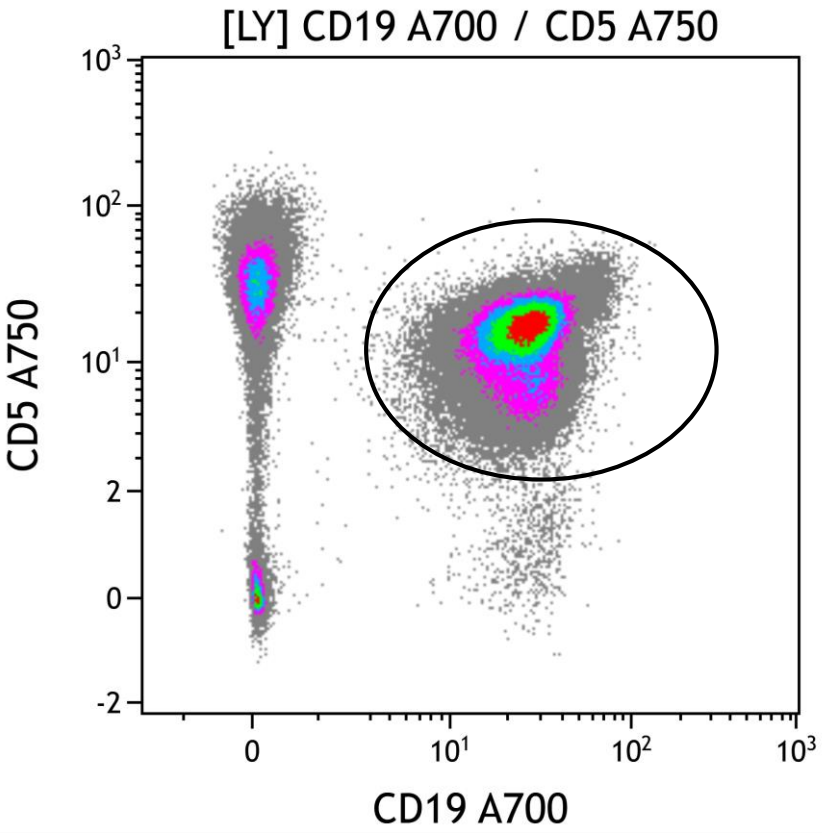
Morphologiquement, on visualise 30% de prolymphocytes parmi les lymphocytes et la présence de lymphocytes de plus grande taille, avec cytoplasme basophile, évoquant une transformation lymphomateuse ou une transformation prolymphocytaire.

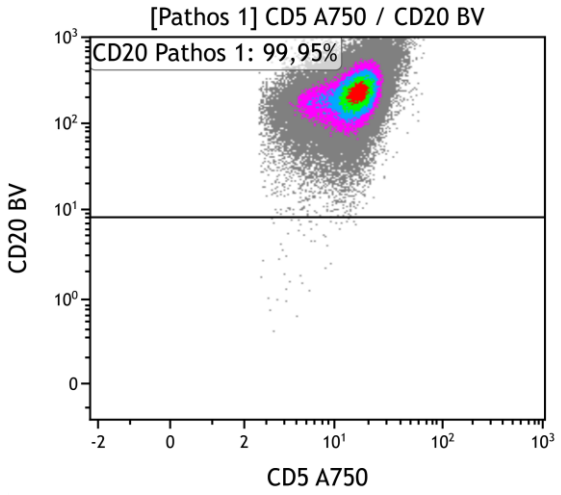
Cas clinique n°II

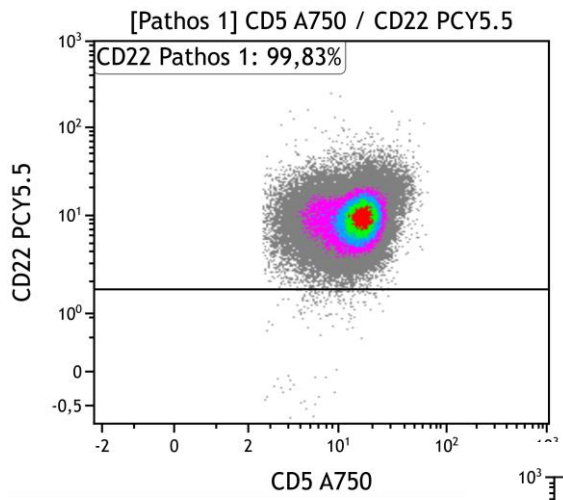
de Lagardère (1857)

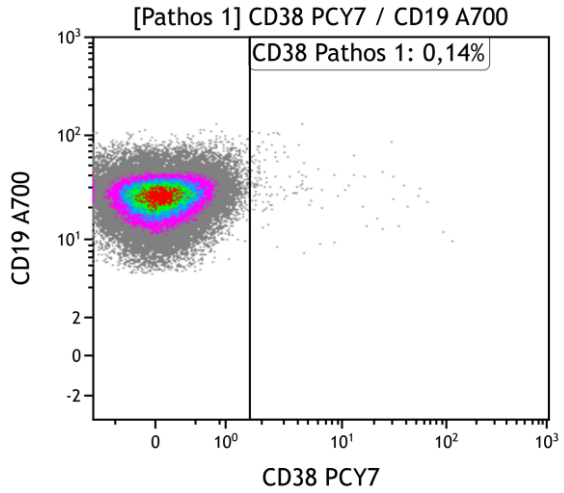


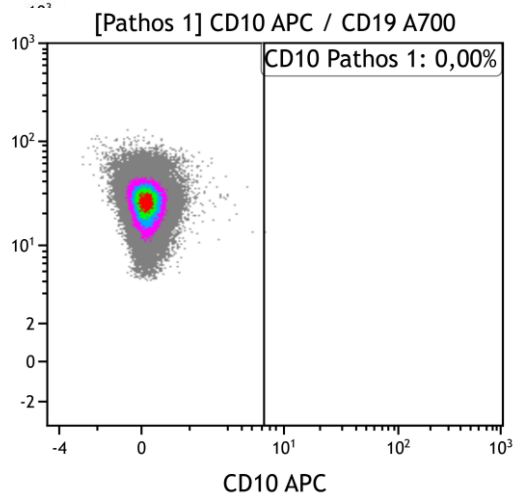

[LY] CD19 A700 / CD5 A750
CD5 A750
CD19 A700
10^3
10^2
10^1
2
0
-2
0
10^1
10^2
10^3

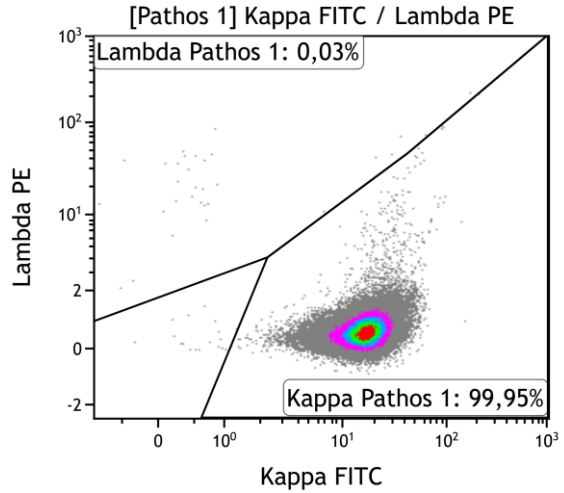

[LY] CD19 A700 / CD5 A750
CD5 A750
CD19 A700

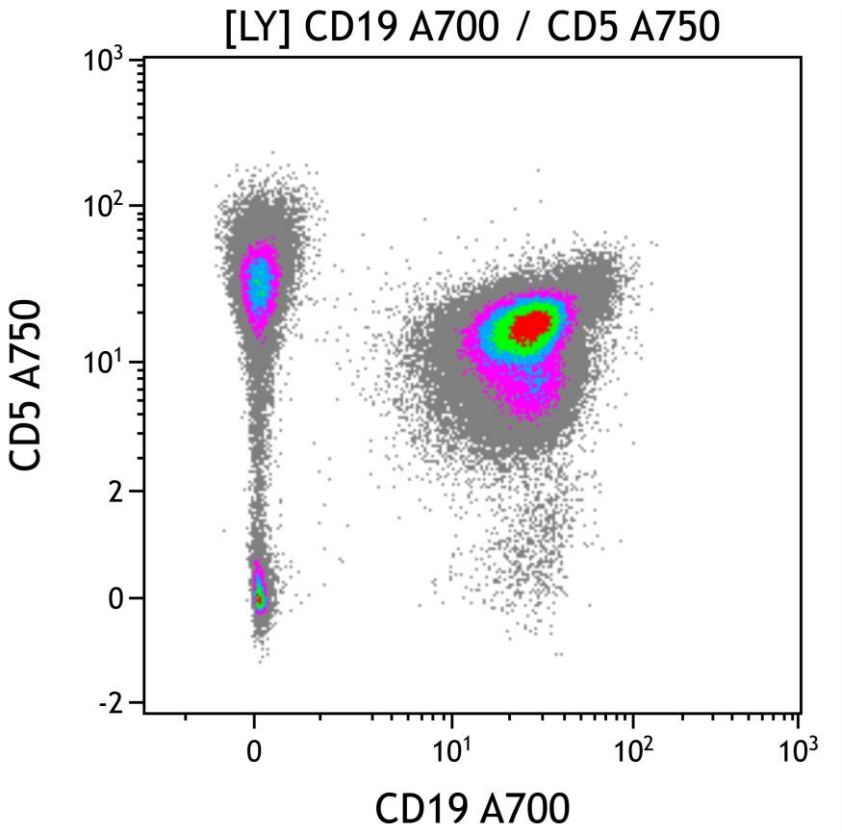

[Pathos 1] CD5 A750 / CD20 BV
CD20 Pathos 1: 99,95%
CD20 BV
CD5 A750

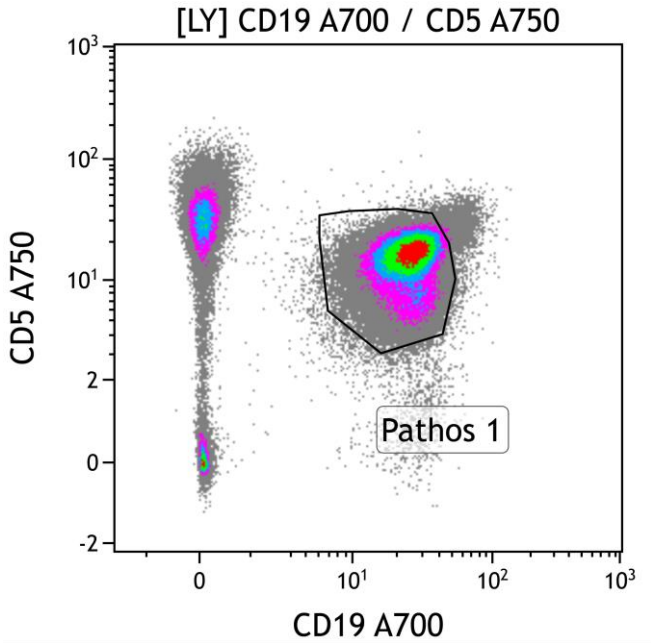

[Pathos 1] CD5 A750 / CD22 PCY5.5
CD22 Pathos 1: 99,83%
CD22 PCY5.5
CD5 A750

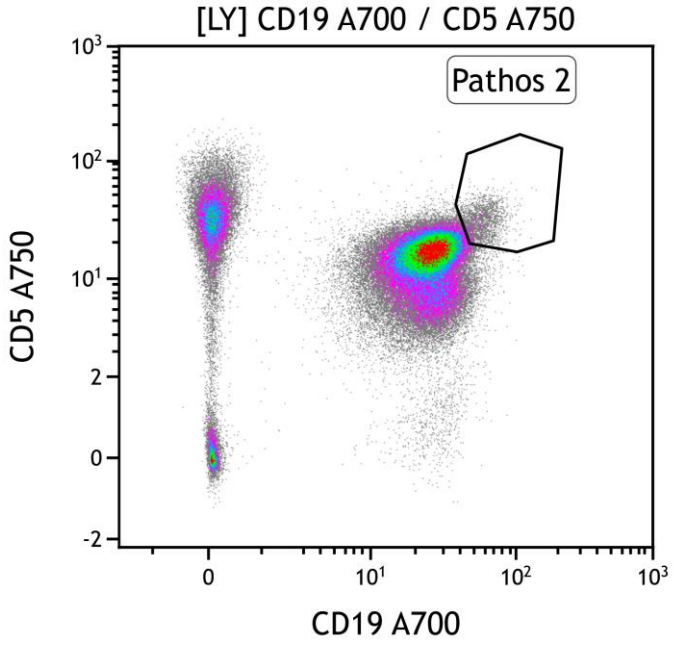

[Pathos 1] CD38 PCY7 / CD19 A700
CD38 Pathos 1: 0,14%
CD19 A700
CD38 PCY7

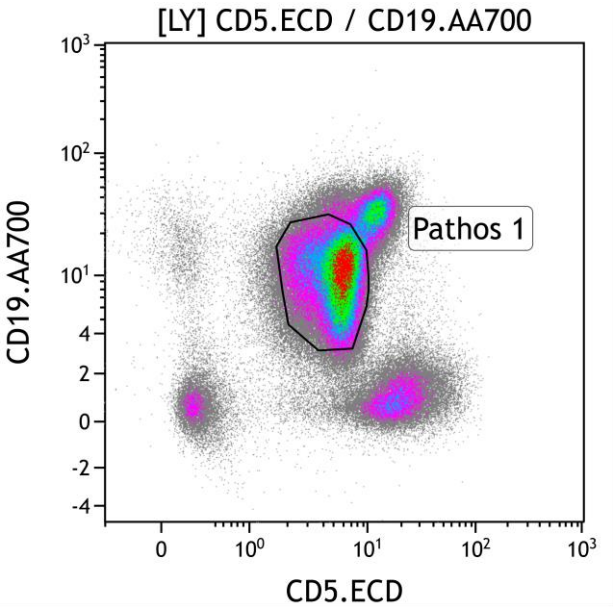

[Pathos 1] CD10 APC / CD19 A700
CD10 Pathos 1: 0,00%
CD19 A700
CD10 APC

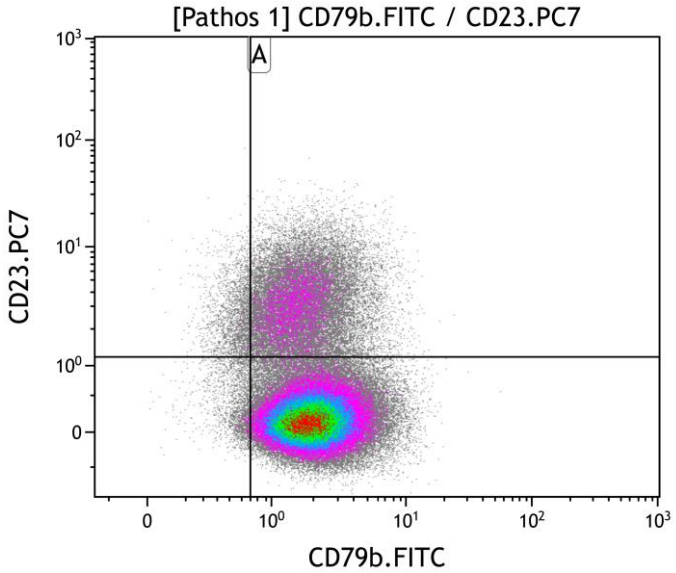

[Pathos 1] Kappa FITC / Lambda PE
Lambda Pathos 1: 0,03%
Kappa Pathos 1: 99,95%
Lambda PE
Kappa FITC

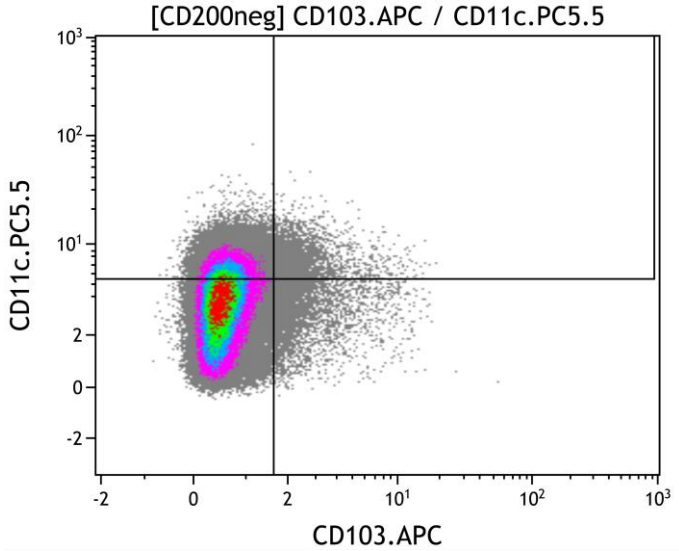

[LY] CD19 A700 / CD5 A750
CD5 A750
CD19 A700
10^3
10^2
10^1
2
0
-2
0
10^1
10^2
10^3

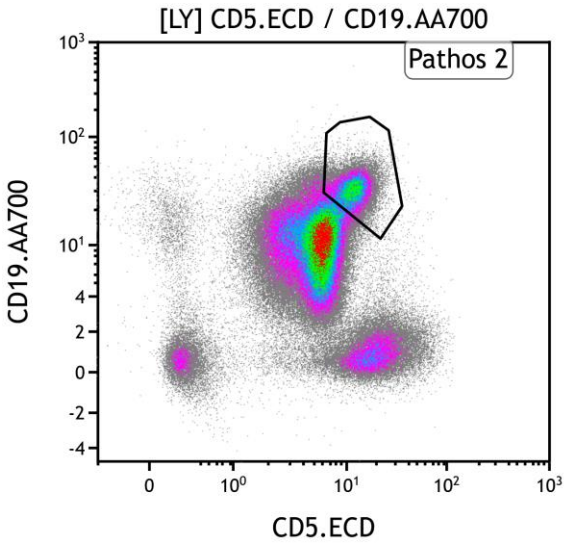

[LY] CD19 A700 / CD5 A750
CD5 A750
CD19 A700
Pathos 1
10^3
10^2
10^1
2
0
-2
0
10^1
10^2
10^3

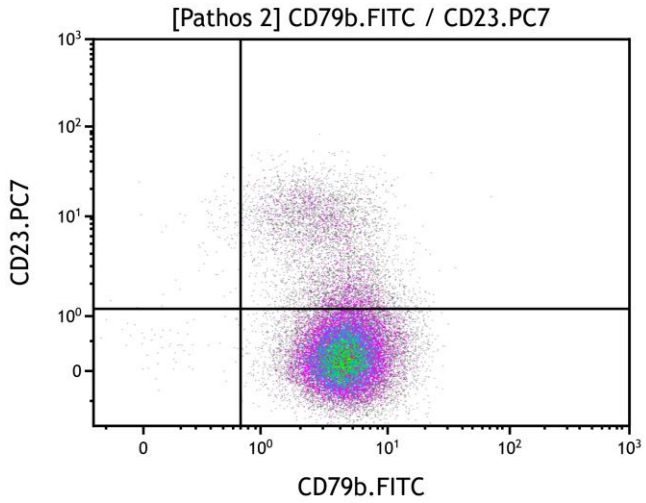

[LY] CD19 A700 / CD5 A750
CD5 A750
CD19 A700
Pathos 2
10^3
10^2
10^1
2
0
-2
0
10^1
10^2
10^3

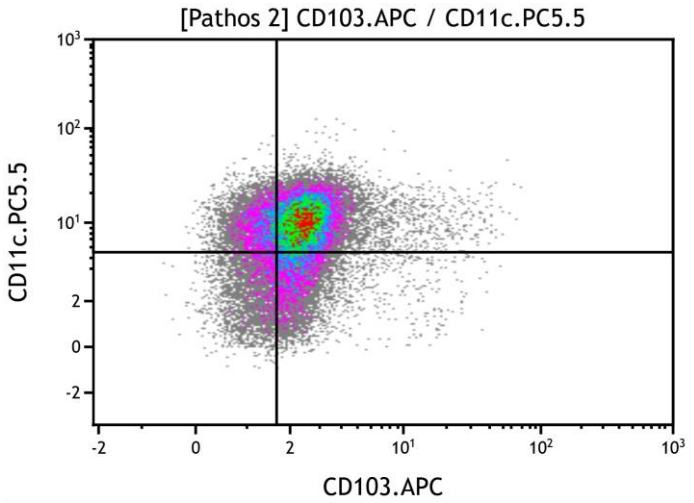

[LY] CD5.ECD / CD19.AA700
Pathos 1
CD19.AA700
CD5.ECD


[Pathos 1] CD79b.FITC / CD23.PC7
A
CD23.PC7
CD79b.FITC


[CD200neg] CD103.APC / CD11c.PC5.5
CD11c.PC5.5
CD103.APC


[LY] CD5.ECD / CD19.AA700
Pathos 2
CD19.AA700
CD5.ECD


[Pathos 2] CD79b.FITC / CD23.PC7
CD23.PC7
CD79b.FITC


[Pathos 2] CD103.APC / CD11c.PC5.5
CD11c.PC5.5
CD103.APC

Cas clinique n°III

Une aiguille dans un botte de foin

A 55-year-old man with a 1-month history of asthenia, right hypochondrial pain, and weight loss was referred to the emergency department for hepatic mass evaluation.

Abdominal computed tomography scan revealed a large necrotic mass in the liver, a second necrotic mass adjacent to the superior mesenteric vessels, and numerous subdiaphragmatic lymph nodes (aorta, right kidney, retro-crural, mesenteric, iliac, and inguinal)

→ Biopsie inguinale

A 55-year-old man with a 1-month history of asthenia, right hypochondrial pain, and weight loss was referred to the emergency department for hepatic mass evaluation.

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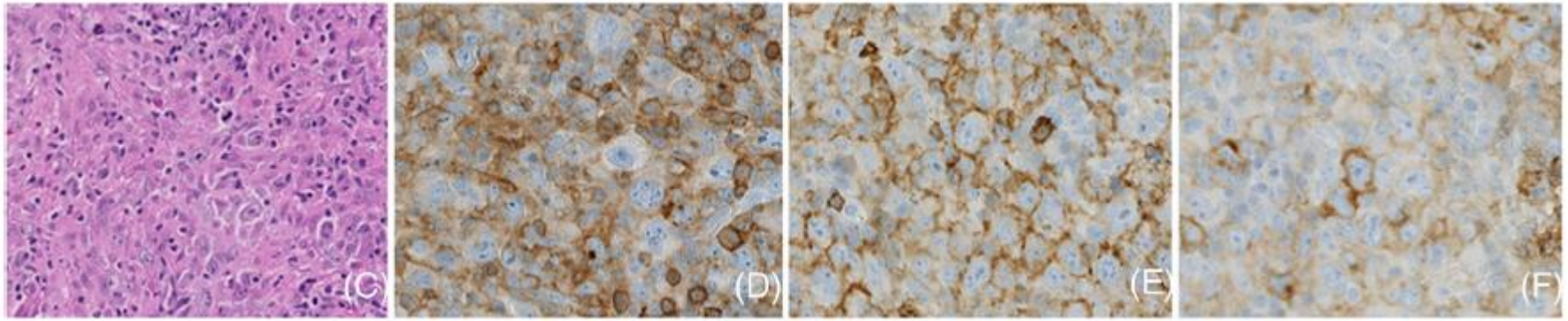

Histological examination revealed infiltration by sheets of large lymphoid cells displaying irregularly shaped nuclei with macronucleoli and moderately abundant, finely granular cytoplasm

cells were positive for common leukocyte antigen (CLA), CD20, and CD30 and negative for anaplastic lymphoma kinase (Figure 1D-F).

Epstein-Barr virus-encoded small RNA was negative.

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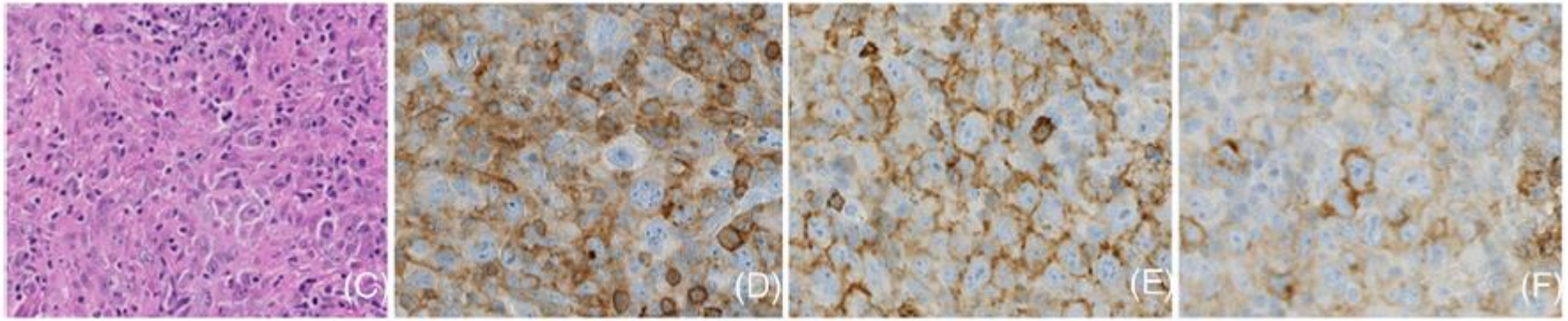

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Epstein-Barr virus-encoded small RNA was negative.

Diffuse large B-cell lymphoma (DLBCL)

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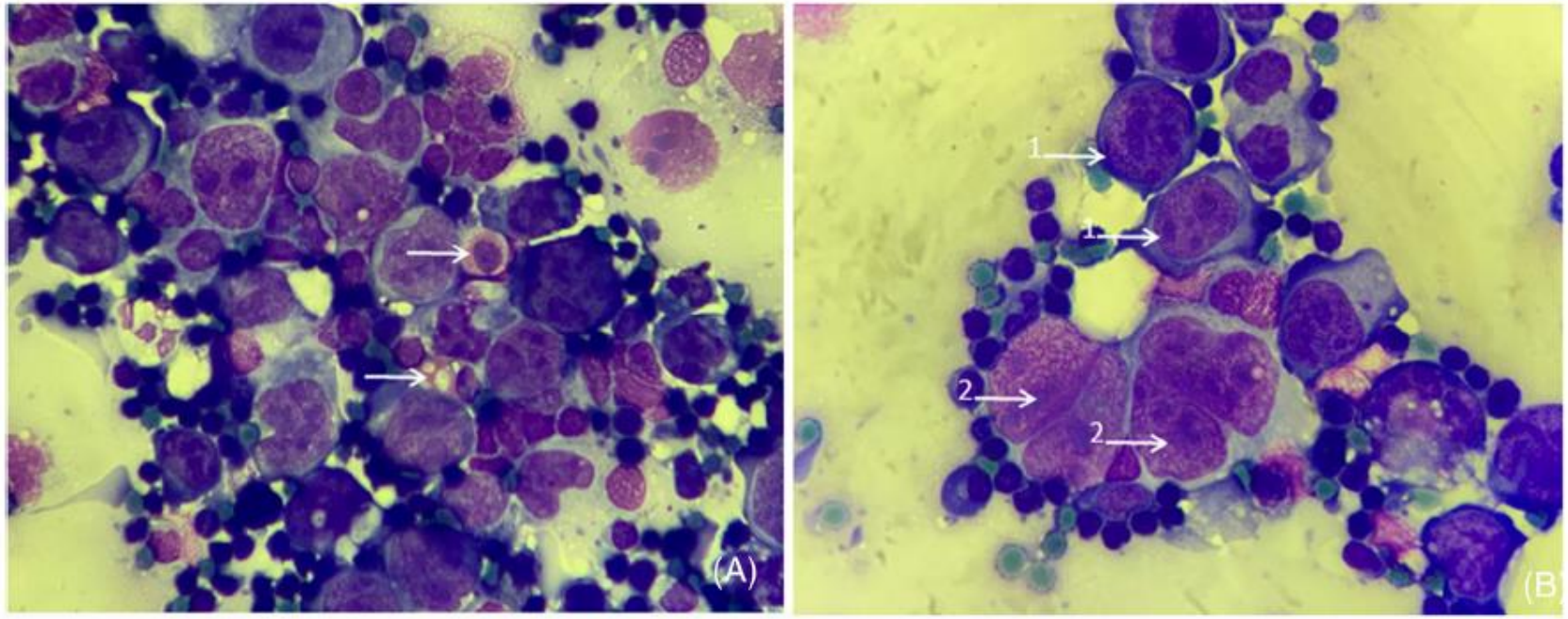
Abdominal computed tomography scan revealed a large necrotic mass in the liver, a second necrotic mass adjacent to the superior mesenteric vessels, and numerous subdiaphragmatic lymph nodes (aorta, right kidney, retro-crural, mesenteric, iliac, and inguinal)

Au labo…

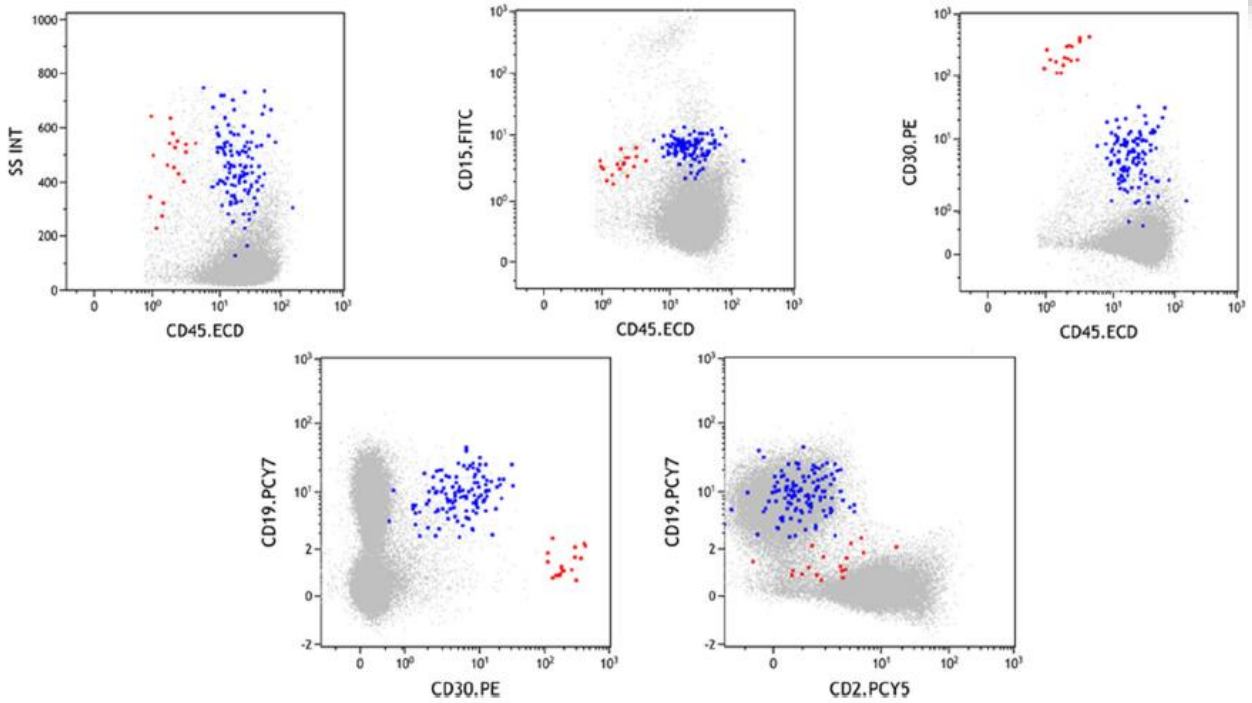


Inguinal lymph node biopsy imprint showing a heterogeneous background containing (A) eosinophils and (B, 1) large cells with various nucleocytoplasmic ratios, occasionally irregular nuclear contour, dispersed and delicately condensed mixed chromatin, multiple prominent nucleoli and deeply basophilic cytoplasm and (B, 2) scattered cells with sternbergoid features and decreased cytoplasmic basophilia

A 55-year-old man with a 1-month history of asthenia, right hypochondrial pain, and weight loss was referred to the emergency department for hepatic mass evaluation.

Abdominal computed tomography scan revealed a large necrotic mass in the liver, a second necrotic mass adjacent to the superior mesenteric vessels, and numerous subdiaphragmatic lymph nodes (aorta, right kidney, retro-crural, mesenteric, iliac, and inguinal)

Au labo…



Flow cytometry charts showing the presence of two cell populations with different immunophenotypes: a population with high side scatter (SSC), CD19+, CD20+, CD22+, CD15+dim, CD30+dim, CD45+ cells (blue population) and a few CD19-, CD20-, CD15+dim, CD30+bright, CD45- cells (red population).

Lauwers M et al. eJHaem 2022

Cas clinique n°VI

Il n’y a pire eau que l’eau qui dort

2018

- Femme – 45 ans
- Nodules sous cutanés (abdomen et membres inférieurs)
- Régressions spontanées

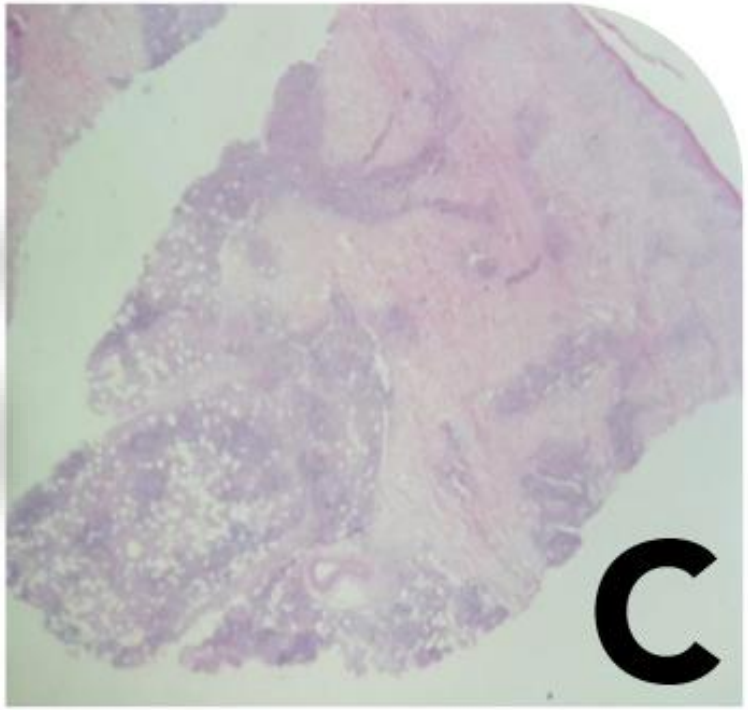
- Femme – 45 ans
- Nodules sous cutanés (abdomen et membres inférieurs)
- Régressions spontanées < 2015

Farhat H et al. Blood. 2018

- Femme – 45 ans
- Nodules sous cutanés (abdomen et membres inférieurs)
- Régressions spontanées < 2015

Cutaneous infiltration that involves both the dermis and the adipose panniculitic tissue.

The epidermis shows marked oedema with basal vacuolization and polymorphic inflammatory reaction, in the absence of tumour infiltration



Biopsie (HE)

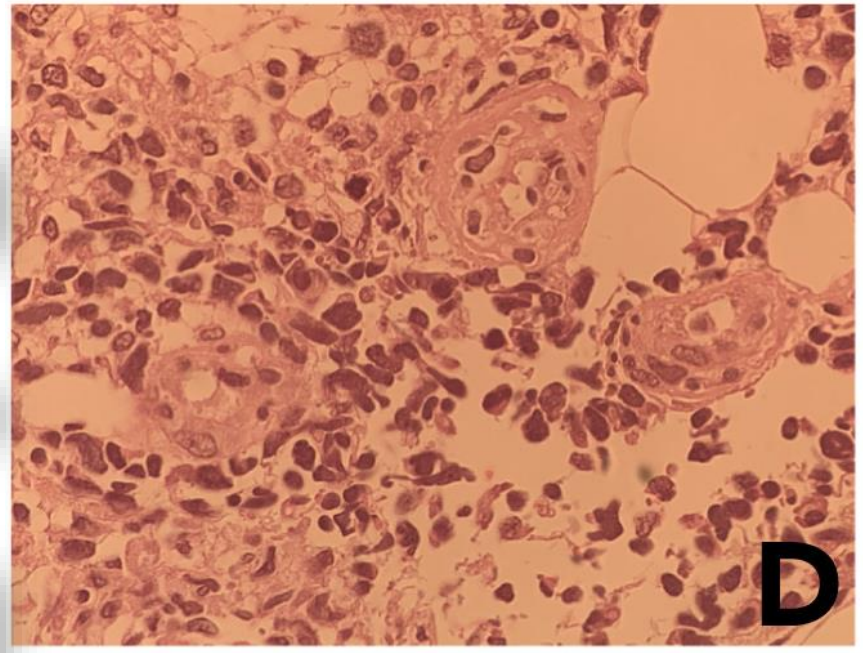
Farhat H et al. Blood. 2018

- Femme – 45 ans
- Nodules sous cutanés (abdomen et membres inférieurs)
- Régressions spontanées < 2015

Median to large size population of neoplastic lymphocytes, with frequent pleomorphic features.

In the dermis these cells show angiotropism with some focus of thrombosis.

In the panniculitic tissue they are located surrounding the adipose tissue cells with a panniculitic-like pattern, frequently associated with destruction of the adipose tissue and a granulomatosis reaction



Biopsie (HE)

Farhat H et al. Blood. 2018

Cytométrie

population:

- *T-cell CD3+ CD45+, phénotype **CD5-** CD7- CD4- CD8- TCR$\gamma\delta$*
- *with cytotoxic/killer marker-associated expression: CD56+, TIA1+, Granzyme B+*
- *in addition: EBER-, CD34-, CD123-, Tdt-, and CD20-*

… au milieu d'un background de lymphocytes T réactionnels CD4+ ou CD8+…

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Cytométrie

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Table 14.05 Differential diagnosis of neoplasms expressing T-cell and NK-cell markers with frequent cutaneous involvement

Disease	Clinical features	CD3	CD4	CD8	Cytotoxic molecules[a]	CD56	EBV	TR genes	Lineage
SPTCL	Tumours (extremities and trunk)	+	–	+	+	–	–	R	T-cell
PCGD-TCL	Tumours, plaques, ulcerated nodules	+	–	–/+	+	+	–	R	T-cell
Extranodal NK/TCL	Nodules, tumours	+	–	–/+	+	+	+	G	NK-cell, sometimes T-cell
Primary C-ALCL	Superficial nodules with epidermal involvement	+	+	–	+	–	–	R	T-cell
Mycosis fungoides	Patches, plaques, tumours late in course	+	+	–	–	–	–	R	T-cell
Blastic PDC neoplasm	Nodules, tumours	–	+	–	–	+	–	G	Precursor of PDC

C-ALCL, cutaneous anaplastic large cell lymphoma; G, in germline configuration; PCGD-TCL, primary cutaneous gamma delta T-cell lymphoma; PDC, plasmacytoid dendritic cell; R, rearranged; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; TCL, T-cell lymphoma; TR, T-cell receptor.

[a] Cytotoxic granule-associated protein(s) TIA1, granzyme B, and/or perforin.
[b] Marked variation in T-cell antigens, including frequent antigen loss of CD3, CD4, CD8

Farhat H et al. Blood. 2018

Cytométrie

population:

- *T-cell CD3+ CD45+, phénotype **CD5-** CD7- CD4- CD8- TCR$\gamma\delta$*
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PCGD-TCL	Tumours, plaques, ulcerated nodules	+	–	–/+	+	+	–	R	T-cell
Extranodal NK/TCL	Nodules, tumours	+	–	–/+	+	+	+	G	NK-cell,
Primary C-ALCL									
Mycosis fungoides	Patches, plaques, tumours late in course	+	+	–	–	–	–	R	T-cell
Blastic PDC neoplasm	Nodules, tumours	–	+	–	–	+	–	G	Precursor of PDC

compatible with infiltration by a primary cutaneous gamma-delta T cell lymphoma, pending confirmation of TCR$\gamma\delta$ expression and rearrangement.

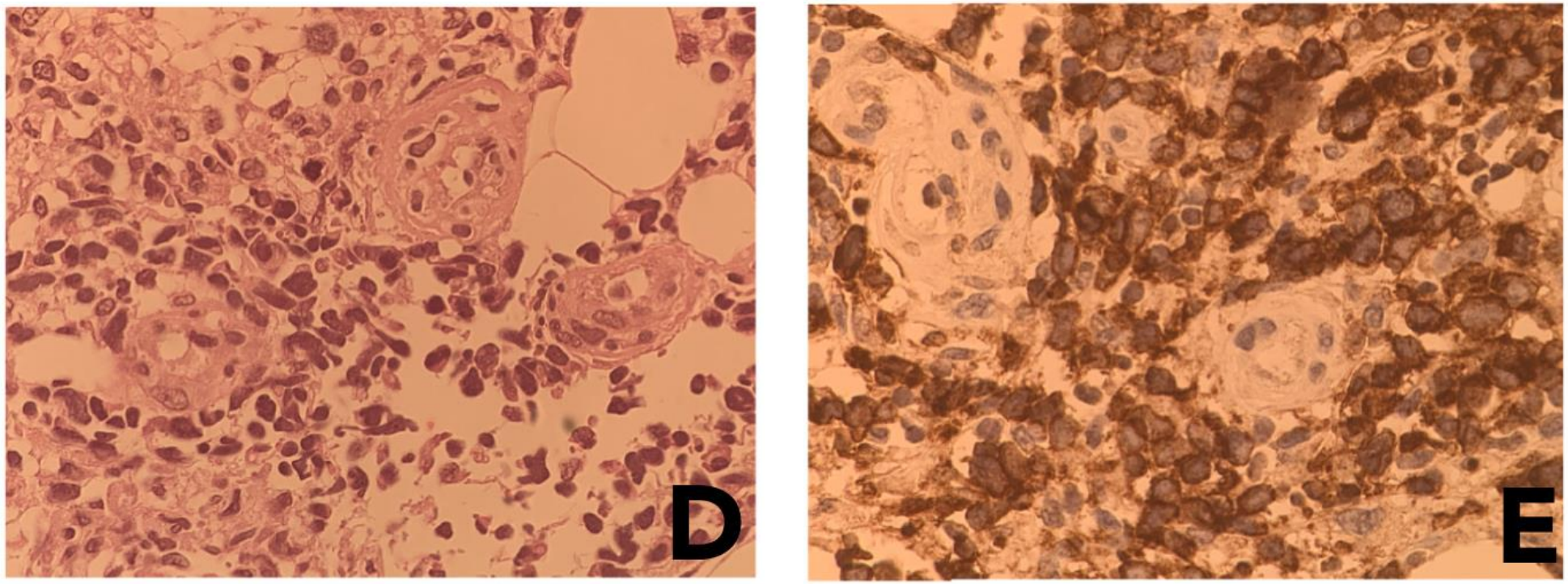
C-ALCL, cutaneous anaplastic large cell lymphoma; G, in germline configuration; PCGD-TCL, primary cutaneous gamma delta T-cell lymphoma; PDC, plasmacytoid dendritic cell; R, rearranged; SPTCL, subcutaneous panniculitis-like T-cell lymphoma; TCL, T-cell lymphoma; TR, T-cell receptor.

[a] Cytotoxic granule-associated protein(s) TIA1, granzyme B, and/or perforin.
[b] Marked variation in T-cell antigens, including frequent antigen loss of CD3, CD4, CD8

Farhat H et al. Blood. 2018

2015 2018

- Anticorps anti-δ (TCRγδ) – MD Anderson Cancer Center (USA)



→ Cellules lymphomateuses peri-vasculaires: positives pour anti-δ (TCRγδ)

Farhat H et al. Blood. 2018

2015 2018

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

14 Mature T- and NK-cell neoplasms

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2015 2018

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

14 Mature T- and NK-cell neoplasms

Primary cutaneous peripheral T-cell lymphomas, rare subtypes

Farhat H et al. Blood. 2018

2015 2018

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

14 Mature T- and NK-cell neoplasms

Primary cutaneous peripheral T-cell lymphomas, rare subtypes

Primary cutaneous gamma delta T–cell lymphoma

Definition

Primary cutaneous gamma delta T–cell lymphoma (PCGD–TCL) is a lymphoma, involving primarily the skin, composed of a clonal proliferation of mature, activated gamma delta T cells with a cytotoxic phenotype.

Prognosis and predictive factors

PCGD–TCLs are usually resistant to multiagent chemotherapy and/or radiation and have a poor prognosis, with a median survival of approximately 15 months {4031,4321}. Patients with subcutaneous fat involvement tend to have a more unfavourable prognosis than do patients with epidermal or dermal disease only {4031}.

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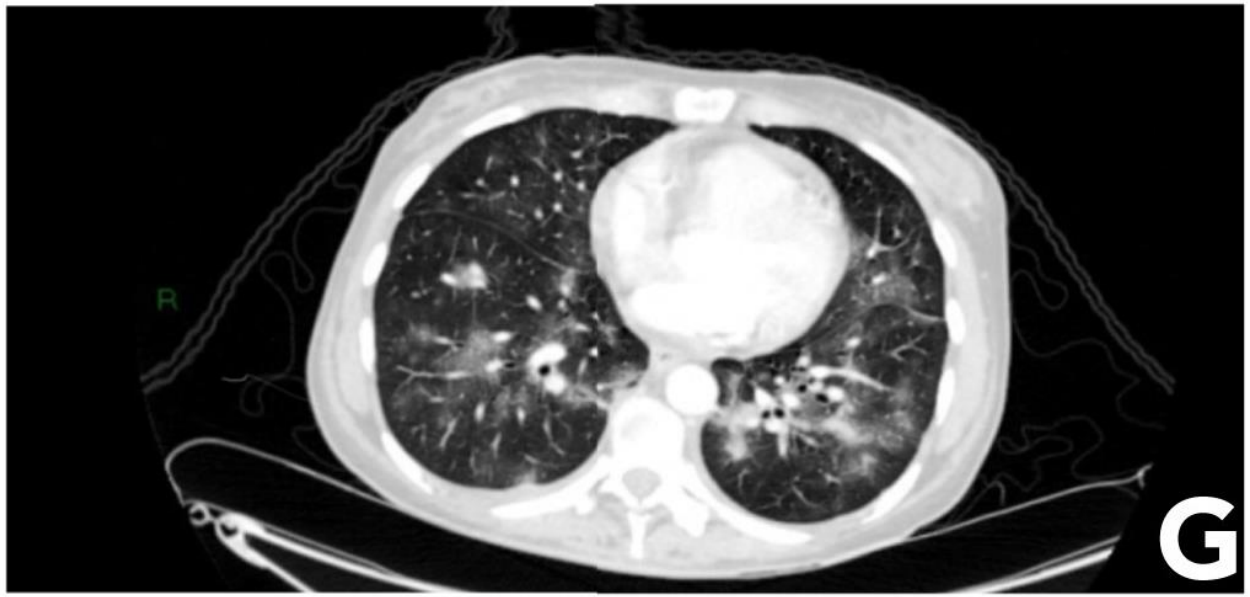
2015 2018 2020

Two years later, the patient was hospitalized for recurrent fever, cough, and severe dyspnea.



Farhat H et al. Blood. 2018

2015 2018 2020

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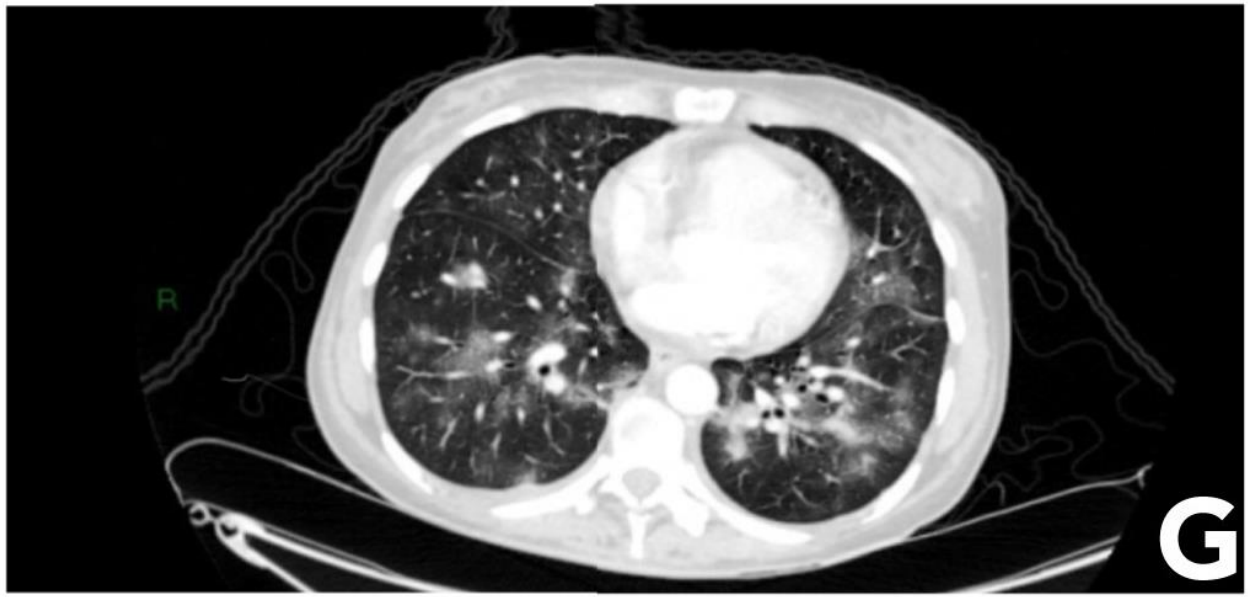
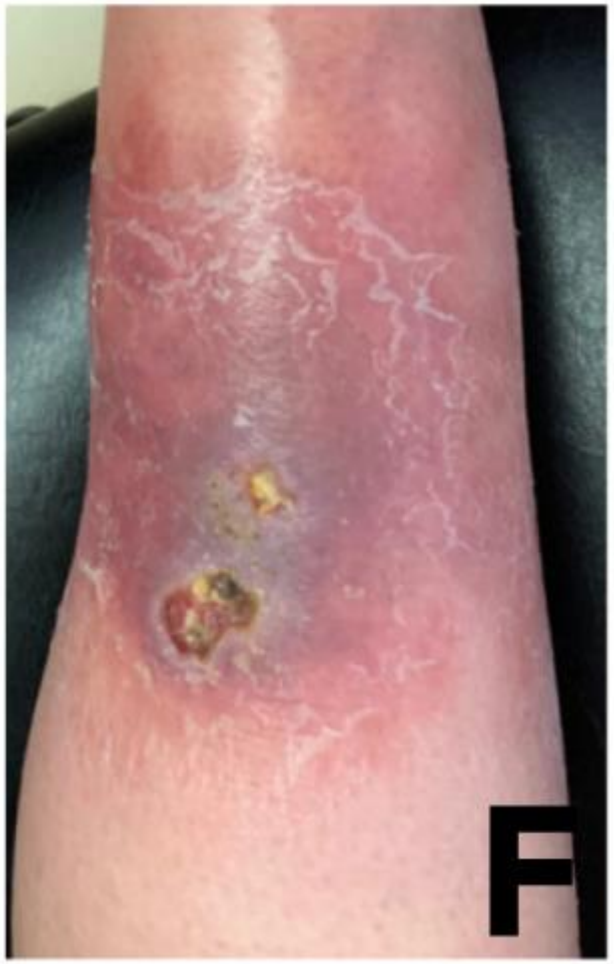
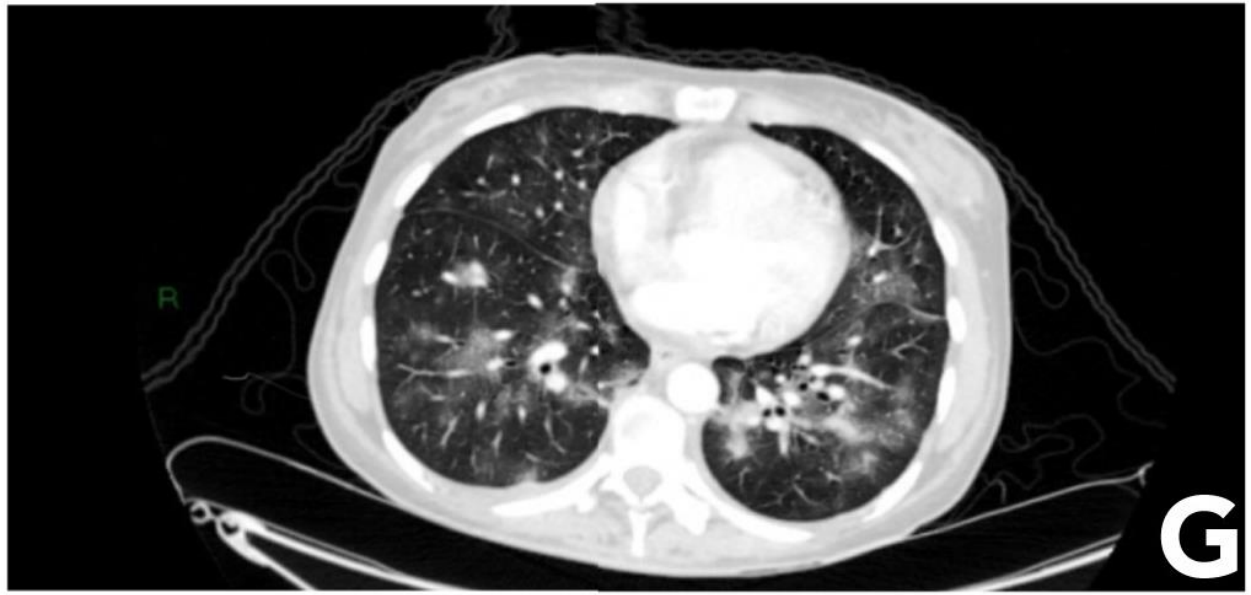


PCR SARS-CoV-2 négative!

Farhat H et al. Blood. 2018

2015 2018 2020

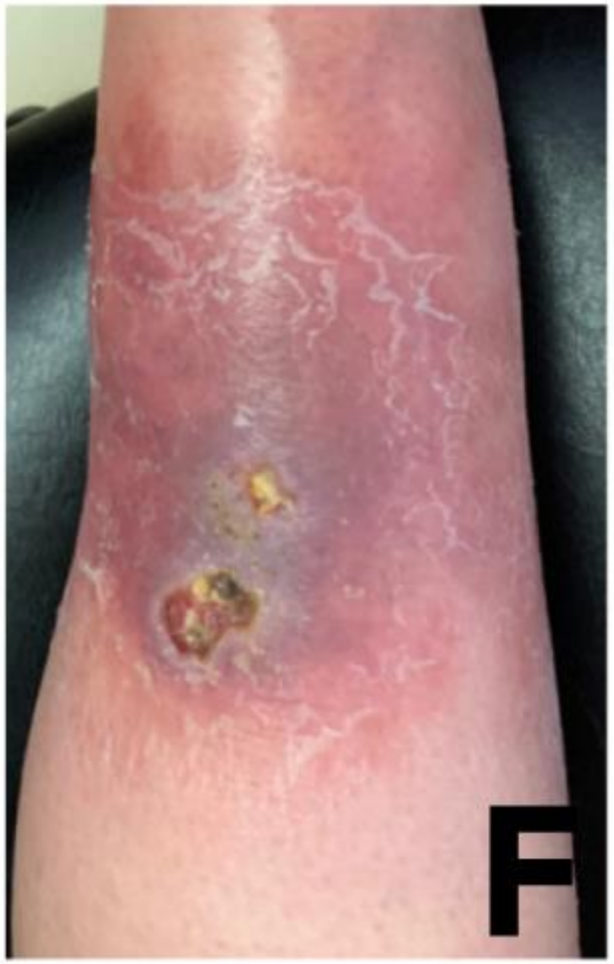


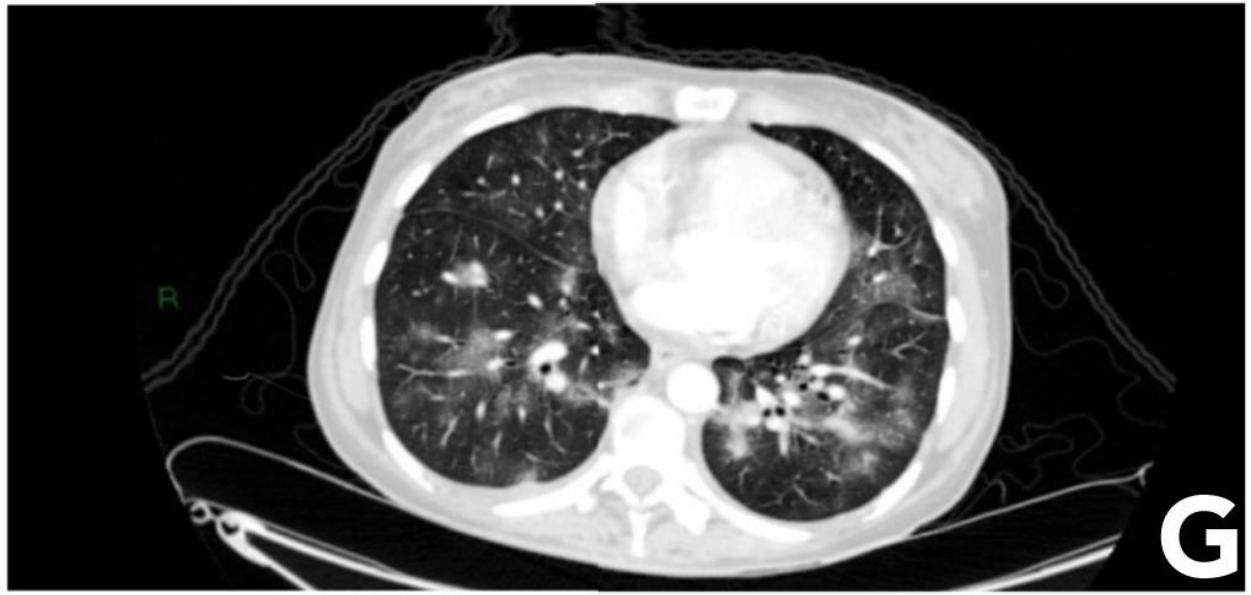


→ Lavage broncho-alvéolaire

Farhat H et al. Blood. 2018

2015 2018 2020



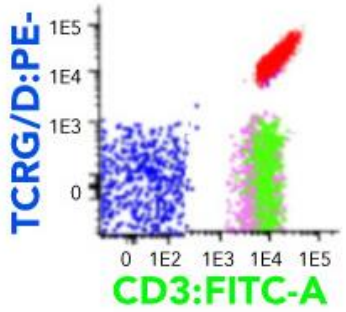


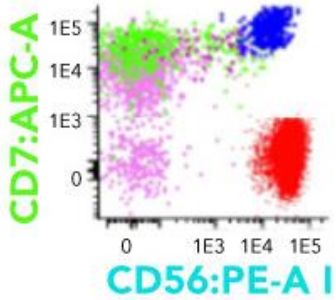
→ Lavage broncho-alvéolaire

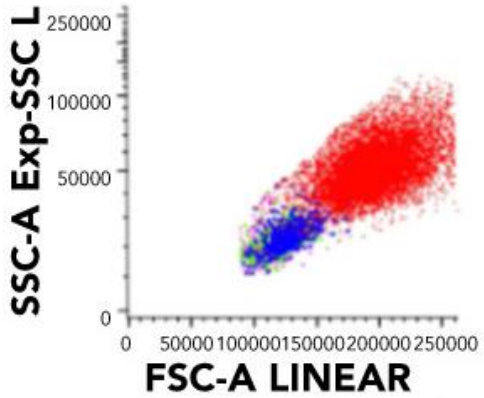
SARS-CoV-2 négatif!

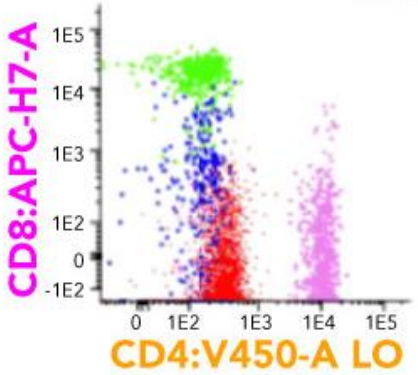
Farhat H et al. Blood. 2018

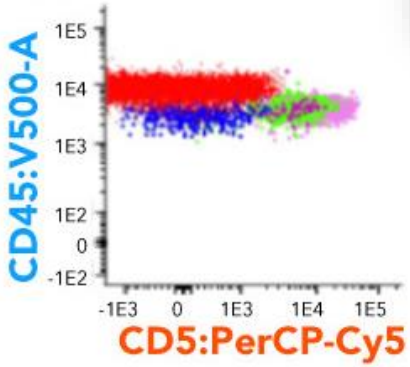
2015 2018 2020

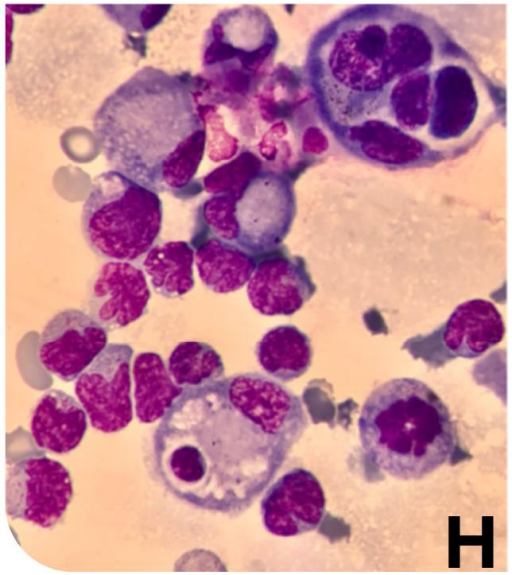








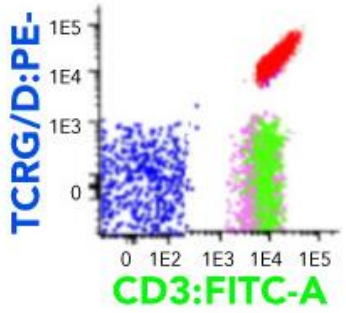


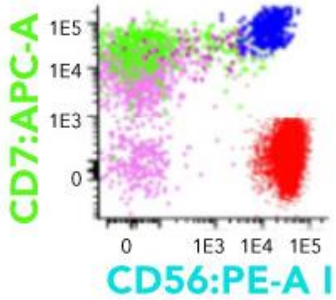


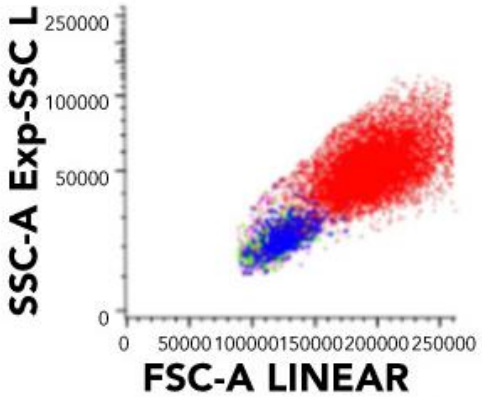
*T-cell CD3+ CD45+, phénotype **CD5-** CD7- CD4- CD8- TCR$\gamma\delta$*

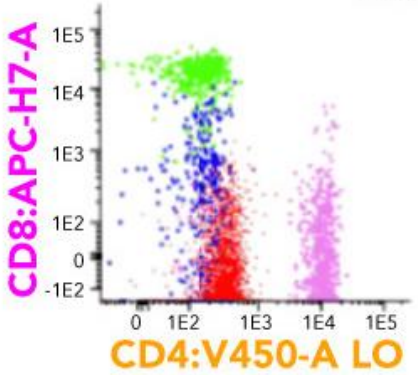
Farhat H et al. Blood. 2018

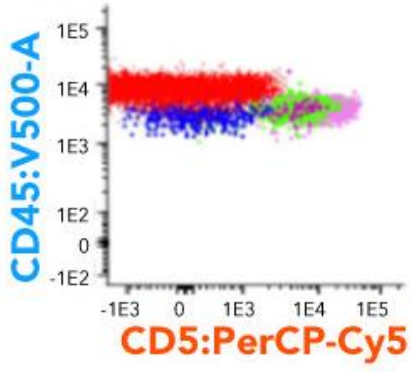
2015 2018 2020

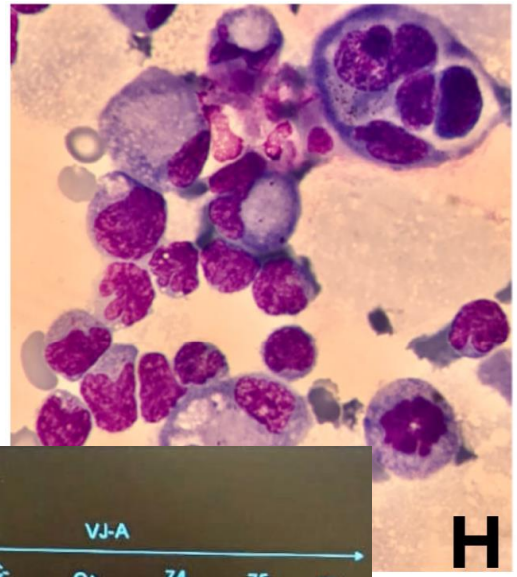


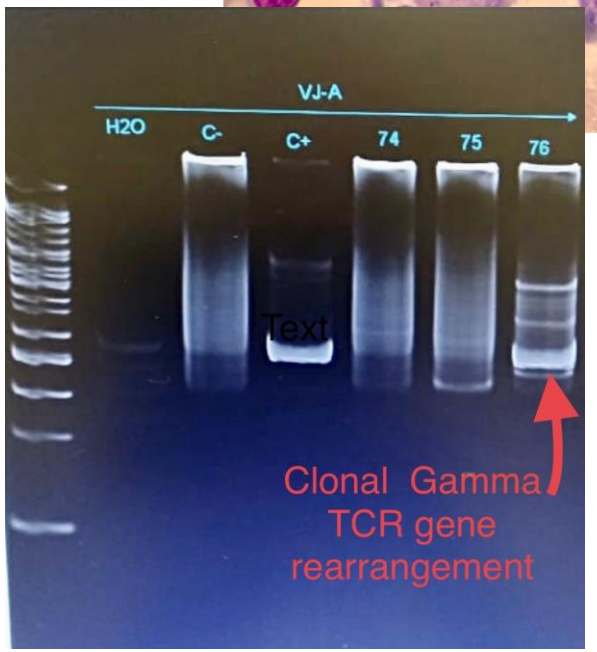






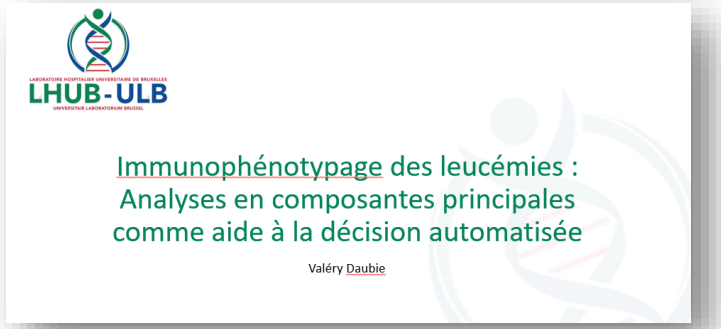






Farhat H et al. Blood. 2018

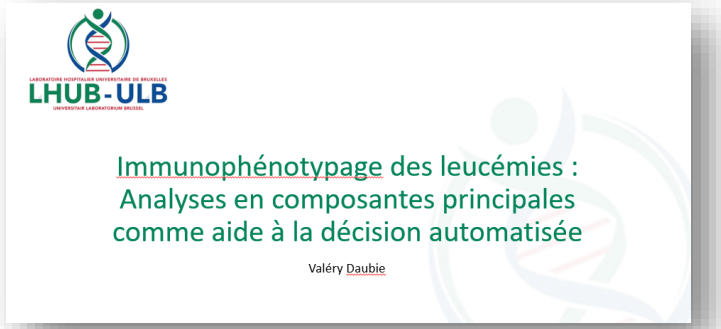
Perspectives



- Régression linéaire
- Fonction multivariée
- ACP

} Bases mathématiques de l'A.I.

Perspectives



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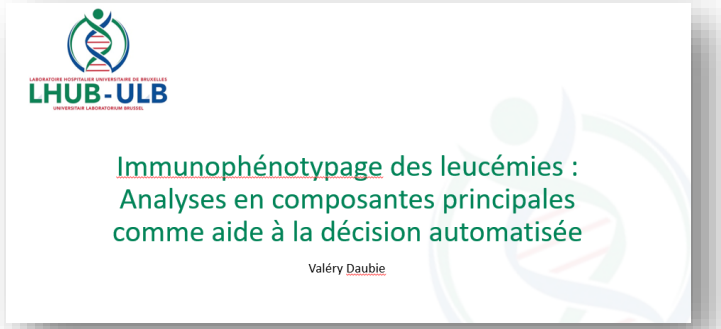
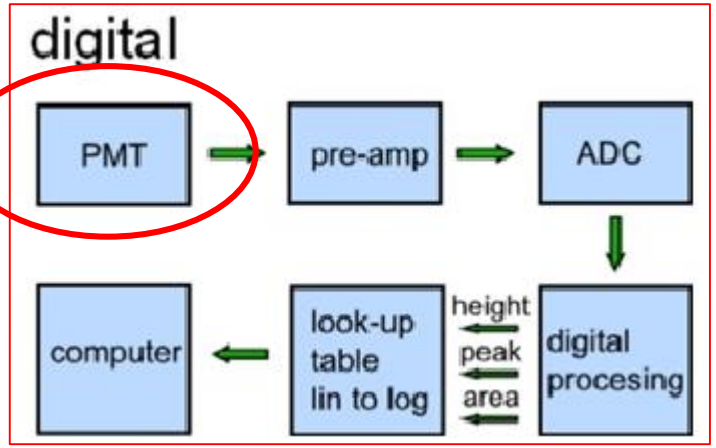
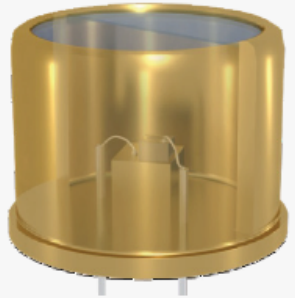




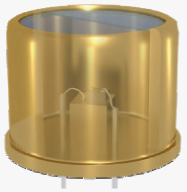


Avalanche Photodiode.
The DxFLEX flow cytometer uses Avalanche Photodiode detectors instead of PMTs. The low electronic noise contributes to the resolution capabilities of the instrument.

Perspectives

Immunophénotypage des leucémies :
Analyses en composantes principales
comme aide à la décision automatisée

Valéry Daubie



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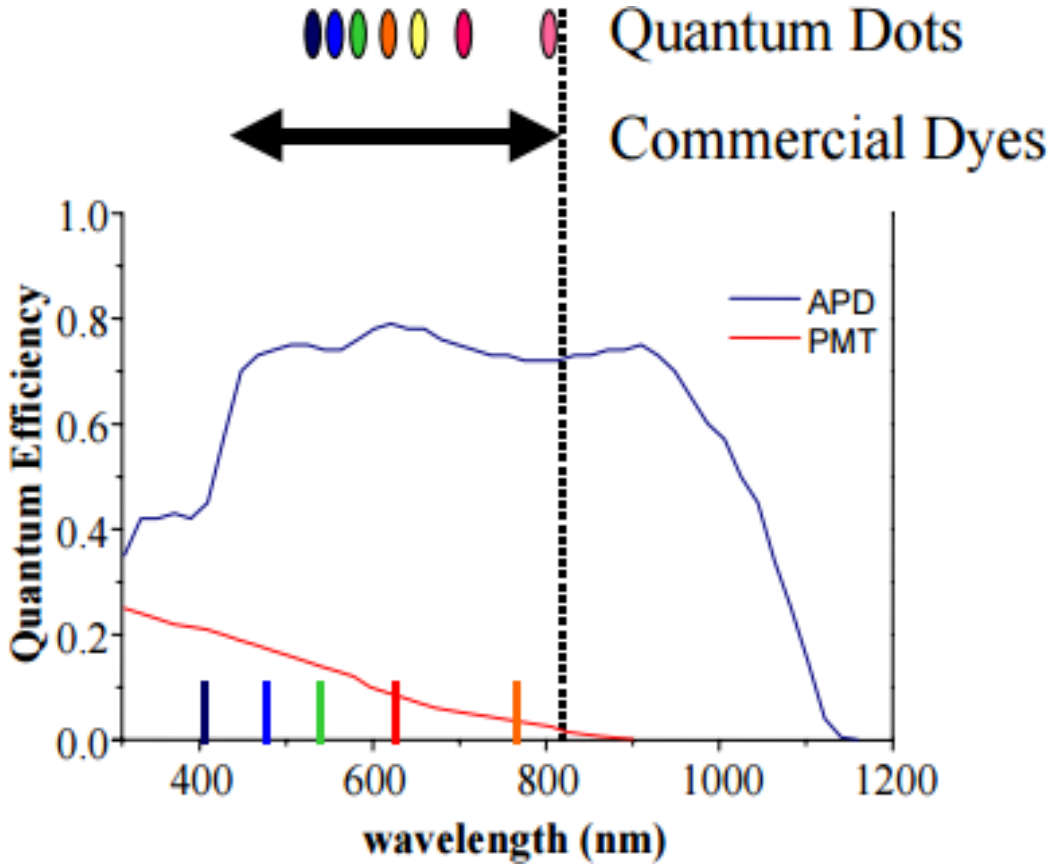
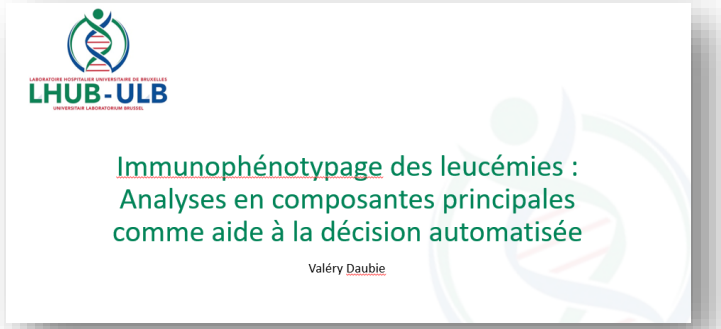


Perspectives



- Régression linéaire
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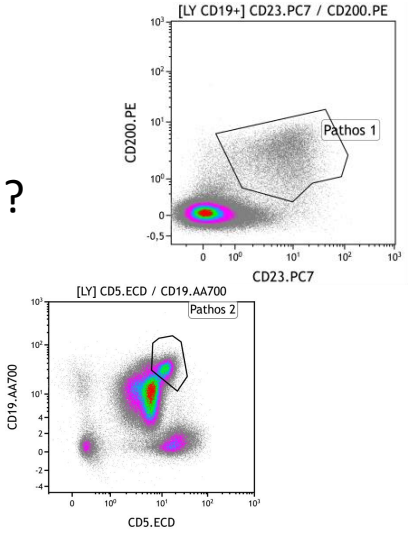
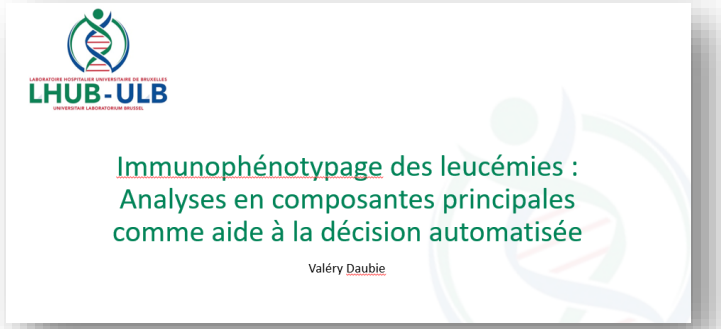
- ACP

Bases mathématiques de l'A.I.

Découverte de "nouveaux profils"?



Perspectives



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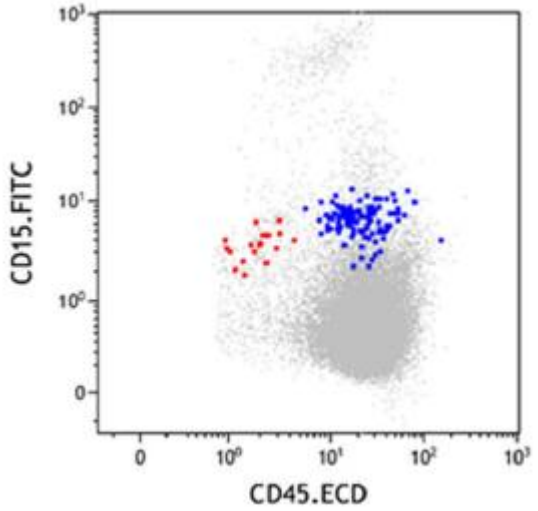
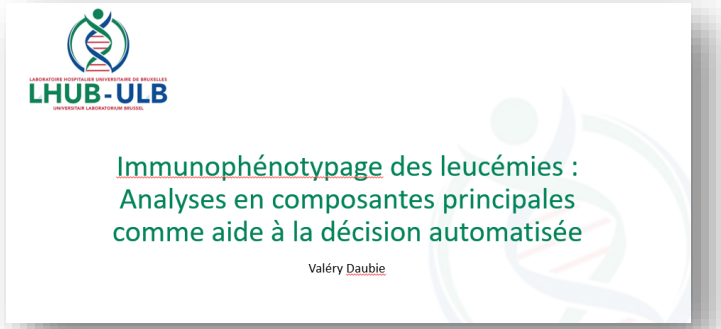


Evénements "rares"

Découverte de "nouveaux profils"?

Perspectives



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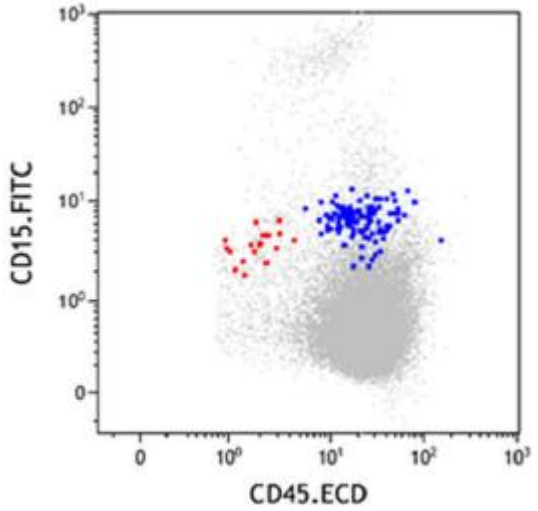
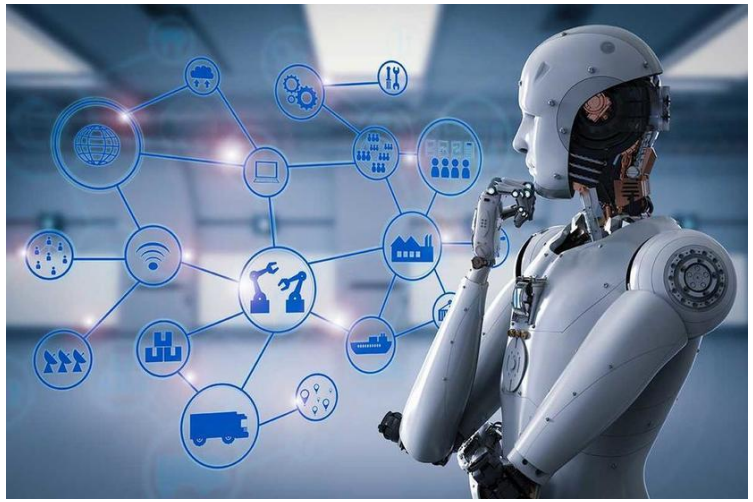


Evénements "rares"

Découverte de "nouveaux profils"?

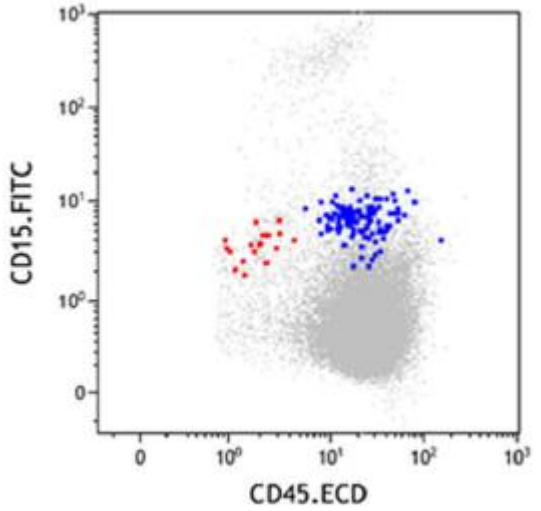
<u>Marqueurs d'évolution et d'agressivité</u>

Perspectives



AI
Machine learning
Big Data

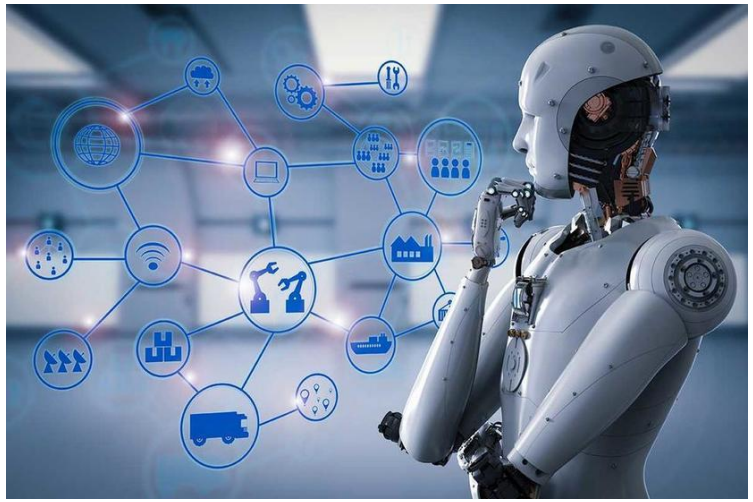
Le macroscopique



Evénements “rares”

Le microscopique

Perspectives



A.I.
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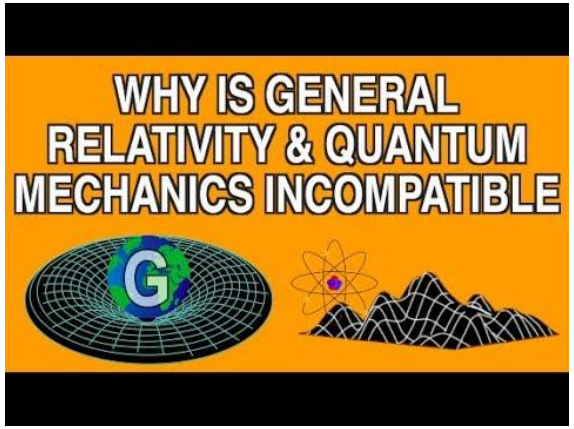

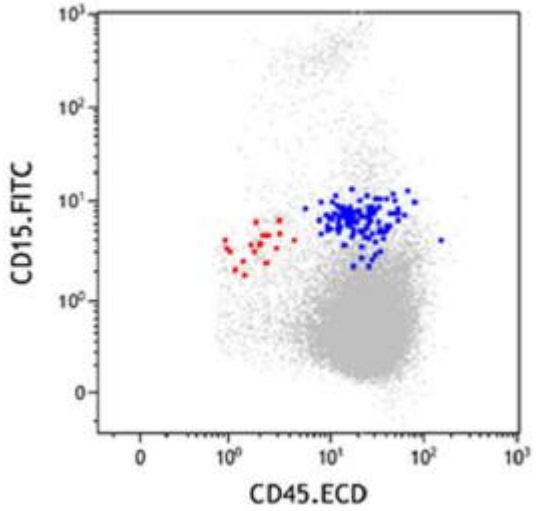

Evénements “rares”

Le microscopique

Background technologique

Clinique

Merci…

…à toute l’équipe Hémato du LHUB-ULB