AML met monocytaire uitrijping

Jaarlijks congres Nederlandse Vereniging voor Cytometrie
& SKML sectie IMCD
“Kwaliteit en ontwikkeling in de flowcytometrie”

Zwolle
November 25th 2020

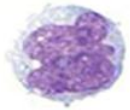
Dr. Apr. Biol. Barbara Depreter

University Hospital Brussels

Universitair Ziekenhuis Brussel

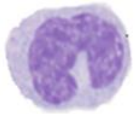
Normal monocytic development

- Monoblasts



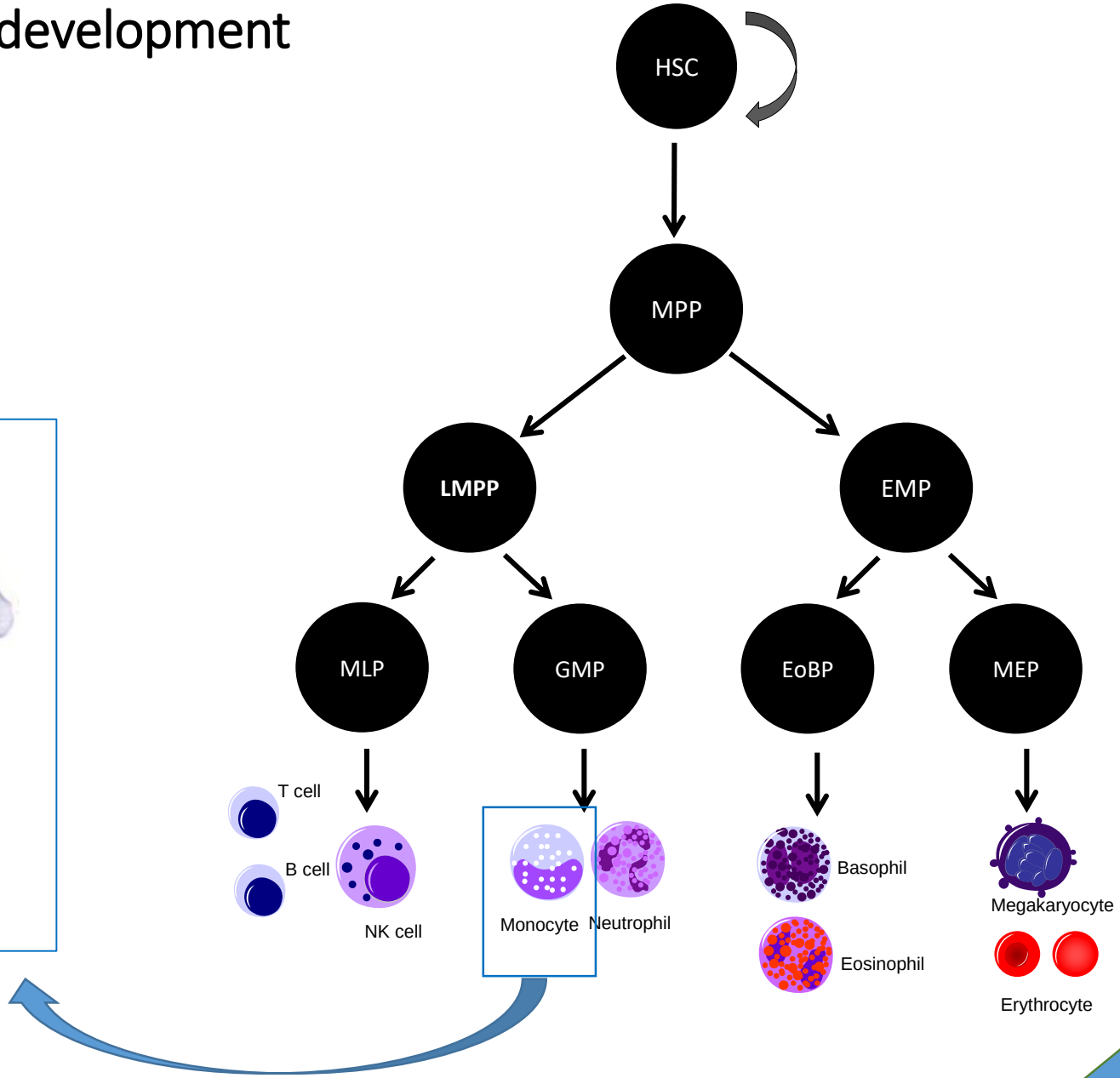
- Promonocytes

- Monocytes



→Macrophages

→Dendritic cells



Normal monocytic development

CD marker	Monoblast	Promonocyte	Monocyte
CD4	++	++	++
CD11b	-	++	+++
CD11c			
CD13	+	+/++	++/+++
CD14	-	+/-	+++
CD15	-	++	+/++
CD16	-	-	-/+
CD33	+++	+++	+++
CD34	+/-	-	-
CD35	-	+/-	+
CD36	-	++	+++
CD45	+	++	+++
CD64	+++	+++	+++
CD117	+	+/-	-
CD300e	-	-	+
HLA-DR	+++	+++	++/+++

Immunohenotypic changes

~ maturation

~ activation

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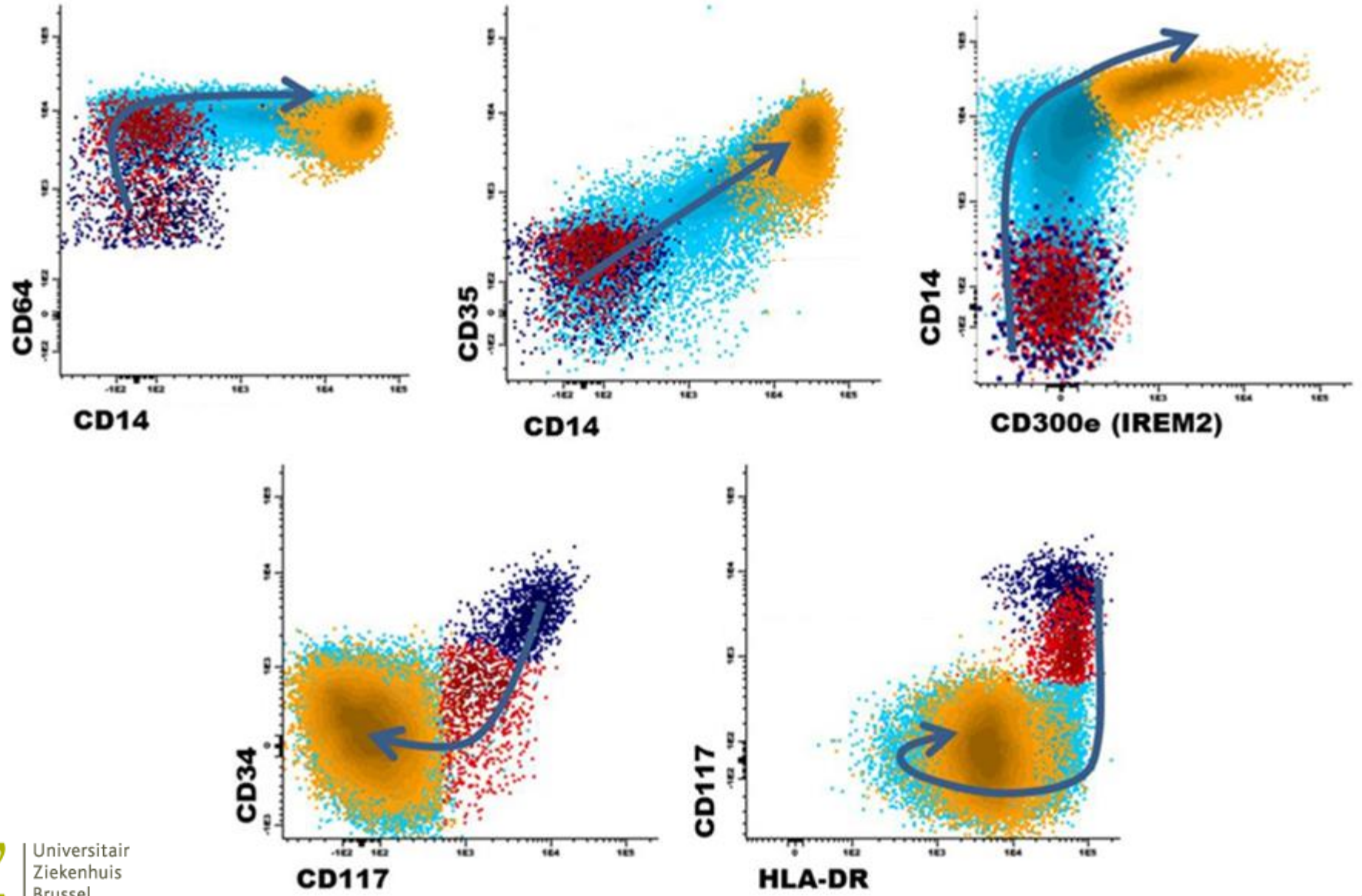
Normal monocytic development



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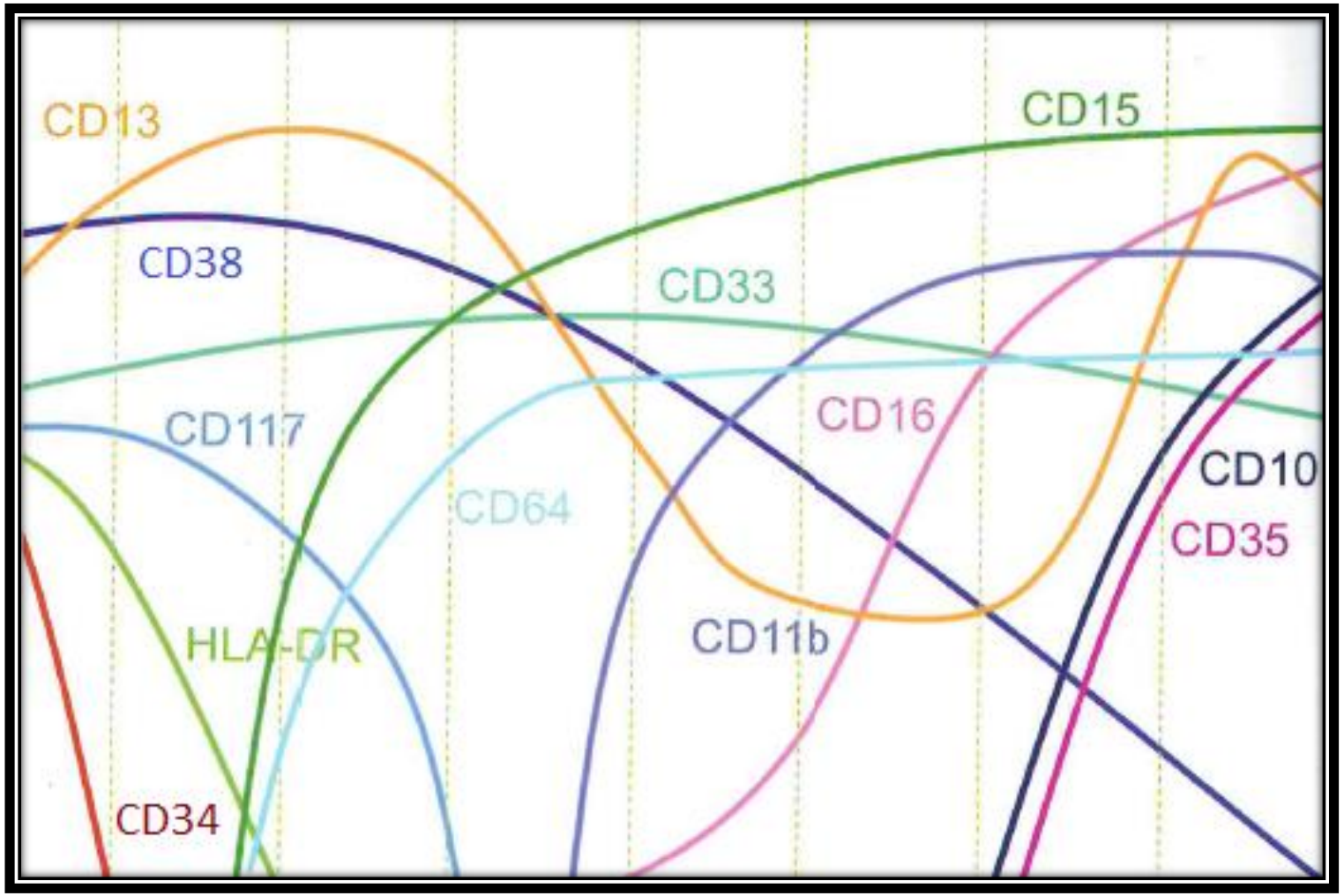
Matarraz, S et al. (2017); van Dongen J et al. (2012)

Normal monocytic development



Nieuwe ontwikkelingen in de medische immunologie 2010 (van Dongen et al.)

Normal monocytic development

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CD11c			
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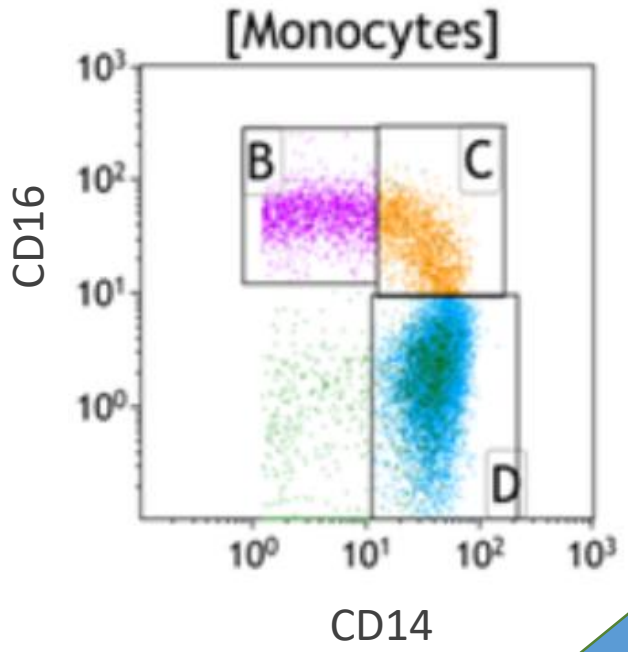
CD14	-	+/-	+++
CD15	-	++	+/++
CD16	-	-	-/+
CD33	+++	+++	+++
CD34	+/-	-	-
CD35	-	+/-	+
CD36	-	++	+++
CD45	+	++	+++
CD64	+++	+++	+++
CD117	+	+/-	-
CD300e	-	-	+
HLA-DR	+++	+++	++/+++

- **3 groups** circulating PB mature monocytic cells
  1. CD14+/CD16- (classical, 80-85%): cMo
  2. CD14+/CD16+ (intermediate, <15%): iMo
  3. CD14lo/CD16+ (non-classical, <15%): ncMo



Mukerjee et al 2015

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Acute myeloid leukemia with monocytosis

- AML NOS subcategories
  - M4: blasts (incl. promonocytes) ≥20%, monocytic cells ≥ 20% and myeloid (precursors) ≥ 20%
  - M5: blasts (incl. promonocytes) ≥20%, monocytic cells ≥ 80% and myeloid (precursors) < 20%
- FCM MRD assessment
  - Establishment of Leukemia-Associated ImmunoPhenotypes (LAIPs)
  - The different-from-normal (DfN) approach

Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party

Gerrit J. Schuurhuis,[1] Michael Heuser,[2,*] Sylvie Freeman,[3,*] Marie-Christine Béné,[4] Francesco Buccisano,[5] Jacqueline Cloos,[1,6] David Grimwade,[7] Torsten Haferlach,[8] Robert K. Hills,[9] Christopher S. Hourigan,[10] Jeffrey L. Jorgensen,[11] Wolfgang Kern,[8] Francis Lacombe,[12] Luca Maurillo,[5] Claude Preudhomme,[13] Bert A. van der Reijden,[14] Christian Thiede,[15] Adriano Venditti,[5] Paresh Vyas,[16] Brent L. Wood,[17,18] Roland B. Walter,[17,19] Konstanze Döhner,[20,†] Gail J. Roboz,[21,†] and Gert J. Ossenkoppele[1]

- Unexpected immunophenotypic changes
  - Neoplastic origin
  - Technical artifacts
  - Regeneration
  - Activation

ELN MRD working party consensus document (2018)

Acute myeloid leukemia with monocytosis

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CD11c			
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CD15	-	++	+/++
CD16	-	-	-/+
CD33	+++	+++	+++
CD34	+/-	-	-
CD35	-	+/-	+
CD36	-	++	+++
CD45	+	++	+++
CD56	+	-	+
CD64	+++	+++	+++
CD117	+	+/-	-
CD300e	-	-	+
HLA-DR	+++	+++	++/+++

CD56 may be aberrantly expressed on neoplastic cells, but is not specific and may be seen in reactive settings

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Acute myeloid leukemia with monocytosis

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CD33	+++	+++	+++
CD34	+/-	-	-
CD35	-	+/-	+
CD36	-	++	+++
CD45	+	++	+++
CD64	+++	+++	+++
CD117	+	+/-	-
CD300e	-	-	+
HLA-DR	+++	+++	++/+++

Some anti-CD14 clones only recognize mature monocytic cells, while others recognize promonocytes and mature monocytes.

Neoplastic monoblasts or promonocytes may not always be CD14 negative
!correlation with morphology

ncMo have decreased expression of CD14 should not be confused with immature monocytic cells.

Yang et al (2005)

Acute myeloid leukemia with monocytosis

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CD34	+/-	-	-
CD35	-	+/-	+
CD36	-	++	+++
CD45	+	++	+++
CD64	+++	+++	+++
CD117	+	+/-	-
CD300e	-	-	+
HLA-DR	+++	+++	++/+++

Activation influences the level of CD15 and HLA-DR expression

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Acute myeloid leukemia with monocytosis

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CD33	+++	+++	+++
CD34	+/-	-	-
CD35	-	-	+
CD36	-	++	+++
CD45	+	++	+++
CD64	+++	+++	+++
CD117	+	+/-	-
CD300e	-	+	+
HLA-DR	+++	+++	++/+++

Asynchronous monocytic maturation (IREM2 expression preceding CD14 and/or CD35) is observed in NPM1m AML with and without monocytic blast cell differentiation

Yang et al (2005), Matarraz S, et al (2019), Alvarez J et al (2019, PB1669 EHA2019)

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Acute myeloid leukemia with monocytosis

- Which of the following 8-color LAIP for FU AML with CD15++ is prone to false-positive MRD interpretation?

  - CD15++/ CD4-/CD11b-/CD13+/CD34+/CD45+/CD64+/HLA-DR+-
  - CD15++/CD4+/CD11b+/CD13+/CD34+/CD45+/CD64+/HLA-DR++

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Acute myeloid leukemia with monocytosis

- Which of the following 8-color LAIP for FU AML with CD15++ is prone to false-positive MRD interpretation?

  - CD15++/ CD4-/CD11b-/CD13+/CD34+/CD45+/CD64+/HLA-DR+-
  - **CD15++/CD4+/CD11b+/CD13+/CD34+/CD45+/CD64+/HLA-DR++**

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Gustafson MP et al. (2010)

Acute myeloid leukemia with monocytosis

- If you would have access to a 10-color FCM panel, do you see an added value for including CD123?
  - No
  - Yes

Acute myeloid leukemia with monocytosis

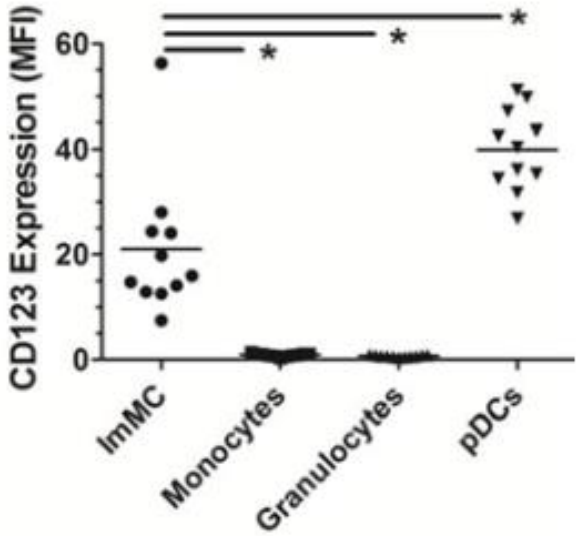
- If you would have access to a 10-color FCM panel, do you see an added value for including CD123?
  - No
  - **Yes**

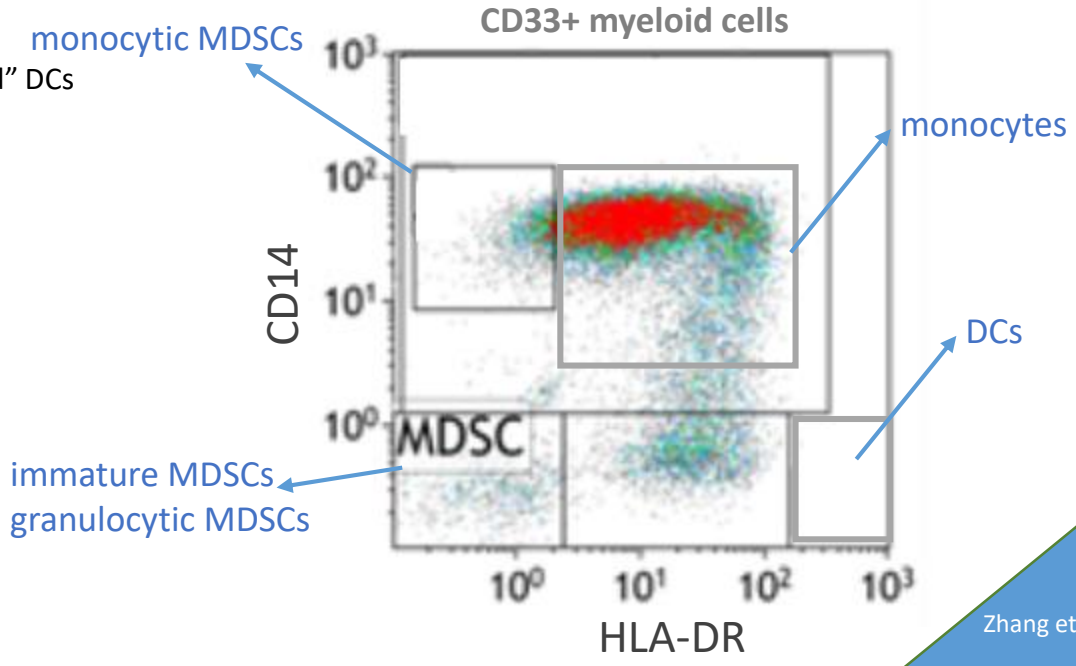
Gustafson MP et al. PLoS One. 2015;10(3):e0121546.

Acute myeloid leukemia with monocytosis

- Myeloid derived suppressor cells (MDSC): CD33+/HLA-DR- cells with variable CD15 expression → 3 classes
  - **Granulocytic** MDSC: CD15+ or CD66b+
  - **Monocytes** MDSC: lost or diminished HLA-DR expression: CD14+/HLA-DRlo (+variable CD16 expression)
  - **Immature** MDSC: activated/degranulated granulocytes lacking CD15 and CD66b expression: CD14-/CD33+/HLA-DR-/**CD123+**:

- Peripheral and BM dendritic cells (DC): CD45+/CD14-/CD13+/CD33+/CD11c+/HLA-DR++/**CD123+** cells
  - BM: 0.082% ± 0.025%; PB ± 0,65%
  - 3 classes
    - CD16-/**CD123-** "myeloid" DCs
    - CD16+/**CD123dim** DCs
    - CD16-/**CD123+** "lymphoid/plasmacytoid" DCs





Zhang et al (2019), Matarraz, S et al. (2017)

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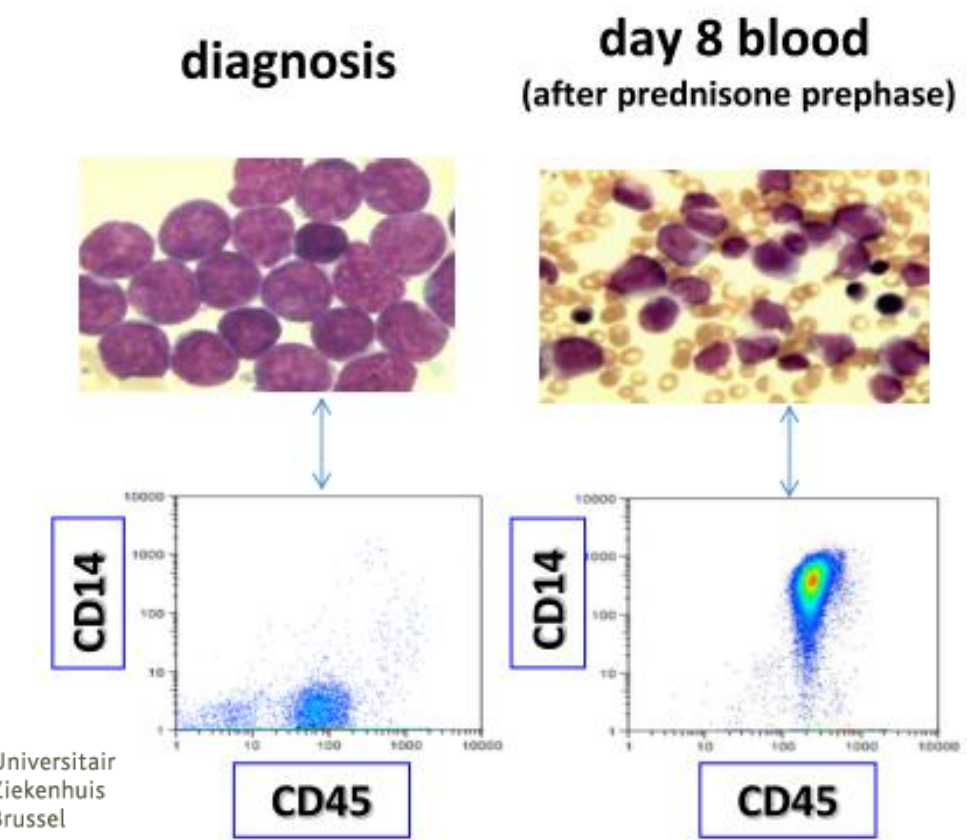
Acute leukemia of ambiguous lineage: Switching leukemia (swALL)

- Subtype of BCP-ALL, 8% pediatric BCP-ALL cases
- CD2 and CD371 (CLEC12A, CLL-1) expression and ↑ *ERG* deletions and *IKZF1* gene alterations at Dx
- CEBPa promotor hypomethylation
- ↑ monocytoid population during early treatment phase (d+8, d+15 or d+33)
  - Transient
  - Identical clone



Leukemia (2014) 28, 609–620
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www.nature.com/leu

ORIGINAL ARTICLE

CD2-positive B-cell precursor acute lymphoblastic leukemia with an early switch to the monocytic lineage

L Slamova[1], J Starkova[1], E Fronkova[1], M Zaliova[1], L Reznickova[1], FW van Delft[2], E Vodickova[3], J Volejnikova[1], Z Zemanova[4], K Polgarova[1], G Cario[5], M Figueroa[6], T Kalina[1], K Fiser[1], JP Bourquin[7], B Bornhauser[7], M Dworzak[8], J Zuna[1], J Trka[1], J Stary[1], O Hrusak[1] and E Mejstrikova[1]

Slamova et al (2014), Clappier et al (2014), Zaliova et al (2014), Lilljebjörn et al (2016), Zhang et ak (2016),

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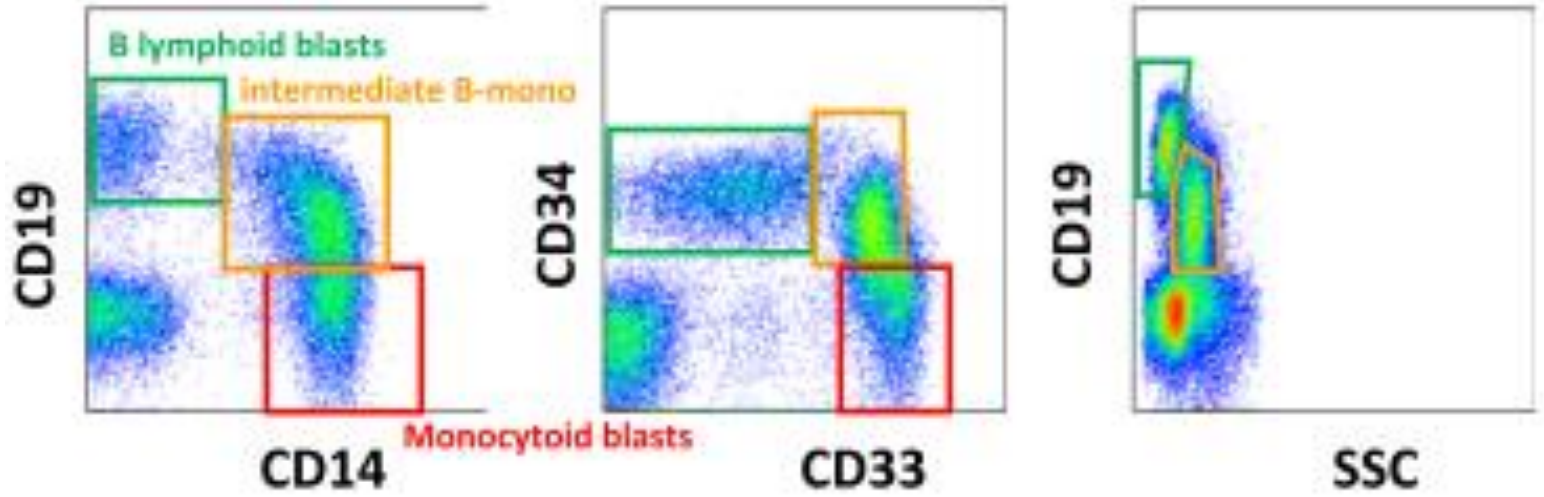
Acute leukemia of ambiguous lineage: Switching leukemia (swALL)

- ↑ monocytoid population during early treatment phase (d+8, d+15 or d33)
  - 'monocytoids': Monocytoid blasts and/or intermediate B-monocytoid population
  - Increased expression of **CD14, CD33 and SSC**; decreased expression of **CD19 and CD34**



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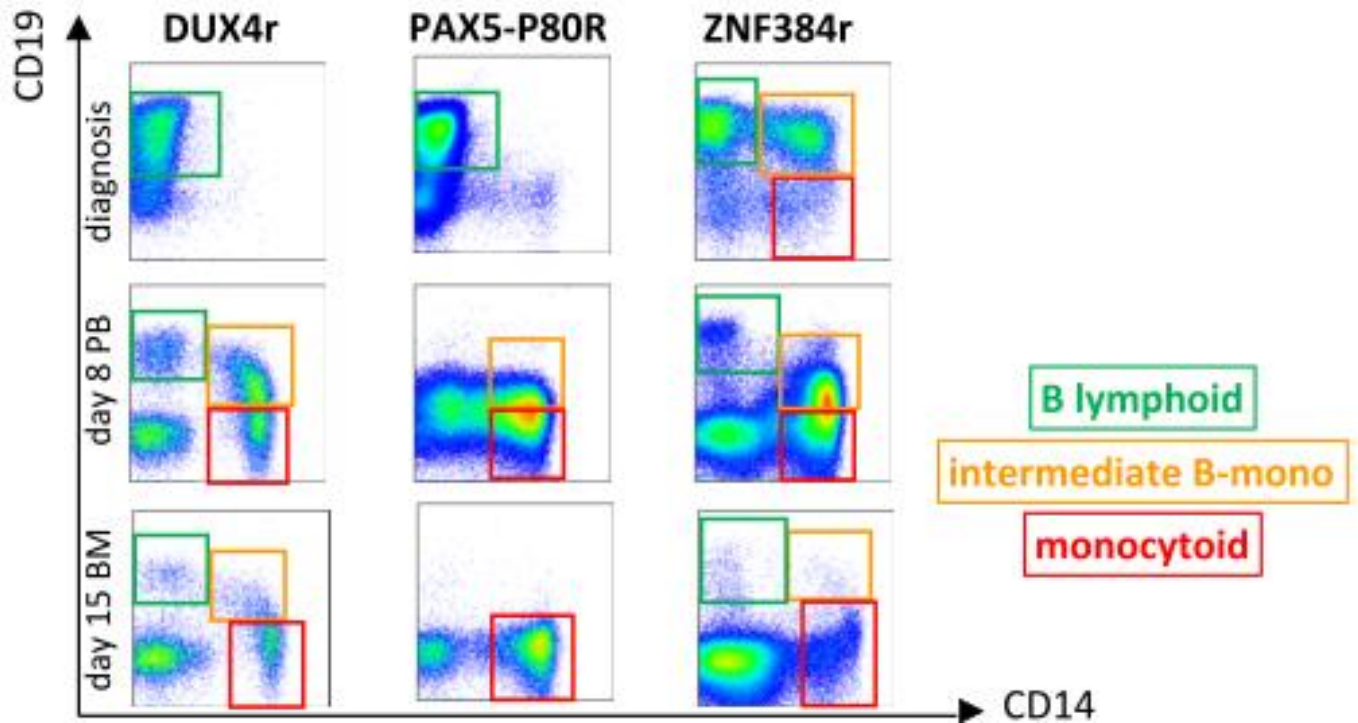
Slamova et al (2014), Clappier et al (2014), Zaliova et al (2014), Lilljebjörn et al (2016), Zhang et al (2016), Novakova et al (2020)

Acute leukemia of ambiguous lineage: Switching leukemia (swALL)

- BCP-ALL with bad prognosis: *BCR-ABL1r* (P<.05), *DUX4r* (P<.0001), *ZNF384r* (P<.01) and *PAX5r* (P<.0001)
- Genetic subtypes prone to switching have a **characteristic immunophenotype** at diagnoses
  - *DUX4*r: CD10low/CD30-/CD34++/**CLL-1++**/**CD2+**
  - *PAX5*-P80R mutation: CD10low/**CD66c++**/**CD2+**/CD4+/CD33+
- **Character of switching** differs between genetic subgroups (i.e. rapid loss of CD19)



Slamova et al (2014), Novakova et al. (2020)

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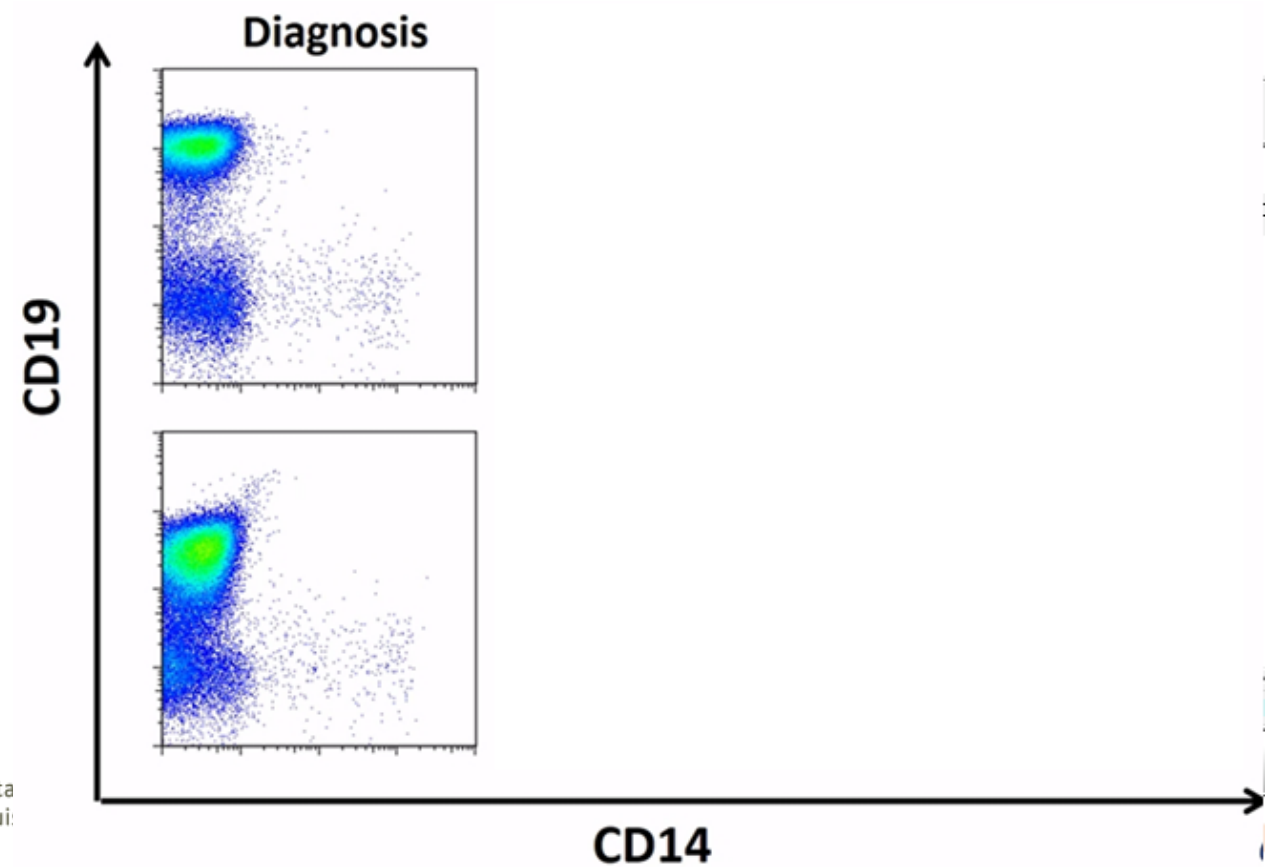
Acute leukemia of ambiguous lineage: Switching leukemia (swALL)

- Discrepancy between FCM MRD (underestimation) and Ig-TCR clonality assessments
  - PAX5-P80R at d+15
  - DUX4r at d+33
- Include other pan-B cell markers (CD22, CD24) next to CD19



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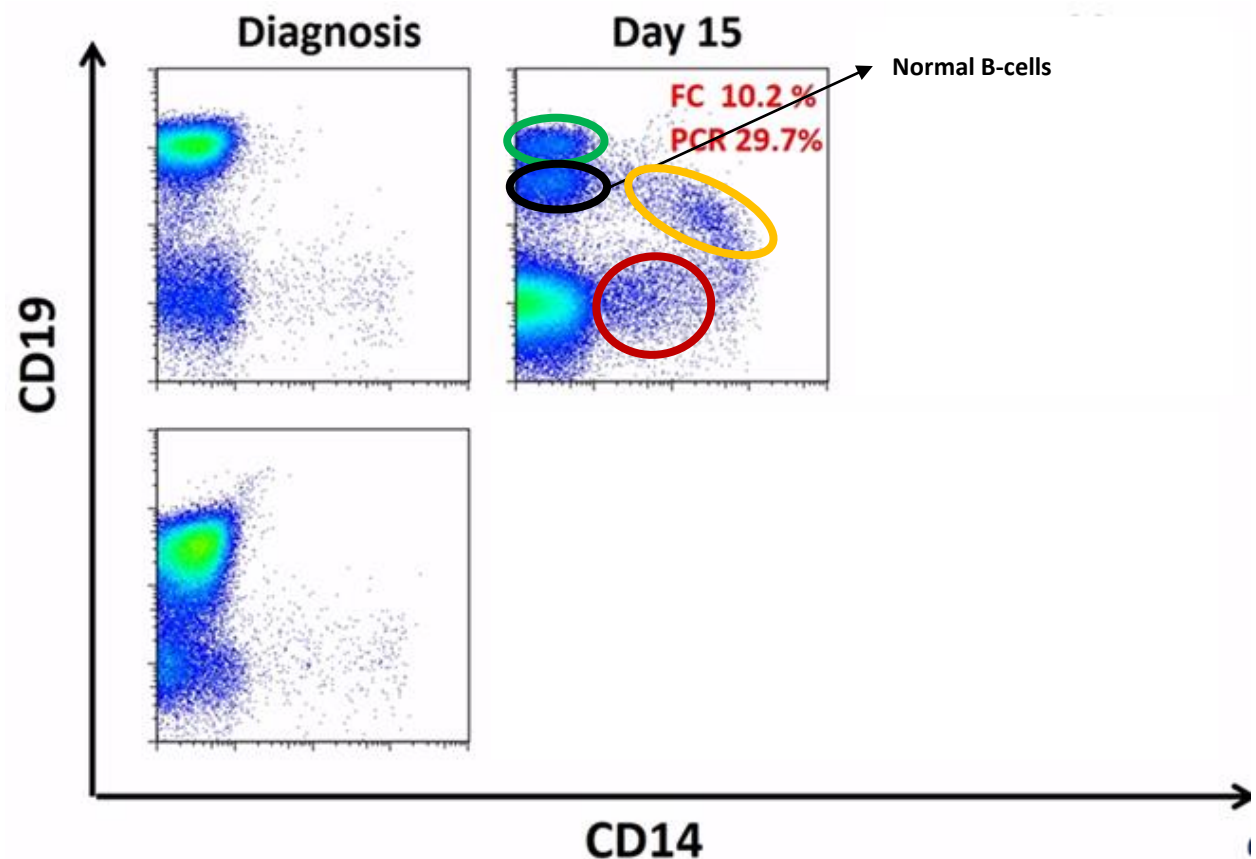

B lymphoid

intermediate B-mono

monocytoid

Slamova et al (2014), Clappier et al (2014), Zaliova et al (2014), Lilljebjörn et al (2016), Zhang et ak (2016), Novakova et al. (2020)

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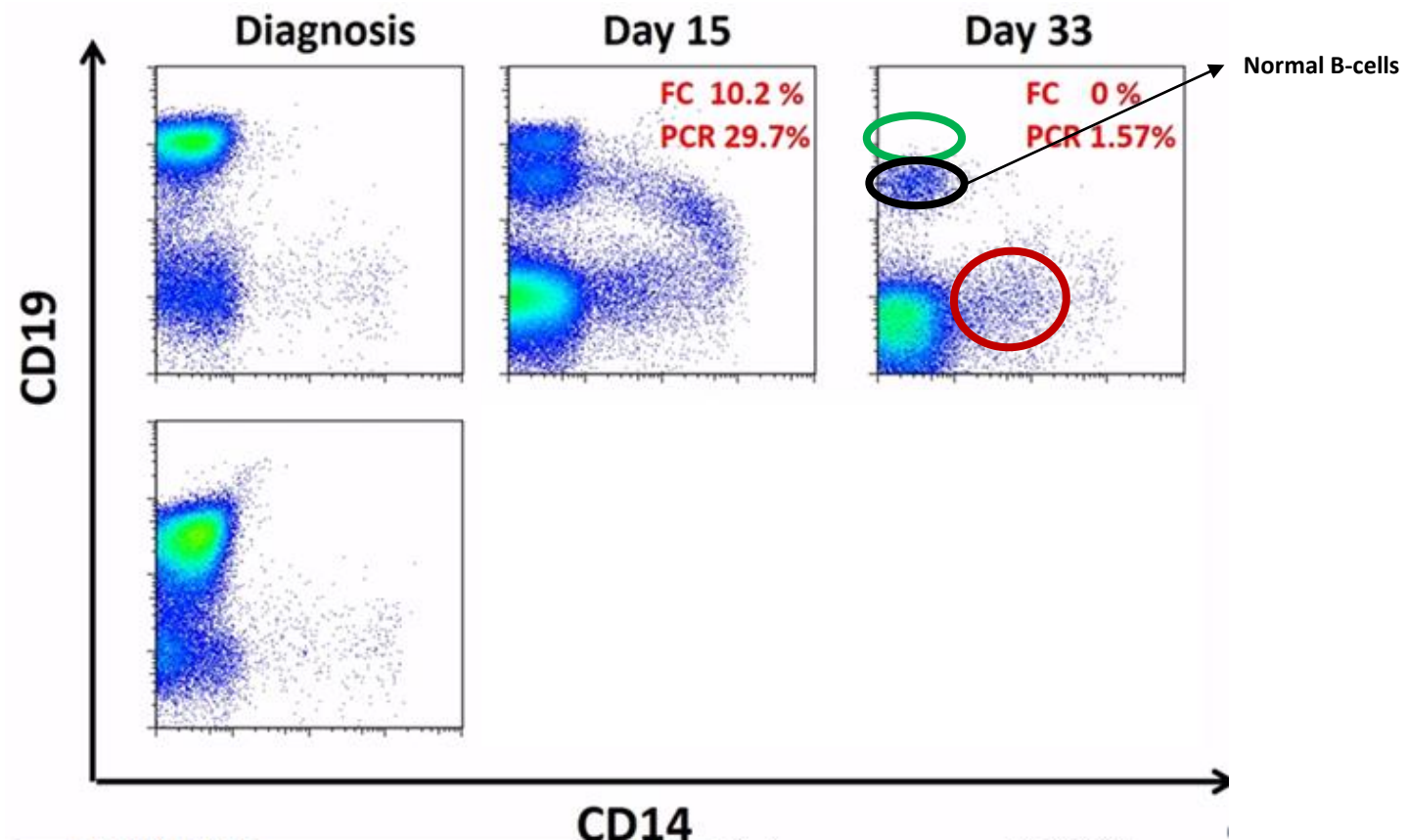

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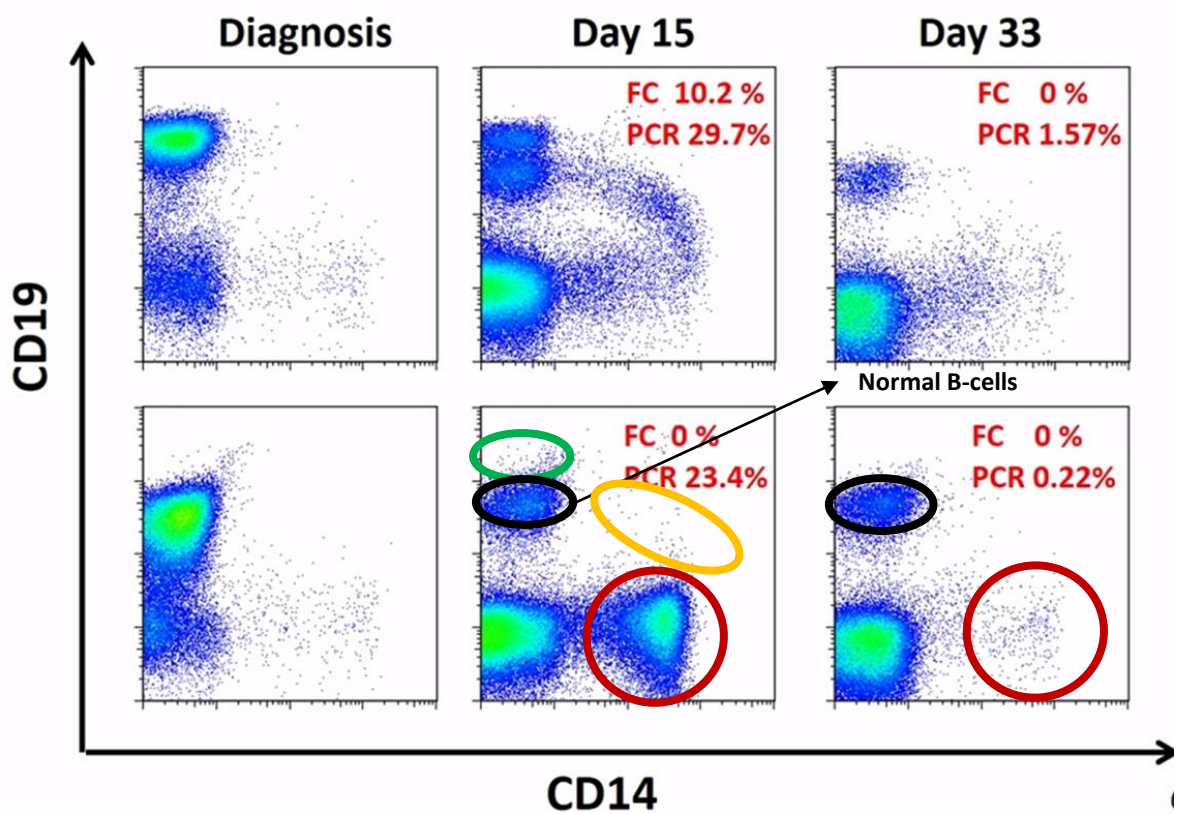

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Chronic myeloid cell neoplasms with monocytosis: CMML

- Classical CMML
  - Diagnosis:
    - Persistent (3 months) absolute (≥1×109L) and relative (≥10% of leukocytes) monocytosis in PB
    - absence of BCR-ABL1, PCM1-JAK2 and rearrangements in PDGFRA, PDGFRB or FGFR1 genes
    - diagnostic dysplasia in ≥ 1 BM cell lineages (≥10%)

      OR
    - CMML-related cytogenetic/molecular lesions AND/OR CMML-related FCM abnormalities
  - Leukocyte count
    - Dysplastic variant (leukocyte count ≤13x10^9/L
    - Proliferative' variant (leukocyte count >13x109/L)
  - % blasts in PB and BM : CMML-0, -1, -2 (! grading should be based on the higher blast cell percentage)

- CMML variants
  - Oligomonocytic CMML (~pre-phase)
    - Abovementioned criteria, except for absolute PB monocyte count 0,5 – 0,9 x109/L, including FCM abnormalities,
    - ~~MDS~~, ~~MDS/MPN-U~~
    - typical morphology of PB and BM, splenomegaly, and CMML-related molecular features
  - CMML associated with systemic mastocytosis (SM-CMML, + *KIT* mutation D816V)
  - CMML (with a concomitant myeloid neoplasm*) expressing a classical MPN- driver
  - CMML with concomitant lymphoid neoplasm

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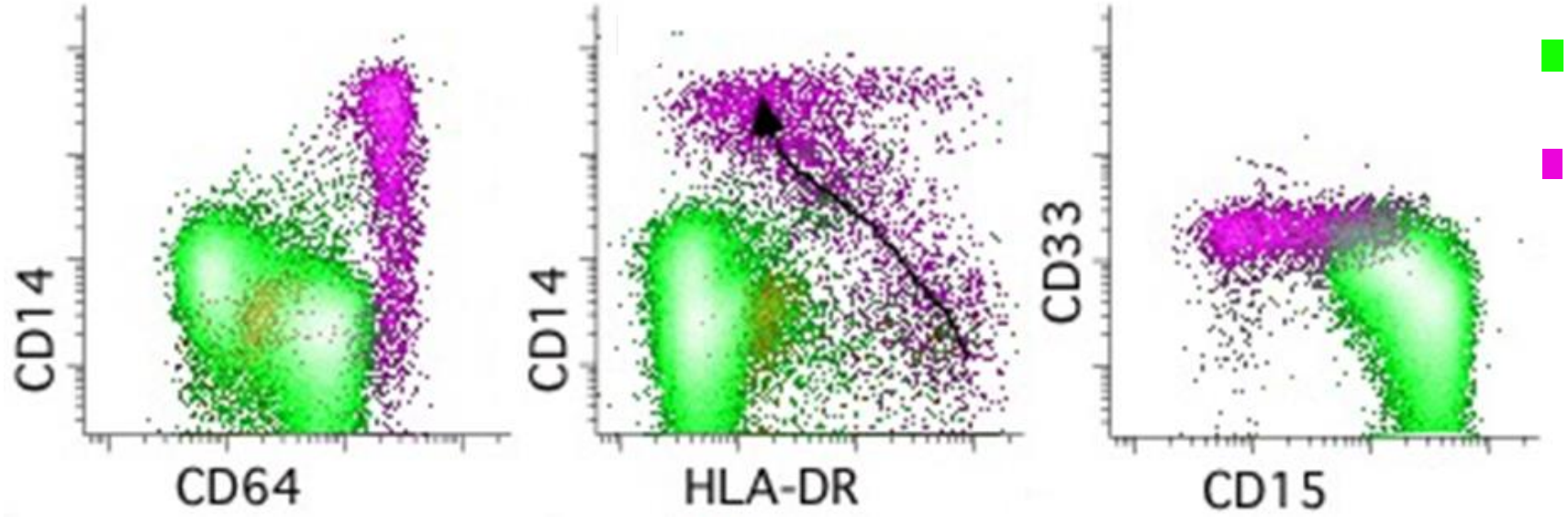
Arber et al (2016), Geyer et al (2017), Valent et al (2019), Calvo et al (2020)

Chronic myeloid cell neoplasms with monocytosis: CMML

- FCM helpful to distinguish **dysplastic myeloid precursors** from **immature monocytic** cells
  - CD14, CD64, HLA-DR, CD15 and SSC
  - BM evaluation is crucial!



- Granulocytic precursors (excl. blasts)
- Mature and immature monocytic precursors

(1) Selimoglu-Buet D et al (2015, (2) Talati et al (2017)

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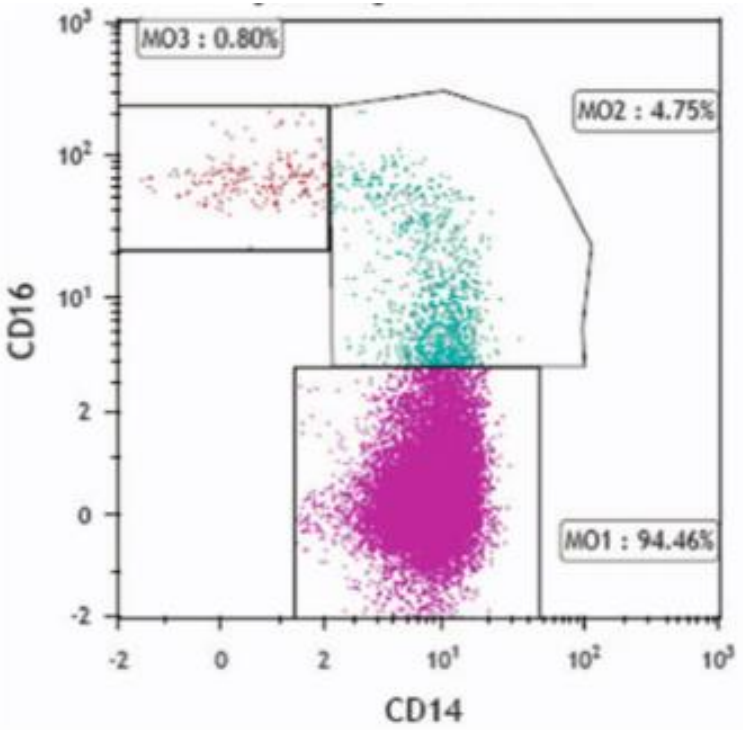
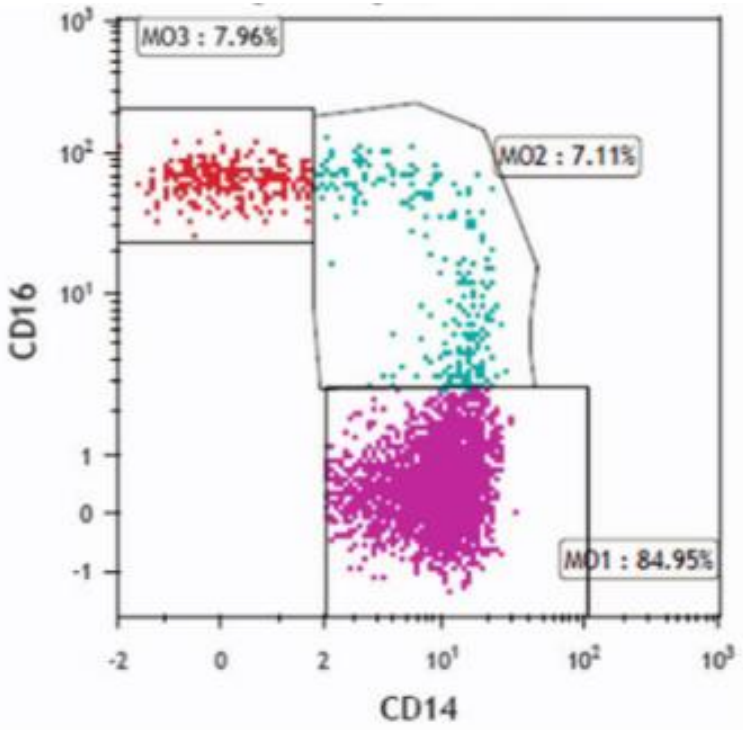
Chronic myeloid cell neoplasms with monocytosis: CMML

- FCM helpful to distinguish **neoplastic** from **reactive** monocytosis
  - **cMo** (CD14+/CD16-) are **increased** in CMML
  - **iMo** (CD14+/CD16+) and **ncMo** (CD14-/CD16+) are relatively **decreased** in CMML

CMML



Reactive monocytosis



cMO⩾94%: CMML
(96% (14/15) and 92% (98/107) of patients)

cMO<92%: other monocytosis

Co-occurence inflammatory disease:
FCM-defined inflammatory CMML
- SLAN+ ncMo <1.7%
- 'bulbous' aspect cMo/iMo

(1) Selimoglu-Buet D et al (2015), (2) Talati et al (2017), Jestin et al (2020)

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Thank you for your attention!

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