From stem cell to blood cell: flow cytometry of the differentiation pathway

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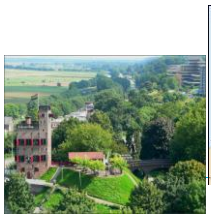

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Conflict of Interest Disclosure

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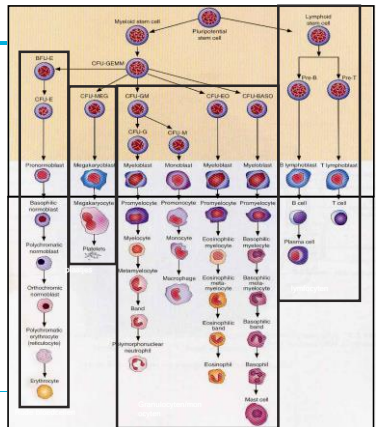
Maturation of Blood cells



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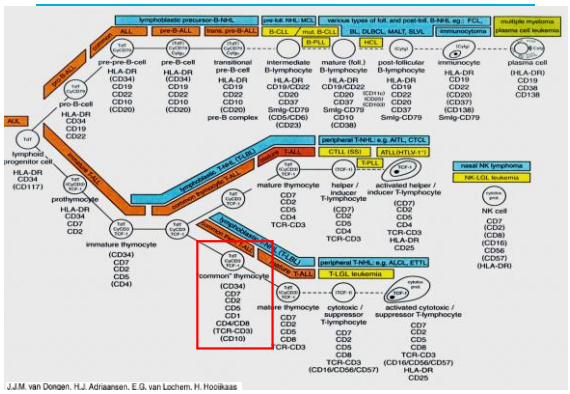
Lymfoid differentiation pathway



J.J.M. van Dongen, H.J. Adriaansen, E.G. van Lochem, H. Hooijkaas

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Essentials for Immunophenotyping of leukemia and lymphoma

How do you differentiate between normal and malignant populations

- Pattern recognition:
  - What is the normal pattern of expression?
  - What is the aberrant pattern of expression?

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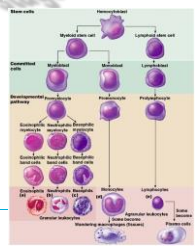
??How to Discriminate between Cells And cell differentiation stages??

How to start??



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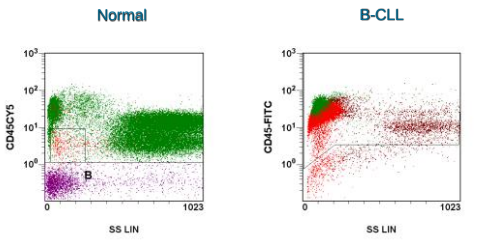
CD45 pattern



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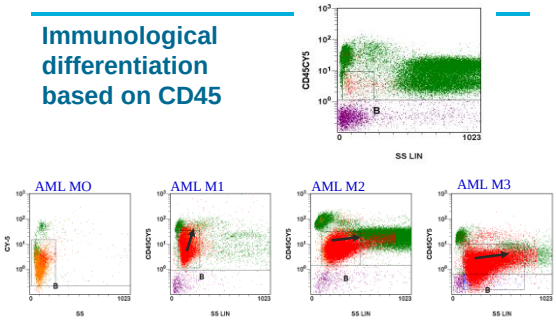
Immunological differentiation based on CD45



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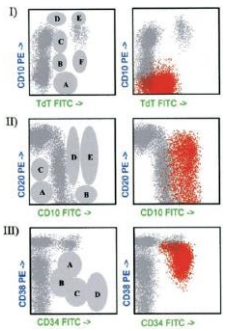
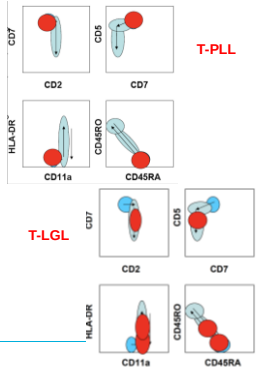
B-cell differentiation



T-cell differentiation



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Phenotyping of the Myeloid Lineage to identify the different maturation stages

Search for the myelo-monocytic progenitor cells

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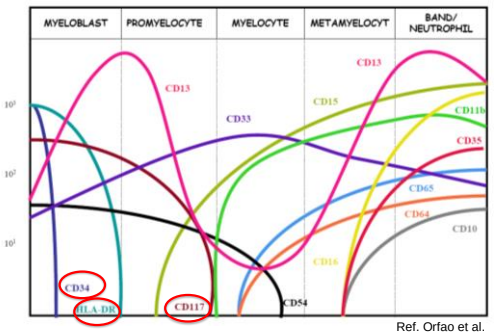
Phenotypic changes in the neutrophilic differentiation pathway



Ref. Orfao et al.

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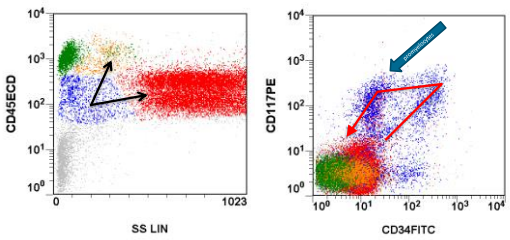
1. Expression pattern of CD34 / CD117 in CD45+ population Normal bone marrow



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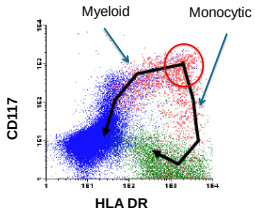
Myelomonocytic differentiation/maturation share the same progenitor cells!



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Phenotyping of the Myeloid Lineages CD11b / CD13 / CD16 / CD45

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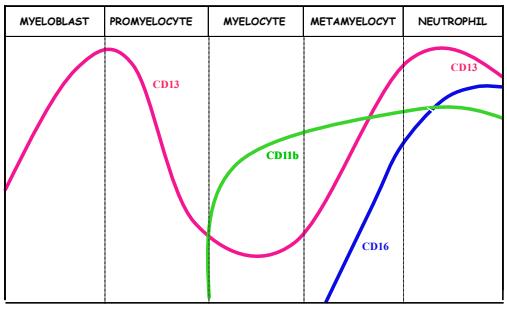
2. Expression pattern of CD13, CD16, CD11b within CD45



Ref. A. Orfao et al

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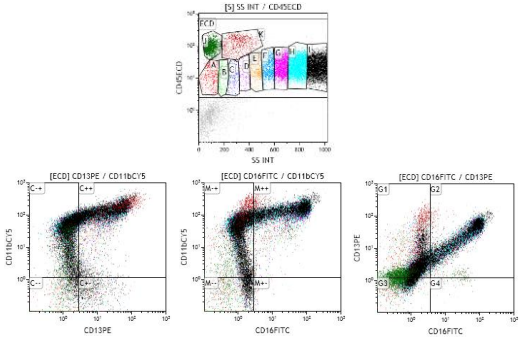
2.a. Expression pattern CD13/CD11b/CD16 (normal BM)



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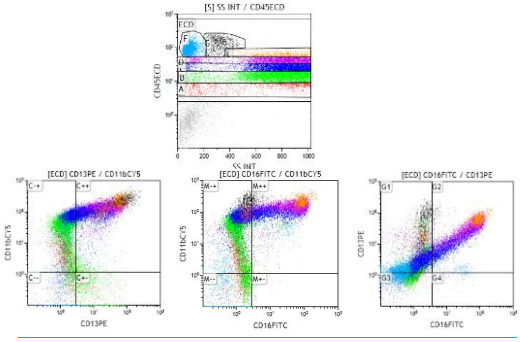
2.b. Expression pattern CD13/CD11b (normal BM)



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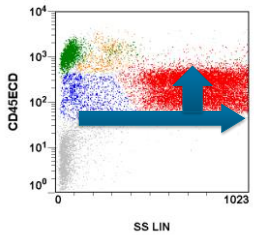
Conclusion

(myeloid differentiation pattern in CD45)



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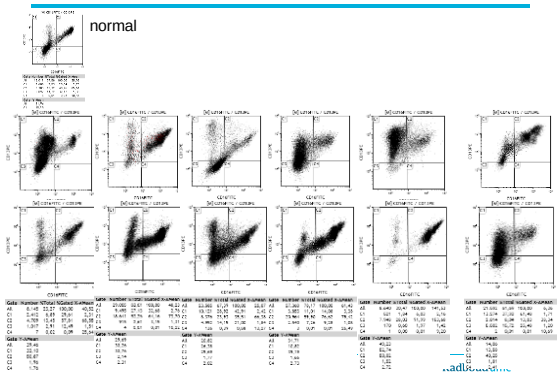
Aberrant pattern of CD13/CD16 in MDS



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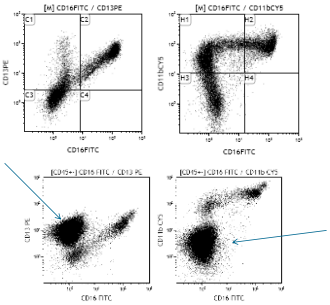
Aberrant pattern of CD13/CD16 in AML



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More detailed phenotyping of the Myeloid and Monocytic Lineages by CD11b / CD15 / HLA-DR / CD45

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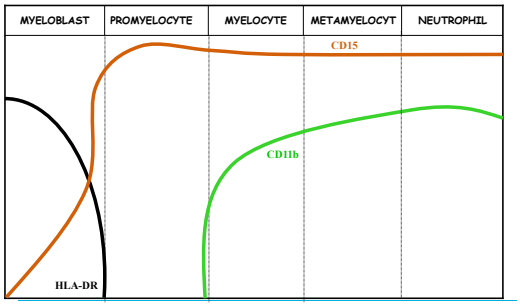
3. Expression pattern CD11b, CD15, HLA-DR



Ref.: A. Orfao et al

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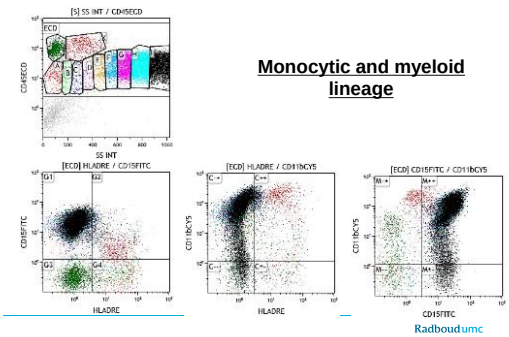
3.a. Expression pattern CD11b/CD15/HLA-DR in CD45+ population (normal BM)



Monocytic and myeloid lineage

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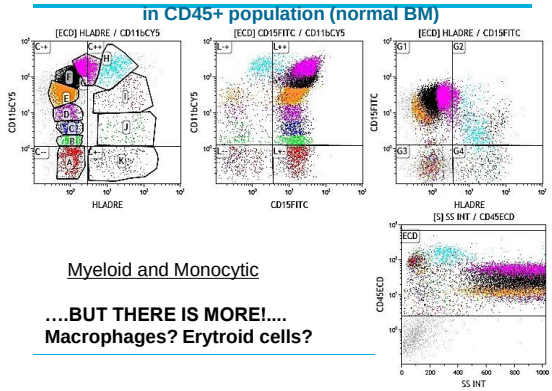
3.b. Expression pattern of CD11b/CD15/HLA-DR in CD45+ population (normal BM)



Myeloid and Monocytic

….BUT THERE IS MORE!.... Macrophages? Erytroid cells?

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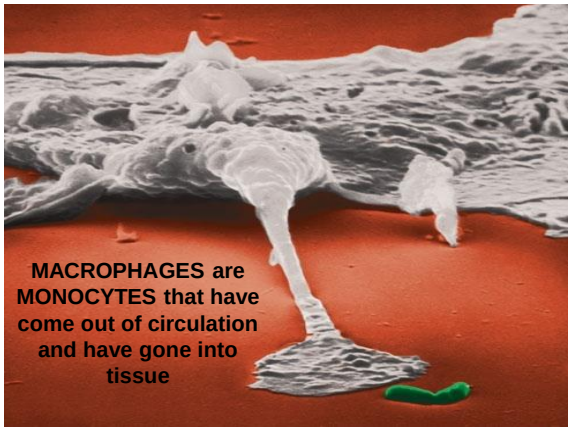

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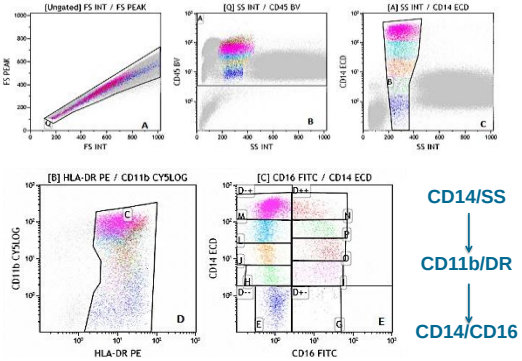
4.a. Where are the Macrophages?
Maturation of the monocytes based on CD14 and CD16



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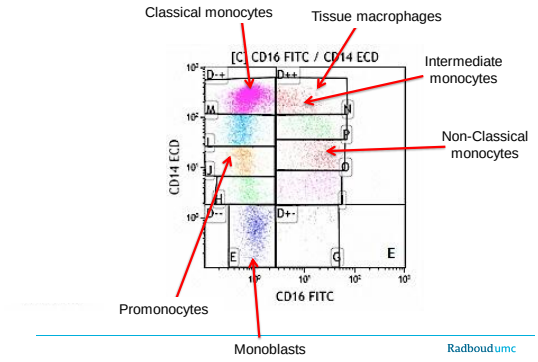
Maturation from monocytes to macrophage



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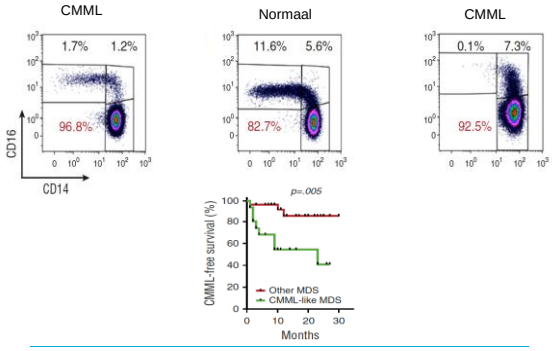
CD14/CD16 in CMML: CD14+CD16- >93%



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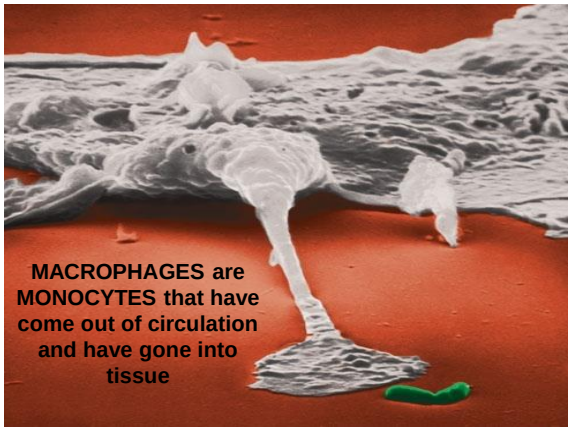

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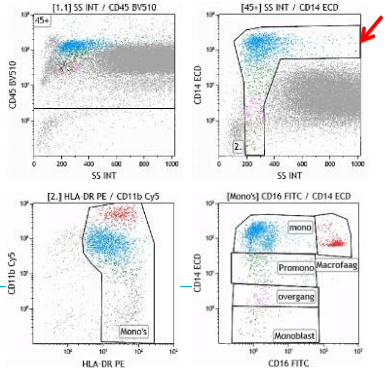
4.b. Maturation from monocytes to macrophages (details)

Macrophages are SS++ CD11b++ HLA-DR++ CD14+ CD16+



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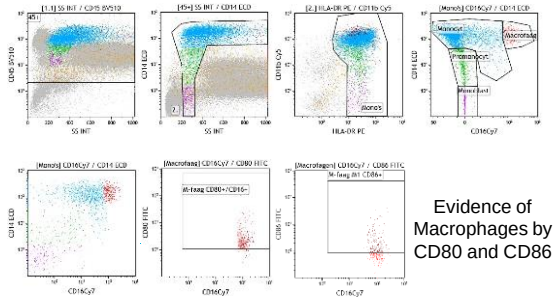
4.c. Maturation from monocytes to macrophages (CD80 and CD86)



Evidence of Macrophages by CD80 and CD86

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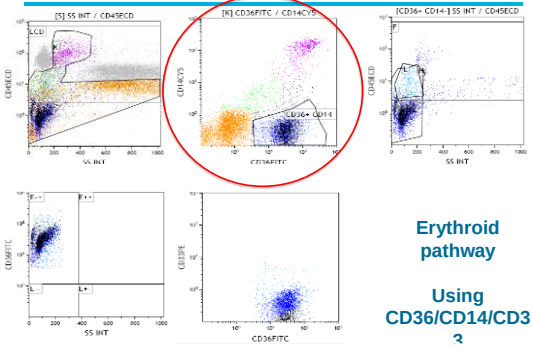
5. Maturation of monocytes. But what about CD36+CD14-



Erythroid pathway

Using CD36/CD14/CD33

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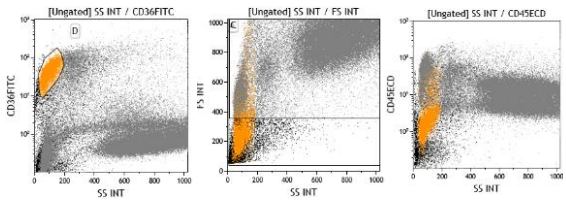
But how can we characterize these CD36+ cells



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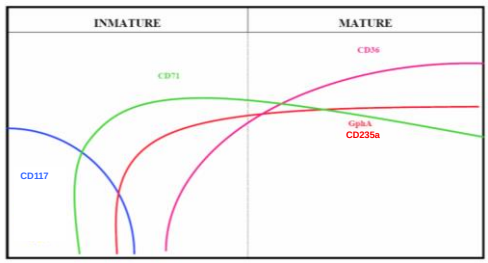
Normal erythroid maturation

CD117 / CD71 / CD235a / CD36



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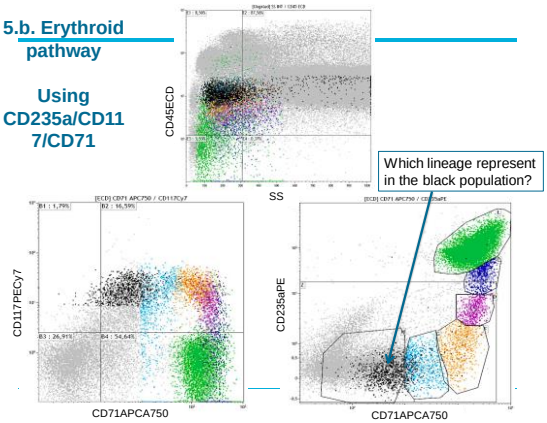
5.b. Erythroid pathway

Using CD235a/CD117/CD71



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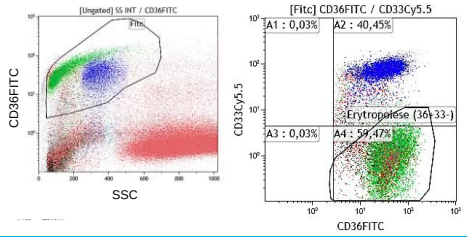
Elimination of myeloid cells from the erythroid lineage (CD36+CD33-)



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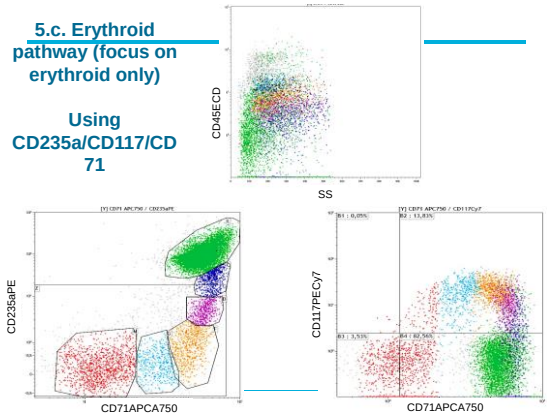
5.c. Erythroid pathway (focus on erythroid only)

Using CD235a/CD117/CD 71



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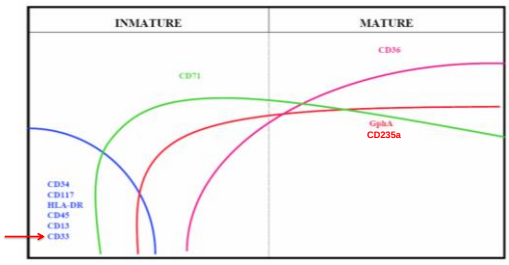
Normal erythroid maturation



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Summary

1. Myeloid differentiation
2. Monocytic differentiation
3. Separation point of myeloid and monocytic
4. Erythroid differentiation

How to show all the lineages in one plot??

The Radar plots

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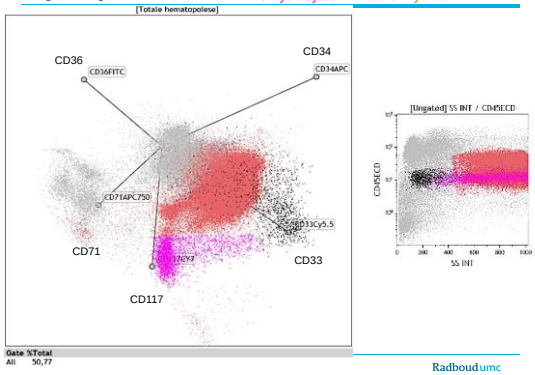
Myelopoiese

CD45+-/CD34+ precursors,
Promyelocytes + Mature myeloid



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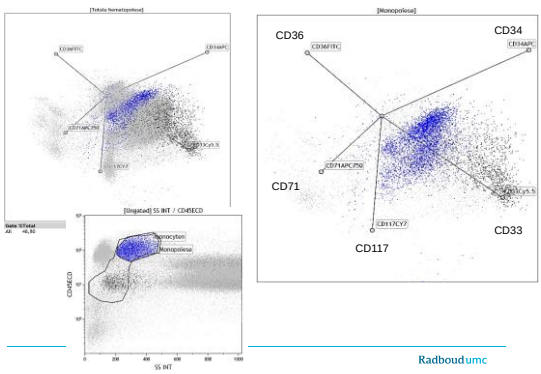
Monopoiese

Mature monocytes and
CD45+-/CD34+ precursors



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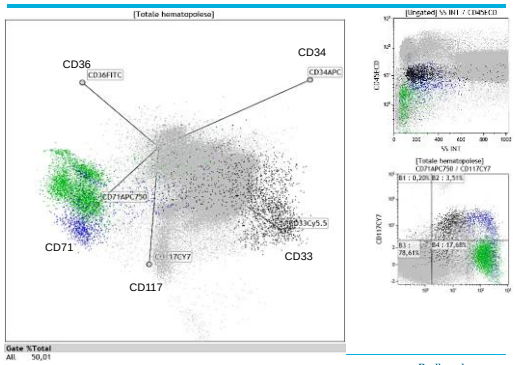
Erythropoiese



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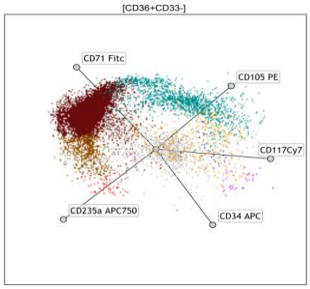
Erythropoiese (CD36+CD33- and CD105/CD117)



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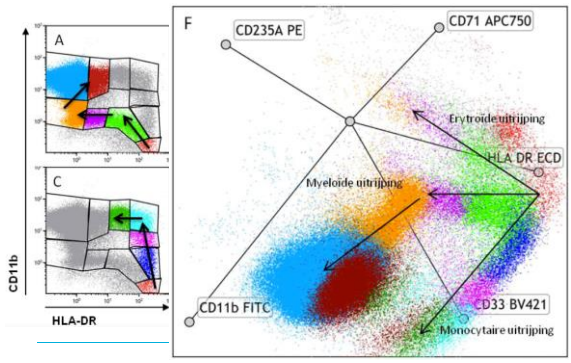
Hematopoiese (Myeloid - Monocytic - Erythroid)



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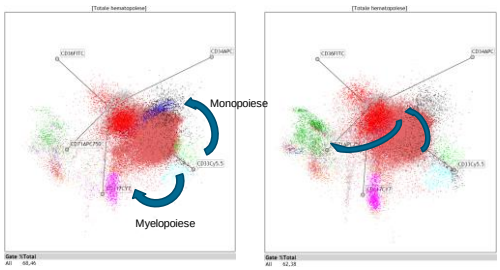
Normal hematopoiese



Myeloid and monocytic hematopoiese

Erythroid hematopoiese

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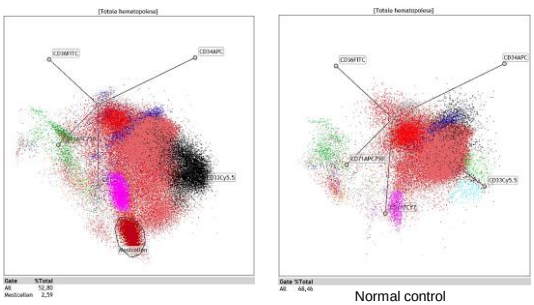
CMML and mastocytosis



Normal control

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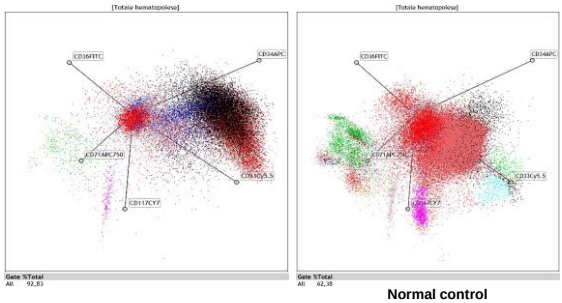
AML without differentiation and monocytic characteristics



Normal control

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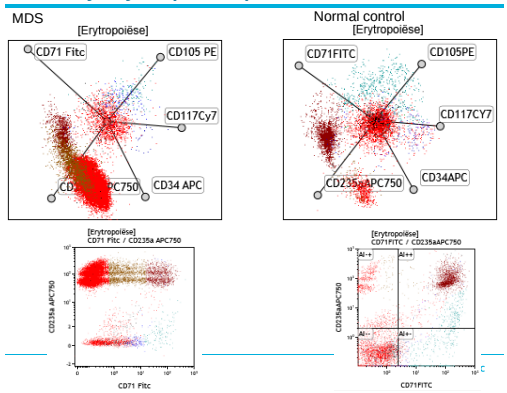
MDS with dyserythropoiesis (increase of CD71dim/CD71CV)



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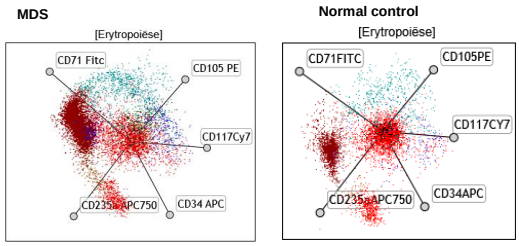
MDS with dyserythropoiesis (increase CD71 CV)



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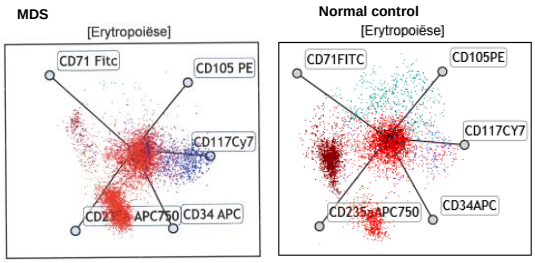
MDS with dyserythropoiesis (decrease immature)



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Conclusions

- CD11b/CD13/CD16 and CD11b/CD15/HLADR are essential marker combinations to study the myelo/monocytic maturation pathways
- In relatively mature myeloid stage, the SSC is expressed from dim to bright with increase in CD45 expression
- CD14/CD36/CD16 are essential marker combinations to study the monocytes and macrophages
- CD36/CD235a/CD117/CD71/CD105 are essential marker combinations to study the erythroid differentiation
- **Radar plots are very useful to study**
  - **The differentiation pathways of the different lineages**
  - **The differentiation variation between normal and aberrant**

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