28 & 29
SEPTIEMBRE
2023

6º CURSO PRÁCTICO
CITOMETRÍA DE FLUJO

Leucemia aguda de linaje ambiguo

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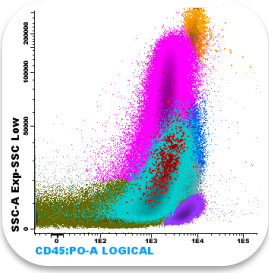
Classification principles

This has not changed much in the last decades

Contemporary classification schemes of hematopoietic neoplasms are based on:



Cellular lineage according to immunophenotype

Clinical attributes eg, acute versus chronic, cytopenias/cytoses

Genetic features, eg, gene fusions, rearrangements, mutations

ALAL: Working definition

This has not changed much in the last decades

Acute leukemias of ambiguous lineage (ALALs) include biologically diverse leukemias that fail to show commitment to either the myeloid, B-, or T-lymphoid lineages (AUL) or show evidence of commitment to more than 1 lineage (MPAL).

ALAL: Rare leukemias with diagnostic and therapeutic challenges

ALAL are not frequent, and maybe less in recent years

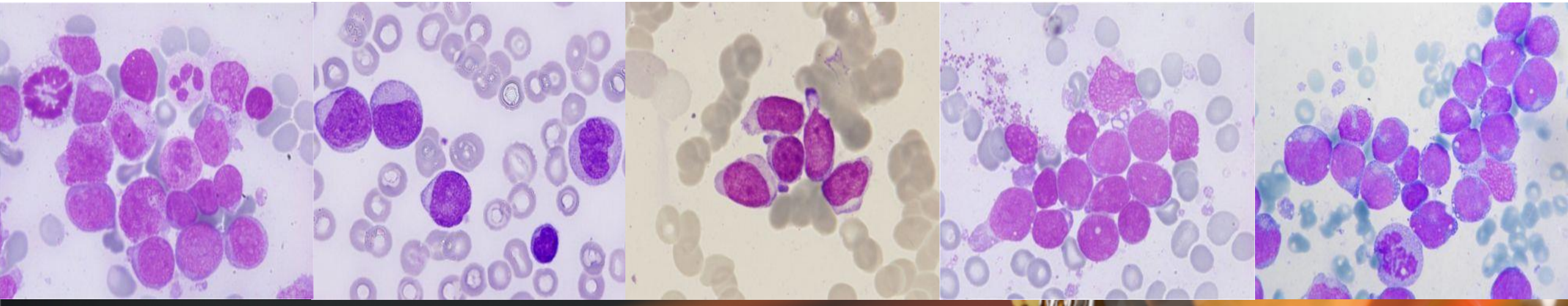
- Both AUL and MPAL need large comprehensive flow cytometry immunophenotyping studies to diagnose and monitor.
- May represent <4% AL cases.
- Some cases (up to 50%) could be better classified in other entities.
  - Overrepresented in historic series

ALAL Morphology

Classical morphology is rather unespecific

Most patients with ALAL show a diffuse infiltration by morphologically diverse blasts. These MPAL blasts may resemble myeloblasts or monoblasts and occasionally lymphoblasts; there may be a dual/ dimorphic population, or blasts may have an undifferentiated appearance. Typically, AUL blasts lack standard myeloid features, such as granules in the cytoplasm or Auer rods.

Matutes E, Pickl WF, Van't Veer M, et al. Mixed-phenotype acute leukemia: clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. Blood. 2011;117:3163-3171.



ALAL conventional genetics

It only discredits our work…. Just joking

Most studies describe over 50% abnormal karyotype

Complex karyotypes more frecuent in T/My (19-30% of all cases)

BCR::ABL more frequent in adults, whereas KMT2A-r is more frequent in infancy. But remember its most B/My MPAL

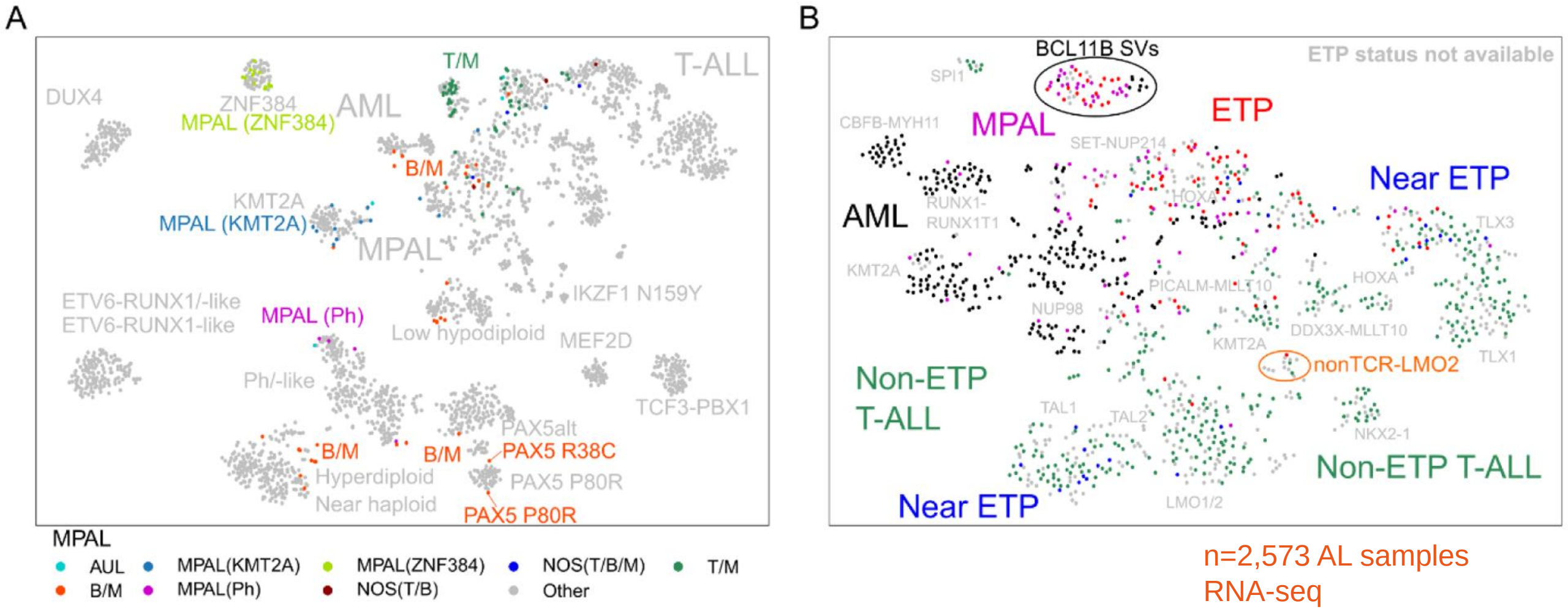
ZNF384-r in nearly 50% pediatric B/My MPAL, but not in adults. Very similar to ZNF384-r B-ALL

BCL11B-r in T/My MPAL

Matutes E, Pickl WF, Van't Veer M, et al. Mixed-phenotype acute leukemia: clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. Blood. 2011;117:3163-3171.
Alexander TB, Gu Z, Iacobucci I, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature 2018;562(7727):373–9.
Takahashi K., Wang F., Morita K., et al: Integrative genomic analysis of adult mixed phenotype acute leukemia delineates lineage associated molecular subtypes. Nat Commun 2018;

ALAL is not a genetically homogeneous disease

Apparently homogeneous diseases are torn appart and apparently different diseases, are quite similar from a genetic viewpoint



n=2,573 AL samples
RNA-seq

Montefiori LE, Bendig S, Gu Z, Chen X, Pölönen P, Ma X, Murison A, Zeng A, Garcia-Prat L, Dickerson K, Iacobucci I, […]M, Haferlach C, Mullighan CG. Enhancer Hijacking Drives Oncogenic BCL11BExpression in Lineage-Ambiguous Stem Cell Leukemia. Cancer Discov. 2021 Nov;11(11):2846-2867. doi: 10.1158/2159-8290.CD-21-0145. Epub 2021 Jun 8. PMID: 34103329; PMCID: PMC8563395.

Montefiori LE, Mullighan CG. Redefining the biological basis of lineage-ambiguous leukemia through genomics: BCL11B deregulation in acute leukemias of ambiguous lineage. Best Pract Res Clin Haematol. 2021 Dec;34(4):101329. doi: 10.1016/j.beha.2021.101329. Epub 2021 Oct 23. PMID: 34865701; PMCID: PMC8649174.

ALAL mutational analysis

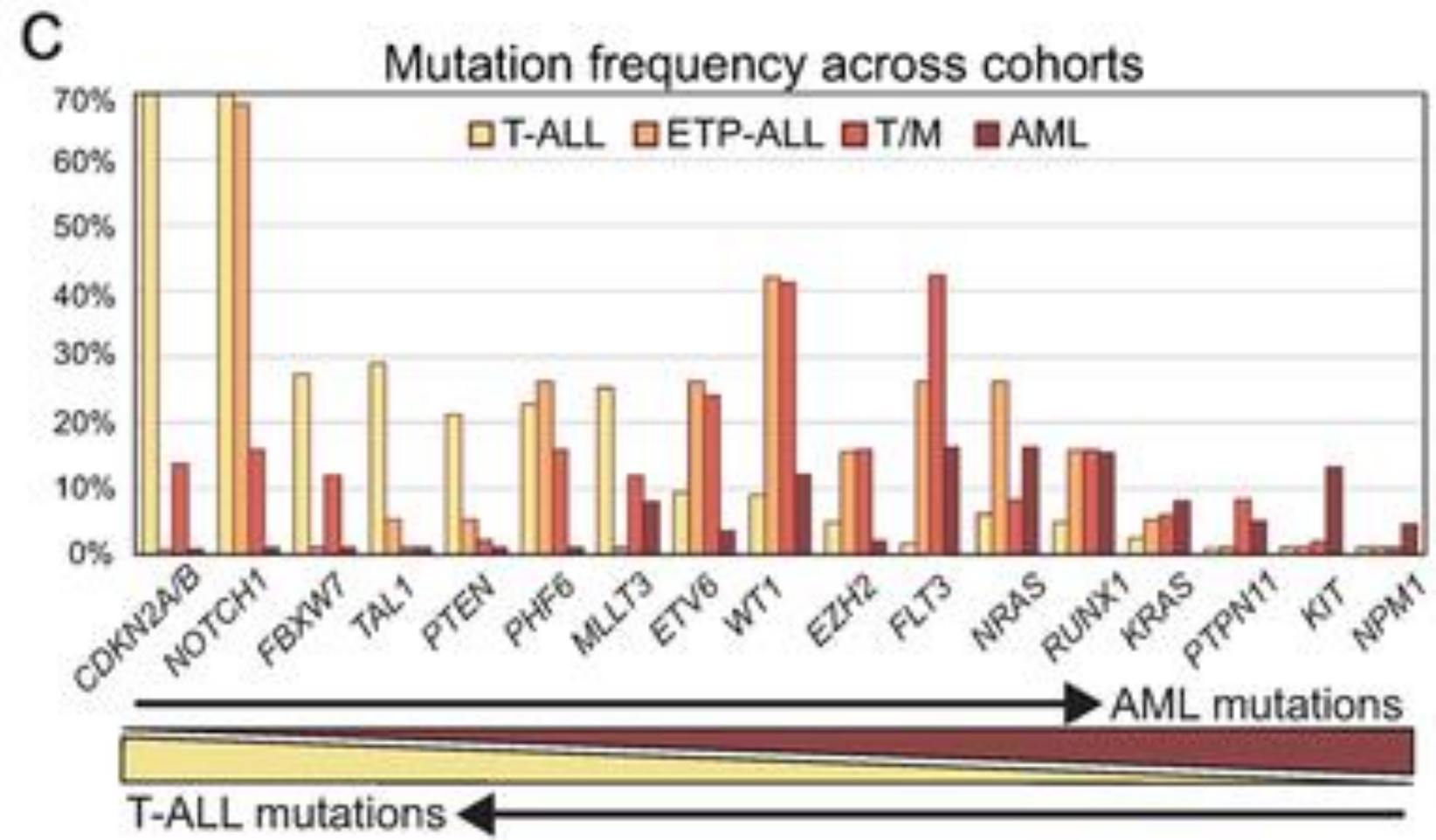
B/My normally has less mutations than T/My cases.

B/Myeloid	T/Myeloid	B/T MPAL	ETP ALL
• IKZF1 deletions • RUNX1 • TET2 • EZH2 • ASLX1	• *EZH2* • *PHF6* • *DNMT3A* • *NOTCH1* • *FBXW7* • *IL7R* • *JAK/STAT* pathway • *WT1* • *IDH2* • *FLT3* • *KRAS* • *NRAS* • *CUX1* • *CEBPA* • *CDKN2A / CDKN2B* • *ETV6* • *VPREB1*	• *PHF6* • *WT1* • *JAK3* • *MED12* • *CTCF* • *IL7R* • *NOTCH1* • *SF3B1* • *PTPN11* • *EZH2* • *DNMT3A* • *TP53*	• *ETV6* • *WT1* • *EZH2* • *FLT3* • NOTCH1

Quesada A.E., Hu Z., Routbort M.J., et al: Mixed phenotype acute leukemia contains heterogeneous genetic mutations by next-generation sequencing. Oncotarget 2018. Alexander TB, Gu Z, Iacobucci I, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature 2018;562(7727):373-9.
Takahashi K., Wang F., Morita K., et al: Integrative genomic analysis of adult mixed phenotype acute leukemia delineates lineage associated molecular subtypes. Nat Commun 2018

ALAL mutational analysis

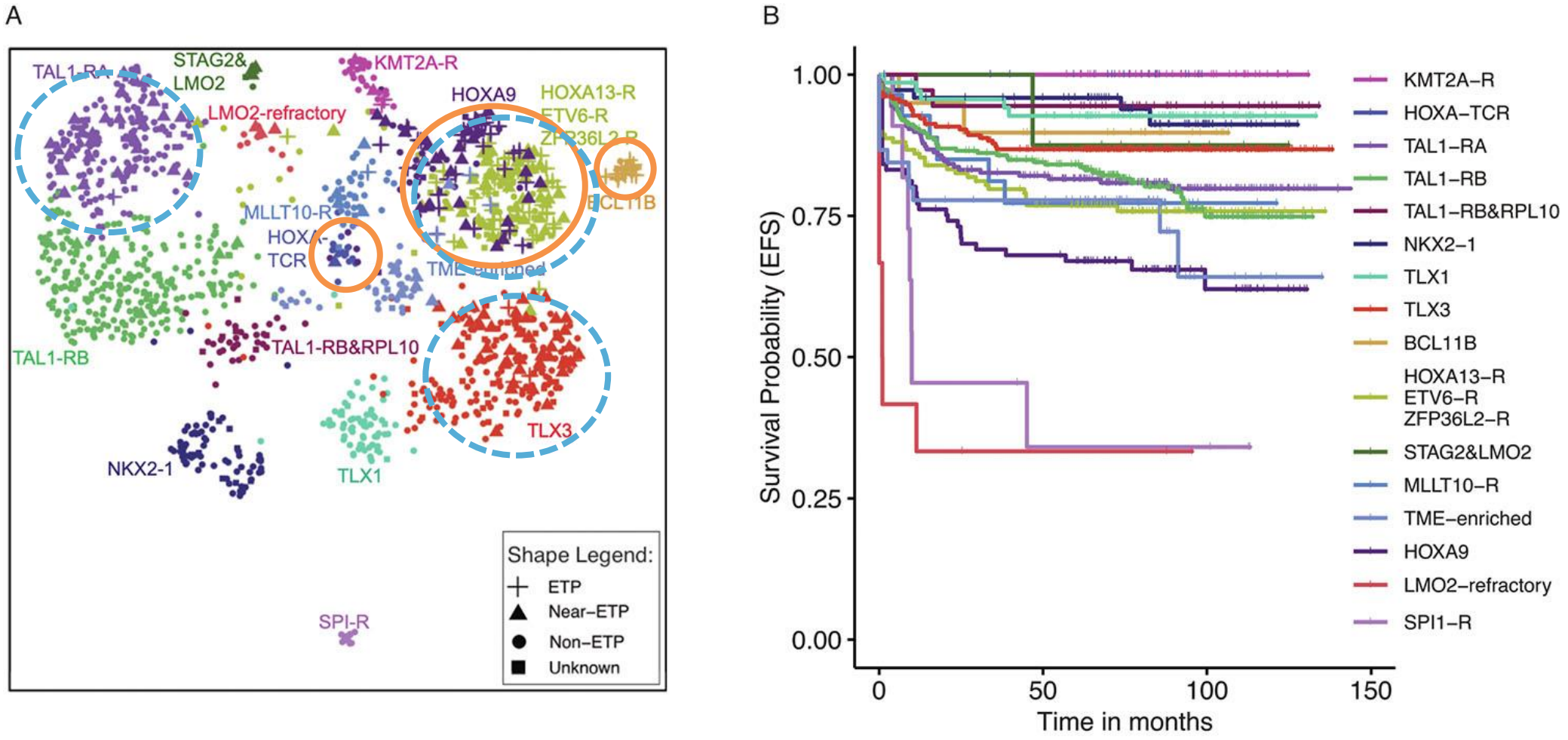
A continuum between not so different leukemias



Alexander TB, Gu Z, Iacobucci I, et al. The genetic basis and cell of origin of mixed phenotype acute leukaemia. Nature 2018;562(7727):373–9.
Takahashi K., Wang F., Morita K., et al: Integrative genomic analysis of adult mixed phenotype acute leukemia delineates lineage associated molecular subtypes. Nat Commun 2018.
Di Giacomo D, K, et al. 14q32 rearrangements deregulating BCL11B mark a distinct subgroup of T-lymphoid and myeloid immature acute leukemia. Blood. 2021 Sep 2;138(9):773-784.

Early T cell precursor leukemia

Transcriptional analysis in pediatric populations

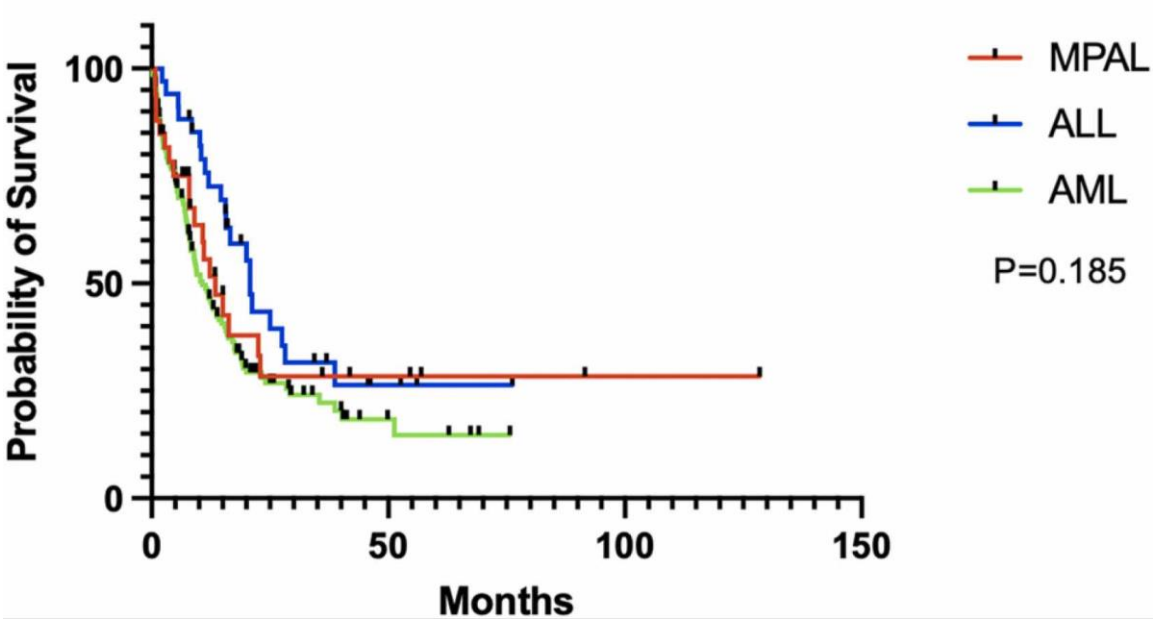


Petri Pölönen, Abdelrahman Elsayed, Danika Di Giacomo, Lindsey Montefiori, Shunsuke Kimura, Jason Myers, Dale Hedges, Jason Xu, Yawei Hui, Zhongshan Cheng, Yiping Fan, Ilaria Iacobucci, Yunchao Chang, Rawan Shraim, Meenakshi Devidas, Stuart S. Winter, Kimberly P. Dunsmore, Jun J.J. Yang, Tiffaney L. Vincent, Kai Tan, Changya Chen, Haley Newman, Mignon L. Loh, Elizabeth A. Raetz, Stephen P. Hunger, Evadnie Rampersaud, Ti-Cheng Chang, Gang Wu, Stanley B. Pounds, Charles G. Mullighan, David T. Teachey; Comprehensive Genome Characterization of Childhood T-ALL Links Oncogene Activation Mechanism and Subtypes to Prognosis. *ASH* 2022.

The case of complex karyotype

And the basis for classifying these cases in AML-MRC

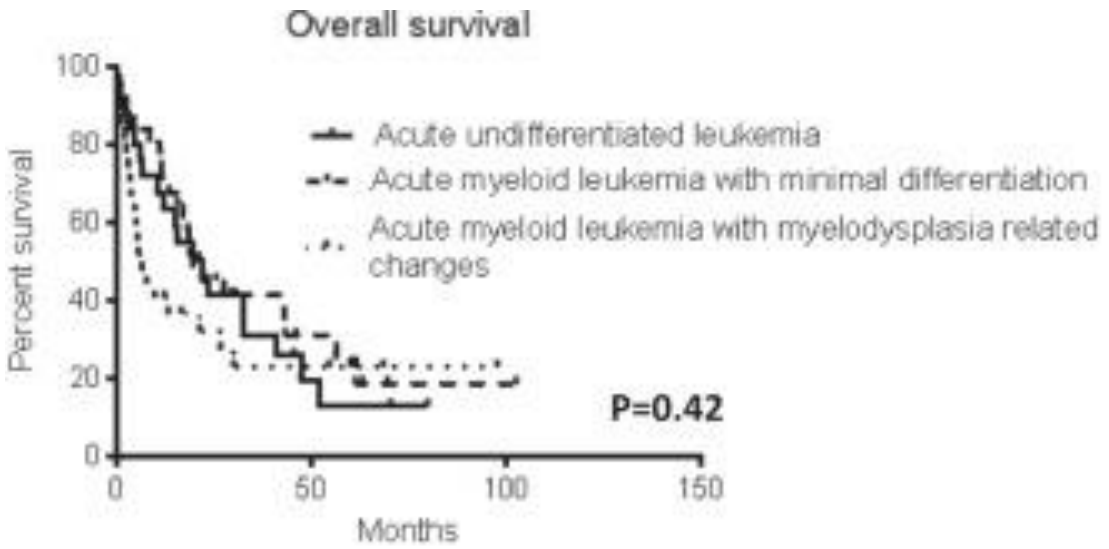
MPAL



Retrospective of AL cases with CK

Not surprisingly, most are AML

AUL



Retrospective of AUL cases.

CK reclassified cases as AML with MRC

Kirtek T, Chen W, Laczko D, Bagg A, Koduru P, Foucar K, Venable E, Nichols M, Rogers HJ, Tam W, Orazi A, Hsi ED, Hasserjian RP, Wang SA, Arber DA, Weinberg OK. Acute leukemias with complex karyotype show a similarly poor outcome independent of mixed, myeloid or lymphoblastic immunophenotype: A study from the Bone Marrow Pathology Group. Leuk Res. 2023 Jul;130:107309. doi: 10.1016/j.leukres.2023.107309. Epub 2023 May 10. PMID: 37210875.

Weinberg, Olga K.; Hasserjian, Robert P.; Baraban, Ezra; Ok, Chi Young; Geyer, Julia T.; Philip, John K. S. S. et al. (2019): Clinical, immunophenotypic, and genomic findings of acute undifferentiated leukemia and comparison to acute myeloid leukemia with minimal differentiation: a study from the bone marrow pathology group. Modern Pathology 32 (9), pág. 1373–1385

The case of complex karyotype

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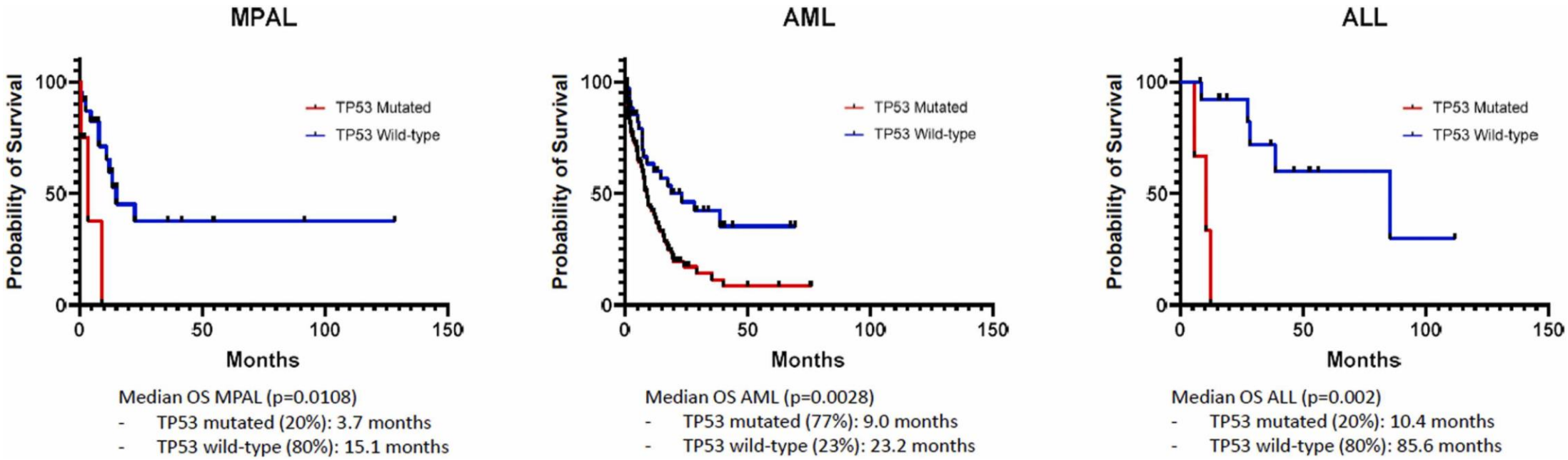


Overlapping features in the biological behavior of MPAL with CK and AML with CK

Supports the exclusion of immunophenotypic MPAL with CK from the MPAL group and their inclusion within AML-MR (WHO HAEM5) and AML with myelodysplasia-related cytogenetics (ICC)

The poor prognosis of TP53 -mutated MPAL with complex karyotype would support its inclusion within the new ICC entity of AML with mutated TP53 .

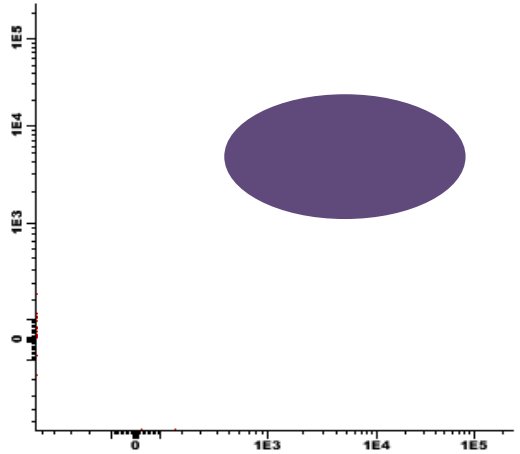
Kirtek T, Chen W, Laczko D, Bagg A, Koduru P, Foucar K, Venable E, Nichols M, Rogers HJ, Tam W, Orazi A, Hsi ED, Hasserjian RP, Wang SA, Arber DA, Weinberg OK. Acute leukemias with complex karyotype show a similarly poor outcome independent of mixed, myeloid or lymphoblastic immunophenotype: A study from the Bone Marrow Pathology Group. Leuk Res. 2023 Jul;130:107309. doi: 10.1016/j.leukres.2023.107309. Epub 2023 May 10. PMID: 37210875.

Pre-EGIL Classification

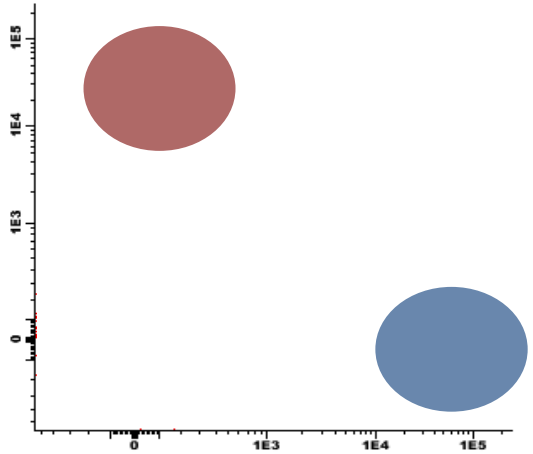
Acute mixed leukemias, acute biphenotypic leukemias,… Working definition

Late 80s. Four scenarios… with 2 color flow

- Typical AML or ALL cases with one aberrant antigen… No prognosis implications.
- Biphenotypic leukemias.
- Bilineal leukemias.
- Lineage switch.



Biphenotypic



Bilineal

Catovsky D, Matutes E, Buccheri V, Shetty V, Hanslip J, Yoshida N, Morilla R. A classification of acute leukaemia for the 1990s. Ann Hematol. 1991 Feb;62(1):16-21. doi: 10.1007/BF01714978. PMID: 2031964.

EGIL Classification

A case is considered biphenotypic when scores for both myeloid and lymphoid lineages are ≥2 points

Points	B-Lineage	T-Lineage	Myeloid
2	cyIgM cyCD22	cyCD3	MPO
1	CD19 CD10 CD24	CD2 CD5 TCR rearrangement (β or δ chain)	CD13 CD33 CD14
0.5	nuTdT IGH rearrangement		AML morphology or ANAE or Sudan Black CD11b CD11c CD15

Catovsky D, Matutes E, Buccheri V, Shetty V, Hanslip J, Yoshida N, Morilla R. A classification of acute leukaemia for the 1990s. Ann Hematol. 1991 Feb;62(1):16-21. doi: 10.1007/BF01714978. PMID: 2031964.

EGIL Classification

A case is considered biphenotypic when scores for both myeloid and lymphoid lineages are >2 points

Points	B-Lineage	T-Lineage	Myeloid
2	cyCD79a* cyIgM cyCD22	CD3 TCRαβ TCRγδ	MPO Lysozyme
1	CD19 CD10 CD20	CD2 CD5 CD8 CD10	CD13 CD33 CD65 CD117
0.5	nuTdT CD24	nuTdT CD7 CD1A	CD14 CD15 CD64

Positivity as expression on at least 20% of blasts for surface markers and at least 10% for cytoplasmic markers compared with an isotype control.

* cyCD79a adds to the B-lineage, EXCEPT when T-cell lineage is considered.

Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9:1783-1786.

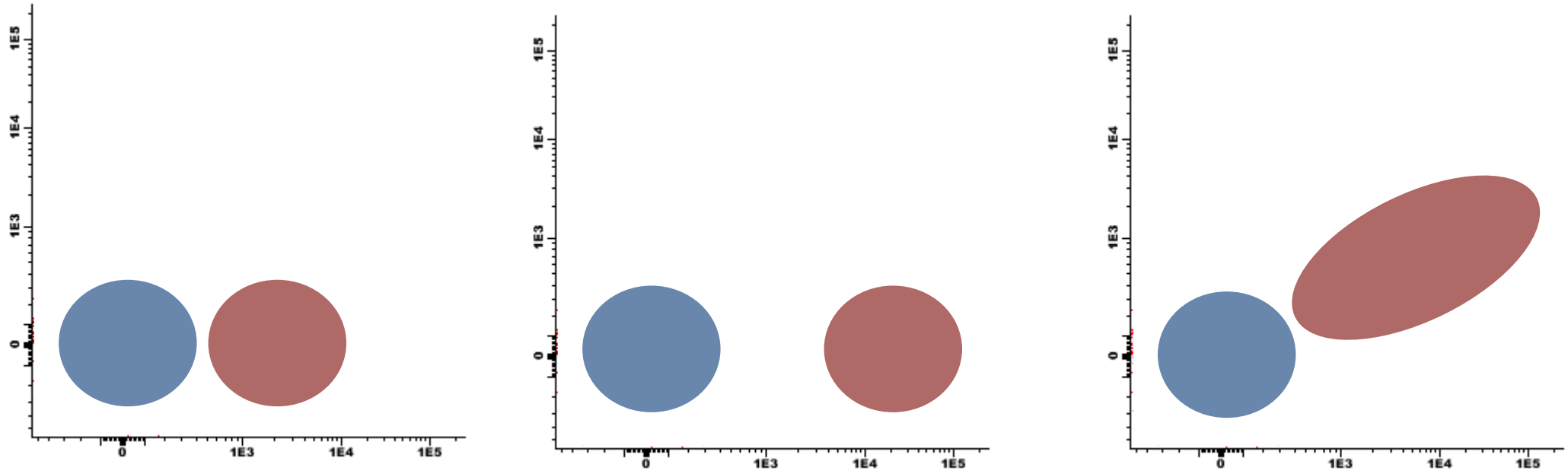
EGIL Classification

Some caveats and practical considerations

A large panel must be used.

Antigens & their relative weight had not been prospectivelly validated.

Who uses and isotypic control?



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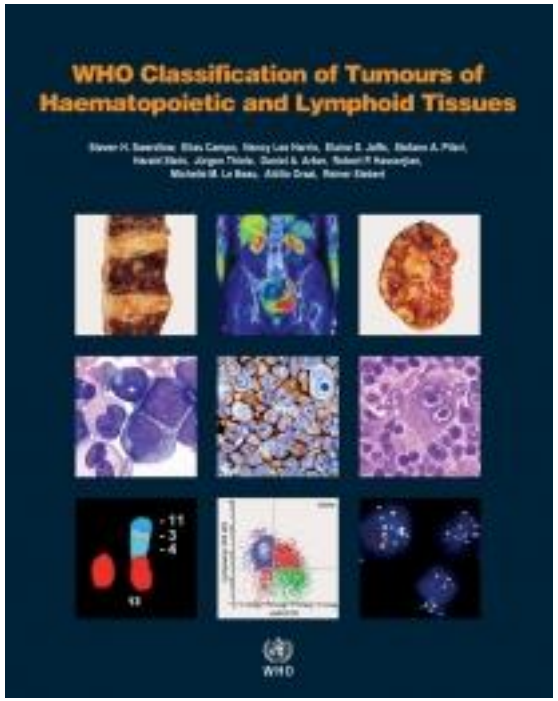
WHO HAEM 4

Aiming for a simplified approach

Exclusion of certain groups based on genetics or clinical data

Distinct populations of leukaemic cells: independently fulfilling the immunophenotypic criteria for AML and other (s) for T- and/or B-lymphoblastic leukaemia (ALL) & Sum of all blast populations ≥ 20% cells OR…



T cell lineage

- CD3. Other markers as CD2, CD5, CD7, CD4 & CD8 can be expressed in other leukemias

Myeloid lineage

- Identification of MPO or evidence of monocytic differentiation (two or more of CD11c, CD14, CD64, NSE or Lysozyme)

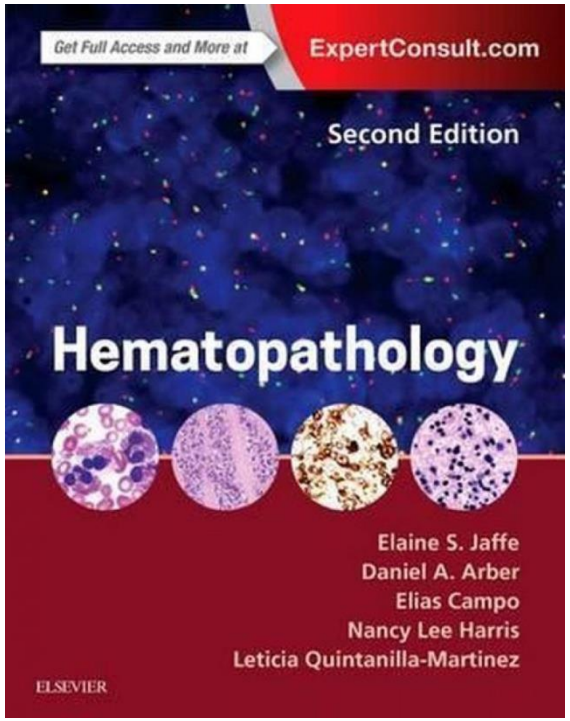
B cell lineage

- CD19 plus 1 (CD19 strong) or 2 (CD19dim) markers (cyCD79a, cyCD22 and/or CD10)

Immunohistochemical stains such as PAX5, although outside the formal definition, may provide helpful supplemental information

WHO HAEM 4

Tricks of the trade



T cell lineage

- cyCD3 with the brightest blasts reaching the level of background normal T cells, or surface CD3 (rare).
- This contrast with T-ALL in which cyCD3 can be dim.
- Careful with CD3-ζ chain (CD247) by IHQ (Also stains NK cells)

Myeloid lineage

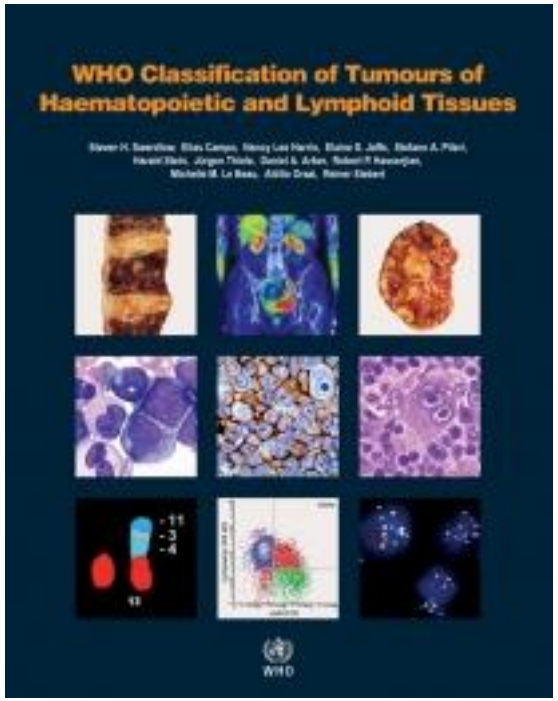
- MPO alone is problematic (MPO+ B-ALL, f.i.)
- Usually positive for non specific markers as CD117, bright CD13 and/or CD33
- What do we do with MPO- myeloid components?

B cell lineage

- CD19 can be seen in AML, so check for additional markers
- Extremelly rare cases lack CD19, but three markers inc PAX5

WHO HAEM 4

Tricks of the trade



Although not formally recognised, bilineal cases may be easier to classify as long all blast populations sum up to 20% and the strict requirements of antigen expression (e.g., MPO, bright cyCD3) do not apply, provided each population independently would meet criteria for acute lymphoid or acute myeloid leukemia.

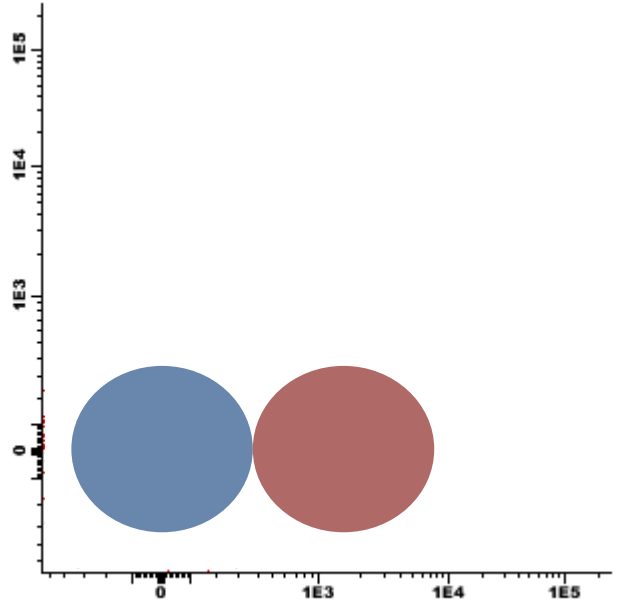
However there is no strict lower limit on how small a population has to be.

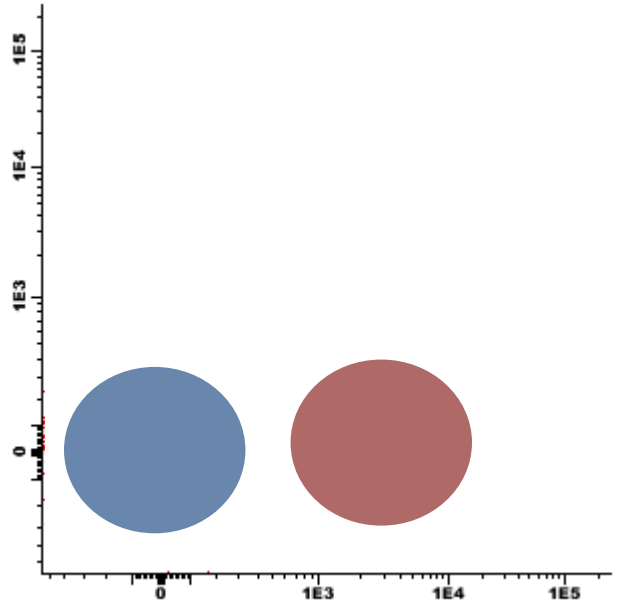
If fewer than 5%, make sure they are not reactive populations.

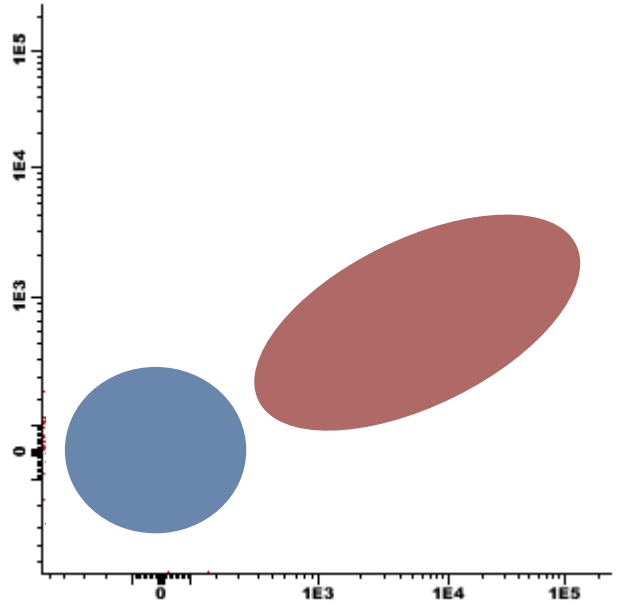
Principles for lineage assignment

The more similar the level and/or the pattern of expression is to normal maturation, the more likely it reflects lineage. Dim intensity may not be very specific

Coordinated expression of more than one antigen improves specificity for lineage, instead of one aberrant single antigen expression.

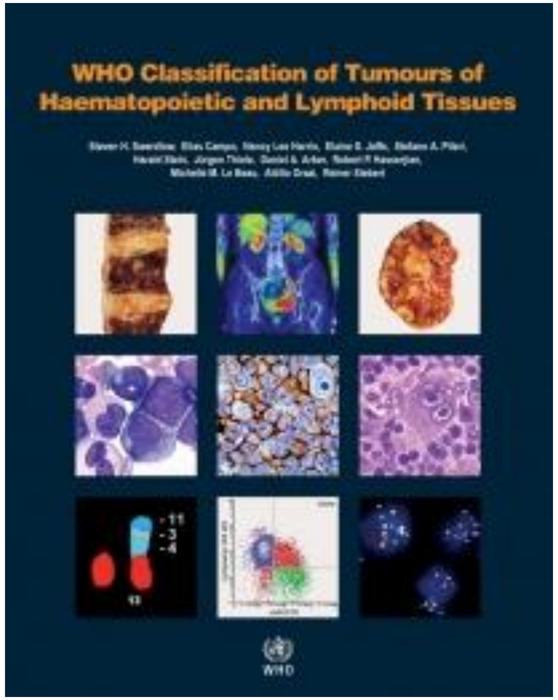


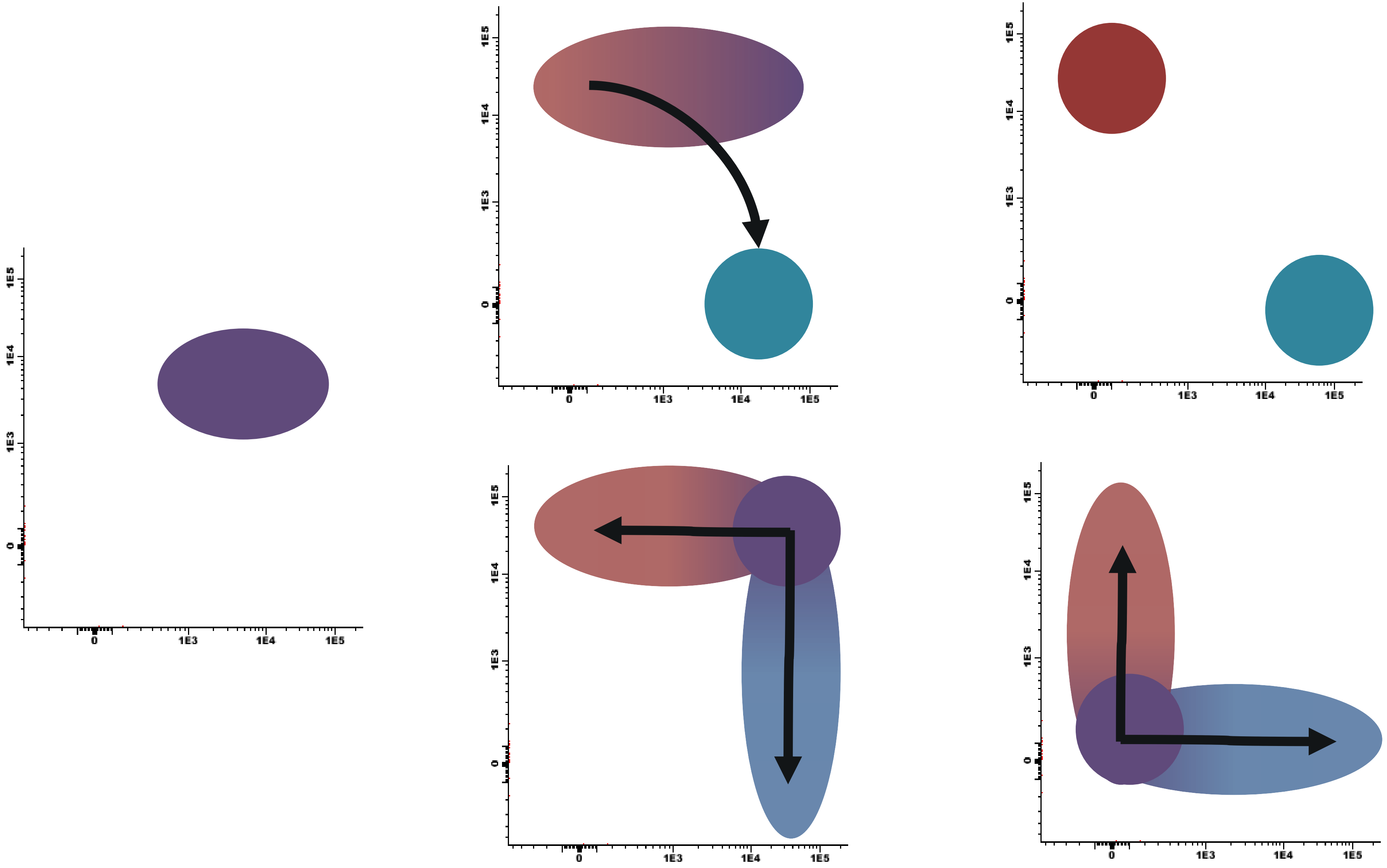




WHO HAEM 4

Tricks of the trade





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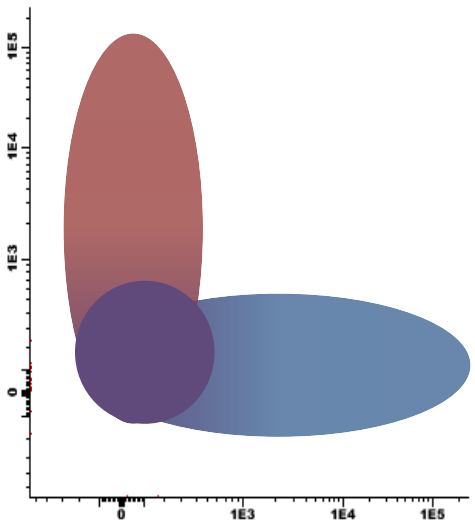
Tricks of the trade



However, the most recognizable feature of MPAL is that nearly all cases display a particular pattern of heterogeneity of antigen expression.

Caution should be exercised when making a diagnosis of MPAL if such heterogeneity cannot be demonstrated.

WHO HAEM 4

Many practical advantages, but still some caveats

A smaller panel can be used.

Criteria for lineage assignment is more strict.

Selected antigens for lineage assignment have had some posterior validation.

The relative weights of the antigens selected.

The intensity of the antigen somehow taken into account for CD3, for example, but it is redacted ambiguously for CD19 and MPO.

MPO cutoff for expression are variable (3% vs 10% vs others)

Matutes E, Pickl WF, Van't Veer M, et al. Mixed-phenotype acute leukemia: clinical and laboratory features and outcome in 100 patients defined according to the WHO 2008 classification. Blood. 2011;117:3163-3171.

Hrusak, Ondrej; Haas, Valerie de; Stancikova, Jitka; Vakrmanova, Barbora; Janotova, Iveta; Mejstrikova, Ester et al. (2018): International cooperative study identifies treatment strategy in childhood ambiguous lineage leukemia. En: Blood 132 (3), pág. 264-276. DOI: 10.1182/blood-2017-12-821363.

Myeloperoxidase

Standardizaton, please

Study	Tecnhique	Recommended cutoff
Manivannan et al,2015	Flow cytometry BD Biosciences, clone 5B8, Ms IgG1 (flow)-FITC conjugated	5.4%
Arber et al,2001	IHC 1:1000 dilution, Dako, Carpinteria, CA), polyclonal and monoclonal MPO antibody (clone MPO-7;1:100 dilution; Dako)	≥5%
Ahuja et al, 2017, studied patients with AML and their MPO reactivity	HC: anti-human MPO (Thermo Scientific, United Kingdom) Cytochemistry and flow cytometry: not mentioned	3% for IHC and cytochemistry and 10% flow cytometry
Oberley et al,2017	Flow cytometry: MPO (clone 8E6; Life Tech, Waltham, MA) IHC (clone 59A5; Leica Biosystems, Newcastle, United Kingdom) plus cytochemistry	>20% for Flow 3% for cytochemistry
Van den Ancker et al, 2013	Flow cytometry (clone used not reported)	>10%
Guy et al, 2013	Flow cytometry: monoclonal antibody (Dako or Immunotech), FITC	>13% (if using isotype control) >28% (if using internal control: lymphocytes)
Matutes et al, 2011	Flow cytometry: monoclonal MPO (clone used not reported)	>10%

Classification systems evolved over time

WHO HAEM3 (2001)	• Included in AML • EGIL as a base lineage assignment • AUL was poorly defined (rare lineages for instance) • Byphenotypic & bilineal ALAL
WHO HAEM4 (2008)	• AUL has to exclude rare lineages (pDC, Ba, NK, no Hematolymphoid f.i.) • MPAL with BCR::ABL and MPAL with MLL-r (11q23) • Exclusion of other entities (CBF AML, FGFR1 My-Ly neoplasms, CML-BP, MDS related AML and t-AML) • Remaining cases are defined by Flow
WHO HAEM4 revised (2016)	• MLL becomes KMT2A • More clarification in lineage assessment, but essentially the same as WHO HAEM4.

However, debate on how best classify certain cases still remains

ICC

Acute leukaemias of ambiguous lineage (ALAL) were not initially addressed, so a CAC subgroup was created afterwards.

There are some clarifications to WHO HAEM4R

- Contrary to other leukemias, classification based primarily on driver mutations does not explain the phenotypic diversity. However, in the future they might be better classified as single genetic entities with variable expression of differentiation-related markers.
- In cases with separate blast population, each population needs to be classified according to AML and ALL criteria
- In cases with small aberrant clones of divergent lineage are identified (>5%), a diagnosis of MPAL could be rendered if clear immunophenotypic aberrancies are identified.
- **However, with clones are less than 5% of all cells, a diagnosis should be based on the major leukemic population with a descriptive modifier**, for example, “Predominantly ALL with a small leukemic population of myeloid lineage detected of uncertain significance

Weinberg, Olga K.; Arber, Daniel A.; Döhner, Hartmut; Mullighan, Charles G.; Orgel, Etan; Porwit, Anna et al. (2023): The International Consensus Classification of Acute Leukemias of Ambiguous Lineage. Blood *26 (6), pág. 914-918.*

ICC

Acute leukaemias of ambiguous lineage (ALAL) were not initally addressed, so a CAC subgroup was created afterwards.

There are some clarifications to WHO HAEM4R

- *Acute leukemia of ambiguous lineage MPAL with defining genetic alterations*
  - MPAL with *BCR::ABL1*
  - MPAL, with t(v;11q23.3); *KMT2A rearranged*
  - MPAL with *ZNF384 rearrangement* *(50% B/My Pediatric cases)*
  - *MPAL with BCL11B activation* *(30% T/My MPAL. Similar gene expression profile as ETP with BCL11B activation and some AMLs with BCL11B activation)*
- MPAL with defining immunophenotypic changes
  - B/Myeloid MPAL
  - T/Myeloid MPAL
  - B/T/Myeloid MPAL
  - B/T MPAL
- Acute undifferentiated leukemia, AUL
- ALAL, NOS

Weinberg, Olga K.; Arber, Daniel A.; Döhner, Hartmut; Mullighan, Charles G.; Orgel, Etan; Porwit, Anna et al. (2023): The International Consensus Classification of Acute Leukemias of Ambiguous Lineage. Blood 26 (6), pág. 914–918.
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Di Giacomo D, K, et al. 14q32 rearrangements deregulating BCL11B mark a distinct subgroup of T-lymphoid and myeloid immature acute leukemia. Blood. 2021 Sep 2;138(9):773-784.

WHO HAEM5

Acute leukaemias of ambiguous lineage (ALAL) are leukaemias composed of >= 20% abnormal progenitors that do not show differentiation along a single lineage

There are some (not so) subtle changes

	Criterion
B lineage	Strong: intensity in part exceeds 50% of normal B cell progenitor
CD19 strong	1 or more also strongly expressed: CD10, CD22, or CD79a
CD19 weak	2 or more also strongly expressed: CD10, CD22, or CD79a
T lineage	
CD3 (cytoplasmic or surface)	Intensity in part exceeds 50% of mature T cells by flow cytometry OR Immunocytochemistry positive with non-zeta chain reagent
Myeloid lineage	
Myeloperoxidase (MPO)	Intensity in part exceeds 50% of mature neutrophil level OR
Monocytic differentiation	2 or more expressed: Non-specific esterase, CD11c, CD14, CD64 or lysozyme

WHO HAEM5

Sum of all blast populations ≥ 20% cells.

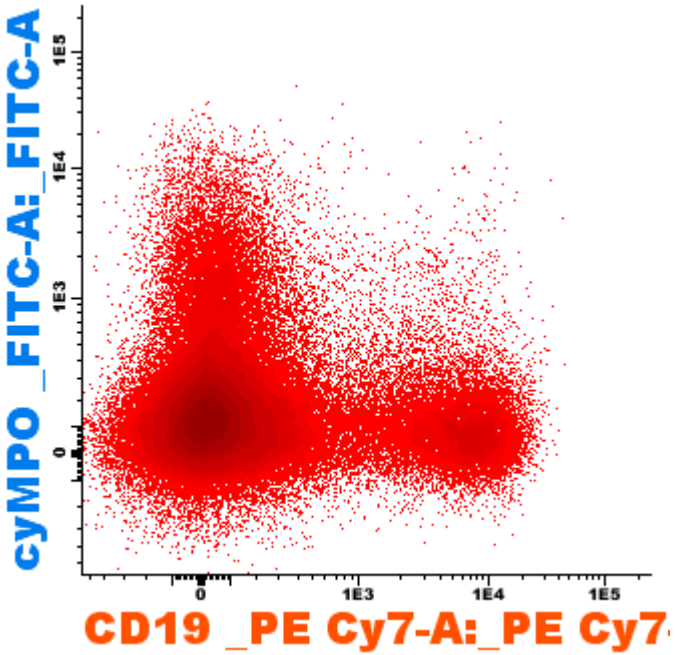
Check both leukemic components to avoid misinterpretation of reactivity

A role for IHQ, but be careful of misattribution of expression

The assignment of lineage by immunophenotyping is dependent on the strength of association between each antigen and the lineage being assessed.

Coordinated expression of multiple antigens more robust than a single antigen

”[…] a level of expression exceeding/that excedes 50%of normal […]”



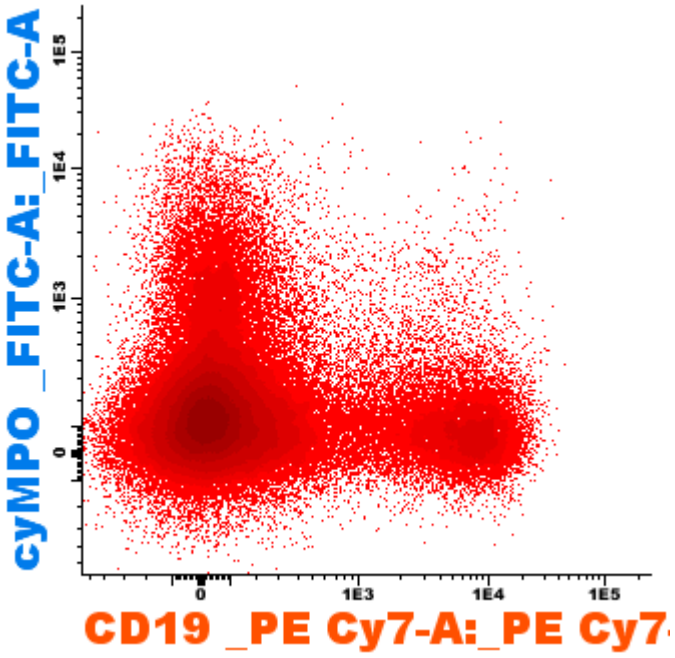
WHO HAEM5

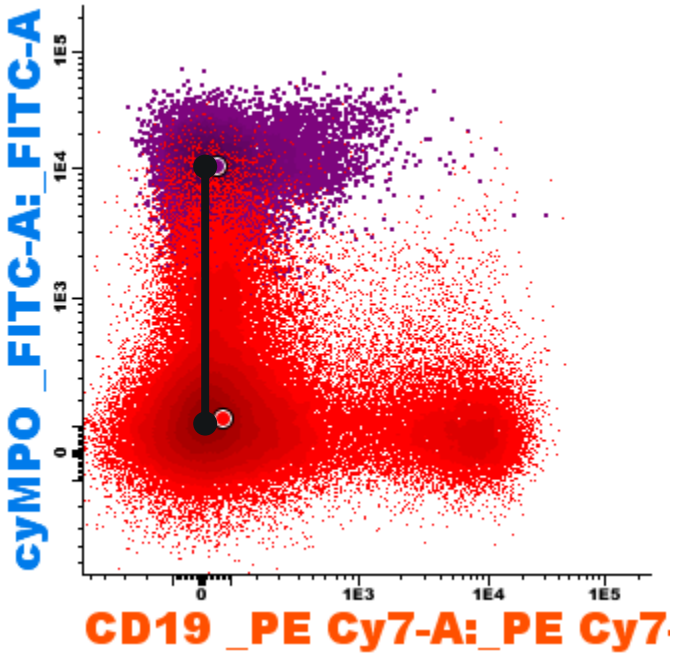
A level of expression exceeding 50% of the level seen in normal….

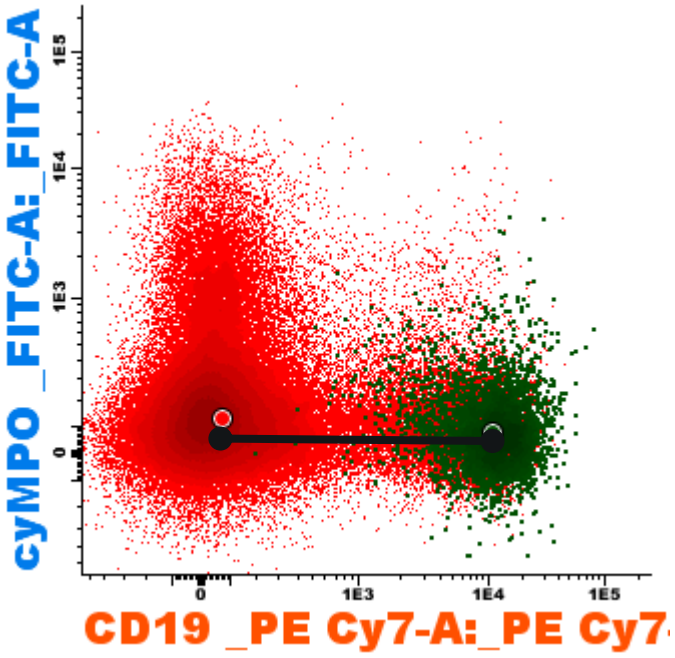
What the heck does this mean?! (MFI?, should it reach or surpass median expression in the reference population?,…)

NO, it´s just visual range

Gets rid of the “negative” cutoff…







52801

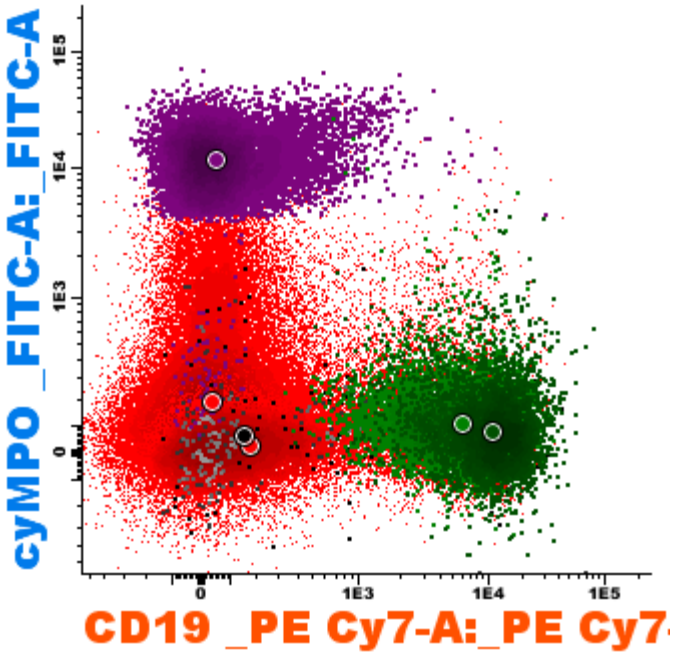
EuroFlow™ database guide analysis on the same case

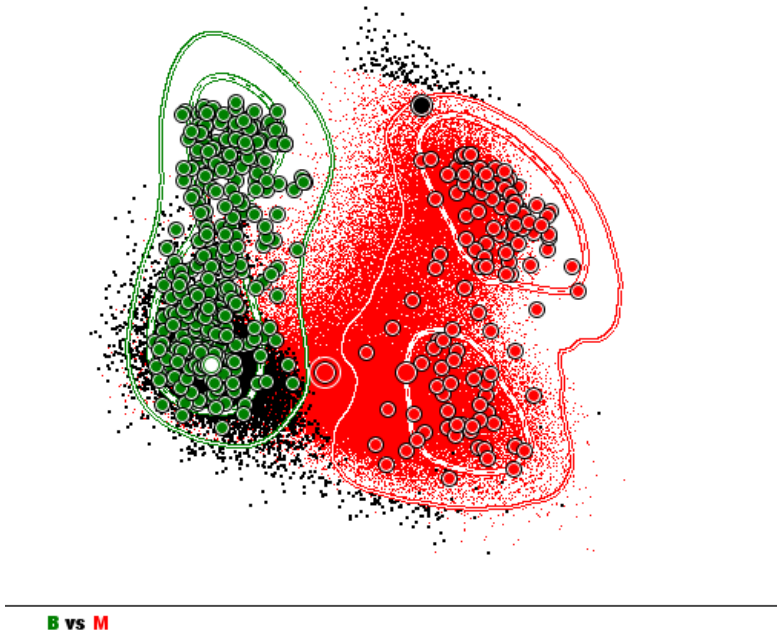
More robust

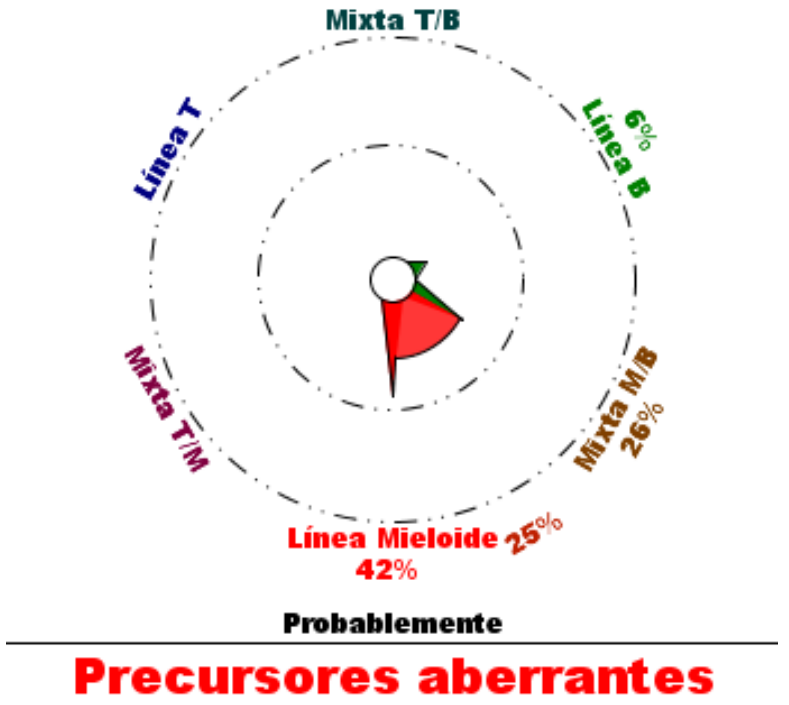
More evidence based

No limitation on “normal”/residual populations

QA for techniqual aspects







Lhermitte L, Barreau S, Morf D, Fernandez P, Grigore G, Barrena S, de Bie M, Flores-Montero J, Brüggemann M, Mejstrikova E, Nierkens S, Burgos L, Caetano J, Gaipa G, Buracchi C, da Costa ES, Sedek L, Szczepański T, Aanei CM, van der Sluijs-Gelling A, Delgado AH, Fluxa R, Lecrevisse Q, Pedreira CE, van Dongen JJM, Orfao A, van der Velden VHJ; EuroFlow Consortium. Automated identification of leukocyte subsets improves standardization of database-guided expert-supervised diagnostic orientation in acute leukemia: a EuroFlow study. Mod Pathol. 2021 Jan;34(1):59-69.

WHO HAEM5

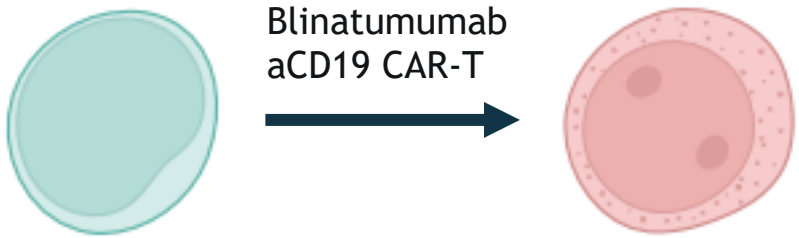
Genetic Abnormalities Associated with Lineage Infidelity.

Genetic Abnormality	Classification	Lineage switch
KMT2A	MPAL B/Myeloid	AML
BCR::ABL1	MPAL B/Myeloid	
ZNF384 fusions	B-LL/LBL , MPAL B/Myeloid	Monocytic*
DUX4 rearrangement	B-LL/LBL	Monocytic*
PAX5 p.P80R	B-LL/LBL	Monocytic*
BCL11B	AUL, MPAL T/Myeloid	
Biallelic WT1 alterations	MPAL T/Myeloid	
FLT3 alterations	MPAL T/Myeloid	
PHF6 mutation	AUL, MPAL T/Myeloid	
RUNX1 mutation	MPAL T/Myeloid	
LMO2 rearrangement non-TCR	MPAL T/Myeloid	
PICALM::MLLT10 rearrangement	MPAL T/Myeloid	
CBFA2T3::GLIS2 and CBFA2T3::GLIS3	MPAL T/Mk	
SET::NUP214	AUL	

* Denotes not associated with a higher incidence of relapse or poor prognosis

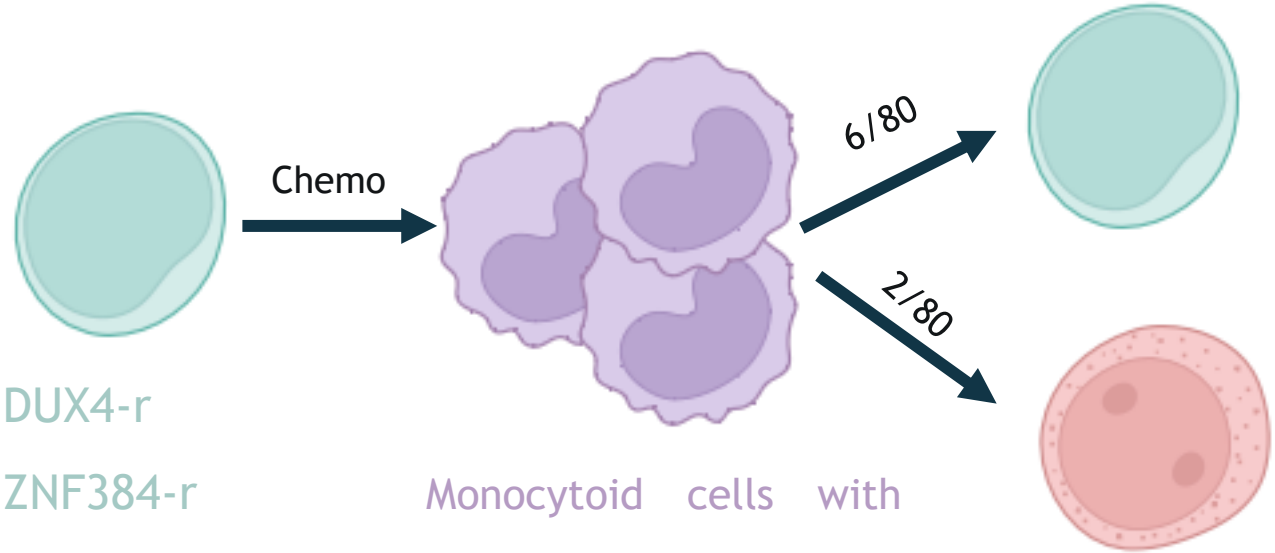
Lineage switch vs switch ALL

Myeloid switch after CD19-targeted therapy



- Case series
  - Rossi et al. Am J Hematology 2012
  - Lee et al Am J Hematology 2022
- 80% KMT2A-r
- Biphenotypic potential of CD19- leukemia stem cells?
- Selection of sublones?
- Need better MRD strategies

"Switch ALL" Novakova Haematologica 2021



- Monocytic switch may lead to uncertainty about the continuation of ALL-type therapy
- Monocytic switch not only creates discordance between MRD levels determined by FC and MRD levels determined by PCR but also affects the availability of CD19 as a therapeutic target.

AUL

AUL is **rare**, and it´s incidence is decreasing.

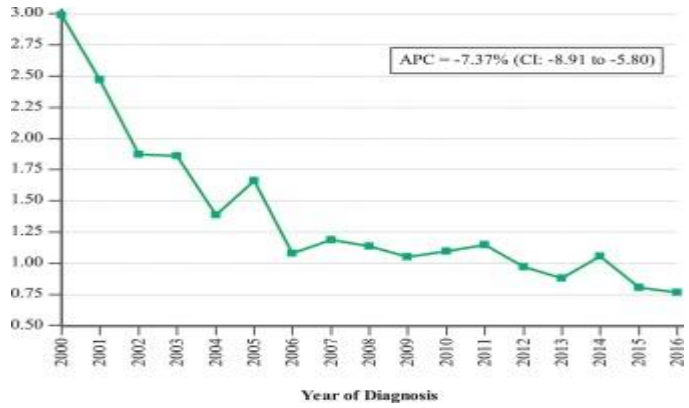
1888 cases from SEER registry from 2000 to 2016 (444 excluded due to previous tumor)

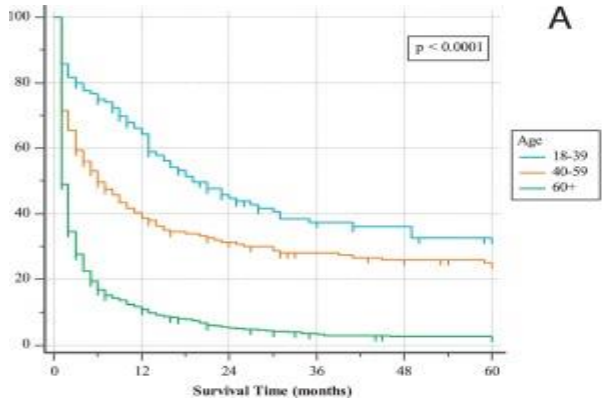
Different prognosis in children vs adults

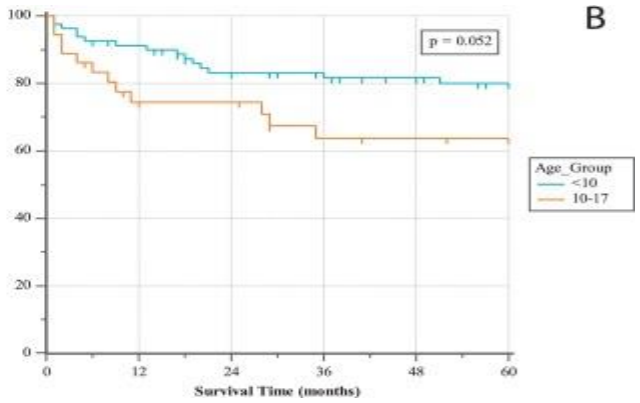
Compared to acute myeloid leukemia with minimal differentiation, acute undifferentiated leukemia cases were characterized by more frequent mutations in PHF6 (5/15) and more frequent expression of TdT on blasts.

PHF6 mutated acute undifferentiated leukemia cases did not show a higher frequency of aberrant lymphoid markers as compared to the remaining acute undifferentiated leukemia cases.

PHF6 mutations may be early events (part of ETP & T/My MPAL spectrum?)







Qasrawi, Ayman; Gomes, Victor; Chacko, Charles Andrew; Mansour, Akila; Kesler, Melissa; Arora, Ranjana et al. (2020): Acute undifferentiated leukemia: data on incidence and outcomes from a large population-based database. Leukemia research 89, pág. 106301.
Weinberg, Olga K.; Hasserjian, Robert P.; Baraban, Ezra; Ok, Chi Young; Geyer, Julia T.; Philip, John K. S. S. et al. (2019): Clinical, immunophenotypic, and genomic findings of acute undifferentiated leukemia and comparison to acute myeloid leukemia with minimal differentiation: a study from the bone marrow pathology group. Modern Pathology 32 (9), pág. 1373–1385.

AUL Therapy & prognosis

it is very difficult to predict — especially the future

However there are some important points for practice

- Treatment bias in published series.
- Usually, not elegible for trials.
- AML-type chemotherapy may be the standard in adults.
- AUL seems to do as well as AML with no differentiation.
  - Some reports indicated that AML M0 would do worse than other FAB subtypes.
- Acute myeloid leukemia with myelodysplasia-related changes patients (as defined by WHO-HAEM4) simulating AUL may have shorter survival when censoring for bone marrow transplant as compared to acute undifferentiated leukemia, but not when patients are transplanted.
- Conflicting data in PHF6 mutated cases.
- iBCL2 are promising

Weinberg, Olga K.; Hasserjian, Robert P.; Baraban, Ezra; Ok, Chi Young; Geyer, Julia T.; Philip, John K. S. S. et al. (2019): Clinical, immunophenotypic, and genomic findings of acute undifferentiated leukemia and comparison to acute myeloid leukemia with minimal differentiation: a study from the bone marrow pathology group. Modern Pathology 32 (9), pág. 1373–1385

Kurzer JH, Weinberg OK. PHF6 Mutations in Hematologic Malignancies. Front Oncol. 2021 Jul 26;11:704471. doi: 10.3389/fonc.2021.704471. PMID: 34381727; PMCID: PMC8350393.

ALAL Therapy & prognosis

In MPAL, it could be less complicate

Targeted therapy

- BCR::ABL1 – Imatinib
- KMT2A R – Menin inhibitors?
- FLT3- ITD or IDH1 or IDH2
- αCD19, αCD22,…
- Nelarabine? T/My MPAL

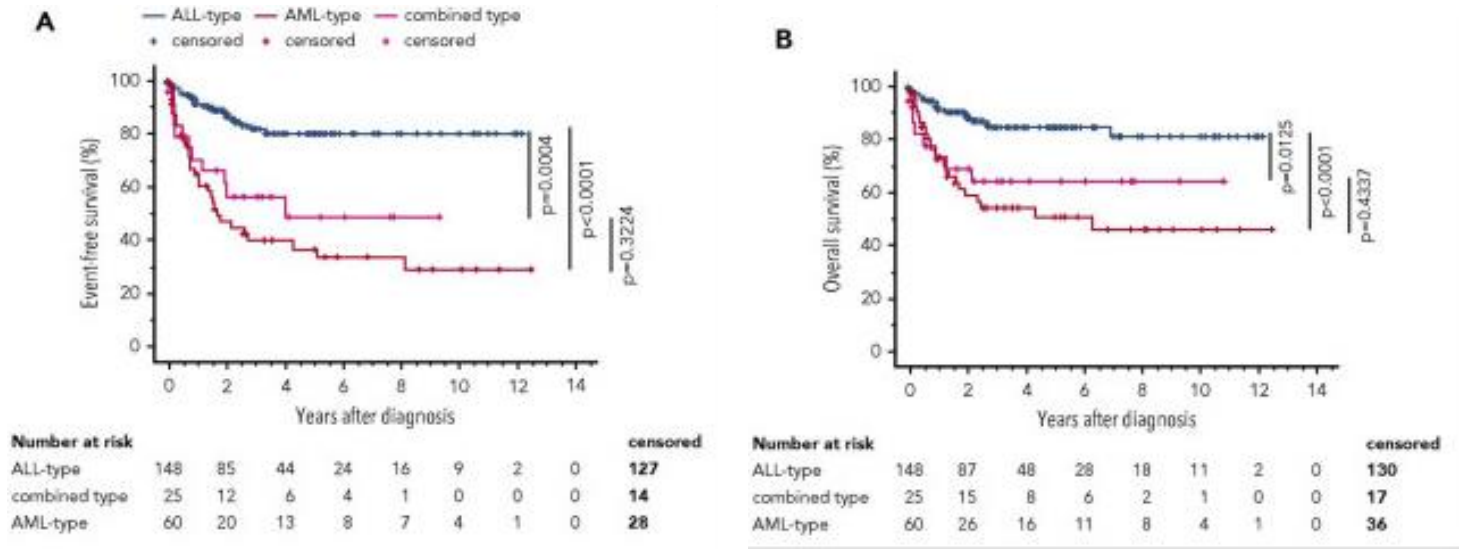
Maruffi M., Sposto R., Oberley M.J., Kysh L., Orgel E. Therapy for children and adults with mixed phenotype acute leukemia: A systematic review and meta-analysis. *Leukemia.* 2018;32:1515–1528

ALAL Therapy & prognosis

In MPAL, it could be less complicate

However there are some important points for practice

- Genetics as the major driver for therapy
- Transplant can be omitted in children providing they achieve MRD negativity



Maruffi M, Sposto R, Oberley MJ, Kysh L, Orgel E. Therapy for children and adults with mixed phenotype acute leukemia: a systematic review and meta-analysis. Leukemia. 2018 Jul;32(7):1515-1528. doi: 10.1038/s41375-018-0058-4. Epub 2018 Feb 27.

Lazzarotto D, Tanasi I, Vitale A, Piccini M, Dargenio M, Giglio F, Forghieri F, et al. Multicenter retrospective analysis of clinical outcome of adult patients with mixed-phenotype acute leukemia treated with acute myeloid leukemia-like or acute lymphoblastic leukemia-like chemotherapy and impact of allogeneic stem cell transplantation: a Campus ALL study. Ann Hematol. 2023 May;102(5):1099-1109. doi: 10.1007/s00277-023-05162-0

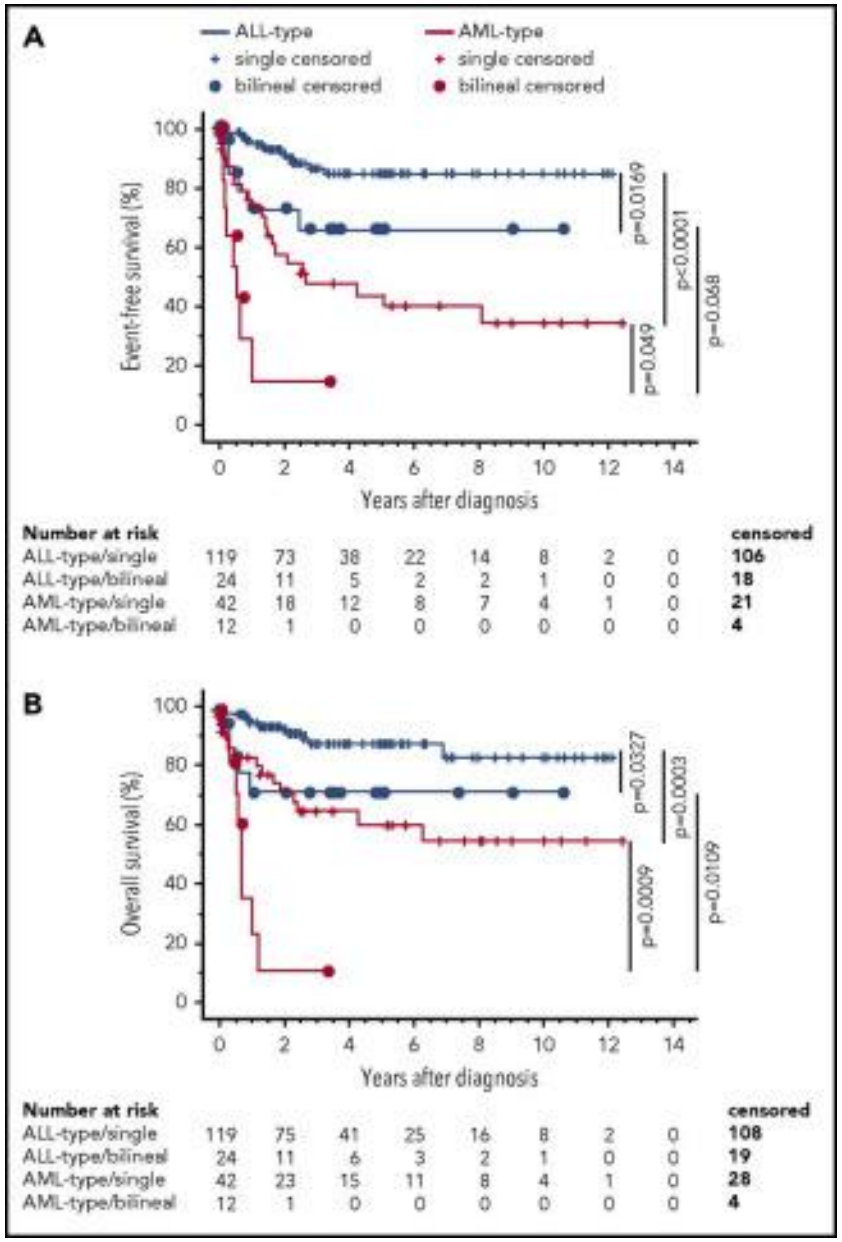
Oberley MJ, Raikar SS, Wertheim GB, et al. Significance of minimal residual disease in pediatric mixed phenotype acute leukemia: a multicenter cohort study. Leukemia 2020;34(7):1741–50.

Hrusak, Ondrej; Haas, Valerie de; Stancikova, Jitka; Vakrmanova, Barbora; Janotova, Iveta; Mejstrikova, Ester et al. (2018): International cooperative study identifies treatment strategy in childhood ambiguous lineage leukemia. En: Blood 132 (3), pág. 264–276. DOI: 10.1182/blood-2017-12-821363.

Orgel, Etan; Alexander, Thomas B.; Wood, Brent L.; Kahwash, Samir B.; Devidas, Meenakshi; Dai, Yunfeng et al. (2020): Mixed-phenotype acute leukemia: A cohort and consensus research strategy from the Children's Oncology Group Acute Leukemia of Ambiguous Lineage Task Force. Cancer 126 (3), pág. 593–601..

ALAL Therapy & prognosis

In MPAL, it could be less complicate



Children with MPAL

- Bilineal vs biphenotypic may also have an impact, but not in multivariate analysis
- Changing from AML type therapy to ALL therapy did not improve prognosis
- CD19+ MPAL cases on AML-type therapy had a dismal prognosis… (13/21 died of leukemia, 1/21 was due to toxicity and 1/21 of unknown cause) even if CD19+ clones were small or had many “myeloid markers”

- Blinatumumab and CART therapy reported in refractory/relapsed cases.

Hrusak, Ondrej; Haas, Valerie de; Stancikova, Jitka; Vakrmanova, Barbora; Janotova, Iveta; Mejstrikova, Ester et al. (2018): International cooperative study identifies treatment strategy in childhood ambiguous lineage leukemia. En: Blood 132 (3), pág. 264–276. DOI: 10.1182/blood-2017-12-821363.

ALAL Therapy & prognosis

In MPAL, it could be less complicate

Adults with MPAL

- Worse outcomes compared to children
- Allogeneic transplantation whenever possible
- Strict MRD follow up
- Doubt about best regime (AL-chemo may be better than AML or “hybrid therapy”)
  - Retrospective in Italy 77 patients AML-like induction yields poorer result than an ALL-like induction (proportion of refractory disease 35.7% vs 11.6%, P = 0.029) and most patients in AML-chemo had fludarabine.
  - No prognostic factors other than age which reflects transplantation… but retrospective & 35% patients with CK
- In PETHEMA, FLAG-Ida, although very low evidence.
- FLAG or DNMT1 inhibitors plus venetoclax show promising activity

Maruffi M., Sposto R., Oberley M.J., Kysh L., Orgel E. Therapy for children and adults with mixed phenotype acute leukemia: A systematic review and meta-analysis. *Leukemia*. 2018;32:1515–1528

Lazzarotto D, Tanasi I, Vitale A, Piccini M, Dargenio M, Giglio F, Forghieri F, Fracchiolla N, Cerrano M, Todisco E, Papayannidis C, Leoncin M, Defina M, Guolo F, Pasciolla C, Delia M, Chiusolo P, Mulè A, Candoni A, Bonifacio M, Pizzolo G, Foà R. Multicenter retrospective analysis of clinical outcome of adult patients with mixed-phenotype acute leukemia treated with acute myeloid leukemia-like or acute lymphoblastic leukemia-like chemotherapy and impact of allogeneic stem cell transplantation: a Campus ALL study. Ann Hematol. 2023 May;102(5):1099-1109. doi: 10.1007/s00277-023-05162-0. Epub 2023 Mar 24. PMID: 36959485.

ALAL: Working definition

This has not changed much in the last decades

Acute leukemias of ambiguous lineage (ALALs) include biologically diverse leukemias that fail to show commitment to either the myeloid, B-, or T-lymphoid lineages (AUL) or show evidence of commitment to more than 1 lineage (MPAL).


Do you guys ever think about…
the role of cytogenetics or molecular biology in ALAL diagnosis?
the role of MPO in defining lineage?
the best approach AML or ALL cases with small aberrant clones?
how to choose therapy based in lab results?
how to accuratelly identify small populations suspicious of bilineal blasts?
my suboptimal MRD studies?
the many markers I need to call a case AUL?