Report of the First National Consensus Meeting for Acute Leukemias Immunophenotyping

Lourdes Arriaga-Pizano,¹ Dalia Ramírez-Ramírez,² Jessica Prieto-Chávez,¹ Rosana Pelayo,² Alejandro Ruiz-Argüelles,³ Guillermo José Ruiz-Delgado⁴ and Rafael Antonio Marín-López⁵

¹Instituto Mexicano del Seguro Social, Centro Médico Nacional Siglo XXI, Specialty Hospital, Ciudad de México; ²Instituto Mexicano del Seguro Social, Centro de Investigación Biomédica de Oriente, Onco-immunology Hospital, Puebla; ³Laboratorios Clínicos de Puebla, Departamento de Inmunología, Puebla; ⁴Director Médico Centro de Hematología y Medicina Interna, Puebla; ⁵Director Académico de Laboratorios Ruiz/ Médica Sur, Puebla, Puebla. México.

Abstract

Recommendations for the typing of hematological malignancies in Latin America were published in 2005. Carrying out a national meeting to update them was deemed necessary. Ninety-five professional experts on the subject were invited in order to analyze and contrast alternatives and reach a consensus. Consensus opinions were reached regarding indications, sample types and processing, antibodies, nomenclature and reporting of results for the diagnosis and monitoring of acute leukemias. This paper describes the recommendations and emphasizes on the need for national laboratories to adhere to them.

KEY WORDS: Leukemia. Immunophenotype. Consensus.

Introduction

Oncological diseases have been defined as a global health priority, especially in low and middle-income countries, where the demographic factor can substantially limit their long-term control.

Acute leukemias are the most common childhood malignancies in the world and continue to be among the main causes of mortality in pediatric and adult patients with relapses. The highest incidence of cases with unfavorable evolution is documented in the Hispanic population.^{1,2} Although survival rates have increased in recent years due to the development of efficient treatments, late diagnosis, resistance to chemotherapy, extramedullary infiltration, residual disease and relapses often significantly worsen the disease outcome, which underscores the need for new diagnostic strategies.³

Uncontrolled oligoclonal production of hematopoietic precursor cells of the lymphoid or myeloid series in the bone marrow at the expense of their normal differentiation is the main characteristic of acute leukemias.⁴ Among them, acute lymphoblastic leukemia

(ALL) accounts for 23 % of malignant tumors and 85 % of leukemia cases in the pediatric population, while acute myeloid leukemia accounts for 15 %.^{1,5,6}

According to lineage and maturation stage international classifications based on the number and degree of specificity of antigens expressed by leukemic cells,⁴ 80 to 85 % of cases have B-cell immunophenotype, whereas nearly 10 to 15 % show T-cell immunophenotype⁷. Congenital leukemia, an entity that is different from typical ALL, accounts for only 3 % of acute leukemia cases, while mixed lineage leukemias, with two-lineage cell properties, account for approximately 2 %.

The largest contribution to disease-free survival is provided by an adequate treatment of the disease, based on patient stratification by risk groups and relapse factors identification.^{8,9} Currently, the most useful prognostic indicators are the immunophenotype, residual disease detection and the response to induction therapy. Leukemic cells immunophenotype is one of the factors that establish the risk of relapse, which occurs in 20 % to 30 % of patients, particularly in T- and mixed lineage leukemias.¹⁰⁻¹²

Correspondence:

Alejandro Ruiz-Argüelles
E-mail: aruiz@clinicaruiz.com

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Both their origin and progression result from multiple factors, including genetic, micro-environmental, macro-environmental and immune factors. The hierarchical theory of cancer development maintains that stem cells or cancer initiators are responsible for the onset and maintenance of tumors and possibly for ultimate invasion and development of metastatic foci.^{13,14} Despite the biological, molecular and clinical heterogeneity of leukemia, the occurrence of alterations is clear since the beginning of the hematopoietic differentiation program. However, while for acute and chronic myeloid leukemia a population of leukemic stem cells is responsible for their development and recurrence,^{15,16} for lymphoblastic leukemias, their undifferentiated nature appears not to be phenotypic and may be related to the tumor microenvironment,^{17,18} which contributes to the disease pathogenesis.

Malignant blood diseases immunophenotyping has been shown to be useful in the study of acute leukemia; however, given the large variety of reagents, instruments and analysis platforms, regulating various criteria concerning the indication for carrying out the testing is necessary, as well as for selecting the reagents and methods for the processing and analysis of samples and interpretation of results. Hence, working groups or consensus conferences have been organized, which allow the comparability and compatibility of the information generated by different laboratories or institutions where these investigations are carried out. Two Latin American Consensus Conferences have been organized for the Immunological Typing of Malignant Blood Diseases,^{19,20} and although the recommendations of the second one can still be considered pertinent, their updating is highly desirable, in accordance with the emergence of different entities and the progress in precision strategies and technologies.

On March 23, 2018, the First National Consensus Meeting for Acute Leukemias Immunophenotyping was carried out, convened by the Mexican Society of Immunology and endorsed by the National Academy of Medicine of Mexico. Several immunophenotyping aspects of these diseases were reviewed and discussed, with the recommendations contained in this document emanating therefrom.

Participation

Ninety-five health professionals participated, including laboratory technicians, clinical specialists and researchers, all experts in leukemia immunophenotyping

by flow cytometry. The participating federal entities were Baja California, Mexico City, Colima, State of Mexico, Guerrero, Jalisco, Michoacán, Morelos, Nuevo León, Oaxaca, Puebla and Yucatán. Doctors Arriaga Pizano, Pelayo and Ruiz-Argüelles were in charge of the consensus chair.

Agenda

The discussion included aspects on medical indications, sample preparation, as well as antigen relevance and selection (identified by the corresponding monoclonal antibodies) for B-cell precursor ALL, T-cell precursor ALL, acute myeloid leukemia and measurable residual disease. In the present consensus, changing the term “minimal” to “measurable” for residual disease was decided, with the purpose to denote that the analytical system sensitivity is the limiting factor for quantifying blasts in the samples. Although hematological disorders include, in addition to acute leukemias, chronic lymphoproliferative disorders, monoclonal gammopathies and myelodysplastic syndromes, these entities were not discussed during this meeting; they will be sequentially addressed in future meetings.

Medical indications

During the consensus, it was agreed that acute leukemia immunophenotyping is useful for diagnosis, classification, prognosis and follow-up. The importance of communication between pathologists, oncologists, hematologists and flow cytometry professionals to determine an accurate interpretation of data that drives to a reliable diagnosis was highlighted.

Sample preparation

Ideally, samples should be processed within 48 hours after having been obtained, and preferably within the first 24. However, taking into account that meeting this requirement sometimes is not possible for various reasons, processing all received samples for cytometry analysis was strongly recommended, accepting that the only valid reason to reject them and request a second sample is insufficient information obtained from the assay; i.e., when the loss of sample quality due to prolonged storage interferes with the certainty of the test interpretation.

Erythrocyte lysis and sample fixation should be carried out after incubation with fluorescent antibodies.

Table 1. Processing of samples for immunophenotyping by flow cytometry

Sample	Time ^a	Washing	Stabilization ^e	Density gradient enrichment ^g	Anticoagulant ^h	Transportation in SS/PBS ⁱ
Peripheral blood	Up to 24 hours ^{b,c,d}	Yes	No	No	EDTA or heparin	No
Bone marrow	Up to 24 hours	Yes	No	No ^k	EDTA or heparin	No
Umbilical cord blood	Up to 24 hours	No	No	No	ACD-A	No
Leukapheresis products	Up to 24 hours	No	No	No	ACD-A	No
Solid tissue biopsy	60 minutes	Yes	Yes ^f	No	No	Yes
Solid tissue puncture	60 minutes	Yes	Yes	No	No	Yes
Cerebrospinal fluid	30 minutes	Yes	Yes	Yes	EDTA ^l	No

^aMaximum time between sample collection and processing, including transportation.^bProcessing as soon as possible, maintaining the sample at stable room temperature (16 to 25 °C) and with anticoagulant up to 24 hours.^cAfter taking the sample and at the moment of starting the processing, verify cell viability to confirm sample stability; the viability fluorochrome will be used for the analysis algorithm rather than as a restrictive aspect for accepting the sample. No recommendation was made regarding the minimum viability percentage of the sample to be processed, but the information shall be included in the final report.^dUp to 48 hours' time if the sample is to be transported to the processing laboratory and the temperature remains stable (16 to 25 °C); after this time, keeping sample refrigerated (4 °C) is recommended.^eStabilization is required after collecting the sample and is effective for 24 to 48 hours.^fAfter the recommended time, the samples should be stabilized.^gCell enrichment is recommended for samples with low cellularity (less than 1000 cells/ μ L) or in cerebrospinal fluid samples.^hThe recommendation is to use only one anticoagulant and never mix them.ⁱUse EDTA only if the sample is contaminated with blood from sample collection. Using a cerebrospinal fluid stabilizer is recommended (if the sample is not going to be processed in less than 30 minutes).^jSaline solution (SS) and phosphate buffered saline (PBS). The RPMI medium can be useful.^kExcept in cases where concentrating the sample in order to quantify rare events is required.

Cell separation or enrichment by density gradient should be limited to cases where the samples are lysis-resistant or whose contamination with necrotic, stromal or fat cells may interfere with the interpretation of results. Osmotic hemolysis and washing prior to incubation with antibodies (bulk lysis) or enrichment by density gradients can also be used when looking for rare events, as in measurable residual disease.²¹

Of special interest was to define the optimal time for the processing of the sample in order to preserve cell viability, which was up to 48 hours after the moment of collection. If the sample has to be transported from the place of sampling to the processing laboratory, it must be kept at stable temperature between 16 and 25 °C. Washing, cell stabilization, centrifugation, anticoagulation and transportation were also discussed. The general recommendations are shown in Table 1.

Monoclonal antibodies for acute leukemia

In the consensus, it was concluded that acute leukemia immunophenotyping through panels of essential antibodies should be able to adequately identify ALL precursor B-cells, ALL precursor T-cells, acute myeloid leukemia and measurable residual disease.

The use of multi-parametric panels that combine at least three monoclonal antibodies conjugated to

different fluorochromes was strongly recommended. This strategy should allow the identification of the lineage, stages of maturation or differentiation and acute leukemia sub-classification.

It was emphasized that sample acquisition and data analysis should be carried out immediately after the incubation process with fluorescent antibodies, thereby avoiding cell viability loss, antibody internalization and fluorochrome luminous intensity decrease.

In the cases of B-cell precursor ALL, the use of anti-CD19 and anti-CD79a cytoplasmic antibodies was corroborated as the most potent approach to identify the lineage of neoplastic cells. To determine the degree of differentiation, anti-CD34, CD45, CD20 and CD38 antibodies were included, whereas to sub-classify the variant, investigating CD10, surface immunoglobulin and cytoplasmic μ chains was recommended. Alternatively, anti-CD13 and anti-CD33 antibodies were mentioned for assessing the expression of aberrant markers in leukemic blasts of lymphoid origin, as well as the anti-TdT antibody, which provides additional information on the degree of maturity of leukemic cells (Table 2).

Regarding T-cell lymphoblastic leukemia, it was agreed that the demonstration of the CD3 (cytoplasmic and surface) and CD7 antigens was necessary to define the lineage, while CD34, CD45, CD1d, TdT and CD99 are necessary to establish the degree of

Table 2. Antibodies for acute leukemia immunophenotyping

Indication	Lineage ^a	Differentiation/Maturation	Sub-classification	Optional
B-cell precursor ALL	CD19 ^b	CD34	CD10	TdT
	CD79 ^a (cytoplasmic) ^b	CD45 ^c	Surface Ig	CD13
		CD20 ^d	Cytoplasmic μ chains	CD33
		CD38 ^d		
T-cell precursor ALL	CD3 (cytoplasmic and surface) ^e	CD34	—	CD2
	CD7 ^f	CD45 ^c	—	CD4
		TdT	—	CD5
		CD1a ^g	—	CD8
		CD99 ^h	—	CD13 ⁱ CD33 ⁱ
Acute myeloid leukemia	MPO (cytoplasmic)	HLA-DR ^j	CD64 ^k	CD36 ^o
	CD13	CD34	CD15	CD14
	CD117 ^a	CD45	CD11 ^b	CD123
			CD300 ^l	CD61
			CD105 ^l	
			CD56 ^m	
			CD71 ⁿ	

^aWhen possible, using the combination of antibodies against CD19, CD79a, CD3 (cytoplasmic) and myeloperoxidase (cytoplasmic) is recommended in order to identify myeloid lineage, T or B cells in the same stain.

^bTo define the B-cell lineage in ALL, both CD19 and CD79a should be present in precursor B-cells.

^cUseful for population selection (analysis algorithm) and as a marker when the degree of infiltration is low.

^dInclusion of CD20 and CD38 can aid in the screening for common genetic abnormalities in ALL precursor B-cells.

^eSurface CD3 was added to distinguish early T-cell leukemia (cytoplasmic CD3+ surface CD3- CD2+ CD7- CD5-) from acute T-cell leukemia (cytoplasmic CD3+ surface CD3+ CD2+ CD7+ CD5+). To discriminate between cytoplasmic and surface CD3, performing stains in separate tubes or with different fluorochromes for each one is necessary, starting with surface staining with subsequent fixation to then proceed with intracellular staining.

^fUseful for measurable residual disease evaluation.

^gCD1a was added, since patients with this phenotype are referred for transplantation.

^hCD99 was added because most leukemias with absence of CD34 occur with CD99+.

ⁱUseful for assessing the expression of aberrant markers in leukemic blasts of lymphoid origin.

^jUseful for promyelocytic leukemia screening.

^kBest marker for cells with monocytic differentiation.

^lCD105 and CD300 are newly recognized markers for erythroid and monocytic differentiation, respectively.

^mMarker associated with poor-prognosis leukemia.

ⁿUseful marker to define erythroid differentiation.

^oUseful for identifying erythroid and megakaryocytic cell lineages or dendritic cells.

differentiation (Table 2). Alternatively, anti CD2, CD4, CD5 and CD8 antibodies were indicated, as well as those against CD13 and CD33 to demonstrate aberrations.

In acute myeloid leukemia, it was agreed that the antigens that define the lineage are cytoplasmic myeloperoxidase and CD13 and CD117 antigens. Maturation of this lineage is based on the investigation of the HLA-DR, CD34 and CD45 antigens, while sub-classification requires investigating the CD11b, CD15, CD56, CD64, CD71 and CD300 antibodies. As optional antibodies to complement sub-classification of these cases, those against CD14, CD36, CD61 and CD123 were indicated (Table 2).

It was confirmed that cases meeting the staining criteria in two or more cell lineages should be defined as mixed leukemias. The combination of anti-CD79a, CD3 and MPO antibodies was highly recommended as a basic panel to identify this group of leukemias.

New entities of clinical interest

Recently, the existence of an early T-cell precursor (ETP) acute leukemia variant has been recognized. This variant, with phenotypic characteristics different to those of T-cell lymphoblastic leukemia, appears to be of a more unfavorable prognosis and it is speculated that it may be the result of a very early arrest in

Table 3. New entities of clinical interest in acute leukemia

ETP-ALL	Present	Absent	Optional
	CD2 ^a	CD1	CD5
	Surface CD3 ^a	CD8	CD11 ^b
	CD4 ^a		CD44
	CD10 ^a		CD65
	CD13		CD117
	CD33		
	CD34		
	CD56 ^b		
	HLA-DR		
Ph-like ALL	Present	Absent	Optional
	CD19	CD4	CRLF2 ^c
	CD34		CD43
	CD10		CD20
	CD45		

ALL = acute lymphoblastic leukemia, ETP-ALL = early T-cell precursor acute lymphoblastic leukemia, Ph-like ALL = acute lymphoblastic leukemia with the Philadelphia chromosome.

^aMarkers significantly less expressed in ETP-ALL than in typical T-cell ALL.

^bMarkers significantly more expressed in ETP-ALL than in typical T-cell ALL.

^cMarker expressed in at least 50% of patients.

T-lineage cells differentiation the at the stage of poorly compromised hematopoietic precursors or still exhibiting myeloid potential.²²

Another B-cell ALL variant of high risk and poor prognosis is the so-called “Philadelphia chromosome-like acute lymphoblastic leukemia”, which occurs in one fifth of adults, mainly of Hispanic origin and whose timely identification can motivate therapeutic decisions.²³ In order to initiate phenotype and prevalence recognition of these two variants in our region, using the antibodies listed in Table 3 is suggested.

Interpretation

For acute leukemia, interpreting as such the cases that meet the lineage criteria when bone marrow blasts account for more than 20 % of nucleated cells in the sample was agreed. The recommendation to always use the phrase “...phenotype consistent with...” was reiterated.

In the case of B-lymphoid precursor leukemia, the recommendation is to sub-classify into pro-B (B-I), common (B-II), pre-B (B-III) and mature B (B-IV), for their possible implications in prognosis and therapeutic approaches. B-I cells only express CD19 and CD79a, B-II cells also express the CD10 antigen; B-III cells express

cytoplasmic m chains (usually together with CD20 and after losing CD10) and mature B cells exhibit immunoglobulins on the surface and the CD20 antigen.

T-cell acute leukemias, except for the ETP-ALL variant, do not need to be sub-classified.

In myeloid leukemia, it was agreed that if the case meets the criteria of more than 20 % of blasts with two markers of myeloid lineage, then it is consistent with acute myeloid leukemia; if in addition it has HLA-DR, CD64, CD11b, CD14 or CD300 expression, it is consistent with monocytic differentiation; if it expresses CD71 or CD36 and CD105 or CD36 and CD123, it is consistent with erythroid differentiation; if it expresses CD36 and CD61, it is consistent with megakaryocytic differentiation and, finally, in the absence or slight expression of CD14, HLA-DR, CD15, CD34, CD117, the phenotype can be interpreted as suggestive of promyelocytic leukemia.

Cases unequivocally denoting co-expression of two markers of each one of the involved lineages shall be interpreted as of mixed lineage.

Acute leukemia immune phenotyping results report

The result of this study must contain as a minimum necessary and sufficient the following sections:

1. Patient demographics: full name, age, gender and origin.
2. Date and time of sample taking.
3. Type of sample and analytical quality thereof.
4. Antibodies and number of fluorescent parameters used in the assay.
5. Description of findings. As a narrative, describe neoplastic cells characteristics and phenotype, as well as the proportion of nucleated cells (CD45+).
6. Interpretation. Following the aforementioned indications and using the following terms:
 - a. Precursor B-cell (I to IV) acute leukemia.
 - b. Precursor T-cell acute leukemia.
 - c. Acute myeloid leukemia....
 - i. ...with monocytic differentiation
 - ii. ...with erythroid differentiation
 - iii. ...with megakaryocytic differentiation
 - d. Promyelocytic leukemia.
7. Suggestions. Related to additional diagnostic tests, never to therapeutic procedures.
8. Histograms representative of the findings. These will be highly useful if assessment for measurable residual disease is carried out in another laboratory.
9. Comments or observations.

Measurable residual disease

It is the neoplastic population remaining after remission induction treatment in negative hematological neoplasms at the cytomorphological level. Early response to induction therapy is one of the most important prognostic factors in patients affected with acute leukemia. Currently, cytological remission is achieved in a large proportion of these patients; however, an important percentage will subsequently relapse. It is necessary to use techniques that are more sensitive for the measurement of residual leukemia and a new concept of complete remission has to be established.

Consensus recommendations

- a) Given the importance of detecting low numbers of neoplastic cells amid abundant normal cells, the viability of the sample is crucial. The assay should be processed as soon as possible after the sample is collected, taking care of sample temperature stability (16 to 25 °C) during its transportation.
- b) Perform the study in bone marrow aspirates or peripheral blood.
- c) Concentrate or enrich the nucleated cell component of the sample.
- d) If possible, when bone marrow is employed, use the first sampled tube for immunophenotyping.
- e) It is highly recommendable for the phenotype of the patient's initial diagnosis and possibly images of representative cytograms to be available.
- f) To the extent possible, use the same approach (combinations of antibodies and fluorochromes, selection or gating strategies, gains, etc.) by means of which the neoplastic cells were best discriminated at diagnosis.
- g) Have a minimum number of 500,000 nucleated cells (CD45+) to establish the frequency of neoplastic cells in the sample.
- h) Report the measurable residual disease (MRD) immunophenotype, taking into account that the phenotype of the leukemic clone that reemerged as MRD+ may have a different phenotype from that the patient started with. This may be due to antigenic modulation by the treatment, but it may also be indicative of the presence of another clone or neoplasm.
- i) Report of results. The report must contain the same sections as the immunophenotype report at diagnosis, but the study interpretation should be:

- I. Positive for measurable residual disease, followed by the amount in proportion (%) of CD45+ nucleated cells, as well as their absolute number per microliter. This estimate can be carried out using one or two platforms.
- II. Undetectable or below the limit of detection, by means of which using the concept of absence is avoided, which only depends on the state of the art.
- III. In addition, for every case, the lower limit of detection must be reported for each instrument, at each laboratory and for each one of the three major variants of acute leukemia (precursor B or T-cell or myeloid).

Although there is certain consistency in the interpretation of the importance of measurable residual disease detection in children with ALL, where > 1 blast/ μ L has been described on days 33 and 83 of treatment, which predicts 100 % of relapses, the flow cytometry laboratory should be restricted to describe the numerical quantity (if measurable) of neoplastic cells, without interfering in the decisions of the treating physician.

As supplementary material, suggestions were generated for antibody panels with 6, 8 and 10-color platforms, which are only intended to serve as general guidelines (Appendix 1).

Final considerations

By definition, a consensus is an "agreement between two or more parties", i.e. an opinion approved by a majority, which does not imply an active consent of each one, but an acceptance in the sense of non-negation. Thus, the consensus recommendations for immune typing of hematological neoplasms only reflect the ideas where there is agreement between the specialists involved, but they are not necessarily entirely satisfactory for all their signatories. Despite this limitation, guidelines and consensus recommendations play a decisive role in facilitating communication and strengthening the compatibility and comparability of information derived from studies whose conclusions rely on flow cytometry-determined immunophenotype. Given the velocity at which new CD specificities appear, and the accelerated development of new and more powerful analytical capabilities in flow cytometry, it is expected and desirable that consensus meetings continue to be organized in order for these guidelines to be updated. The community of users will always appreciate these efforts.

List of participants

Adrián Eduardo Acosta Sansén, Luz María Aguilar Anguiano, Juana Wendy Aguilera Caldera, Monserrat Aguirre, Carlos Alonso Muñoz, Lourdes Arriaga Pizano, Karen Amalinalli Ayala Contreras, Juan Carlos Balandrán, Rocío Baños Lara, Olga Verónica Barrales Benítez, Georgina Barrera Lumbreras, Andrea Bedoya, Omar Cano Jiménez, Antonieta Chávez González, Juan Carlos Chong Melchor, Sergio De Alba Camacho, Oxana Dobrovinskaya, Carlos Leonardo Duarte Martínez, Erick Espíndola, Karla A. Espinosa Bautista, Eduardo Ferat Osorio, María del Carmen Garza González, Miguel Alejandro Guerra Raphael, María Lourdes Gutiérrez, Sergio Gutiérrez Castilledos, Arely Eunice Hernández Alcántara, Ana Karen Hernández Colín, Hilda Elizabeth Hernández Juárez, Elva Jiménez Hernández, Gerardo Juárez Avendaño, Angélica Juárez Gómez, Lizeth Linares Sotelo, Briseida López Martínez, Felipe Alfonso Lule B, Ismael Mancilla Herrera, Juan Carlos Marín Corte, Orlando Martínez Loya, Alejandro Martínez Sánchez, Antonio Marín López, Juan Manuel Mejía Aranguré, Nereida Méndez Ramírez, Libertad Meza Arias, Carlos Mojica Cardoso, Adrián Morales Maravilla, Dafne Moreno Lorenzana, Lorena Nava Villegas, Juan Carlos Núñez Enríquez, Adriana Núñez Valencia, Roxana Olguín, Erick Olvera Juárez, Israel Parra Ortega, Rosana Pelayo, Elías Pérez Becerra, Beatriz Pérez Romano, Heriberto Prado García, Jessica Lakshmi Prieto Chávez, Dalia Ramírez, Heidi Ramírez Rodríguez, Alma B. Rodríguez Gallegos, Víctor Hugo Rosales, Héctor Alejandro Rosete Hernández, Alejandro Ruiz Argüelles, Mónica Saavedra, Stephanie Saint Remy Hernández, Guillermo José Ruiz Delgado, Jessica Sánchez Vargas, Antonio Sandoval Cabrera, Eliana Silva Casoluengo, Gloria Soldevila Melgarejo, Raúl Solís, Elena Soto Vega, Karina Suárez Álvarez, Patricia Rangel, Wilfrido David Tapia Sánchez, Gabriela Vargas Zaragoza, Esperanza Velasco Pérez, Armando Vilchis Ordoñez, Jairo Ricardo Villanueva Toldo, Pedro Arturo Zárate Rodríguez.

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Appendix 1

Suggestions for the construction of panels

Below, suggestions for antibody panels for 6, 8 and 10-color platforms are presented, which aim to serve as general guidelines. More information about markers (clones, availability) is described in the table “List of proposed markers”.

B-cell precursor ALL immunophenotyping			
Fluorochrome	10 colors	8 colors	6 colors
FITC	Ig	Ig	CD45
PE	CD79a	CD79a	CD79a
PE-Dazzle 594	CD10	—	—
PerCP-Cy5.5	CD34	CD34	CD34
PE-Cy7	CD19	CD19	CD19
APC	CD38	CD38	CD38
APC-R700	CD13	—	—
APC-Cy7	CD20	CD20	CD20
Brilliant violet 421	TdT	CD10	—
Pacific orange	CD45	CD45	—

T-cell precursor ALL immunophenotyping			
Fluorochrome	10 colors	8 colors	6 colors
FITC	CD7	CD7	CD7
PE	CD99	CD99	CD3
PE-Dazzle 594	CD4	—	CD34
PerCP-Cy5.5	CD34	CD34	—
PE-Cy7	CD1a	CD1a	CD45
APC	TdT	TdT	TdT
Alexa fluor 700	CD2	—	—
APC-Cy7	CD3	CD3	CD3
Brilliant violet 421	CD3	CD3	—
Pacific orange	CD45	CD45	—

AML immunophenotyping, 10 colors		
Fluorochrome	T1	T2
FITC	CD64	CD15
PE	CD71	CD16
PE-Dazzle 594	CD19	CD123
PE-Cy5	CD117	CD10
PE-Cy7	CD13	CD13
APC	CD34	CD34
Alexa fluor 700	MPO	CD38
APC-Cy7	CD11b	CD33
Brilliant violet 421	HLA DR	HLA DR
Pacific orange	CD45	CD45

AML immunophenotyping, 8 colors		
Fluorochrome	T1	T2
FITC	CD64	CD15
PE	CD71	CD16
PE-Cy5	CD117	CD10
PE-Cy7	CD13	CD13
APC	CD34	MPO
APC-Cy7	CD11b	CD33
Brilliant violet 421	HLA DR	HLA DR
Pacific orange	CD45	CD45

AML immunophenotyping, 6 colors			
Fluorochrome	T1	T2	T2
FITC	CD64	CD15	CD71
PE	HLA DR	HLA DR	HLA DR
PE-Cy5	CD117	CD10	CD11b
PE-Cy7	CD13	CD13	CD13
APC	CD34	CD33	MPO
APC-Cy7	CD45	CD45	CD45

List of proposed markers

Marker	Fluorochrome	Clone	Company	Catalogue
CD1a	PE-Cy7	HI149	BioLegend	300122
CD2	Alexa fluor 700	TS1/8	BioLegend	309228
CD3	APC-Cy7	SK7	BioLegend	344818
CD3	Pacific blue	UCHT1	BioLegend	300431
CD3	PE	UCHT1	BioLegend	300441
CD4	PE-Dazzle 594	SK3	BioLegend	344640
CD7	FITC	4H9	eBioscience	11-0078-42
CD10	PE-Dazzle 594	HI10a	BioLegend	312228
CD10	Brilliant violet 421	HI10a	BioLegend	312218
CD10	PE-Cy5	HI10a	BioLegend	312206
CD11b	APC-Cy7	ICRF44	BioLegend	301342
CD11b	PE-Cy5	ICRF44	BioLegend	301308
CD13	APC-R700	WM15	BD Biosciences	565124
CD13	PE-Cy7	L138 (Leu-M7)	BD Biosciences	338425
CD15	FITC	MMA	BD Biosciences	340703
CD16	PE	3G8	BioLegend	980102
CD19	PE-Cy7	HIB19	BioLegend	302216
CD19	PE-Dazzle 594	SJ25-C1	BioLegend	363032
CD20	APC-Cy7	2H7	BioLegend	302314
CD33	APC	P67.6	BioLegend	366606
CD33	APC-Cy7	P67.6	BioLegend	366614
CD34	PerCP-Cy5.5	8G12	BD Biosciences	347203
CD34	APC	8G12 (HPCA2)	BD Biosciences	340441
CD38	Alexa fluor 700	HB-7	BioLegend	356624
CD38	APC	HB-7	BioLegend	356606
CD45	APC-Cy7	HI30	BioLegend	304014
CD45	FITC	HI30	BioLegend	304038
CD45	Pacific orange	HI30	Thermo Fisher Scientific	MHCD4530
CD45	PE-Cy7	HI30	BioLegend	304016
CD64	FITC	10.1	BioLegend	305006
CD71	FITC	M-A712	BD Biosciences	555536
CD71	PE	M-A712	BD Biosciences	555537
CD79a	PE	HM57	BD Biosciences	563777
CD99	PE	3B2/TA8	BioLegend	371306
CD117	PE-Cy5	104D2	BioLegend	313210
CD123	PE-Dazzle 594	6H6	BioLegend	306034
HLA DR	Pacific blue	L243	BioLegend	980404
HLA DR	PE	L243	BioLegend	307606
Ig	FITC	Policlonal	Thermo Fisher Scientific	H17001
MPO	Alexa fluor 700	2C7	Novus	NB100-64803AF700
MPO	APC	2C7	Novus	NB100-64803APC
TdT	Brilliant violet 421	41A	BioLegend	368806
TdT	APC	41A	BioLegend	368808