

Submission ID #: 71304

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21747768>

Title: Multicenter Flow Cytometry for Acute Myeloid Leukemia Measurable Residual Disease with LAIP/DfN and CD34+CD38– Leukemic Stem Cell-enriched Populations

Authors and Affiliations:

Adriana Plesa^{1,35*}, Florent Dumezy², Stéphanie Mathis³, Isabelle Arnoux⁴, Veronique Saada⁵, Valerie Bardet⁶, Iole Oster¹, Bouchra Badaoui⁷, Elsa Bera⁸, Edouard Bonneville⁹, Bouthaina Bouslama¹⁰, Nicolas Chapuis¹¹, Clementine Chauvel³, Edouard Cornet¹², Louis Thomas Dannus¹³, Camille Debord¹⁴, Frédéric Feger¹⁵, Jean Feuillard¹⁶, Thomas Fournet¹⁷, Francine Garnache¹⁷, Franck Geneviève¹⁸, Delphine Gerard¹⁹, Valerie Goncalves Monteiro²⁰, Julien Guy²¹, Veronique Harrivel²², Claire Hemar²³, Cassandra Jadeau¹⁶, Caroline Mayeur-Rousse¹⁴, Hélène Lapillonne¹⁰, Veronique Latger-Cannard¹⁹, Magali Le Garff-Tavernier²⁴, Pierre Lemaire³, Remi Letestu²⁵, Anne Catherine Lhoumeau²⁶, Delphine Manzoni¹, Aguirre Mimoun²⁷, Vanessa Nivaggioni⁴, Jennifer Osman²⁸, Victoria Raggueneau²⁸, Hubert Rambaud¹⁸, Tatiana Raskovalova⁹, Anne Roggy¹⁷, Mikael Roussel²⁹, Didier Sciortino²⁶, Valerie Soenen², François Vergez³⁰, Richard Veyrat Masson¹³, Jean Philippe Vial²⁷, Manon Vogrig³¹, Orianne Wagner-Ballon^{7,37}, Jaja Zhu⁶, Karine Celli-Lebras³², Raphaël Itzykson^{33,38}, Christian Récher³⁴, Hervé Dombret^{32,33}, Claude Preudhomme^{2,36}, Christophe Roumier^{2,36*}

* Coordinators of the Acute Leukemia French Intergroup AML MRDflow & LSC network

¹Laboratory of Hematology and Flow cytometry, Lyon-Sud Hospital, Hospices Civils de Lyon, CHU-Lyon

²Laboratory of Hematology and Flow cytometry, CHU-Lille

³Laboratory of Hematology and Flow cytometry, Saint Louis Hospital, APHP

⁴Laboratory of Hematology and Flow cytometry, APHM-Marseille

⁵Laboratory of Hematology and Flow cytometry, IGR

⁶Laboratory of Hematology and Flow cytometry, Ambroise Pare University Hospital, APHP, Paris Saclay

⁷Département d'Hématologie et Immunologie Biologiques, Hôpitaux Universitaires Henri Mondor, APHP

⁸Laboratory of Hematology and Flow cytometry, CHU-Rouen

⁹Laboratory of Immunology and Flow cytometry, CHU-Grenoble

¹⁰Laboratory of Hematology and Flow cytometry, Trousseau Hospital, APHP

¹¹Laboratory of Hematology and Flow cytometry, Cochin Hospital, APHP

¹²Laboratory of Hematology and Flow cytometry, CHU-Caen

¹³Laboratory of Hematology and Flow cytometry, CHU-Clermont Ferrand

¹⁴Laboratory of Hematology and Flow cytometry, CHU-Nantes

¹⁵Laboratory of Hematology and Flow cytometry, Saint Antoine Hospital, APHP

¹⁶Laboratory of Hematology and Flow cytometry, CHU-Limoges

¹⁷Laboratory of Hematology and Flow cytometry, CHU-Besancon

¹⁸Laboratory of Hematology and Flow cytometry, CHU-Angers

¹⁹Laboratory of Hematology and Flow cytometry, CHU-Nancy

²⁰Laboratory of Hematology and Flow cytometry, CHU-Reims

²¹Laboratory of Hematology and Flow cytometry, CHU-Dijon

²²Laboratory of Hematology and Flow cytometry, CHU-Amiens

²³Laboratory of Hematology and Flow cytometry, CH-Valenciennes

²⁴Laboratory of Hematology and Flow cytometry, Pitie Salpetriere Hospital, APHP

²⁵Laboratory of Hematology and Flow cytometry, Avicenne Hospital, APHP

²⁶Laboratory of Hematology and Flow cytometry, IPC-Marseille

²⁷Laboratory of Hematology and Flow cytometry, CHU-Bordeaux

²⁸Laboratory of Hematology and Flow cytometry, Versailles Hospital

²⁹Laboratory of Hematology and Flow cytometry, CHU-Rennes

³⁰Laboratory of Hematology and Flow cytometry, CHU-Toulouse

³¹Laboratory of Hematology and Flow cytometry, CHU-Saint Etienne

³²Acute Leukemia French Association Group Coordination, IRSL

³³Department of Hematology, Saint Louis Hospital, APHP

³⁴Department of Hematology, CHU Toulouse, Université de Toulouse, Institut Universitaire du Cancer de Toulouse Oncopole

³⁵CRCL INSERM 1052/CNRS 5286, University of Lyon

³⁶UMR9020 CNRS-UMR-S1277 INSERM, University of Lille

³⁷Université Paris-Est Créteil (UPEC), INSERM U955 IMRB

³⁸Service Hématologie Adultes, Hôpital Saint Louis, APHP Paris, Université Paris Cité, INSERM, CNRS, Institut de Recherche Saint Louis (IRSL)

Corresponding Author:

Adriana Plesa adriana.plesa@chu-lyon.fr

Email Addresses for All Authors:

Florent Dumezy florent.dumezy@chu-lille.fr
Stéphanie Mathis stephanie.mathis@aphp.fr
Isabelle Arnoux isabelle.arnoux@mail.ap-hm.fr
Veronique Saada veronique.saada@igr.fr
Valerie Bardet valerie.bardet@aphp.fr
Iole Oster iole.oster@chu-lyon.fr
Bouchra Badaoui bouchra.badaoui@aphp.fr
Elsa Bera Elsa.Bera@chu-rouen.fr
Edouard Bonneville ebonneville@chu-grenoble.fr
Boutheina Bouslama boutheina.bouslama@aphp.fr
Nicolas Chapuis nicolas.chapuis@aphp.fr
Clementine Chauvel clementine.chauvel@aphp.fr
Edouard Cornet cornet-e@chu-caen.fr
Louis Thomas Dannus ltdannus@chu-clermontferrand.fr
Camille Debord camille.debord@chu-nantes.fr
Frédéric Feger frederic.feger@aphp.fr
Jean Feuillard jean.feuard@unilim.fr
Thomas Fournet tfournet@chu-besancon.fr
Francine Garnache fgarnache@chu-besancon.fr
Franck Geneviève FrGenevieve@chu-angers.fr
Delphine Gerard D.GERARD@chru-nancy.fr
Valerie Goncalves Monteiro vgoncalves-monteiro@chu-reims.fr
Julien Guy julien.guy@chu-dijon.fr
Veronique Harrivel Harrivel.Veronique@chu-amiens.fr
Claire Hemar hemar-c@ch-valenciennes.fr
Cassandra Jadeau Cassandra.JADEAU@chu-limoges.fr
Caroline Mayeur-Rousse caroline.mayeurrousse@chu-nantes.fr
Hélène Lapillonne helene.lapillonne@aphp.fr
Veronique Latger-Cannard v.cannard@chru-nancy.fr
Magali Le Garff-Tavernier magali.legarff@aphp.fr
Pierre Lemaire pierre.lemaire@aphp.fr

Remi Letestu	remi.letestu@aphp.fr
Anne Catherine Lhoumeau	LHOUMEAUAC@ipc.unicancer.fr
Delphine Manzoni	delphine.manzoni@chu-lyon.fr
Aguirre Mimoun	aguirre.mimoun@chu-bordeaux.fr
Vanessa Nivaggioni	Vanessa.NIVAGGIONI@ap-hm.fr
Jennifer Osman	josman@ch-versailles.fr
Victoria Raggueneau	vraggueneau@ch-versailles.fr
Hubert Rambaud	HuRambaud@chu-angers.fr
Tatiana Raskovalova	TRaskovalova@chu-grenoble.fr
Anne Roggy	aroggy@chu-besancon.fr
Mikael Roussel	Mikael.ROUSSEL@chu-rennes.fr
Didier Sciortino	SCIORTINOD@ipc.unicancer.fr
Valerie Soenen	Valerie.SOENEN@chu-lille.fr
François Vergez	Vergez.Francois@iuct-oncopole.fr
Richard Veyrat Masson	rveyrat@chu-clermontferrand.fr
Jean Philippe Vial	jean-philippe.vial@chu-bordeaux.fr
Manon Vogrig	Manon.VOGRIG@chu-st-etienne.fr
Orianne Wagner-Ballon	orianne.wagnerballon@aphp.fr
Jaja Zhu	jaja.zhu@aphp.fr
Karine Celli-Lebras	karine.cellilebras@alfa-leukemia.org
Raphaël Itzykson	raphael.itzykson@aphp.fr
Christian Recher	Recher.Christian@iuct-oncopole.fr
Hervé Dombret	herve.dombret@mac.com
Claude Preudhomme	Claude.PREUDHOMME@CHU-LILLE.FR
Christophe Roumier	Christophe.ROUMIER@chu-lille.fr
Adriana Plesa	adriana.plesa@chu-lyon.fr

Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **YES**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES, all done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **NO**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **NO**

Current Protocol Length

Number of Steps: 25

Number of Shots: 55 (35 SC)

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

We have retained 5 best answers as per our journal guidelines

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Adriana Plesa:** Our research focused on accurate quantification of measurable residual disease, including Leukemic Stem Cells, in acute myeloid leukemia patients.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Christophe Roumier:** To implement multicenter and multiplatform monitoring, we tailored the flow cytometer settings to each platform and a common analysis strategy.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Iole Oster:** This protocol allows researchers to measure the Leukemia aberrant immunophenotype identified at diagnosis and leukemic stem cells in the CD34+CD38-enriched fraction.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Herve Dombret:** Choosing a multicenter approach can be challenging. The feasibility of this concept requires mandatory steps before implementing MRD flow in AML clinical trials. (take by SCREEN AP/CR)

~~1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera~~

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Adriana Plesa:** The "crossroads" moment has arrived to move forward and incorporate LSC quantification performed with great accuracy into future AML clinical trials at diagnosis and follow-up.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Human Ethics Committee of CHU Lyon and the Ethics Committee of the Institutional Review Board of Lille CHU

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Cytometer Standardization

Demonstrator: Iole Oster

2.1. To begin, prepare the 8-peak rainbow beads and determine the cytometer-specific target values [1].

2.1.1. WIDE: Talent preparing the 8-peak rainbow beads. ***Videographer:** Please obtain CU shot as well for this shot.*

2.2. Set each machine according to the target values [1] and ensure that each channel's measured value is within 5% of its target [2].

2.2.1. Talent setting the machine according to the target values.

2.2.2. LAB MEDIA: Table 1.

NOTE: Treat this as separate section

Bone Marrow Sample Preparation

2.3. Collect 1 to 2 milliliters of bone marrow in EDTA from acute myeloid leukemia patients at diagnosis, during treatment monitoring, and at relapse, when applicable [1-TXT]. For morphologic examination, place a drop of bone marrow aspirate on a glass slide [2] and gently spread the sample [3]. After 30 minutes, stain the smear with May-Grunewald-Giemsa [4] and examine the sample under a microscope at 100x magnification [5]. Next, add 15 milliliters of ammonium chloride lysing solution to the collected bone marrow sample [6]. Mix gently and incubate for 10 minutes at room temperature [7].

2.3.1. Talent adding bone marrow sample to EDTA tubes and labeling the tubes as 'Diagnosis', MRD-1. **TXT: Treatment monitoring: MRD-1 to 4; MRD: Measurable Residual Disease** **Authors:** This labeling is just for representation

2.3.2. Talent adding a drop of sample on a slide.

2.3.3. Talent spreading then sample.

2.3.4. Talent adding the stain on the sample.

2.3.5. LAB MEDIA: Supplementary figure 10. *Video editor: Label the left panel as 'Healthy regenerating marrow' and right one as "Acute myeloid leukemia*

patient”

- 2.3.6. Talent adding ammonium chloride lysing solution to the bone marrow sample.
- 2.3.7. Talent gently mixing the sample and keeping it aside.

2.4. Then, centrifuge the sample at 450 *g* for 5 minutes at room temperature [1] and discard the supernatant [2]. Resuspend the cell pellet in 10 milliliters of PBS with 0.1% BSA [3]. Repeat the centrifugation step at 450 *g* for 5 minutes [4]. After discarding the supernatant, resuspend the cell pellet at a concentration of 30×10^6 white blood cells per milliliter in PBS [6].

- 2.4.1. Talent placing the sample in the centrifuge.
- 2.4.2. Talent discarding the supernatant.
- 2.4.3. Talent resuspending the cell pellet in PBS with 0.1% bovine serum albumin.
- 2.4.4. Talent placing the sample in the centrifuge.
- 2.4.5. Talent resuspending the cell pellet in PBS.

3. Immunostaining the Bone Marrow Cells

3.1. Distribute the monoclonal antibodies in tubes T1, T2, and T3 according to the platform [1-TXT].

- 3.1.1. Talent distributing monoclonal antibodies into tubes T1, T2, and T3. **TXT: See Table 2 for antibody panel configurations**

3.2. Add 2 to 3×10^6 lysed cells per tube in 100 microliters of cell suspension [1] and incubate the cells with antibodies for 15 minutes in the dark at room temperature [2].

- 3.2.1. Talent adding lysed cells to each tube.
- 3.2.2. Talent placing the tubes in a dark chamber for incubation at room temperature.

3.3. Wash the cells by adding 3 milliliters of PBS [1] and centrifuging at 450 *g* for 5 minutes [2]. Discard the supernatant [3] and resuspend the stained cells in 300 microliters of PBS [4].

- 3.3.1. Talent adding PBS to the cells.
- 3.3.2. Talent placing the tubes in the centrifuge.

3.3.3. Talent discarding the supernatant.

3.3.4. Talent resuspending the stained cells in PBS.

4. Flow Cytometry

4.1. Open the MRD assay or acquisition protocol with the adapted settings and compensation matrix [1].

4.1.1. SCREEN: MRD assay or acquisition protocol being opened with the adapted settings and compensation matrix. **NOTE: Use videographer's footage for 4.1.1 and 4.2.2**

Videographer: Please record the computer screen for the shots labeled as SCREEN as back-up. The screen should be in focus and the fonts should be clearly visible.

4.2. Load the samples and acquire a minimum of 500,000 to 1,000,000 white blood cells [1] in each tube at diagnosis and follow-up [2].

4.2.1. Talent loading the samples.

4.2.2. SCREEN: Acquisition in process.

NOTE: Treat this as a separate section

Leukemia-Associated Immunophenotype (LAIP) Analysis

4.3. Use a common template and dot-plot sequence [1].

4.3.1. SCREEN: SCREEN-4.3 00:00-00:05

4.4. Capture leukemia-associated immunophenotype, patterns in bulk blasts by drawing a single-cell gate based on forward scatter width versus forward scatter height [1].

4.4.1. SCREEN: SCREEN-4.4 00:05-00:21

4.5. Draw a live-cell gate based on forward scatter versus side scatter [1].

4.5.1. SCREEN: SCREEN-4.5 00:07-00:16

- 4.6. Then, draw a blast gate on CD45 dim and side scatter [1], followed by the primitive marker gate using CD34 and CD117 [2].

4.6.1. SCREEN: SCREEN-4.6.1 00:05-00:17

4.6.2. SCREEN: SCREEN-4.6.2 00:05-00:17

- 4.7. Using fixed biparametric dot plots, draw the relevant gates, including LAIP 1 through LAIP 8 [1]. After identifying and excluding relevant physiological and nonspecific populations, apply the Boolean gating strategy for LAIP analysis [2-TXT].

4.7.1. SCREEN: SCREEN-4.7.1 00:06-00:11 AND 00:35-00:38

4.7.2. SCREEN: SCREEN-4.7.2 00:10-00:22. **TXT: See text protocol for the LAIP approach Boolean operators**

5. Different-from-Normal (DfN) Analysis

- 5.1. To capture a DfN (*D-F-N*) pattern in bulk blasts, draw live cells using forward scatter and side scatter [2].

5.1.1. SCREEN: SCREEN-5.1.2 00:05-00:14

- 5.2. Draw single cells by removing doublets based on the forward scatter peak and the side scatter area [1]. Then, draw the mononuclear cell gate using forward scatter and side scatter [2].

5.2.1. SCREEN: SCREEN-5.2.1 00:07-00:20

5.2.2. SCREEN: SCREEN-5.2.2 00:08-00:18

- 5.3. Apply a cleanup gate to remove lymphocytes using CD45, CD33, and side scatter [1]. Next, apply a cleanup gate to remove erythroblasts and debris using CD45 and CD36 [2].

5.3.1. SCREEN: SCREEN-5.3.1 00:06-00:16

5.3.2. SCREEN: SCREEN-5.3.2 00:04-00:06 and 00:13-00:18

- 5.4. Use two input gates, CD34 positive, CD45, and side scatter [1], and CD117 positive, CD45, and side scatter, to define the empty boxes [2].

5.4.1. SCREEN: SCREEN-5.4.1 00:05-00:10

5.4.2. SCREEN: SCREEN-5.4.1 00:13-00:20

5.5. Draw each DfN empty box based on internal control populations, including lymphocytes for cross-lineage markers and normal myeloid precursors for loss, decrease, or overexpression markers [1]. Compare the empty boxes with reference bone marrow samples, including regenerating, inflammatory, and healthy donor bone marrow. Define abnormal populations as clusters of abnormal cells separated from normal cells by at least 0.5 decade on the log scale [2].

5.5.1. SCREEN: SCREEN-5.5.1 00:10-00:25

5.5.2. SCREEN: SCREEN-5.5.2 00:04-00:20

6. Leukemic Stem Cell Analysis and CD38 Threshold Definition

6.1. Define the leukemic stem cell, or LSC, input gate as mononuclear cell CD34-positive [1].

6.1.1. SCREEN: SCREEN-6.1.1 00:01-00:10

6.2. Next, set the CD38 channel high enough to distinguish CD38-negative populations from CD38-positive populations with good resolution [1].

6.2.1. SCREEN: SCREEN-6.2 00:01-00:10 and 00:18-00:19

6.3. Use bright reference populations to establish the CD38 signal range [1]. Identify plasma cells as the brightest population and hematogones according to the measurement range of each platform [2].

6.3.1. SCREEN: SCREEN-6.3.1 00:00-00:05

6.3.2. SCREEN: SCREEN-6.3.2 00:00-00:05 and 00:10-00:12

6.4. Then, define the CD34-positive CD38-negative population P6 using the CD38 fluorescence-minus-one approach validated by an isoclonic CD38 control [1-TXT]. Outline P7, the CD34-positive and CD38-low population, using the hematogone-negative level [2]. Define P8, the CD34-positive and CD38-high population, using the hematogone-positive and plasma-cell negative levels [3].

- 6.4.1. SCREEN: SCREEN-6.4.1 00:03-00:13 **TXT: See text protocol for the Boolean operators**
- 6.4.2. SCREEN: SCREEN-6.4.2
- 6.4.3. SCREEN: SCREEN-6.4.3 00:05-00:16

- 6.5. Apply the same CD38-negative threshold across the harmonized group, with robust methods such as CD38 fluorescence minus one or an isoclonic CD38 control [1].
 - 6.5.1. SCREEN: SCREEN-6.5.1 00:05-00:16

- 6.6. Include at least one aberrant immunophenotype in the CD34-positive CD38-negative fraction to define LSC-enrichment [1]. Use LSC markers or lineage markers for this identification [2-TXT]. Use CD36 to remove nonspecific events from the final MRD LSC gate [3].
 - 6.6.1. SCREEN: SCREEN-6.6.1 00:03-00:08 and 00:11-00:16
 - 6.6.2. SCREEN: SCREEN-6.6.2 00:05-00:13. **TXT: LSC markers: CLL1/TIM3/CD97/CD45RA/CD123**
Lineage markers: CD7/CD19/CD56/CD33
 - 6.6.3. SCREEN: SCREEN-6.1.3 00:03-00:06 and 00:10-00:15

- 6.7. Apply the assay sensitivity thresholds, with a limit of quantification of 10^{-4} and a limit of detection of 10^{-5} [1]. Finally, define LSC positivity at diagnosis as at least 1% of CD45-side-scatter-positive blasts [2], and at MRD follow-up as at least 0.01% of white blood cells. Provide the harmonized clinical flow report [3].
 - 6.7.1. SCREEN: SCREEN-6.7.1 00:05-00:17
 - 6.7.2. SCREEN: SCREEN-6.7.2 00:05-00:14
 - 6.7.3. SCREEN: SCREEN-6.7.2 00:16-00:25

Results

7. Results

- 7.1. After applying the defined settings, the cell staining patterns overlapped across the 4 flow cytometer platforms [1], with the main difference being a translation of the cell clouds along the logarithmic scales [2].
 - 7.1.1. LAB MEDIA: Figure 6.
 - 7.1.2. LAB MEDIA: Figure 6. *Video editor: Emphasize that the cell clouds (with red centers) in 2nd row as an example.*
- 7.2. The wet external quality assessment or EQA showed reproducible distributions of P6 cells [1] and mononuclear cell CD34 positive cells among the white blood CD45 positive cells across the 5 assessment rounds [2].
 - 7.2.1. LAB MEDIA: Figure 7A.
 - 7.2.2. LAB MEDIA: Figure 7B.
- 7.3. More than 90% of the locally analyzed data were included within the centralized interval based on the series mean in Dry-EQA-2017 (20-17) [1] and Dry-EQA-2024 (20-24) [2].
 - 7.3.1. LAB MEDIA: Figure 8A. *Video editor: Highlight the data points clustered around the central reference line*
 - 7.3.2. LAB MEDIA: Figure 8B. *Video editor: Highlight the data points clustered around the central reference line*
- 7.4. The leukemia-associated immunophenotype cartography established at diagnosis [1] was applied to the follow-up sample to quantify measurable residual disease [2].
 - 7.4.1. LAB MEDIA: Figure 9A. *Video editor: Highlight the black cell population marked as the leukemia-associated immunophenotype in the diagnosis panels.*
 - 7.4.2. LAB MEDIA: Figure 9A. *Video editor: Highlight the black measurable residual disease population in the follow-up panels.*
- 7.5. The DfN approach identified abnormal cells in the areas not observed in normal bone marrow [1].

- 7.5.1. LAB MEDIA: Figure 9B. *Video editor: Highlight the black potted regions.*
- 7.6. The leukemic stem cell-enriched cells expressing the defined marker profile were identified at diagnosis within the CD34-positive CD38-negative fraction [1], and the measurable residual disease leukemic stem cell-enriched cells with the same profile persisted at follow-up [2].
- 7.6.1. LAB MEDIA: Figure 10A. *Video editor: Highlight the orange cell population in the diagnosis samples.*
- 7.6.2. LAB MEDIA: Figure 10B. *Video editor: Highlight the orange cell population in the follow-up samples.*
- 7.7. The limit of blank for leukemic stem cell detection was less than 0.0005% [1], corresponding to 14 events in the leukemic stem cell Boolean gate [2].
- 7.7.1. LAB MEDIA: Figure 11A. *Video editor: Highlight the value showing 0.0003%.*
- 7.7.2. LAB MEDIA: Figure 11A. *Video editor: Highlight the boxed cluster labelled with "14" events on the right.*
- 7.8. The serial dilution of leukemic stem cell-enriched cells showed a correlation between the measured measurable residual disease values and the theoretical values [1].
- 7.8.1. LAB MEDIA: Figure 11B. *Video editor: Highlight the points aligned along the correlation line in the graph.*
- 7.9. The serial dilution of blasts with different leukemia-associated immunophenotypes showed a correlation between the measured measurable residual disease values and the theoretical values [1].
- 7.9.1. LAB MEDIA: Figure 12A. *Video editor: Highlight the data points following the trend line in the dilution graphs.*
- 7.10. The limit of quantification reached the 0.1% clinical threshold for all tested leukemia-associated immunophenotypes [1].
- 7.10.1. LAB MEDIA: Figure 12B. *Video editor: Highlight the blue bars and the red horizontal line marking the 0.1% clinical threshold.*

1. Cytometer

Pronunciation link: <https://www.merriam-webster.com/medical/cytometer>

- IPA: /sar'ta:mə'tər/
Phonetic Spelling: sy·taa·muh·ter
2. Myeloid
Pronunciation link: <https://www.merriam-webster.com/dictionary/myeloid>
IPA: /'maɪə'loɪd/
Phonetic Spelling: my·uh·loyd
 3. Leukemia
Pronunciation link: <https://www.merriam-webster.com/dictionary/leukemia>
IPA: /lu:'ki:miə/
Phonetic Spelling: loo·kee·mee·uh
 4. Morphologic
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphologic>
IPA: /,mɔr.fə'lɑ:dʒɪk/
Phonetic Spelling: mor·fuh·laa·jɪk
 5. Aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /'æs.pə.rət/
Phonetic Spelling: as·puh·ruht
 6. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sɛn.trə'fju:dʒ/
Phonetic Spelling: sen·truh·fyooj
 7. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /,su:.pər'nei.tənt/
Phonetic Spelling: soo·per·nay·tuhtnt
 8. Monoclonal
Pronunciation link: <https://www.merriam-webster.com/dictionary/monoclonal>
IPA: /,mɑ:.nə'kloʊ.nəl/
Phonetic Spelling: maa·nuh·kloh·nuhl
 9. Immunophenotype
Pronunciation link: <https://www.merriam-webster.com/medical/immunophenotype>
IPA: /,ɪm.jə.noʊ'fi:.nə'taɪp/
Phonetic Spelling: im·yuh·noh·fee·nuh·type
 10. Biparametric
Pronunciation link: No confirmed link found
IPA: /,baɪ'pærə'metɪk/
Phonetic Spelling: by·pair·uh·meh·trɪk
 11. Lymphocytes
Pronunciation link: <https://www.merriam-webster.com/dictionary/lymphocyte>
IPA: /'lɪm.fə'saɪts/
Phonetic Spelling: lim·fuh·sites
 12. Erythroblasts
Pronunciation link: <https://www.merriam-webster.com/dictionary/erythroblast>

- IPA: /ɪˈrɪθ.rə.blæsts/
Phonetic Spelling: ih·rith·ruh·blasts
13. Mononuclear
Pronunciation link: <https://www.merriam-webster.com/dictionary/mononuclear>
IPA: /ˌmɑː.noʊˈnuː.kli.ər/, /ˌmɑː.noʊˈnjuː.kli.ər/
Phonetic Spelling: maa·noh·noo·klee·er / maa·noh·nyoo·klee·er
14. Hematogones
Pronunciation link: <https://www.merriam-webster.com/medical/hematogone>
IPA: /ˈhiː.mæt.əˌɡoʊnz/, /hɪˈmæt.əˌɡoʊnz/
Phonetic Spelling: hee·muht·uh·gohnz / hi·mat·uh·gohnz
15. Isoclonic
Pronunciation link: No confirmed link found
IPA: /ˌaɪ.souˈklɑː.nɪk/
Phonetic Spelling: eye·soh·klaa·nik
16. Immunophenotypes
Pronunciation link: <https://www.merriam-webster.com/medical/immunophenotype>
IPA: /ˌɪm.jə.noʊˈfiː.nəˌtaɪps/
Phonetic Spelling: im·yuh·noh·fee·nuh·types
17. Cartography
Pronunciation link: <https://www.merriam-webster.com/dictionary/cartography>
IPA: /kɑːˈtɑːɡrəfi/
Phonetic Spelling: kar·taa·gruh·fee
18. Leukemic
Pronunciation link: <https://www.merriam-webster.com/dictionary/leukemia>
IPA: /luːˈkiːmɪk/
Phonetic Spelling: loo·kee·mik
19. Quantification
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantification>
IPA: /ˌkwɑːn.təˈfæːʃən/
Phonetic Spelling: kwaan·tuh·fuh·kay·shuhn
20. Physiological
Pronunciation link: <https://www.merriam-webster.com/dictionary/physiological>
IPA: /ˌfɪziəˈlɑːdʒɪkəl/
Phonetic Spelling: fiz·ee·uh·laa·ji·kuhl
21. Ammonium
Pronunciation link: <https://www.merriam-webster.com/dictionary/ammonium>
IPA: /əˈmouniəm/
Phonetic Spelling: uh·moh·nee·uhm
22. Giemsa
Pronunciation link: <https://www.merriam-webster.com/dictionary/Giemsa>
IPA: /ˈɡiːmzə/
Phonetic Spelling: geem·zuh
23. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>

IPA: /flʊ'reɪsəns/

Phonetic Spelling: floo·res·uhns

24. Immunostaining

Pronunciation link: No confirmed link found

IPA: /,ɪm.jə.noʊ'steɪ.nɪŋ/

Phonetic Spelling: im·yuh·noh·stay·ning

25. Erythroblasts

Pronunciation link: <https://www.merriam-webster.com/dictionary/erythroblast>

IPA: /ɪ'riθ.rə.blæsts/

Phonetic Spelling: ih·rith·ruh·blasts