DOTTORATO DI RICERCA
in "Artificial Intelligence in Medicine and Innovation in Clinical and Methodological Research"

Silvia Mutti
XXXIX ciclo

Coordinatore: Prof. Domenico Russo
Supervisor: Prof.ssa Simona Bernardi

«Development of a step-by-step protocol to gain success in the use of Extracellular vesicles (EVs) as powerful diagnostic and prognostic markers in Chronic Myeloid Leukemia»

UNIVERSITÀ DEGLI STUDI DI BRESCIA

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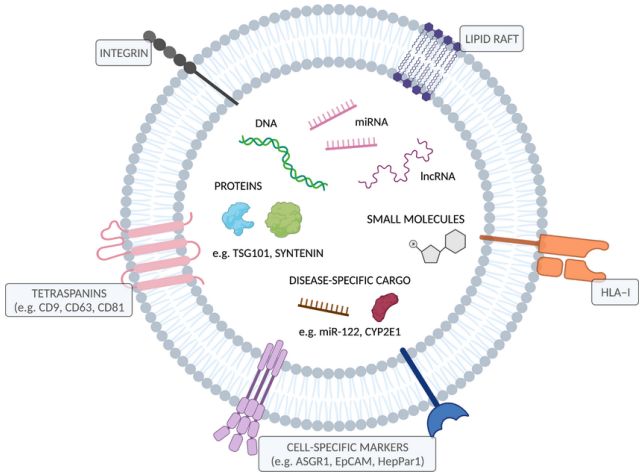
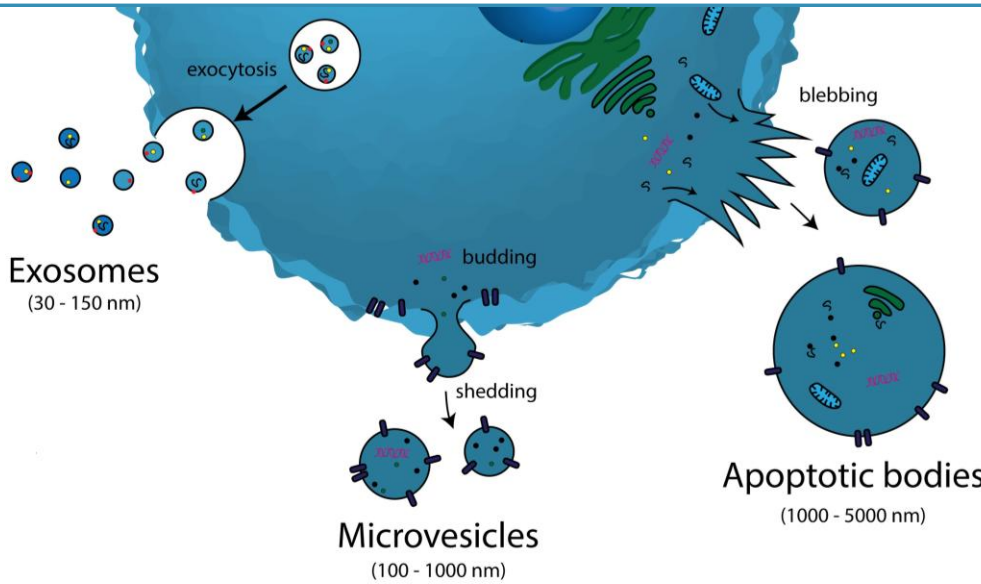
EXTRACELLULAR VESICLES

The term ‘extracellular vesicles’ (EVs) refers to particles that are released from cells, are delimited by a lipid bilayer, and cannot replicate on their own (i.e., do not contain a functional nucleus).

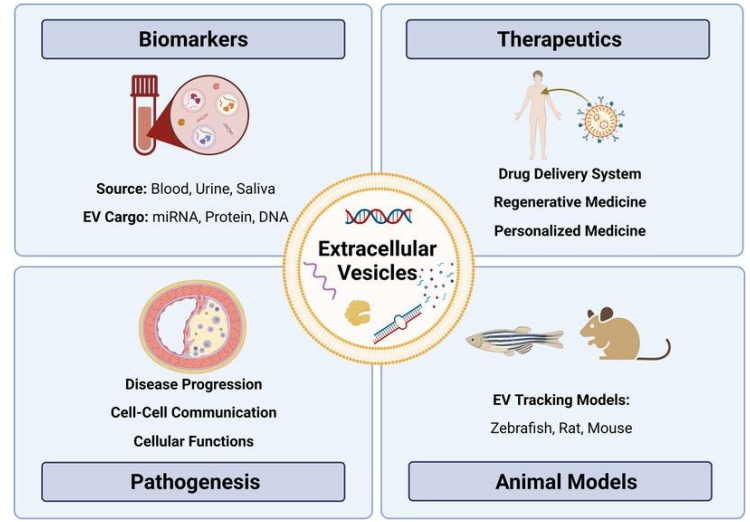
MISEV 2023





EVs play crucial roles as mediators of intercellular communication, facilitating the transfer of bioactive molecules between cells in both physiological and pathological pathways.

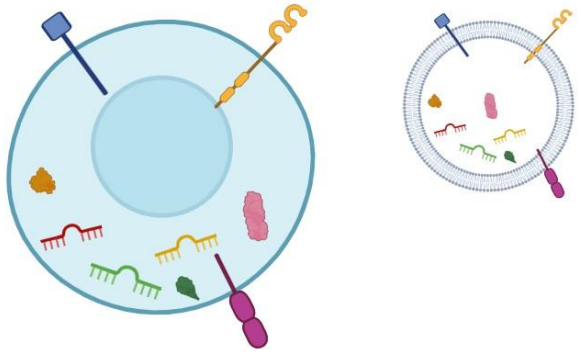
Autocrine **Paracrine** **Endocrine**

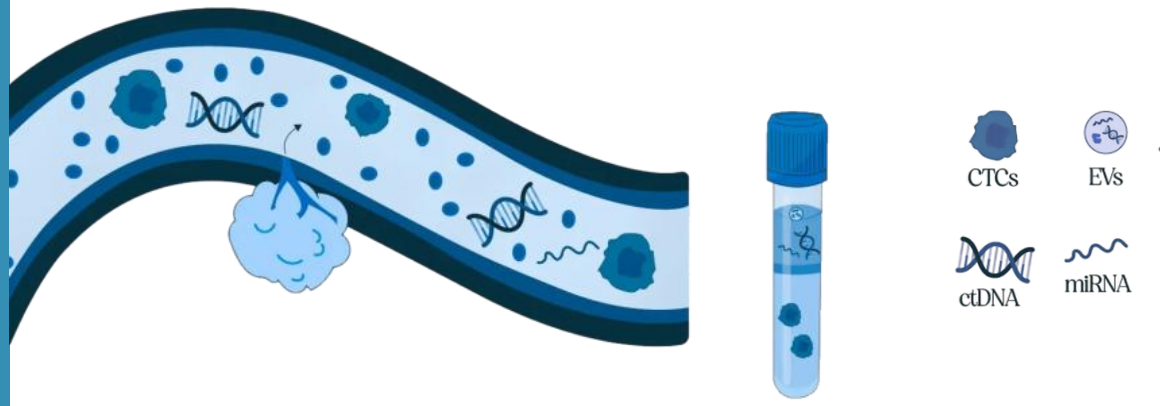


LIQUID BIOPSY

Extracellular Vesicles can be regarded as a stable and accessible *fingerprint* of the parent cell since their composition depends on the type and even status of the producer cell.

Depending on the disease for which the biomarker is being developed, an accessible biofluid, in which the EVs of interest are likely the most concentrated and a liquid biopsy (LB) approach can be easily obtained, should be considered.





LIQUID BIPOSY consists in the isolation/analysis of disease-derived materials in bodily fluids (e.g. peripheral blood, saliva…) and offers interesting opportunities for the identification of novel diagnostic and prognostic biomarker (e.g. EVs).

LYMPHOID NEOPLASIA

Characterization of CLL exosomes reveals a distinct microRNA signature and enhanced secretion by activation of BCR signaling

Yuh-Ying Yeh,[1] Hatice Gulcin Ozer,[2] Amy M. Lehman,[3] Kami Maddocks,[1] Lianbo Yu,[3] Amy J. Johnson,[1,4] and John C. Byrd[1,4]

frontiers in IMMUNOLOGY

ORIGINAL RESEARCH ARTICLE
published: 10 April 2014
doi: 10.3389/fimmu.2014.00160

Plasma exosomes as markers of therapeutic response in patients with acute myeloid leukemia

Chang-Sook Hong[1], Laurent Muller[1], Theresa L. Whiteside[1,2]* and Michael Boyiadzis[3]

[1] Department of Pathology, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[2] Department of Immunology, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[3] Department of Medicine, University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Annals of Hematology (2019) 98:595–603
https://doi.org/10.1007/s00277-019-03608-y

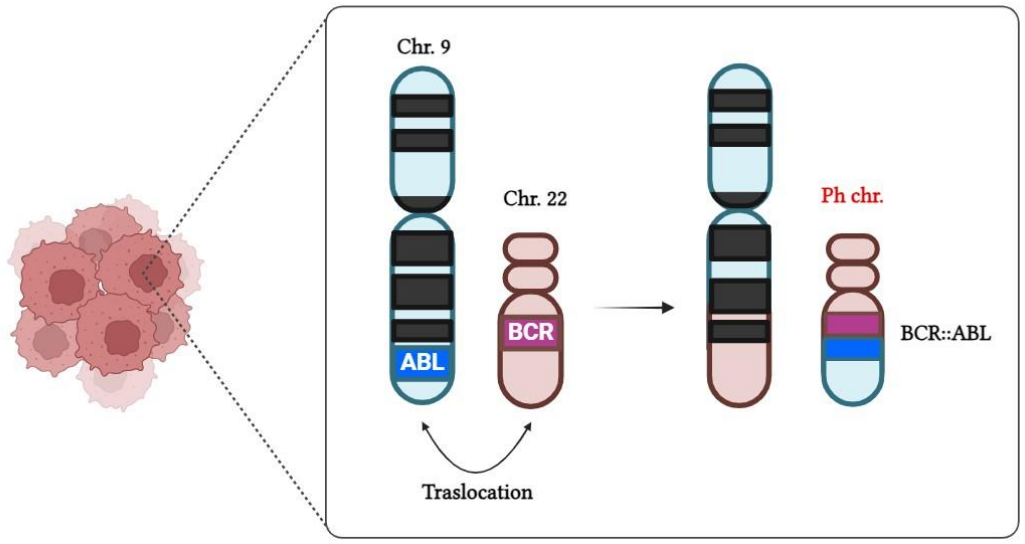
ORIGINAL ARTICLE

CrossMark

Detection of AML-specific mutations in pediatric patient plasma using extracellular vesicle–derived RNA

Fabienne Kunz[1] · Evangelia Kontopoulou[1] · Katarina Reinhardt[1] · Maren Soldierer[1] · Sarah Strachan[1] · Dirk Reinhardt[1] · Basant Kumar Thakur[1]

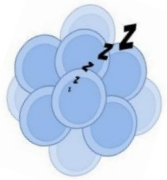
CHRONIC MYELOID LEUKEMIA



Treatment-Free-Remission (TFR), **THE NEW CLINICAL GOAL,** is tested in clinical trials for patients with stable MR or undetectable MRD via RT-qPCR. **HOWEVER,** relapse is experienced in ~40-60% of eligible patients, also in patients with undetectable *BCR::ABL1* levels.

1. **RT-qPCR** is not sensitive enough for precise monitoring
2. **Circulating cells** do not represent the entire disease

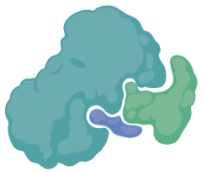
Residual MRD?



Residual leukemic cells resident in the Bone Marrow niche (BM-LSCs) might be responsible for relapse.

Introduction of tyrosine kinase inhibitors (TKIs): The turning point in the management of CML

Active BCR::ABL1



Inactive BCR::ABL1

BCR-ABL1 (%IS)	Log Reduction in *BCR-ABL1*	Reduction in *BCR-ABL1*
100		Baseline
10	1	Approximates MCyR
1	2	Approximates CCyR
0.1	3	MMR
0.01	4	MR[4]
0.0032	4.5	MR[4.5]
0.001	5	MR[5]
0.0001	6	MR[6] (with currently available technology, this level of responses cannot be assessed)

Deep Molecular Response

MYELOID NEOPLASIA

Brief report

Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease

Jean-Claude Chomel,[1,2] Marie-Laure Bonnet,[2] Nathalie Sorel,[1,2] Angelina Bertrand,[2] Marie-Claude Meunier,[2] Serge Fichelson,[3] Michael Melkus,[4] *Annelise Bennaceur-Griscelli,[4] *François Guilhot,[2,5] and Ali G. Turhan[1,2]

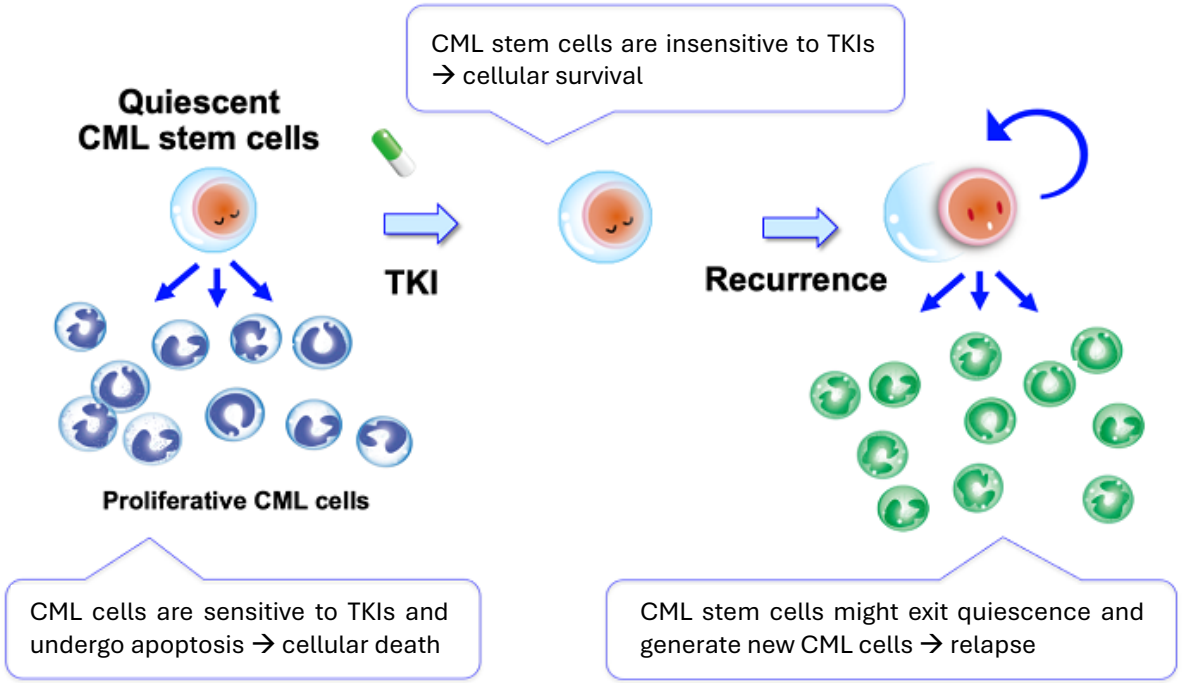
MYELOID NEOPLASIA

Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment

Su Chu,[1,2] Tinisha McDonald,[1,2] Allen Lin,[1,2] Sujata Chakraborty,[1,2] Qin Huang,[3] David S. Snyder,[2] and Ravi Bhatia[1,2]

[1]Division of Hematopoietic Stem Cell and Leukemia Research, [2]Department of Hematology and Hematopoietic Cell Transplantation, and [3]Department of Pathology, City of Hope National Medical Center, Duarte, CA

LEUKEMIC STEM CELLS



- **CD34+CD38-CD26+ LSCs** are considered the reservoir of CML. They are distinguished from normal hematopoietic stem cells by the expression of the CD26 marker (dipeptidyl-peptidase IV), making them identifiable through flow cytometry for rapid and specific CML diagnosis.

- **CD26$^+$ LSCs** are detectable in peripheral blood and bone marrow even during deep molecular remission (DMR) and treatment with tyrosine kinase inhibitors (TKIs). These cells may also persist in patients who discontinue treatment, potentially contributing to disease relapse.

- The presence of **CD26$^+$ LSCs** in peripheral blood at the time of TKI discontinuation or during TFR is not always directly predictive of remission loss.

Residual Peripheral Blood CD26$^+$ Leukemic Stem Cells in Chronic Myeloid Leukemia Patients During TKI Therapy and During Treatment-Free Remission

Bocchia M. et al., doi: 10.3389/fonc.2018.00194. PMID: 29900128; PMCID: PMC5988870.

In Search of Drivers of CD34+/CD38-/CD26+ Leukemia Stem Cells Persistence in CML Patients

Paola Pacelli, Elena Bestoso, Anna Sicuranza, Elisabetta Abruzzese, Luigiana Luciano, Alessandra Iurlo, Marzia Defina, Sara Fredducci, Monica Crugnola, Valentina Giai, Federica Sorà, Sara Galimberti, Donatella Raspadori, Monica Bocchia

EVs FOR CML-LSCs MONITORING

Chronic myeloid leukemia-derived exosomes promote tumor growth through an autocrine mechanism

Stefania Raimondo[1], Laura Saieva[1], Chiara Corrado[1], Simona Fontana[1], Anna Flugy[1], Aroldo Rizzo[2], Giacomo De Leo[1] and Riccardo Alessandro[1*]

Published in final edited form as:
Int J Cancer. 2012 May 1; 130(9): 2033–2043. doi:10.1002/ijc.26217.

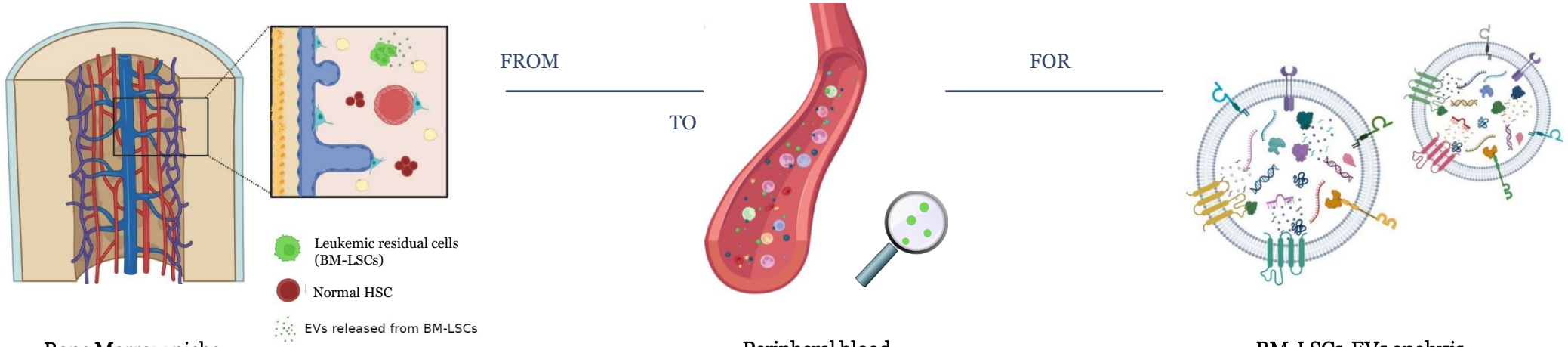
Role of exosomes released by chronic myelogenous leukemia cells in angiogenesis

Simona Taverna[1,*], Anna Flugy[1,*], Laura Saieva[1,*], Elise C Kohn[2], Alessandra Santoro[3], Serena Meraviglia[1], Giacomo De Leo[1], and Riccardo Alessandro[1,°]

INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 44: 2133-2144, 2019

Feasibility of tumor-derived exosome enrichment in the onco-hematology leukemic model of chronic myeloid leukemia

SIMONA BERNARDI[1,2*], CHIARA FORONI[1,2*], CAMILLA ZANAGLIO[1,2], FEDERICA RE[1,2], NICOLA POLVERELLI[1], ALESSANDRO TURRA[1], ENRICO MORELLO[1], MIRKO FARINA[1], FEDERICA CATTINA[1], LISA GANDOLFI[1], TATIANA ZOLLNER[1], EUGENIA ACCORSI BUTTINI[1], MICHELE MALAGOLA[1] and DOMENICO RUSSO[1]



OBJECTIVES

1. **Detect** EVs released by $CD26^+$ Leukemia stem cells in CML using advanced immunocapture techniques to isolate highly pure and specific EVs.

2. **Identify** molecular markers associated with $CD26^+$ cells

3. **Integrate** the protocol into clinical practice: Enabling more sensitive and personalized monitoring for patients in Treatment-Free Remission (TFR).

STRATEGIES

1. **Specific isolation of EVs** using peptides or antibodies to select vesicles specifically released by $CD26^+$ cells.

2. **Advanced EVs characterization:** EVs quantification and analysis of membrane markers through technologies such as nanoparticle tracking analysis (NTA), Flow Cytometry (FC) and Western Blot.

3. **Molecular content analysis**: Evaluating the intra vesicular RNA and protein to identify disease-related biomarkers by means of sensitive techniques such as digitalPCR.

WORKFLOW

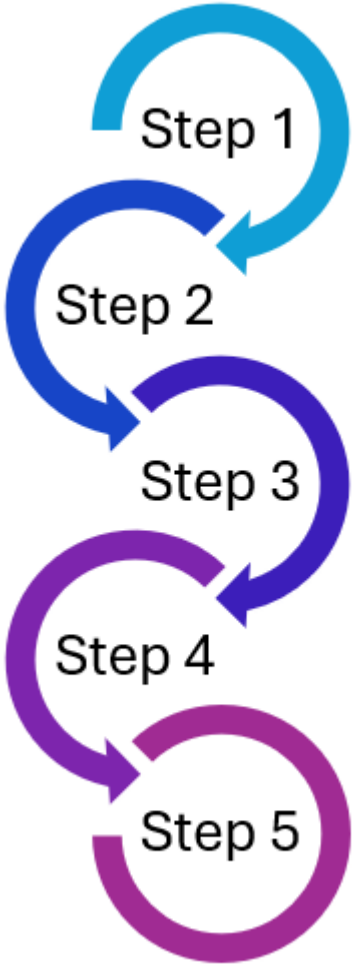
1. **Feasibility Study and Semi-Quantitative Analysis of EVs:** Isolation and preliminary characterization of EVs derived from CML-LSCs in a cohort of patients at various disease stages, using specific markers (CD34+, CD38-, CD26+) and analysis via Flow Cytometry.

2. **High throughout put analysis of CD26+ stem cells and their EVs**: Isolation of CD26+ cells from a small cohort of patients and EVs analysis

3. **Development of a Prototype for Selective EV Isolation:** Design and testing of an innovative method based on affinity for surface markers, validated on cell lines and *ex vivo* samples.

4. **Feasibility Testing on Patient Cohorts:** Validation of the optimized workflow on patients at different stages of CML, aiming to improve disease monitoring and propose new clinical strategies.

5. **Retrospective study and possible Prospective study.**



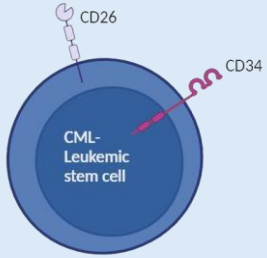
FIRST YEAR ACHIEVEMENTS

1

Design the **research study**, establishing theoretical and methodological foundations.

2

Conduct an in-depth **literature review** on residual leukemic stem cells in CML.

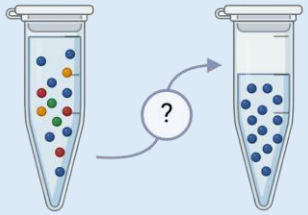


3

Understand **CML-LSCs characteristics** and functions to develop a solid workflow.

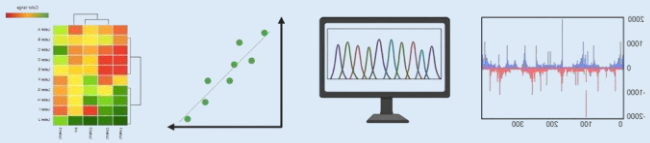
4

Explore extracellular vesicle **(EVs) isolation methods** with a focus on affinity selection, examining different techniques and their effectiveness in isolating EVs based on specific membrane markers.



5

Assemble a **specific and reproducible protocol** for isolating EVs that preserves their integrity and functionality, ensuring they remain suitable for subsequent analysis.



6

Assess the **translational potential** of selected methodologies for clinical applications.

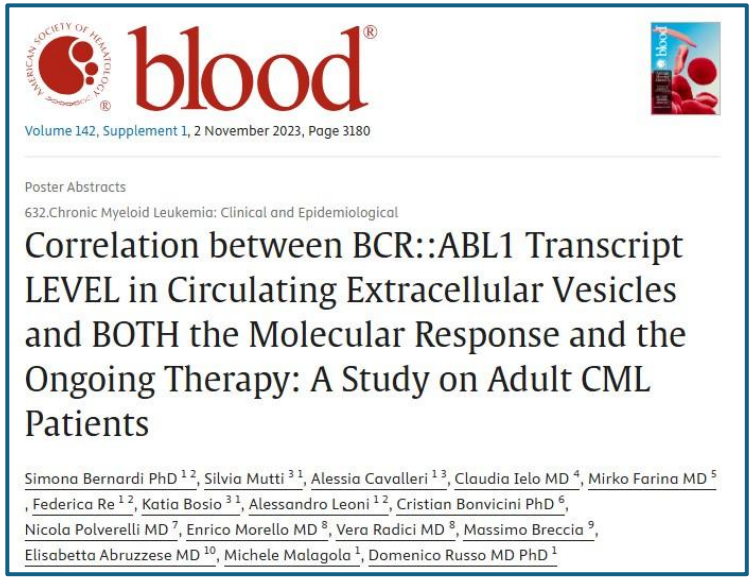
FIRST YEAR ACHIEVEMENTS

Evaluate *BCR::ABL1* transcript within EVs from CML patients at least in major molecular response (MMR), with the goal of investigating its correlation with clinical and biological parameters, including circulating CD26+ cells. Preliminary findings, presented at several scientific conferences, have yielded promising insights and initiated multiple ongoing in vitro experiments.

Results cannot be disclosed prior to publication.

Presented as poster:

- The European Hematology Association: EHA 2023
- John Goldman Conference on Chronic Myeloid Leukemia: ESH 2023
- American Society of Hematology: ASH 2023
- European LeukemiaNet: ELN Symposium 2024



Presented as oral communications:

- Società Italiana di Ematologia: SIE 2023 by Prof. Simona Bernardi
- Gruppo Italiano di Ematologia Sperimentale: SIES 2024 by Dott. **Silvia Mutti**

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