**EXTRACELLULAR VESICLES RELEASED BY DIFFERENT ACUTE MYELOID LEUKEMIA DERIVED CELL LINES: ANALYSIS OF THEIR PROTEIN CARGO**

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**Introduction**

Clonal expansion of undifferentiated **Myeloid Precursors** leading to:
- Impaired Hematopoiesis
- Bone Marrow Failure [3]

**Acute Myeloid Leukemia (AML)**

- 12% of hematological diseases [2]
- 5-year survival of less than 50% [2]

High Frequency of **Post-Treatment Relapse**

Current MRD detection. **Bone Marrow Aspirate**:
- Invasive Technique
- Lacking real-time monitoring [3]

Innovative liquid biopsy-based biomarkers of MRD, using **Peripheral Blood**, would allow minimally invasive monitoring

**EXTRACELLULAR VESICLES (EVs):**

- Cell released particles
- 30-1000 nm
- Isolated from body fluids (including blood)
- Reflect the pathological state of the cell of origin [4]

Potential source of cancer biomarkers

**Methodology**

**EVS ISOLATION FROM HL-60 AND NB4 LEUKEMIC CELL LINES USING ULTRACENTRIFUGATION**

**DAY 0:**
- Cells Expansion

**DAY 1:**
- Seeding of 1 x 10^6 cells/ml in 24 ml in medium supplemented with EVs-depleted serum
- EVs Quantification for:
  - Proteomic Analysis*
  - Western Blot*
  - Transmission Electron Microscopy (TEM) for morphology analysis
  - Nanoparticle tracking analysis (NTA) to estimate particles size and quantity
  * To be performed.

**DAY 2:**
- Cell Lysis
- Protein Extraction and Quantification
- Pellet wash with PBS Citrate
  - Then, 2nd Ultracentrifugation
  - 100 000g
  - 1.5h
  - 4 °C
- Centrifugation at 2000g, 30 minutes, 4°C to remove cell debris

**Culture medium collection**

**Results**

**Protein Quantification EVs Pellet**

![Protein Concentration](image1)

**Mean Size of EVs released by cells (nm)**

![Mean Size of EVs released by cells (nm)](image2)

**EVs Size**

![EVs Size](image3)

**Figure 2: Mean size of EVs released by HL-60 and NB4 (N=3)**

**EVs Quantity**

![EVs Quantity](image4)

**Figure 3: Number of EVs released by HL-60 and NB4 (N=3)**

**Conclusions**

- EVs isolation was well succeeded in both cell lines
- Include more cell lines in the study, representative of other AML subtypes
- High-throughput proteomic analysis of EVs and their cells of origin, to identify potential novel biomarkers of this disease
- Western Blot validation of the proteomic results
- Confirm the presence of the previously identified biomarkers in EVs isolated from the peripheral blood of patients with AML

**EVs Morphology**

![EVs Morphology](image5)

**Figure 4: TEM Images of EVs isolated from the cell lines HL-60 and NB4**

**Future Perspectives**

- Evs were isolated from the cell lines HL-60 and NB4

**References**


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