MISSIONE 4
ISTRUZIONE
RICERCA

Finanziato dall'Unione europea
NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

Bioinspired lipid nanocarriers and their payloads: antiproliferative/anticancer effects and regenerative medicine applications

Università degli Studi di Urbino
23 maggio 2024

WP 3.2 Dr. Mariele Montanari

- Bioinspired lipid nanocarriers
- Results
- Conclusion I

- Regenerative medicine applications
- Results
- Conclusion II

VITALITY

MISSIONE 4
ISTRUZIONE
RICERCA

Finanziato dall'Unione europea
NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

VITALITY

1506 UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

frontiers | Frontiers in Oncology

Bacterial toxins exert anticancer effects by affecting the cell cycle and apoptotic pathways and regulating tumorigenesis. Chimeric toxins, which are recombinant derivatives of bacterial toxins, have been developed to address the low specificity of their conventional peers. Khoshnood S. et al, Sec. Cancer Molecular Targets and Therapeutics, Volume 12 - 2022 | https://doi.org/10.3389/fonc.2022.953678

ORIGINAL PAPER

Extracellular vesicles from *Aggregatibacter actinomycetemcomitans* exhibit potential antitumorigenic effects in oral cancer: a comparative in vitro study

Metsäniitty M, et al, Archives of microbiology **2024**;206(6):244 doi:10.1007/s00203-024-03976-8

REVIEW

Cytolethal distending toxin: from genotoxin to a potential biomarker and anti-tumor target

Kailoo S., et al, World Journal of Microbiology and Biotechnology **2021** 37:150; doi.org/10.1007/s11274-021-03117-z

REVIEW

ADVANCED SCIENCE Open Access
www.advancedscience.com

Bacterial-Mediated Tumor Therapy: Old Treatment in a New Context

Liu, Y., et al,. Advanced science **2023**, 10(12), e2205641. https://doi.org/10.1002/advs.202205641

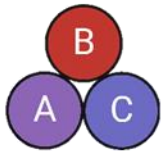
toxins

MDPI

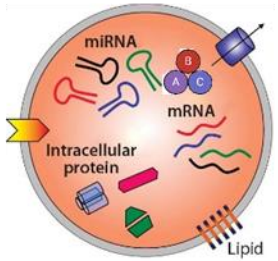
Article

Engineering of Cytolethal Distending Toxin B by Its Reducing Immunogenicity and Maintaining Stability as a New Drug Candidate for Tumor Therapy; an In Silico Study

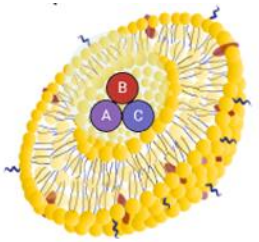
Keshtvarz M et al, Toxins **2021**, 13, 785. doi.org/10.3390/toxins13110785



***Campylobacter jejuni* CDT**



Evs from infected cells contain CDT



"Our"

CDT Liposomes

MISSIONE 4
ISTRUZIONE
RICERCA

Finanziato dall'Unione europea
NextGenerationEU

Ministero dell'Università e della Ricerca

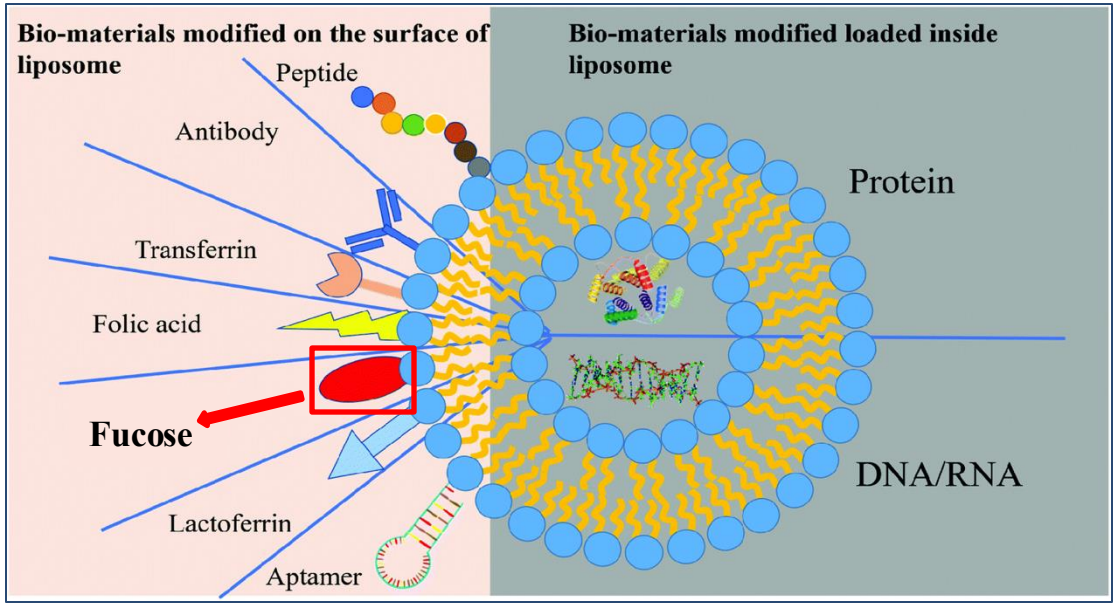
Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

VITALITY

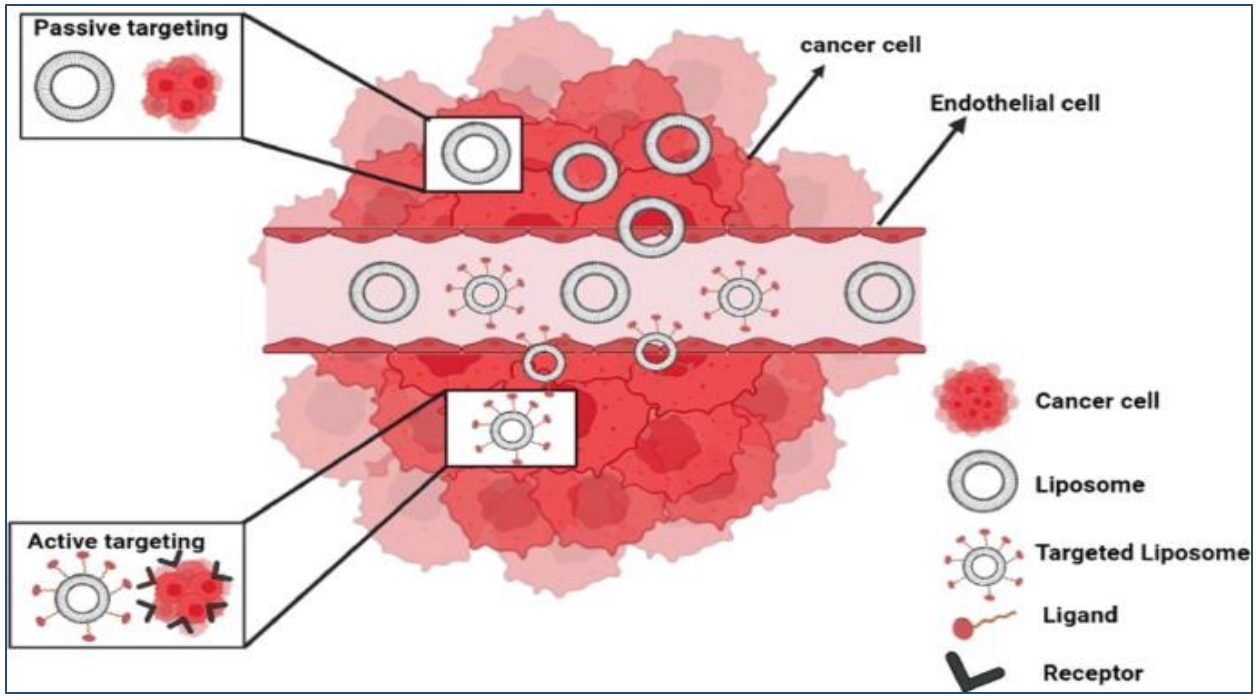
1506
UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

Fucosylated Liposomes as pilot antitumor drug delivery system

The mechanism of fucosylation has been observed to facilitate targeting in many cancer cells.



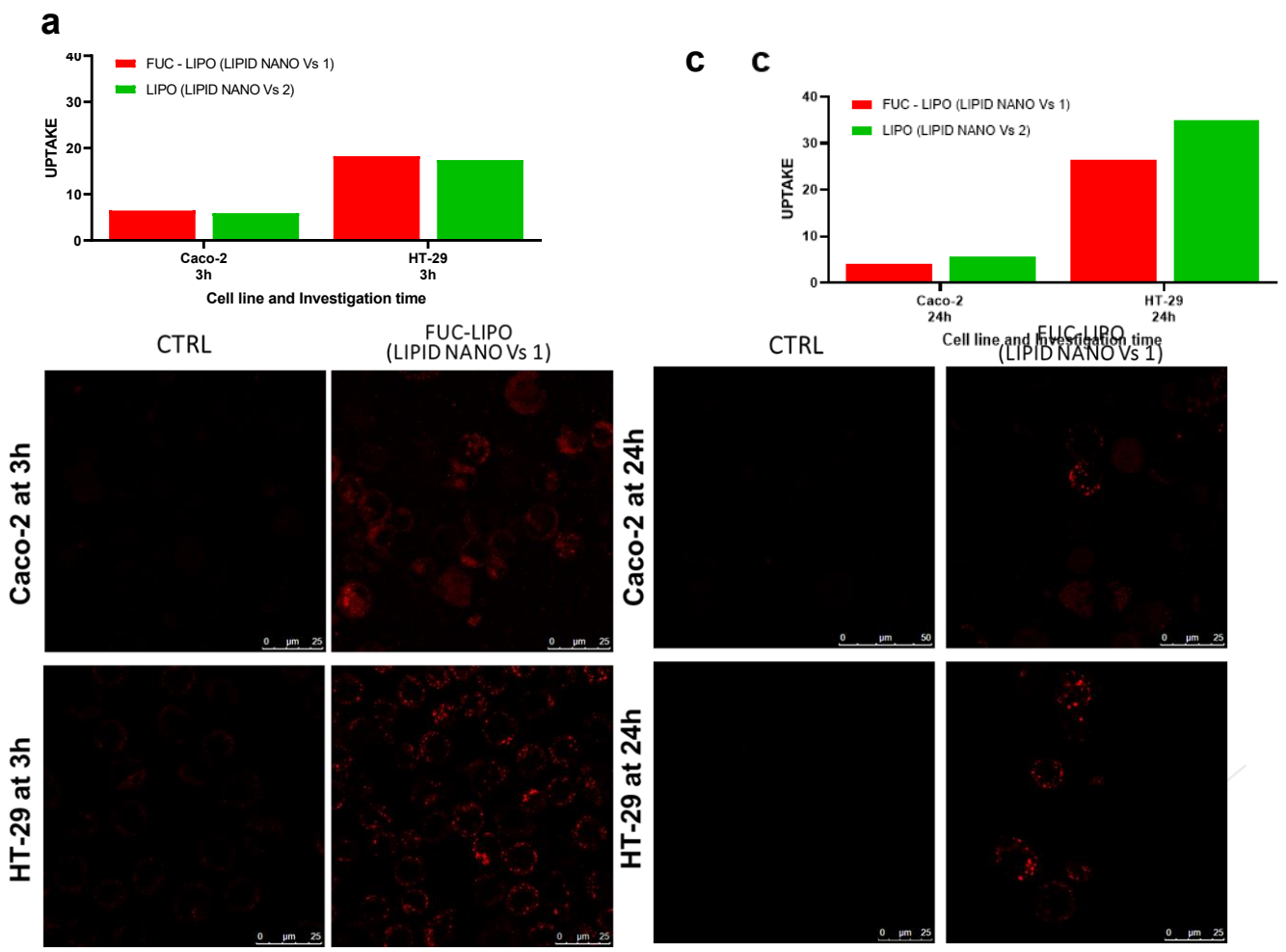
Li et al., Biomaterials science **2020**, 8(23), 6442-6468.



Kumar et al., In Advanced Drug Delivery: Methods and Applications **2023**

Regarding these aspects, we investigated fucose-decorated and undecorated liposomes as a carrier for delivering targeted anticancer drugs to cancer cells.

UPTAKE OF DIFFERENTLY-DECORATED LIPOSOMES



Liposomes were labelled by Rhodamine and the uptake was evaluated after 3h and 24h by flow cytometry and confocal microscope in two different colon cancer cells, highlighting that in Caco-2 the uptake is moderate after 3h and it is similarly maintained after 24h, whereas HT-29 relevantly increase the liposomal uptake (for both formulations) from 3h to 24h.

Confocal microscopic analysis shows the uptake of Rhodamine fucosylated liposomes (FUC-LIPO) in both cell lines Caco-2 and HT-29, at 3h and 24h compared to control. At 24h, the bright red dots indicate different liposomes uptake, demonstrating that liposomes are still retained inside the cells.

MISSIONE 4
ISTRUZIONE
RICERCA

Finanziato dall'Unione europea NextGenerationEU

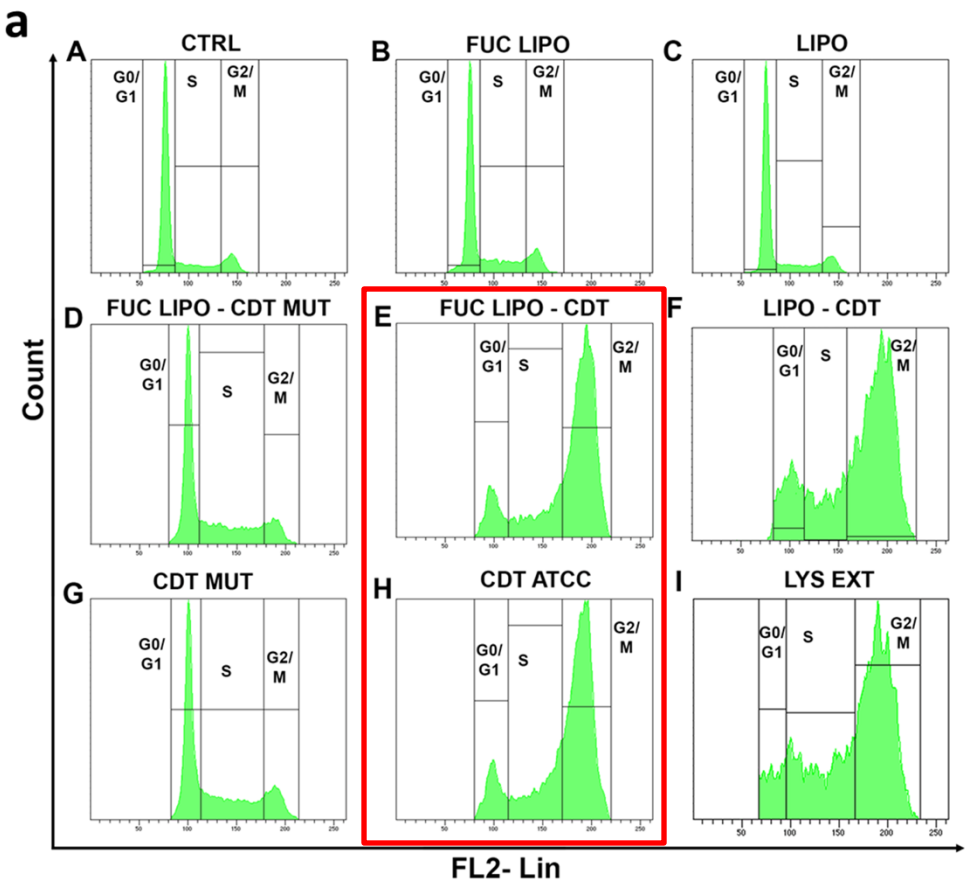
Ministero dell'Università e della Ricerca

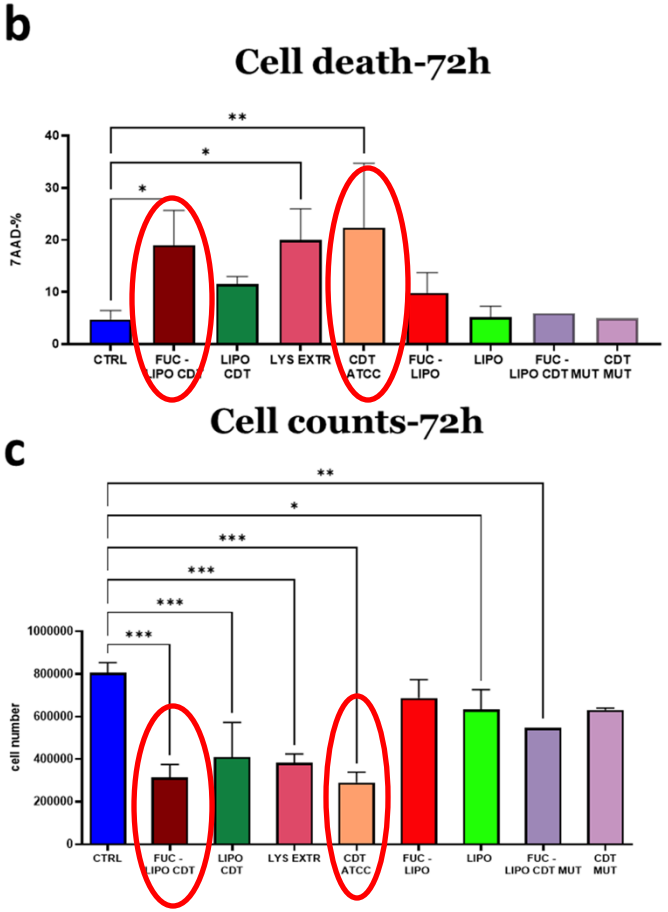
Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

VITALITY

1506 UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

LIPOSOME CONTAINING CDT: EFFECTS ON CELL PROLIFERATION AND DEATH

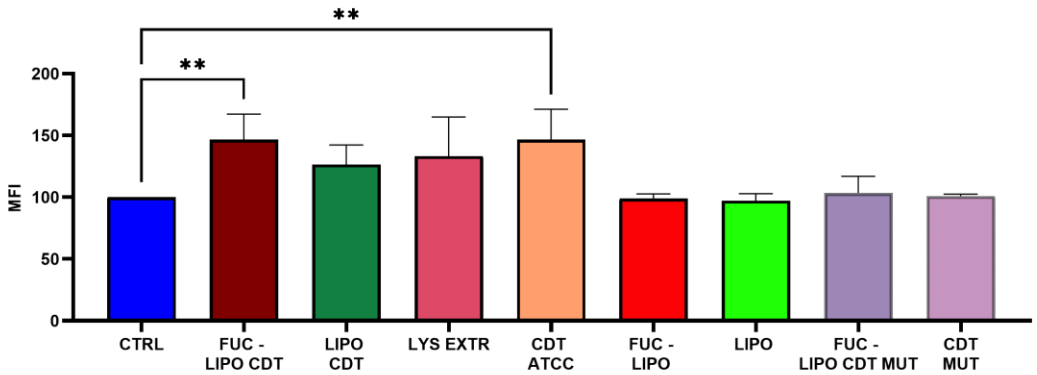




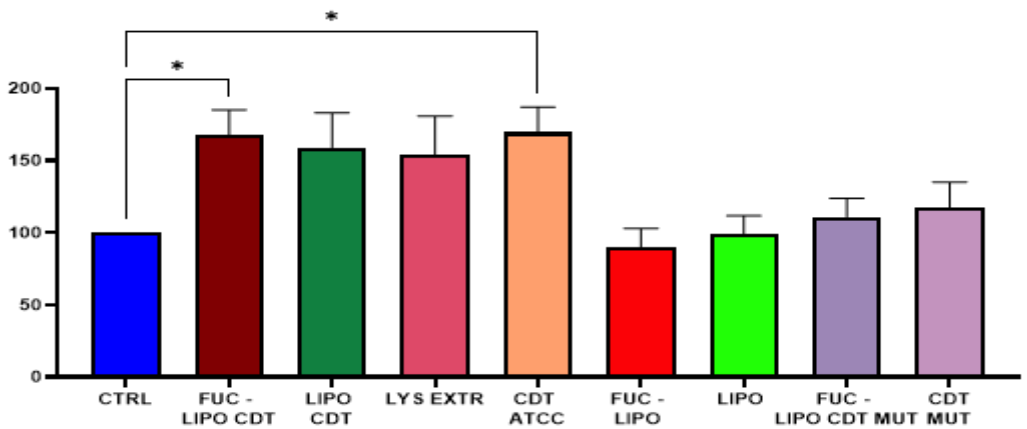
Our data demonstrate that liposomes containing CDT, can enter in epithelial intestinal tumor cells (Caco-2) and exert a strong antiproliferative effect, with differences related to the specific formulation: fucose-decorated (FUC LIPO) and undecorated (LIPO), reveal the same behavior for cell numbers stationing in the cycle phases G0/G1 and G2/M, and this is also shown by CDT ATCC lysate. Differences appear in the regulation of the S phase. These findings must be coupled with data from cell death and absolute cell counts.

LIPOSOME CONTAINING CDT: EFFECTS ON DIFFERENT PATHWAYS

Hydrogen peroxide content - 72h



Autophagic vacuole content - 72h



Since ROS can regulate several signalling pathways affecting many cellular functions and ultimately influencing cell survival or cell death, we studied the intracellular content of Hydrogen peroxide (H_2O_2).
Fucosylated liposomes and ATCC CDT lysate showed the most significant increase in H_2O_2 levels.

Increased levels of Hydrogen peroxide (H_2O_2) have been linked to the induction of autophagic cell death in human colon cancer cells since ROS is a signalling molecule that induces autophagy in cancer cells. (Azmanova, M., & Pitto-Barry, A. 2022. Chembiochem : a European journal of chemical biology, 23(10), e202100641. https://doi.org/10.1002/cbic.202100641)

In fact, we found a significant increase of autophagic vacuole in fucose-decorated liposomes and ATCC CDT lysate.

MISSIONE 4 ISTRUZIONE RICERCA

Finanziato dall'Unione europea NextGenerationEU

Ministero dell'Università e della Ricerca

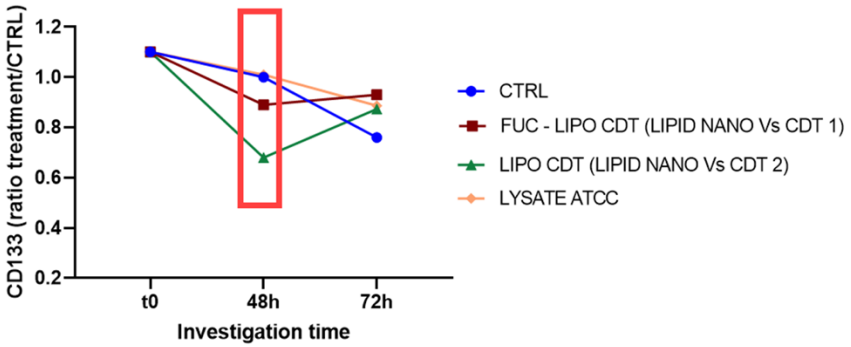
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA

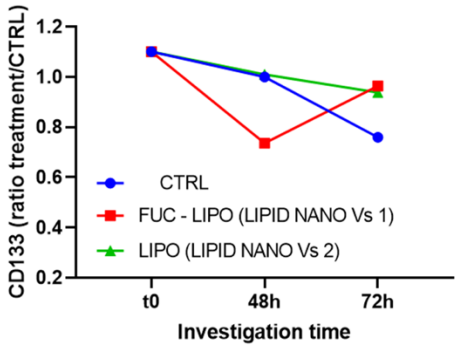
VITALITY

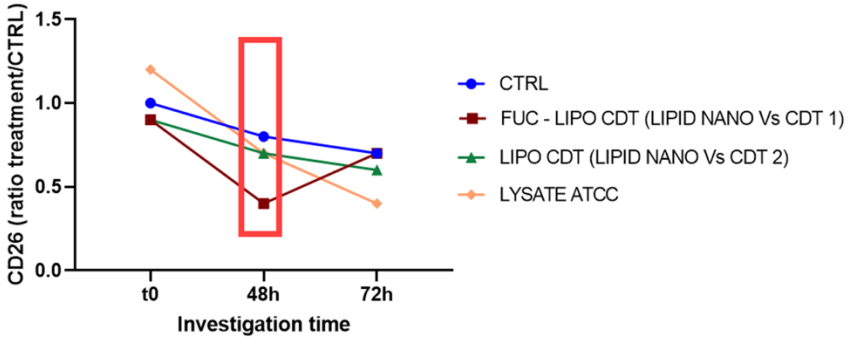
1506 UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO

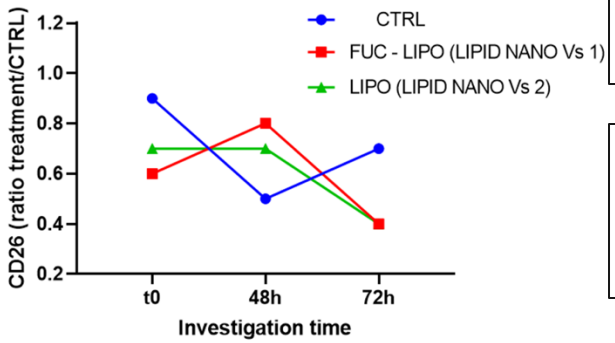
LIPOSOME CONTAINING CDT: Targeting Colorectal Cancer Stem Cells

Name of Marker	Cancer	Function
CD133	Colon, lung, gastric, ovarian, pancreatic and liver cancer	Self-renewal, tumorigenesis, invasiveness, proliferation, differentiation, angiogenesis, resistant to apoptosis
CD26	Colon cancer	Self-renewal, tumorigenesis, invasiveness









Preliminary data highlight CD133 and CD26 modulation by the different treatments. Such findings are currently not significant.

Further investigations are needed to explore the possible impact of CDT treatment on Caco-2 cells since the two liposome formulations seem to decrease CD133 and CD26 expression compared to control cells after 48h.

Independently of CDT loading, the two formulations seem to impact surface CD133 and CD26.

Conclusion I

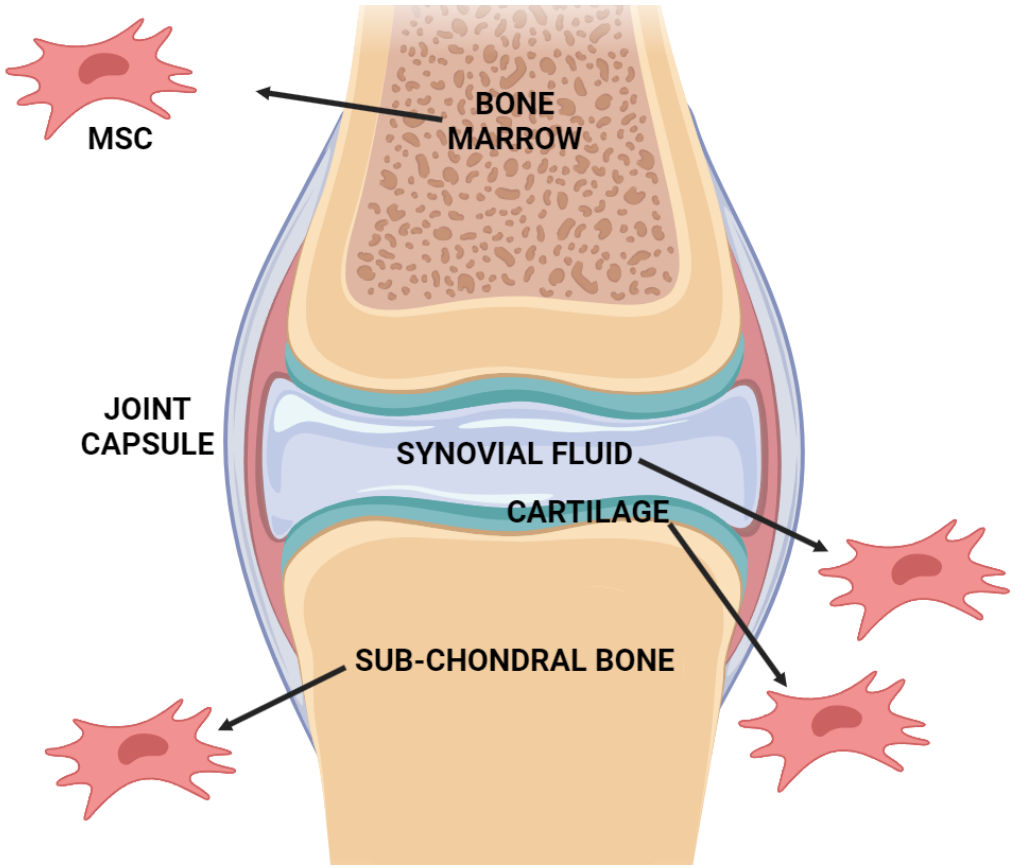
Liposome uptake shows different behaviour in the different investigated colorectal cancer cells.

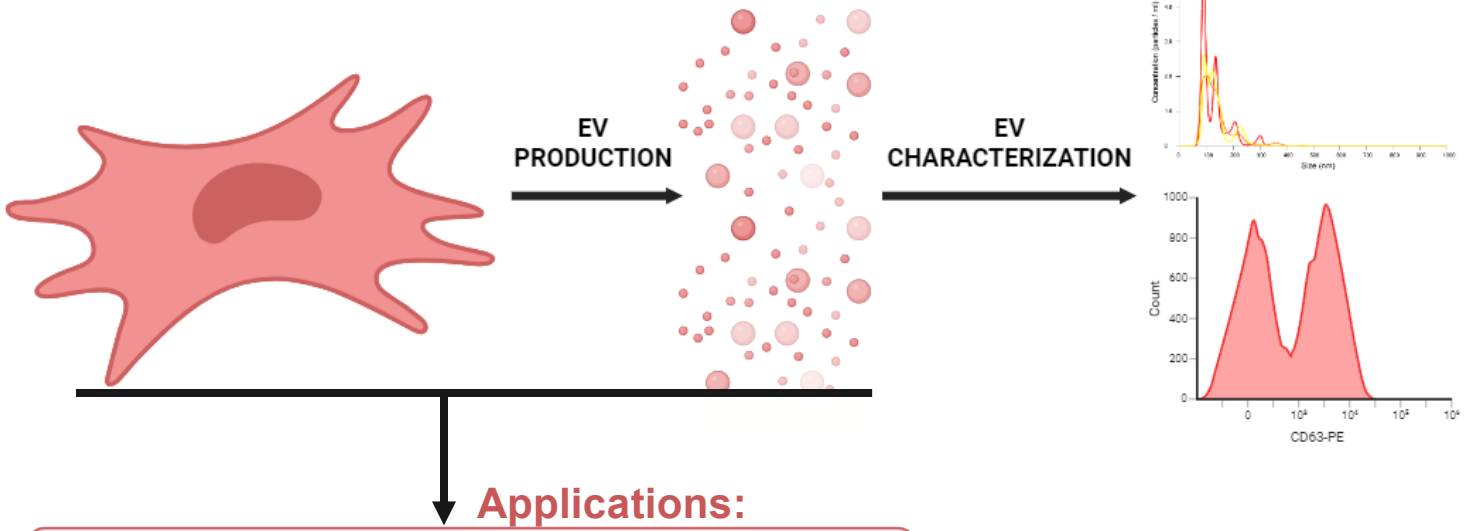
Liposomes containing CDT cause after 72h the typically CDT G2/M block, a decrease in the number of cells, and an increase in cell death; the activation of autophagy via ROS increase.

Fucosylated and undecorated liposomes prime a different cellular response to CDT (more effective for fucose-decorated liposomes).

CDT containing liposome seems to modulate at 48h the expression of markers of Colorectal Cancer Stem Cells.

Regenerative medicine application





Applications:

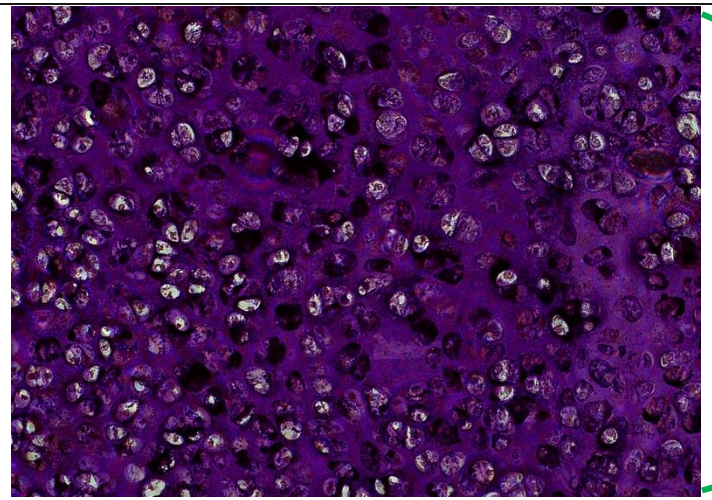
- Bone and cartilage regeneration
- Treatment of liver diseases (hepatitis, fatty liver)
- Treatment of kidney diseases (acute and chronic)
- Treatment of cardiovascular diseases
- Wound healing

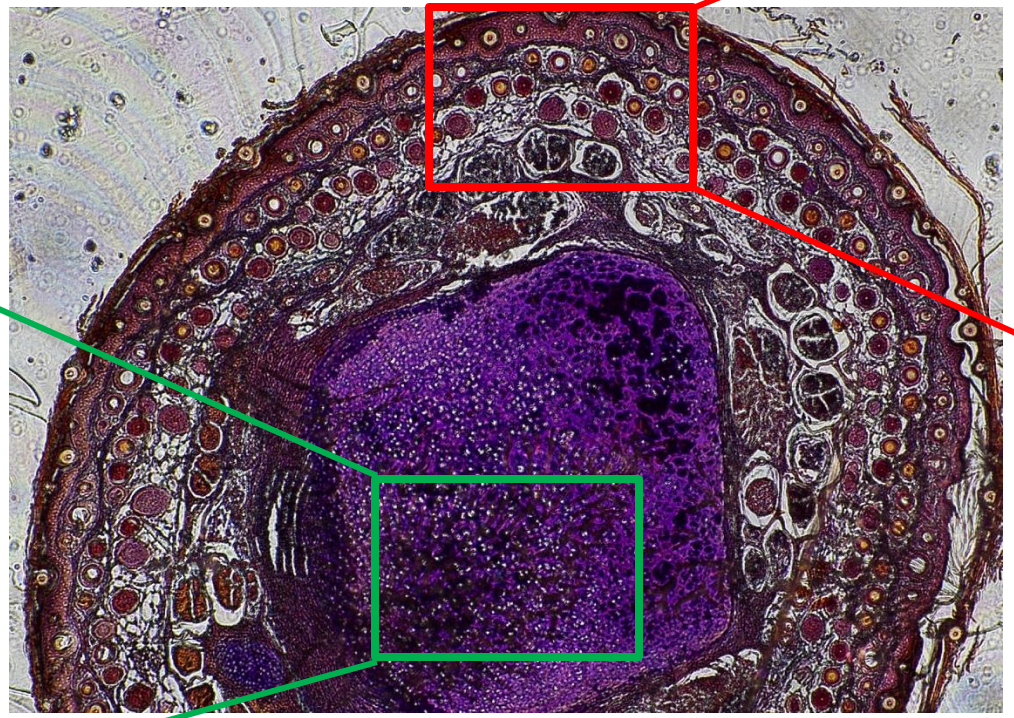
For applications in regenerative medicine, the first step is the isolation of mesenchymal stem cells derived not only from the bone marrow. In addition, bone marrow-MSC and other MSCs can be present in multiple areas within the joint: the synovial fluid, cartilage, and subchondral bone.

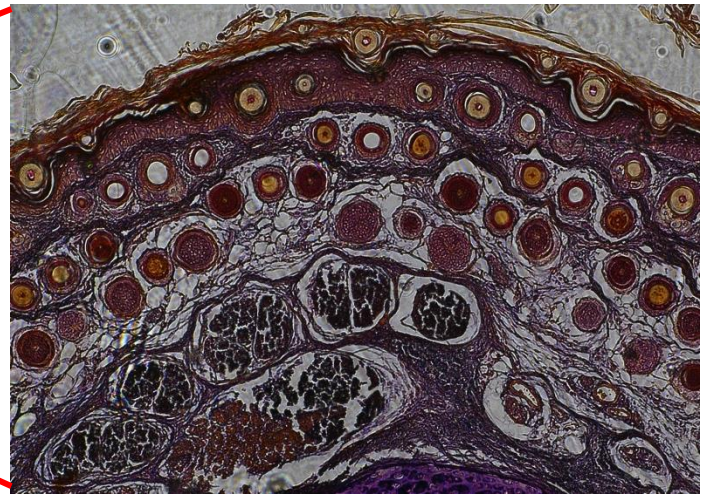
ISOLATION AND CHARACTERIZATION OF CELLS FROM MICE CARTILAGE and BONE

The mouse tail is composed of chondroid tissue, bone, tendon, muscle, and skin with hair follicles, as well as nerves and blood vessels. The tail was washed with 70% ethanol and PBS, and the tail's superficial dermis (red square) was removed using forceps to target chondroid tissue and bone.

Bruneau, Amélie et al. Journal of visualized experiments : JoVE ,41 2176. 30 Jul. 2010, doi:10.3791/2176



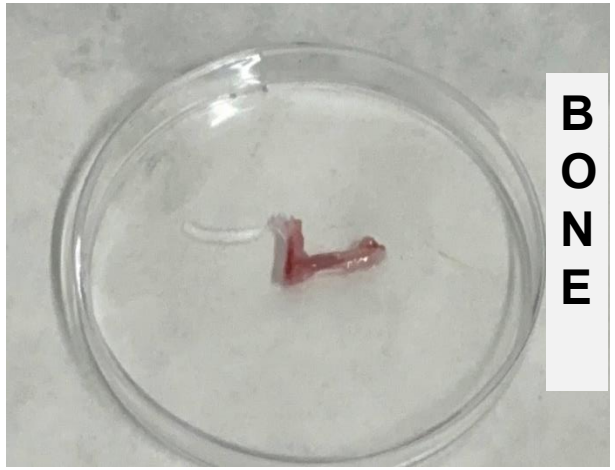


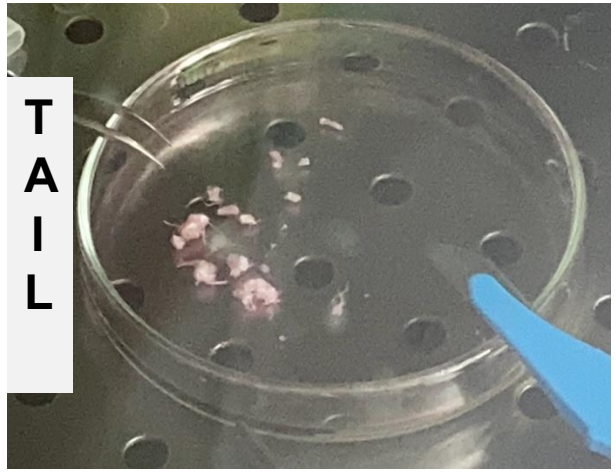


The bone tissues of the femur and tibia were deprived of bone marrow.

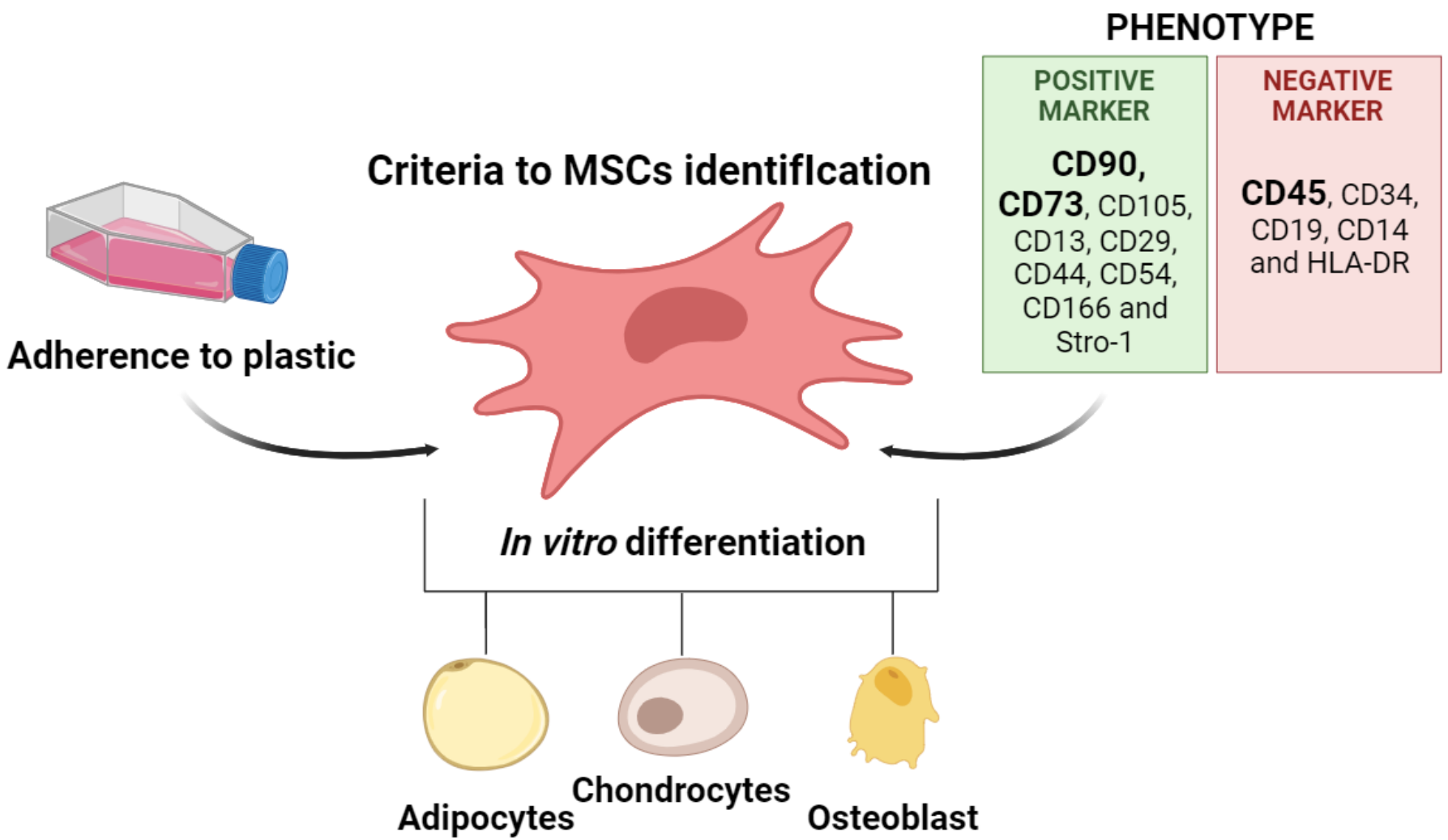
ISOLATION AND CHARACTERIZATION OF CELLS FROM MICE CARTILAGE and BONE

The tail and bone tissues were cut into 1-mm pieces using a scalpel. Automated mechanical disaggregation by Medimachine II was used to process four or five pieces.
The cell suspension was filtered from 70μm to 30μm.



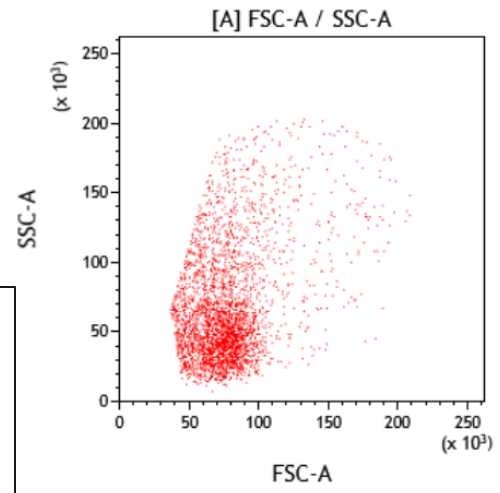


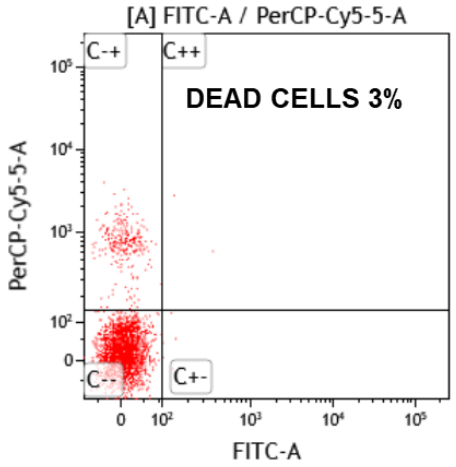
IDENTIFICATION OF MESENCHYMAL STEM CELLS BY INTERNATIONAL SOCIETY FOR CELLULAR THERAPY

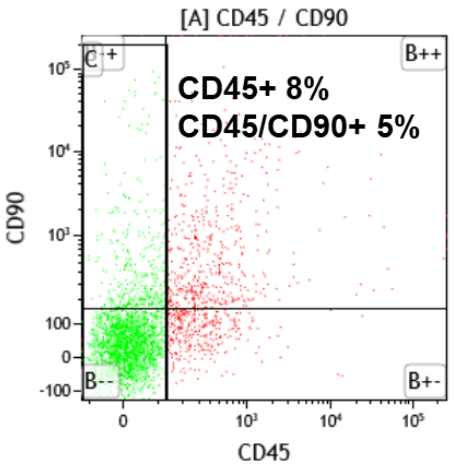


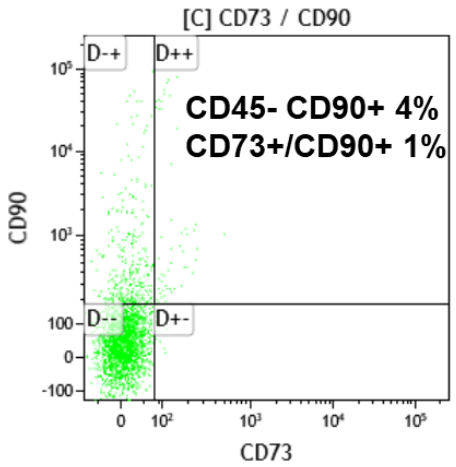
ISOLATION AND CHARACTERIZATION OF CELLS FROM MICE TAIL

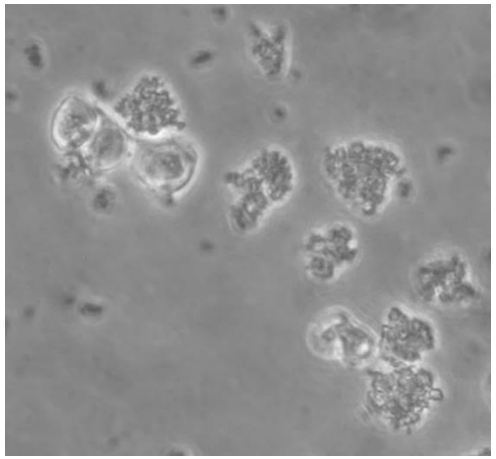
The cell suspension obtained from the tail shows high cell viability. Only 3% was positive for Propidium iodide.

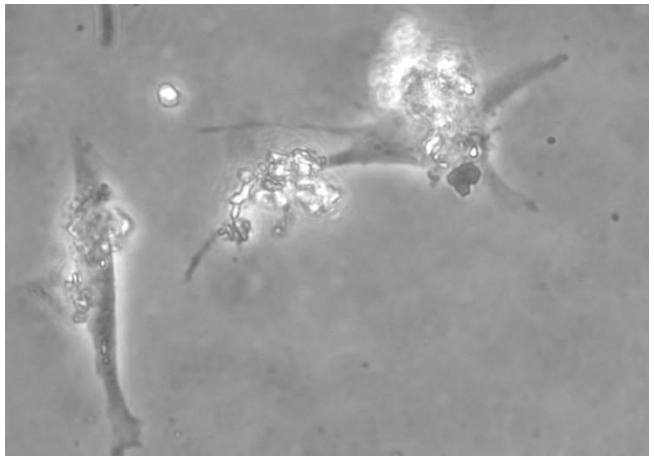






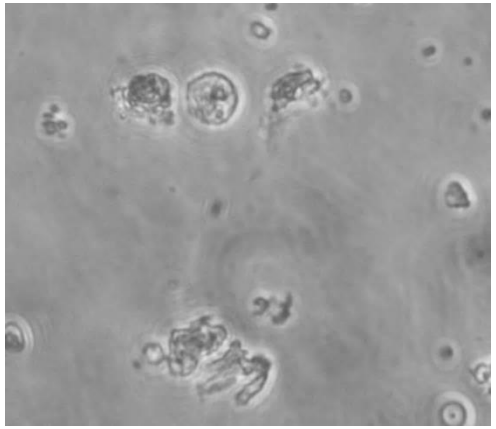


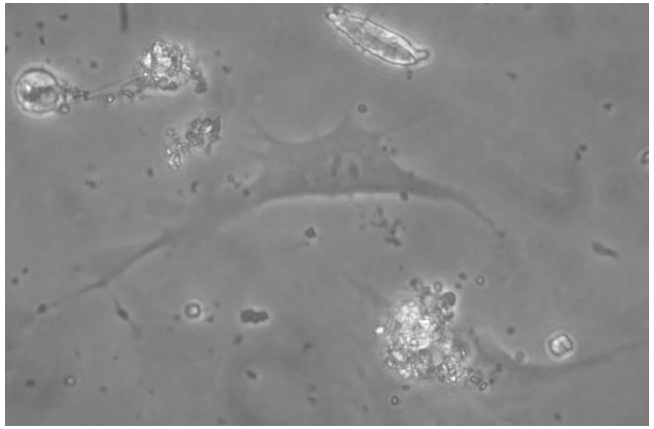




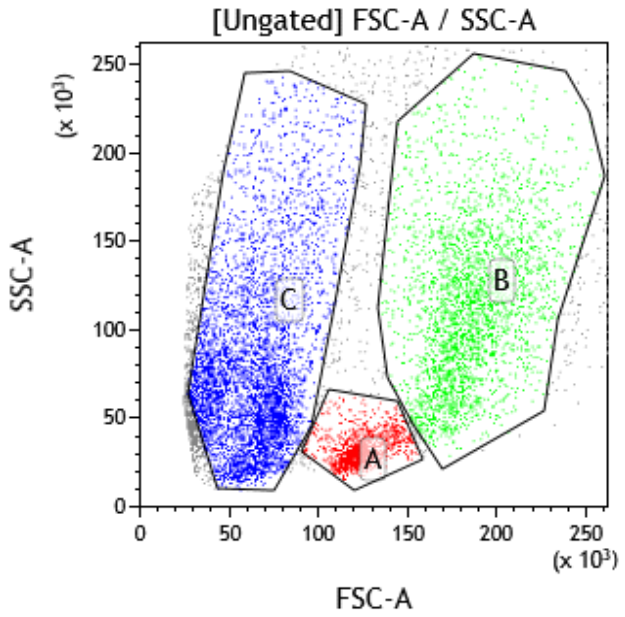
T0

T1

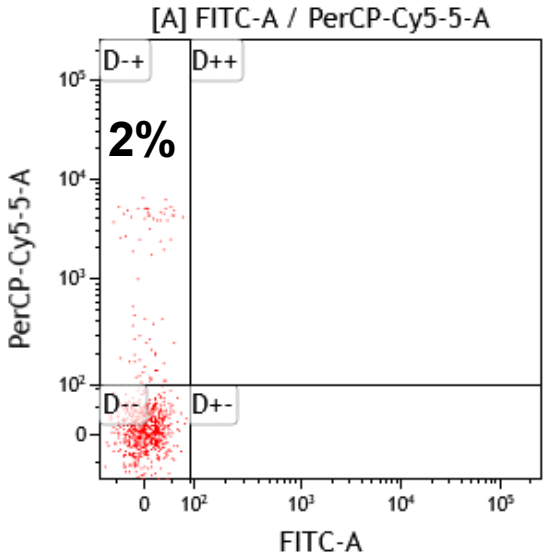




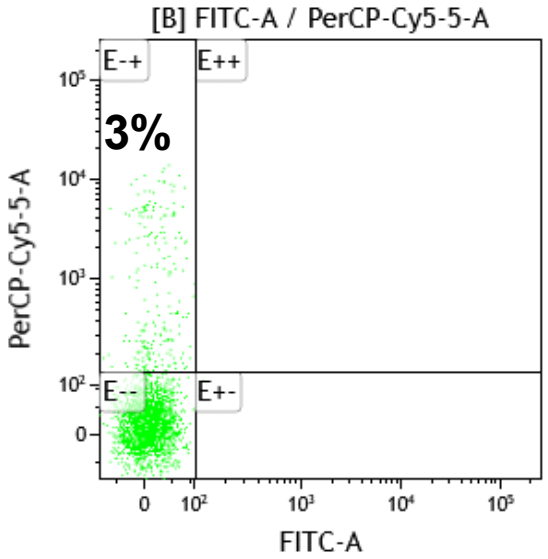
ISOLATION AND CHARACTERIZATION OF CELLS FROM MICE BONE



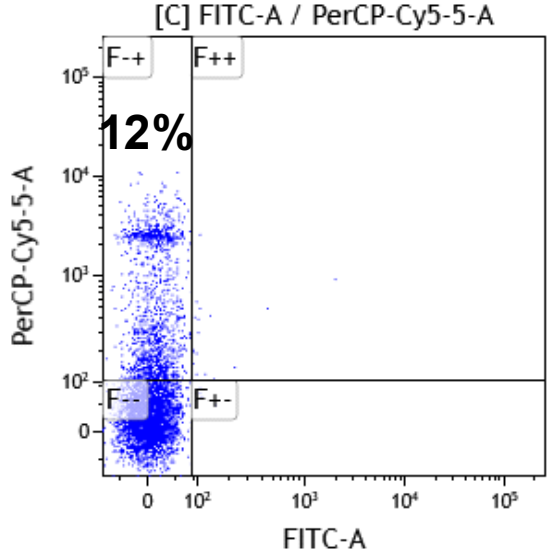
LEUKOCYTES



VIABLE CELLS



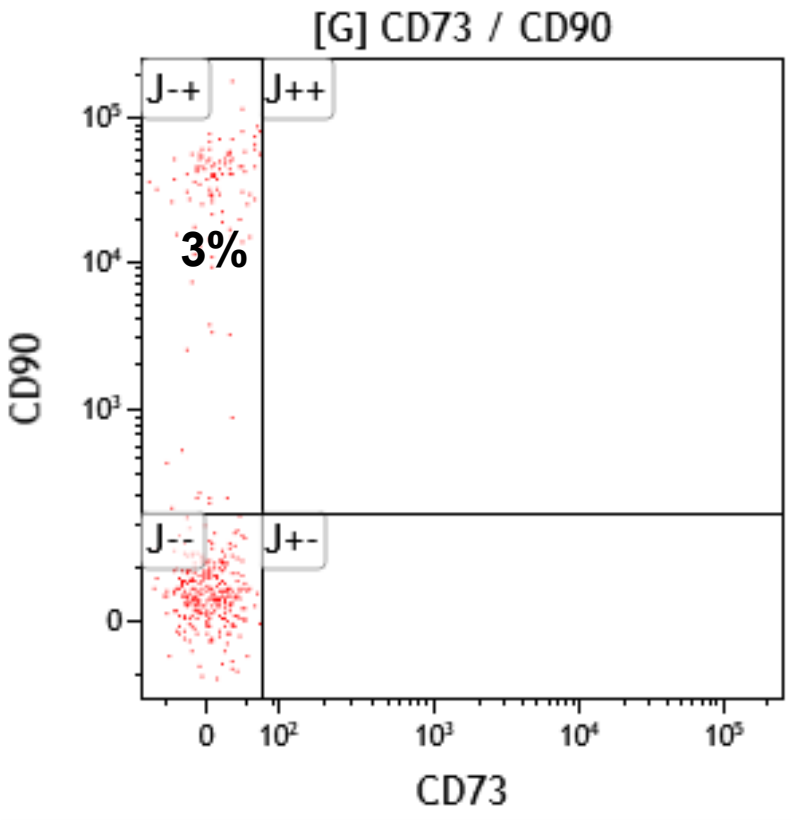
SHRUNKEN CELLS



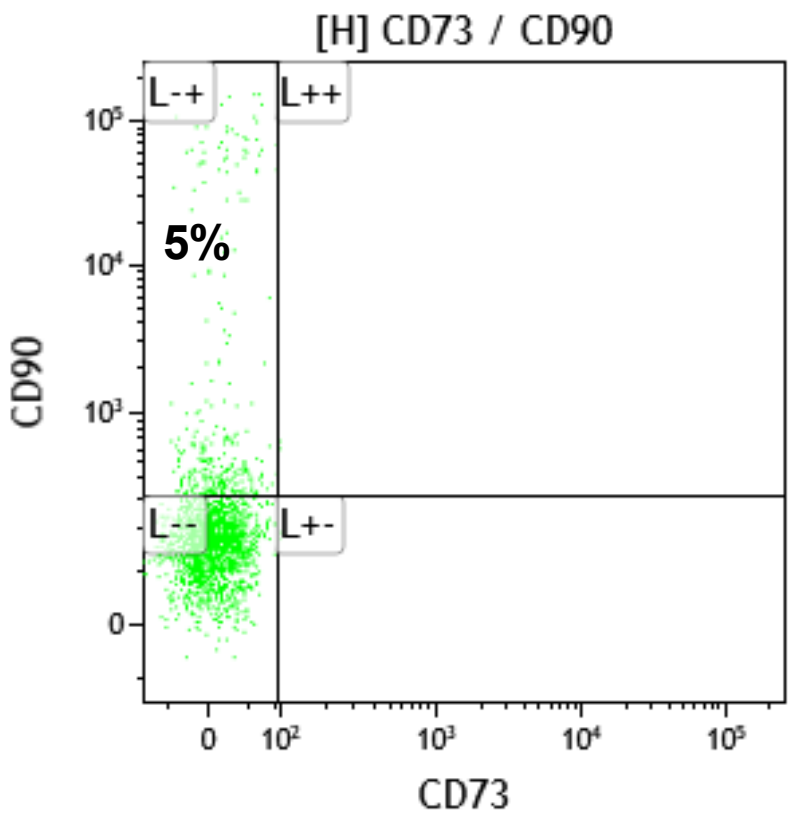
TOTAL DEAD CELLS 17%

FLOW CYTOMETRIC STEM CHARACTERIZATION OF CELLS FROM MICE BONE

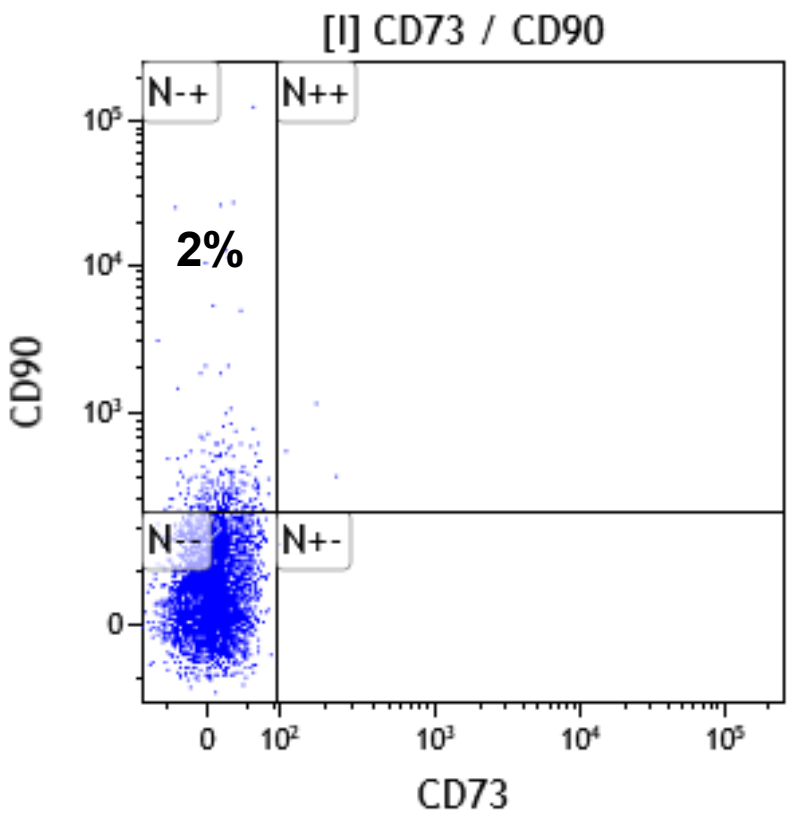
LEUKOCYTES



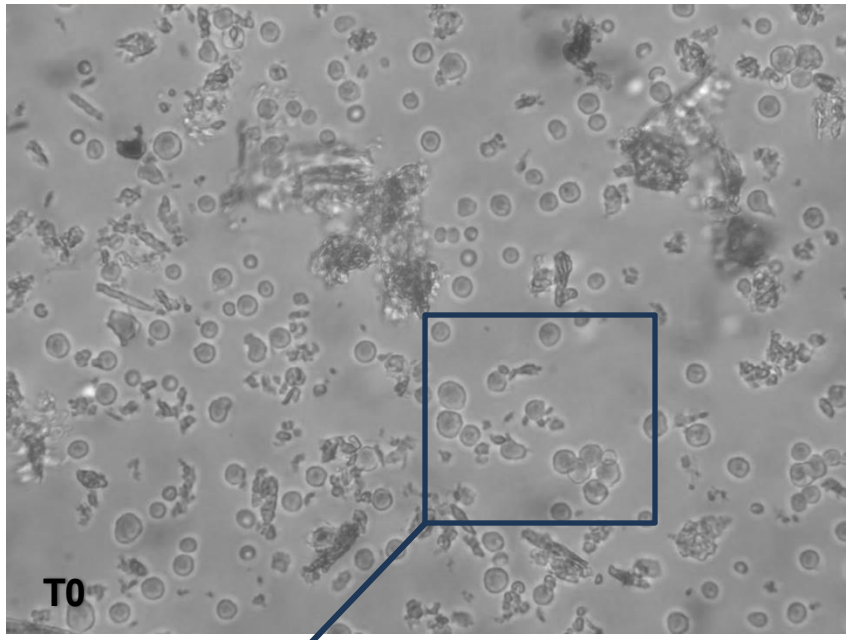
VIABLE CELLS

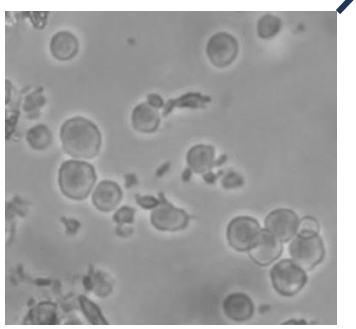


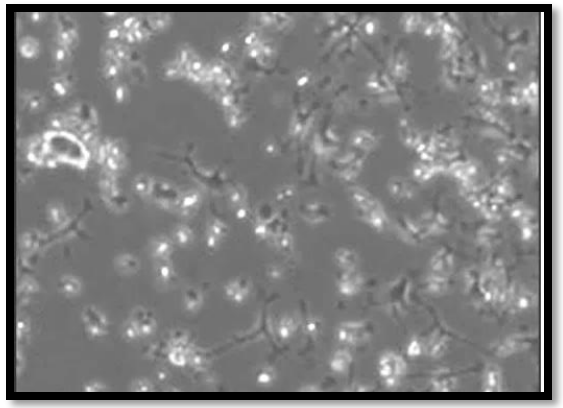
SHRUNKEN CELLS

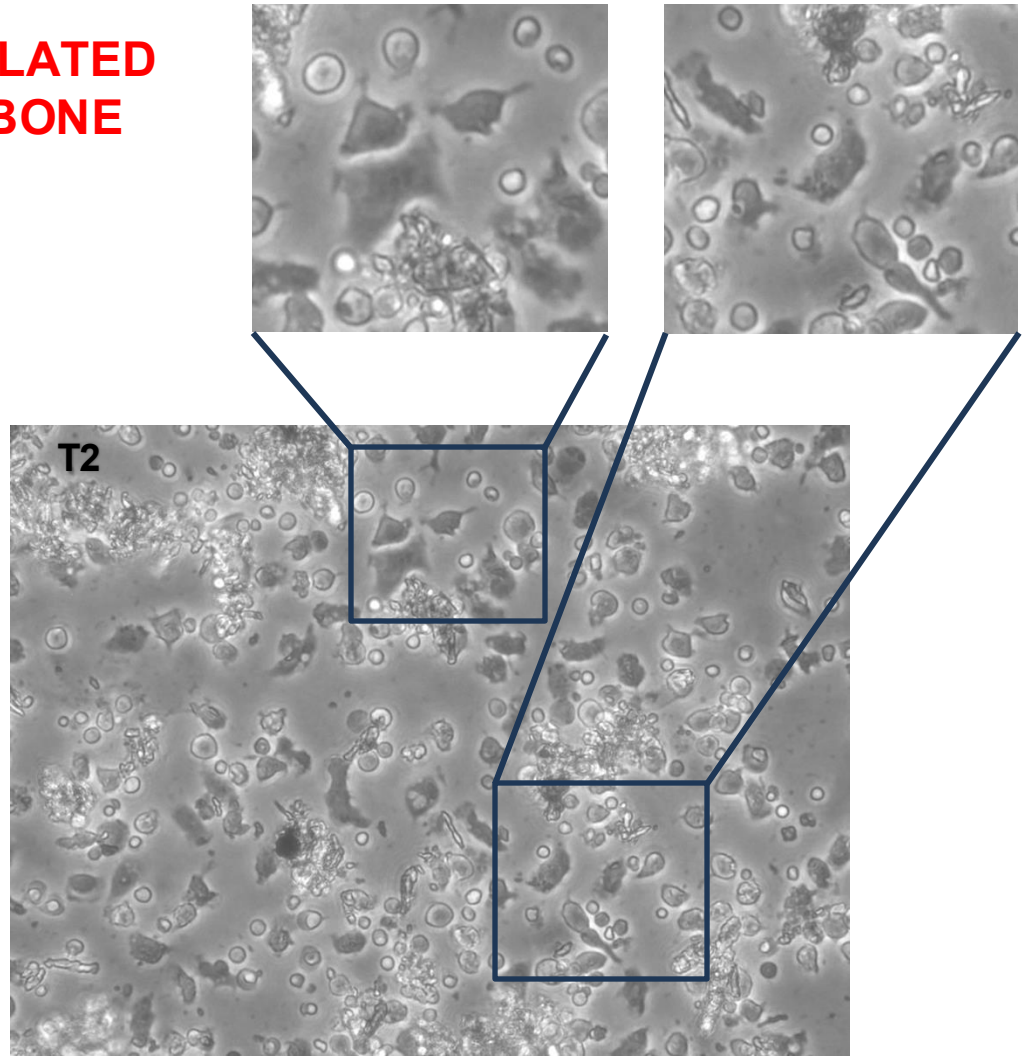


MICROSCOPY OF ISOLATED CELLS FROM MICE BONE







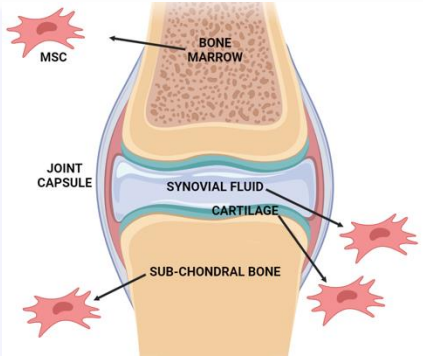


Sun, Q., Gu, Y., Zhang, W. et al. Bone Research 3, 15026 (2015).
https://doi.org/10.1038/boneres.2015.26

Conclusion II

Our preliminary results are encouraging but need the implementation of the mechanical technique (by MediMachine II) due to the peculiar features of these connective tissues and their extracellular matrix.

Indeed, we are working on moving to the human model, taking into account the application of both cells and Evs in regenerative medicine.



MISSIONE 4
ISTRUZIONE
RICERCA

Finanziato dall'Unione europea
NextGenerationEU

Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

VITALITY

Acknowledgments

Prof. Barbara Canonico

Prof. Luca Casettari

Prof. Michele Guescini

Prof. Stefano Papa

Dr. Mattia Tiboni

Dr. Daniele Lopez

Dr. Giovanna Panza

1506 UNIVERSITÀ DEGLI STUDI DI URBINO CARLO BO
DISB DIPARTIMENTO DI SCIENZE BIOMOLECOLARI

Prof. Rita Crinelli

Prof. Alessandra Fraternale

Dr. Francesca Pierigè

Laboratorio di Citometria

Pharma Tech Lab
Formulation and Delivery Sciences @ University of Urbino Carlo Bo