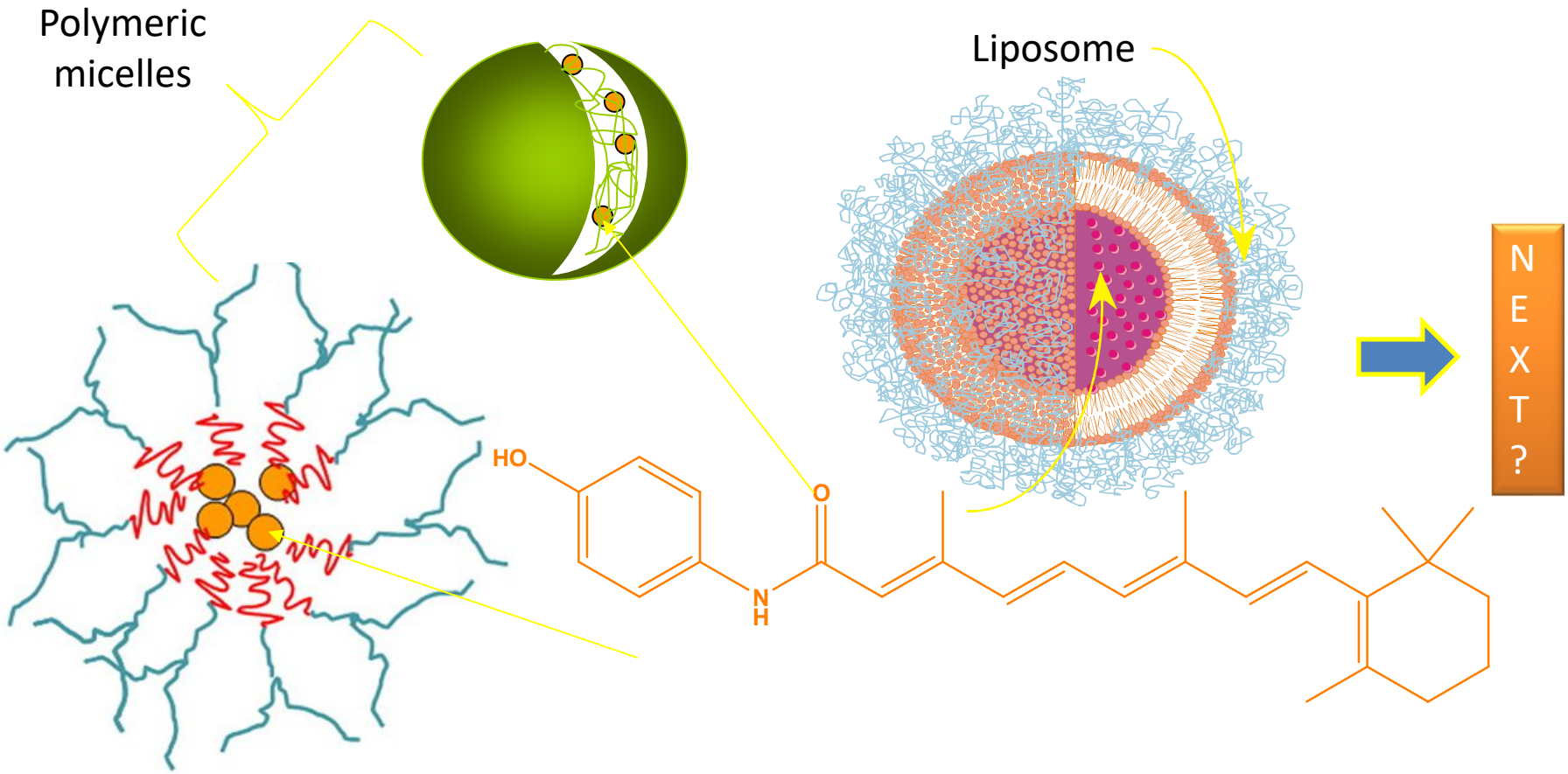
Guendalina Zuccari[1,*], Carla Villa [1], and Eleonora Russo [1]

[1] Department of Pharmacy, University of Genoa, Viale Cembrano 4, 16145 Genoa, Italy.

* Corresponding author: guendalina.zuccari@unige.it

Encapsulating Fenretinide into Nanoparticles: Where we are and Where we are going

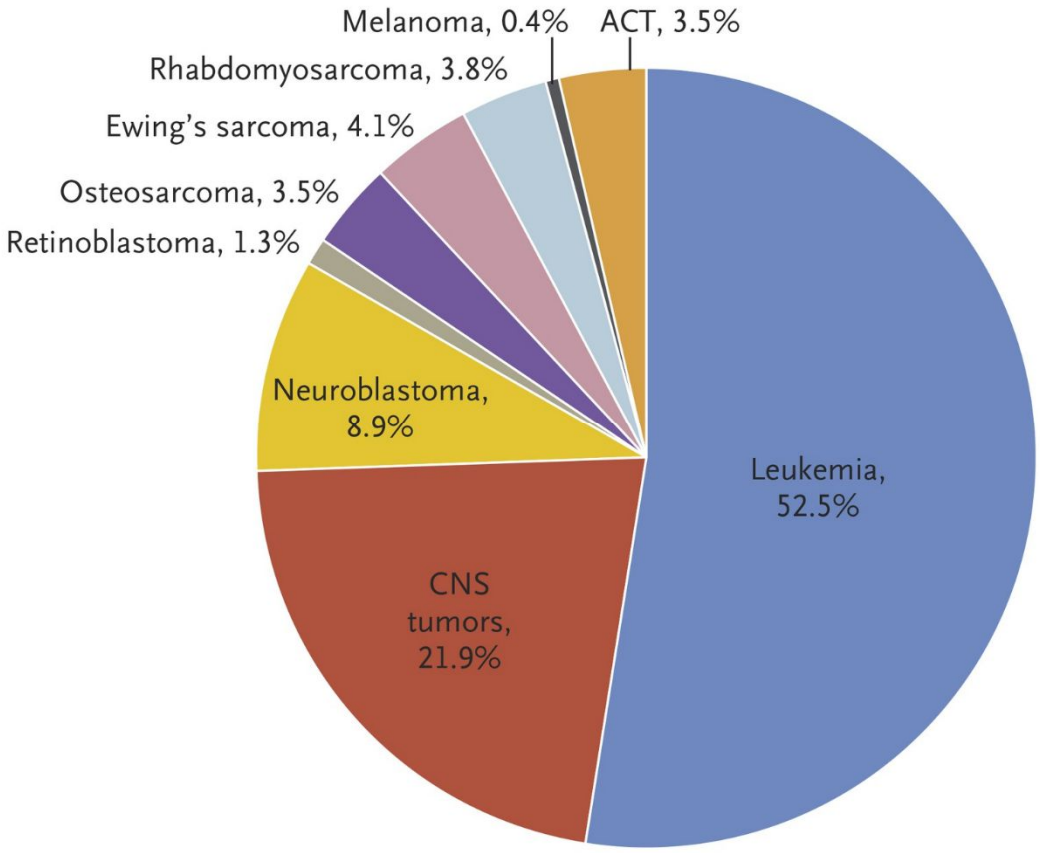


Abstract: Fenretinide (N-(4-hydroxyphenyl)-retinamide, 4-HPR) is a synthetic retinoid with fewer adverse effects than natural retinoids, effective against ovarian, prostate, small cell lung, brain, neuroectodermal-derived tumors. Clinical responses in adult and pediatric patients are often partial, revealing a limited activity of 4-HPR against existing disease. The underlying causes of this slight therapeutic efficacy consist in 4-HPR poor water solubility, low bioavailability and high first-pass hepatic effect. To overcome these drawbacks, nanomedicine could represent a valid alternative. We have already developed nanostructured drug delivery systems able to encapsulate 4-HPR. Indeed, polymeric micelles made of branched polyethylene glycol or amphiphilic dextrin have been prepared and investigated for their effectiveness both in vitro and in vivo. We have also designed a liposomal 4-HPR endowed with an active targeting moiety. Recently, we have focused our attention on a more physiological and not immunogenic drug delivery system. With this in mind 4-HPR-loaded mesenchymal stem cells-derived extracellular vesicles have been prepared. The drug amount encapsulated into the vesicles was determined by HPLC. Briefly, prior 4-HPR quantification an extraction procedure was optimized and, to estimate the analyte recovery an internal standard was employed. Since for this purpose, N-(4-ethoxyphenyl)-retinamide (4-EPR) has been reported, we developed a new operator-friendly one-step procedure to synthetize highly pure 4-EPR in quantitative yield. Studies aim to establish the best drug loading conditions are ongoing.

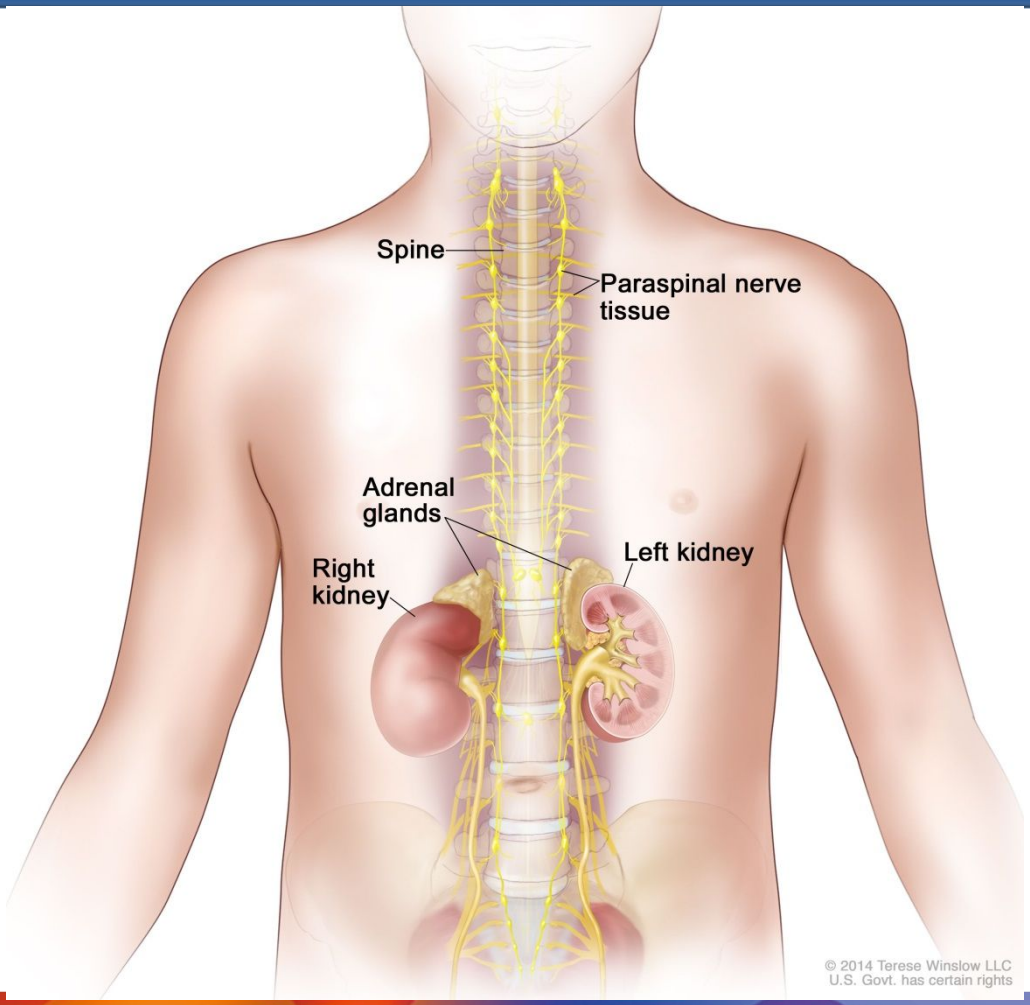
Keywords: Nanomedicine; Fenretinide; Drug Delivery Systems; Cancer; Retinoids

Frequency of Paediatric Cancer Types

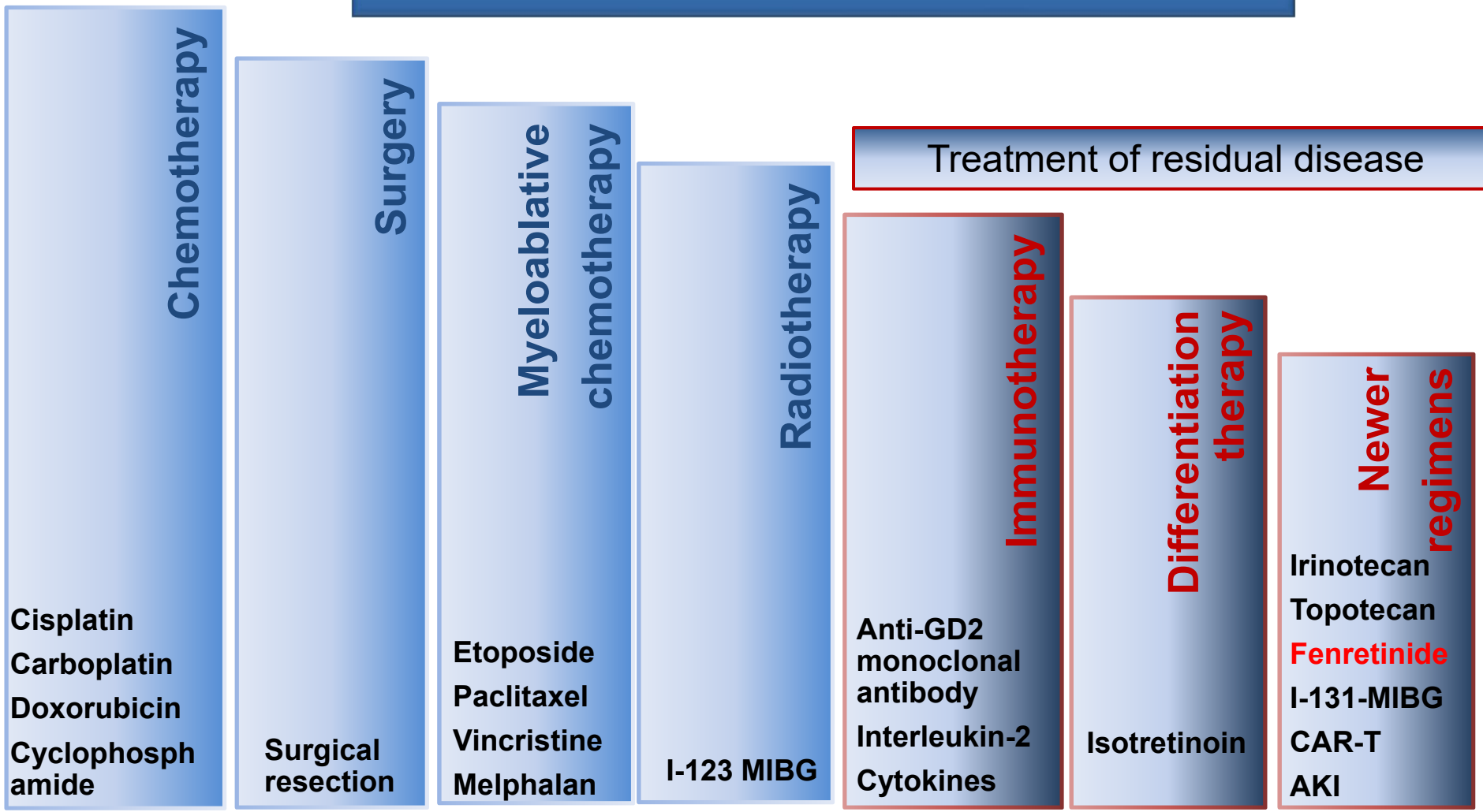
1 in 7,000 children



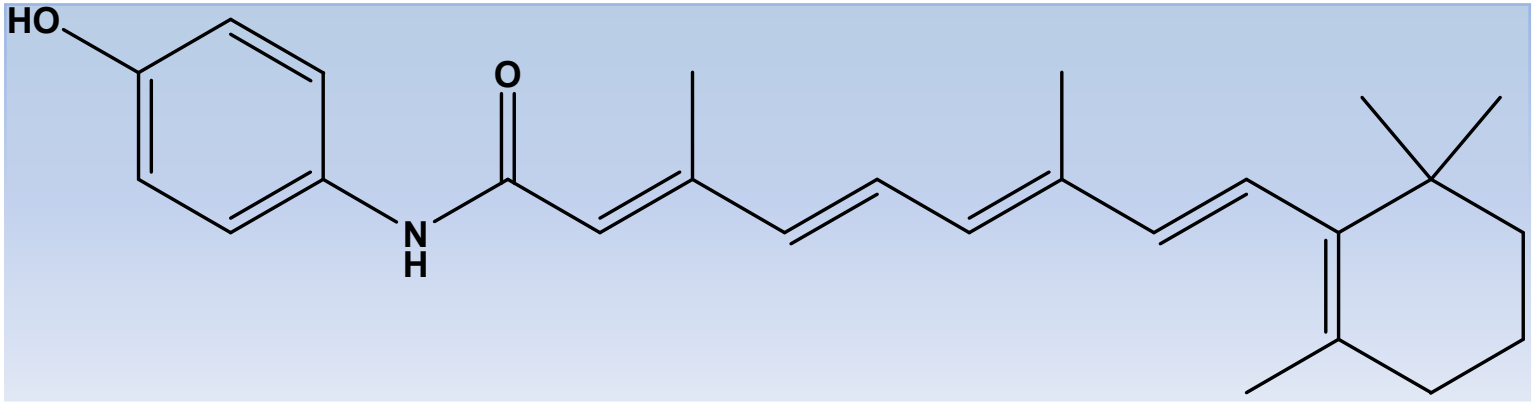
Primary distribution of Neuroblastoma



Therapy for Neuroblastoma



Fenretinide



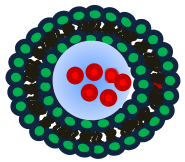
N-(4-hydroxyphenyl)retinamide (4-HPR)

PROS	CONS
HIGH ANTITUMOR ACTIVITY	LOW SOLUBILITY
FAVORABLE TOXICOLOGICAL PROFILE	POOR BIOAVALABILITY
NO INDUCTION OF RESISTANCE	CLINICAL TRIALS WITH HIGH VARIABILITY IN RESULTS

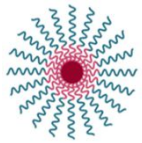
Nanomedicines for Neuroblastoma

Nanostructured Drug Delivery Systems

- Liposomes

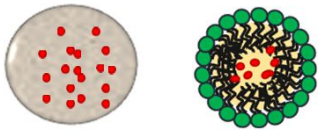


- Polymeric Micelles



- Polymer-Drug Conjugates

- Polymeric/Lipid Nanoparticles



- Complexes

BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine

Research Article

nanomedjournal.com

Novel micelles based on amphiphilic branched PEG as carriers for fenretinide

Isabella Orienti, PhD[a,*], Guendalina Zuccari, PhD[a], Mirella Falconi, MD, PhD[b], Gabriella Teti, PhD[b], Nicola A. Illingworth, PhD[c], Gareth J. Veal, PhD[c]

JPP
Journal of Pharmacy
And Pharmacology

Enhanced anti-neuroblastoma activity of a fenretinide complexed form after intravenous administration

Roberta Carosio[a], Vito Pistoia[a], Isabella Orienti[b,*], Franca Formelli[c], Elena Cavadini[c], Salvatore Mangraviti[d], Paolo G. Montaldo[a], Emanuela Ognio[e], Laura Emionite[e] and Guendalina Zuccari[b,*]

Contents lists available at SciVerse ScienceDirect

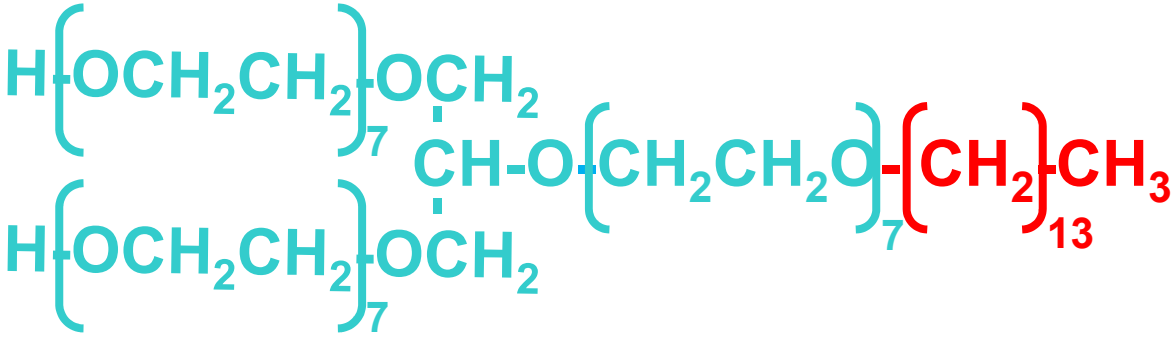
Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

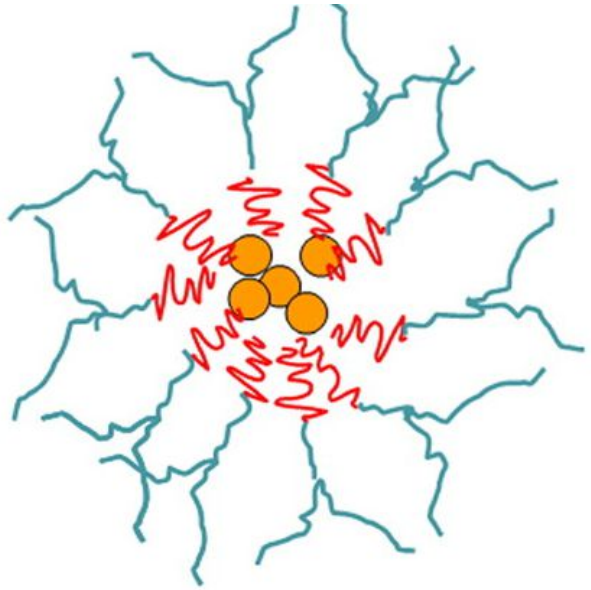
Enhanced anti-tumor and anti-angiogenic efficacy of a novel liposomal fenretinide on human neuroblastoma

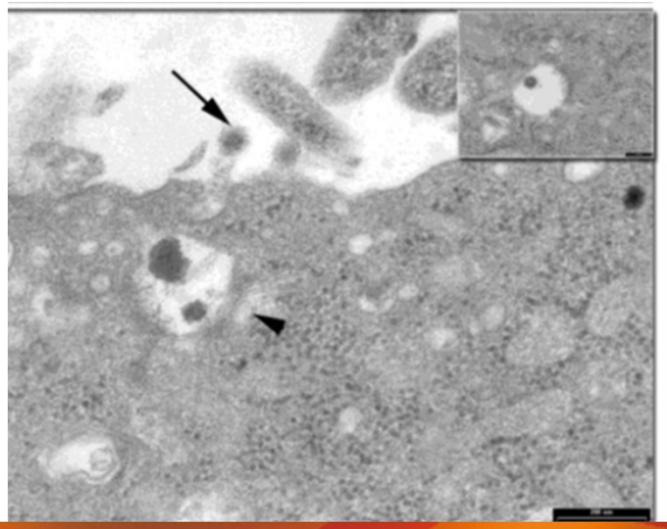
Daniela Di Paolo [a,1], Fabio Pastorino [a,*,1], Guendalina Zuccari [b], Irene Caffa [a,2], Monica Loi [a], Danilo Marimpietri [c], Chiara Brignole [a], Patrizia Perri [a], Michele Cilli [d], Beatrice Nico [e], Domenico Ribatti [e], Vito Pistoia [c], Mirco Ponzoni [a,3], Gabriella Pagnan [a,**,3]

Our Previous Results-1



Glycerol ethoxylate linked to myristyl chain





Micelles entering a neuroblastoma SH-SY5Y cell

Branched PEG

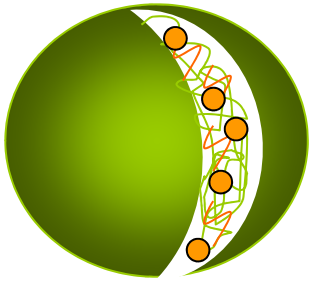
Hydrophobic chain

4-HPR

Our Previous Results-2

Self-Assembly

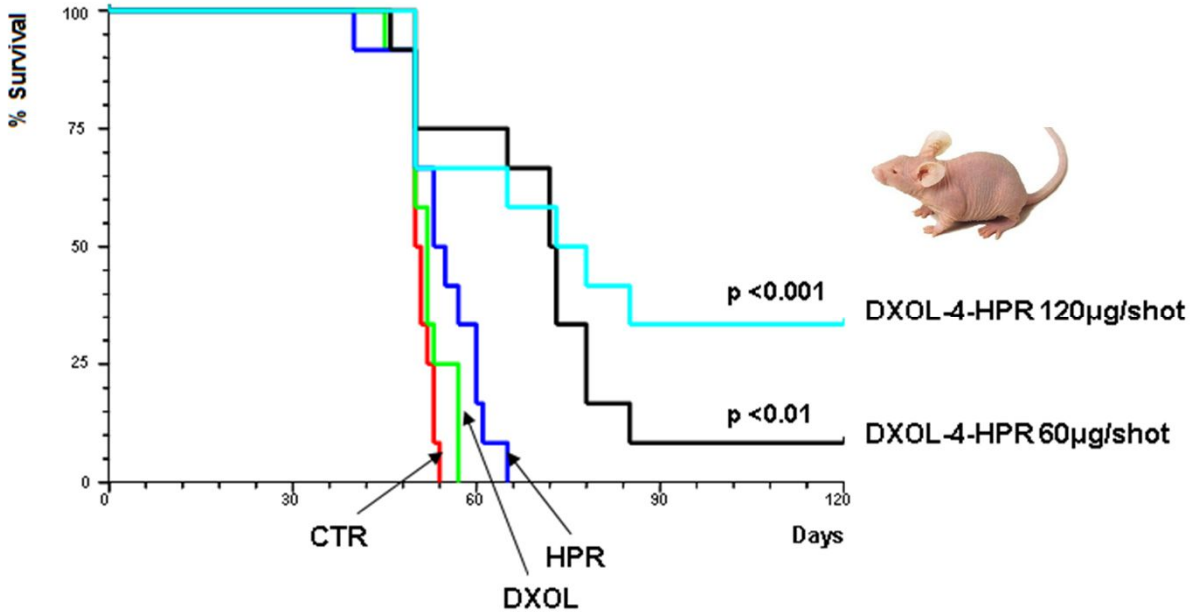
4-HPR



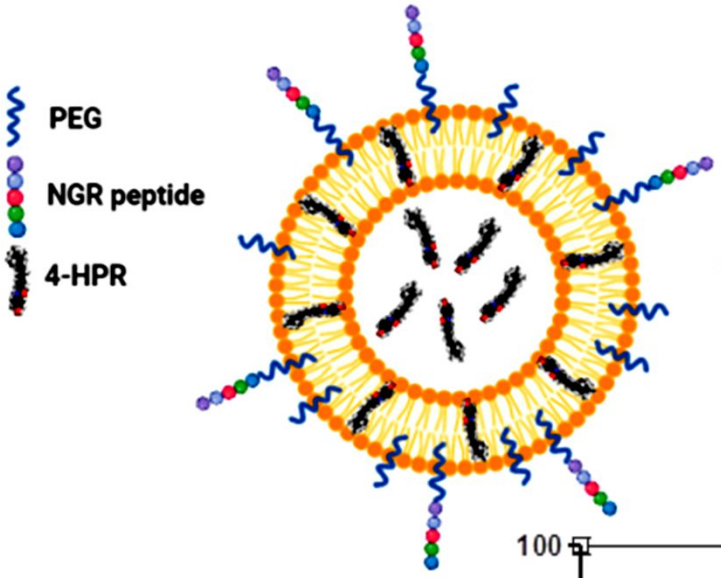
Dextrin

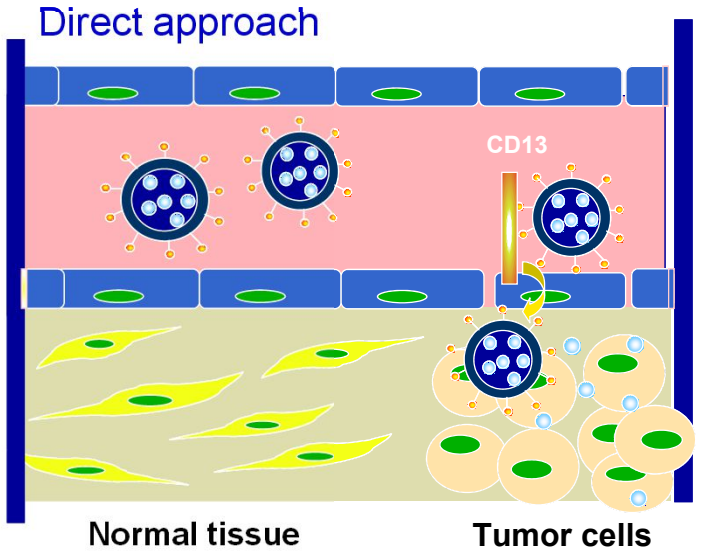
Hydrophobic chain

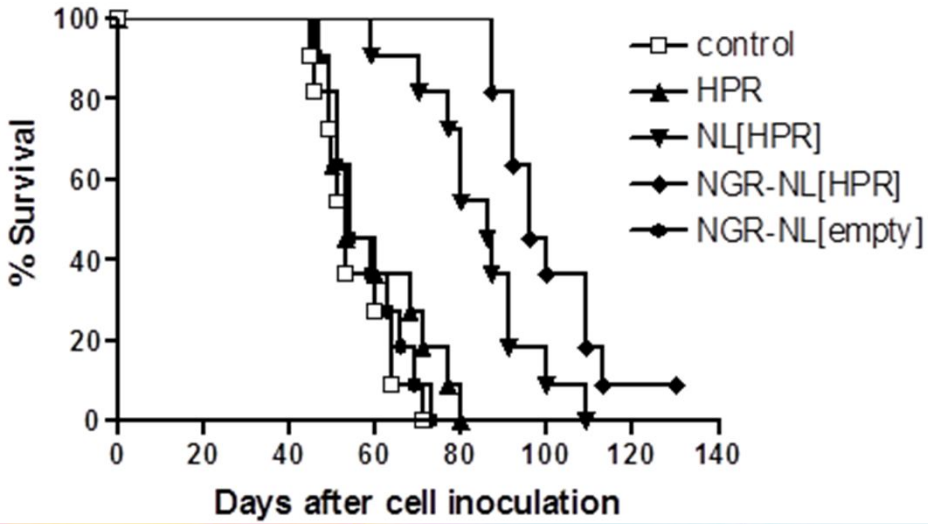
4-HPR



Our Previous Results-3



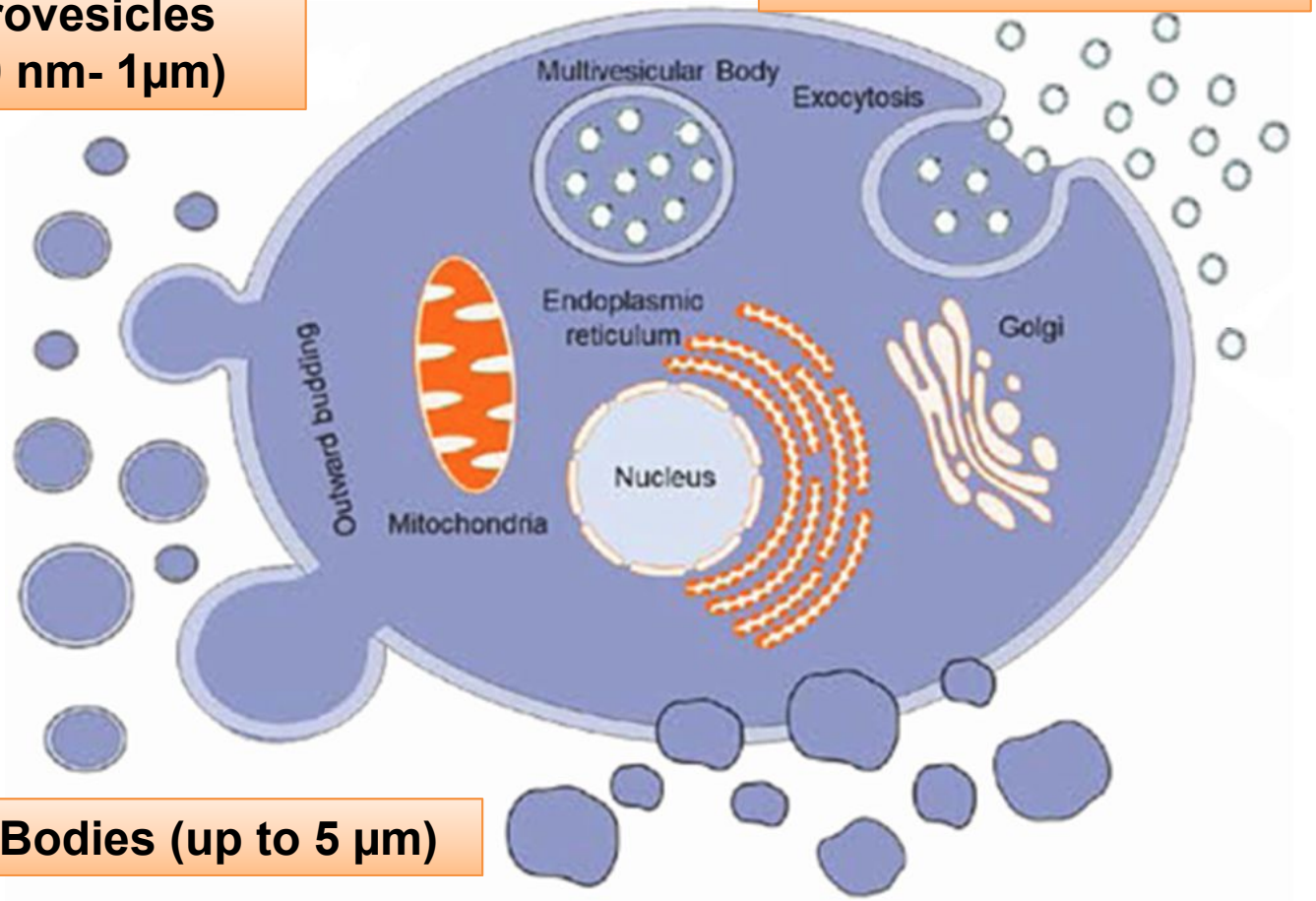




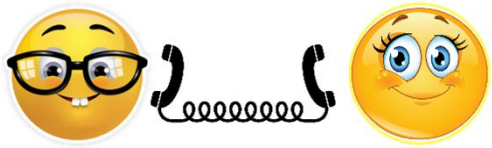
Extracellular Vesicles

Exosomes (30-150 nm)

Microvesicles (100 nm- 1µm)



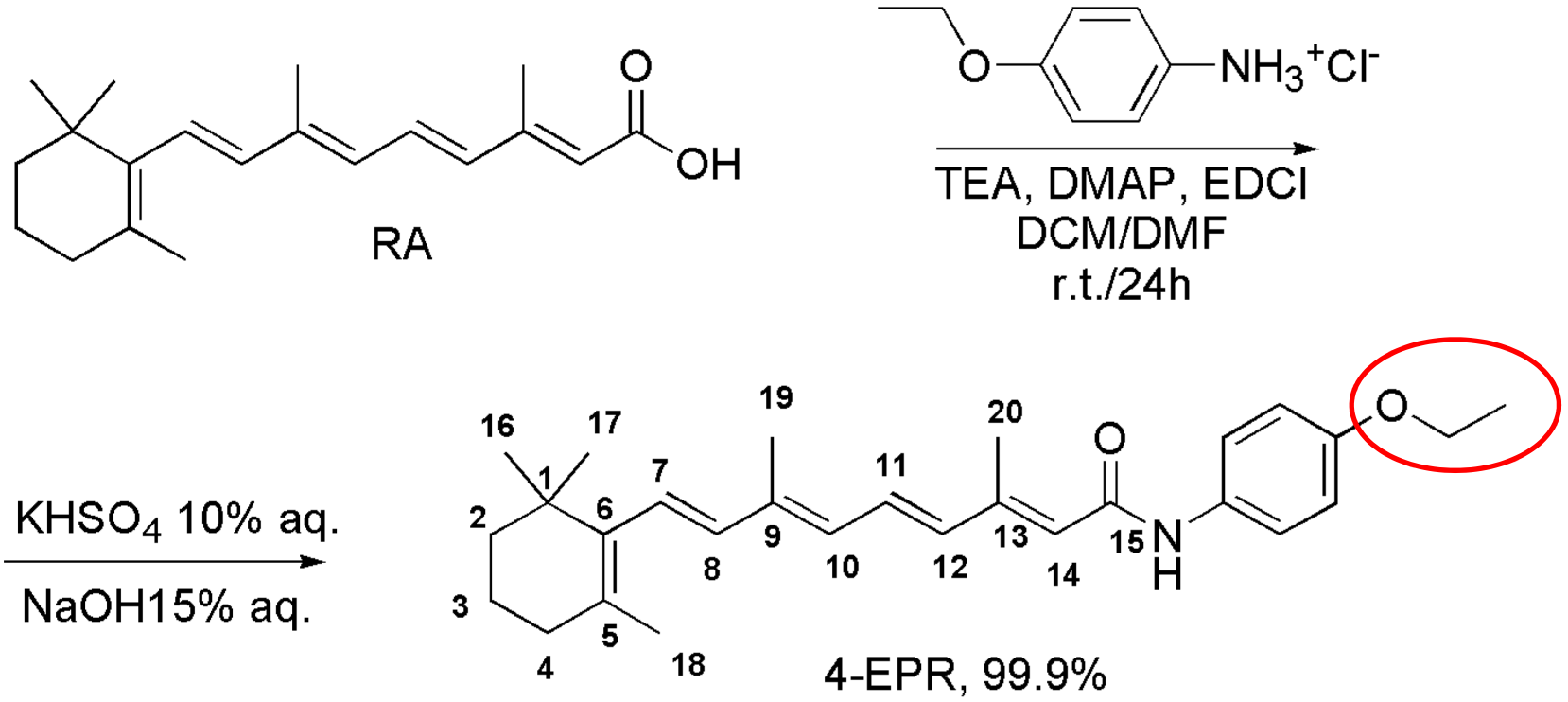
CROSS-TALK



Peptides
RNA
Lipids
Proteins

Apoptotic Bodies (up to 5 µm)

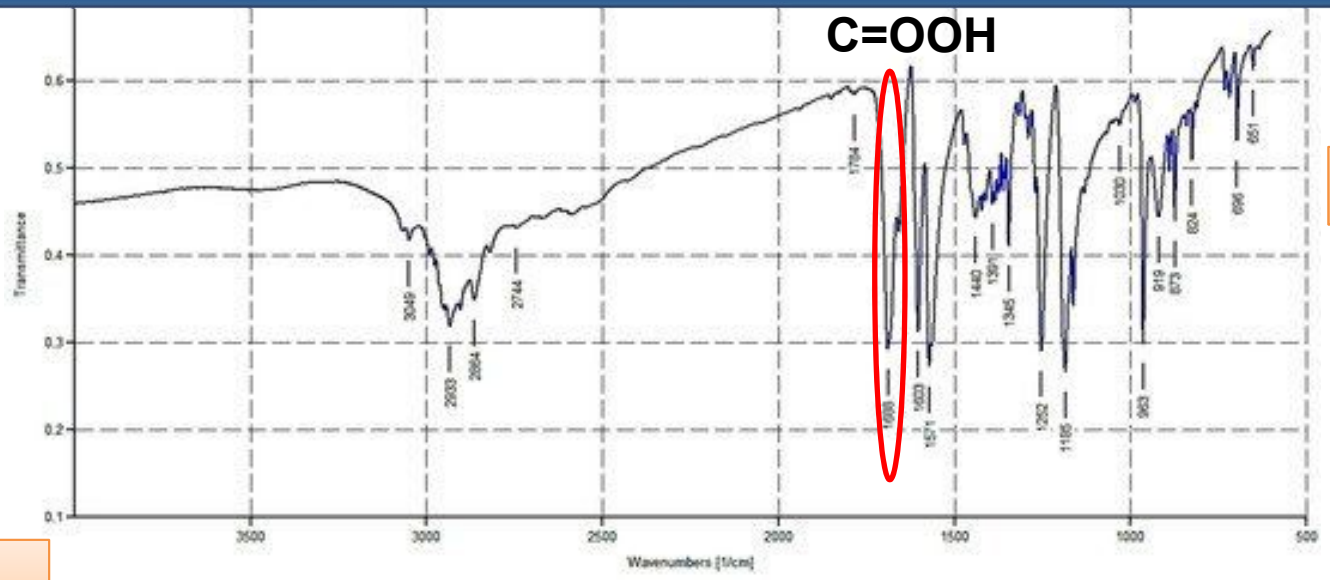
Synthesis of the Internal Standard *N*-(4-ethoxyphenyl)-retinamide (4-EPR)



RA = retinoic acid; TEA = triethylamine; DMAP = 4-dimethylaminopyridine; EDCI = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; DCM = dichloromethane; DMF = *N*,*N*-dimethylformamide

Alfei S, Zuccari G. *Molecules* 2022.

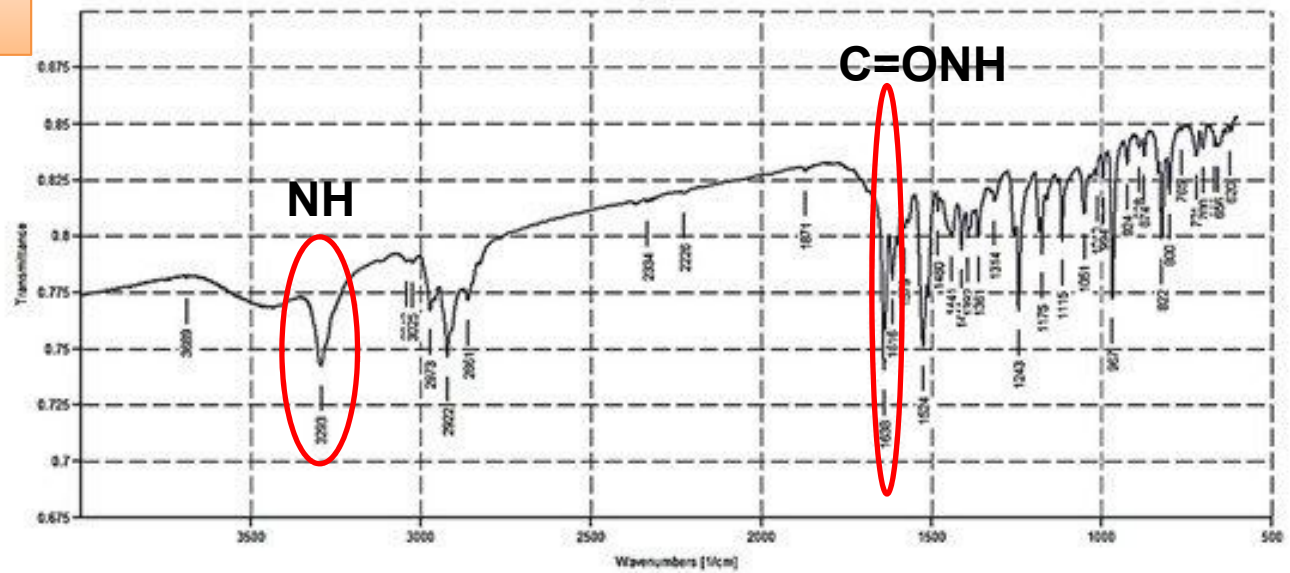
Synthesis of the Internal Standard *N*-(4-ethoxyphenyl)-retinamide (4-EPR)



(a)

RA

✓ FTIR spectra

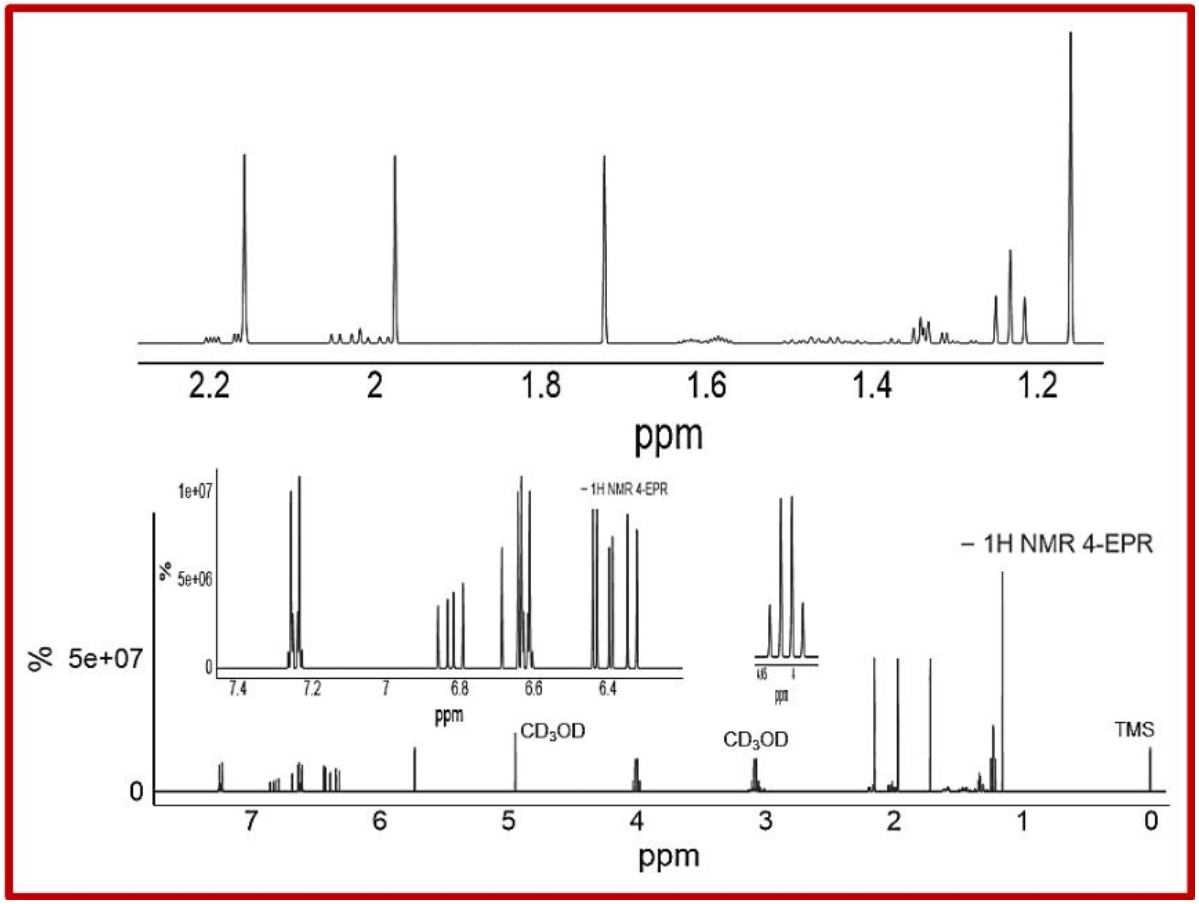


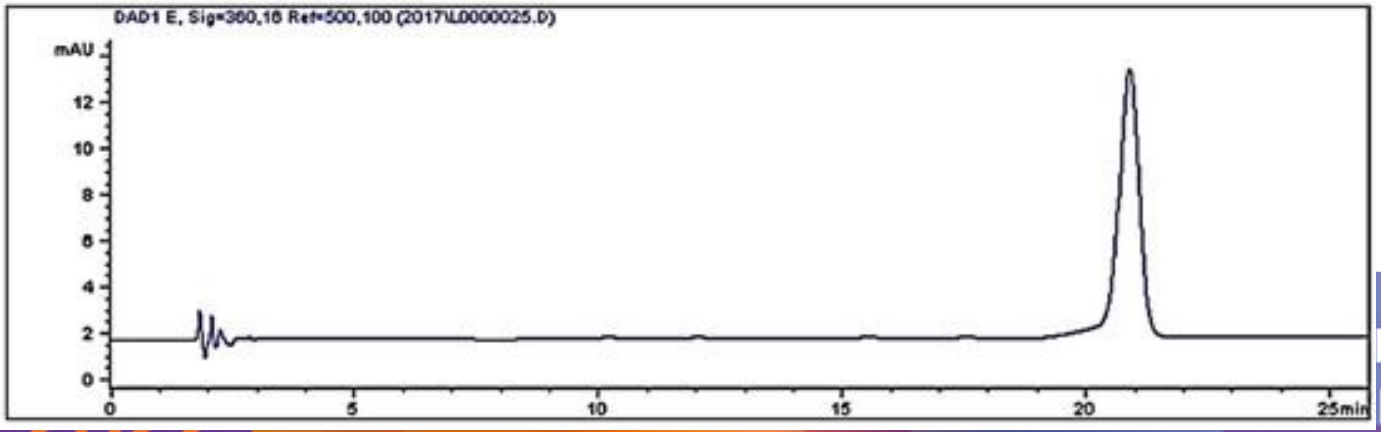
(b)

4-EPR

ECMC 2022

✓ ^{1}H NMR Spectrum

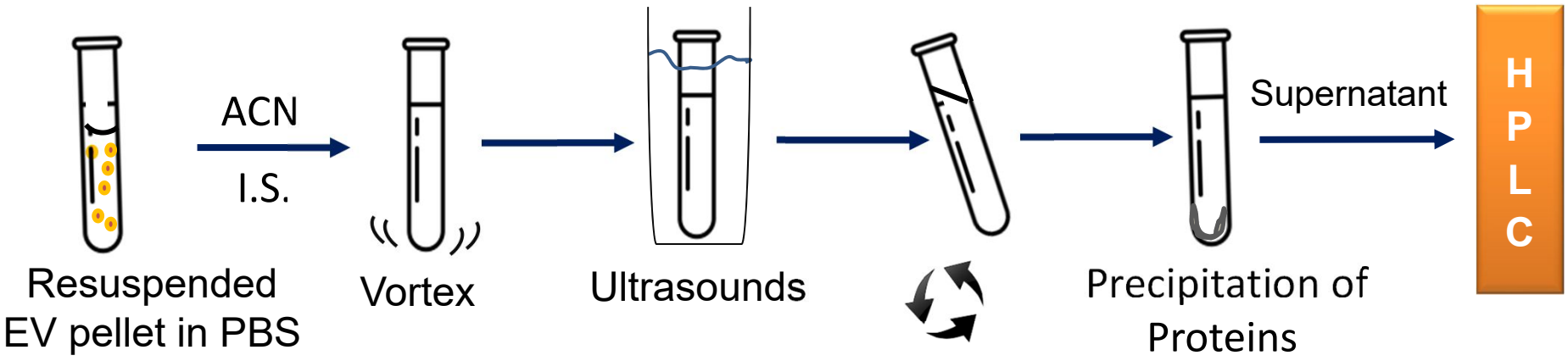


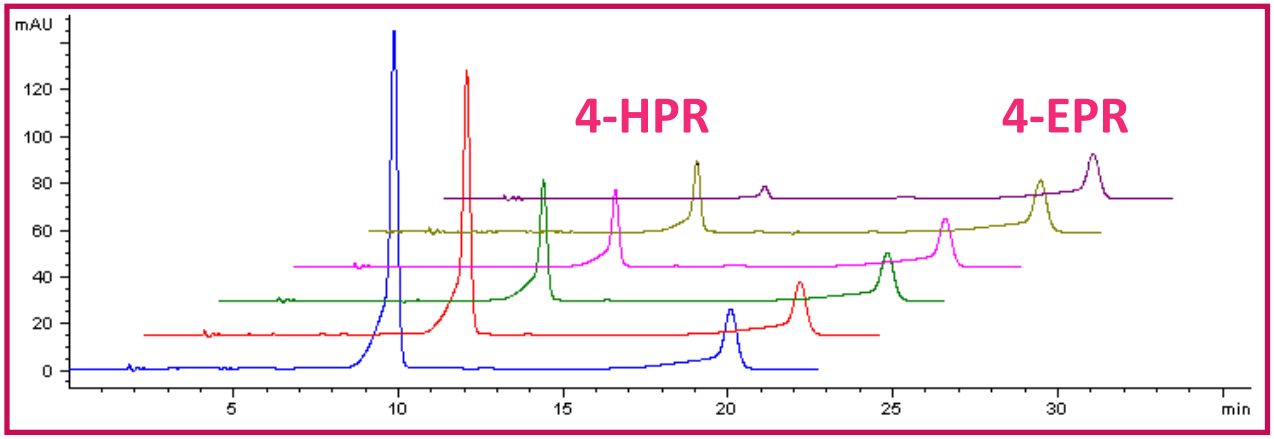


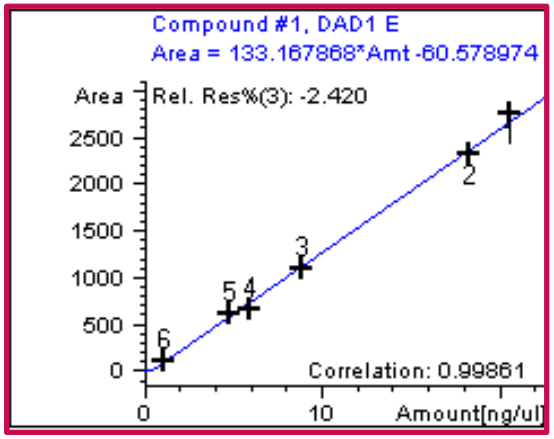
✓ RP HPLC-DAD analysis of 4-EPR

Alfei S, Zuccari G. *Molecules* 2022.

Vesicle Cargo Measurement by HPLC-1







Vesicle Cargo Measurement by HPLC-2

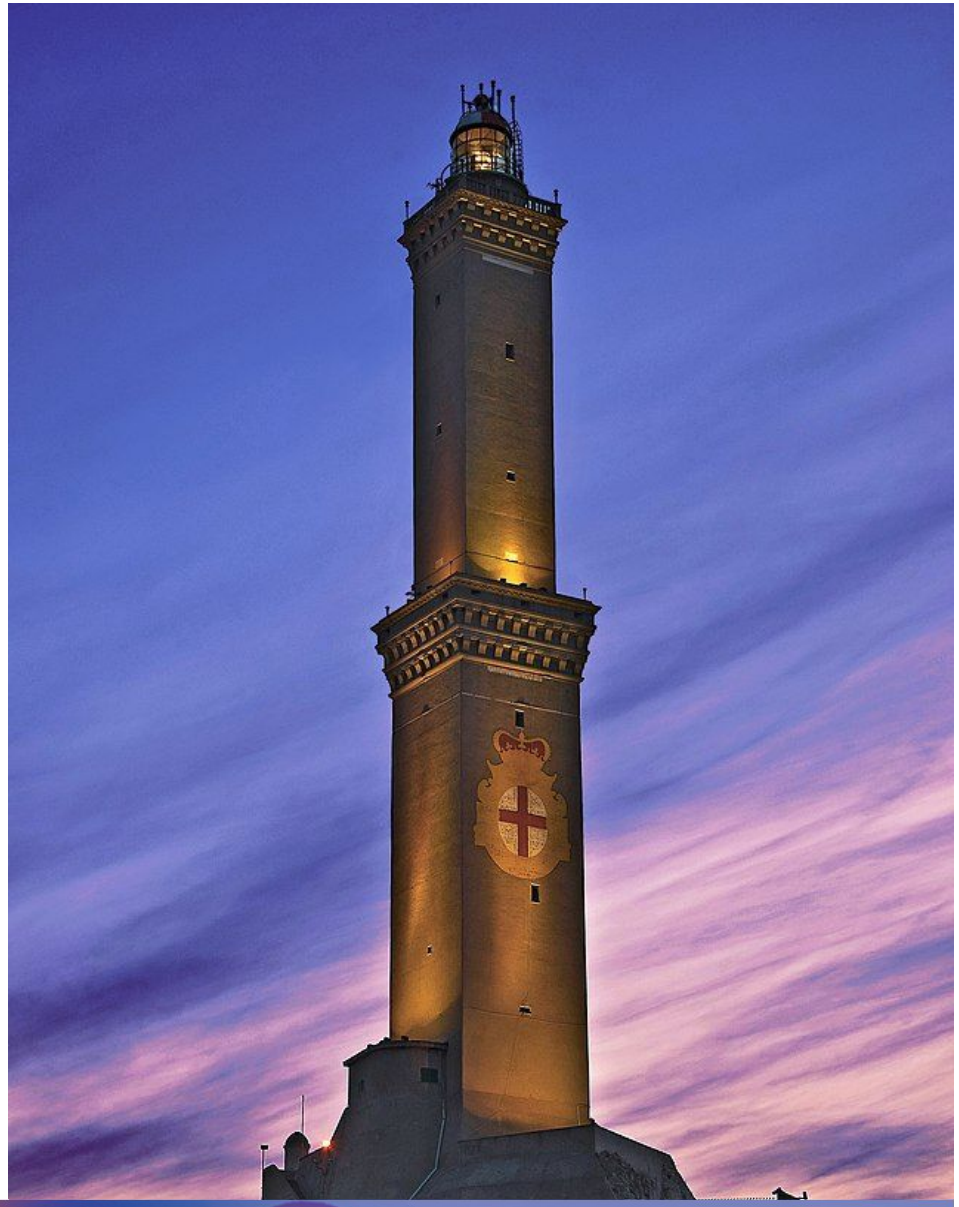
Treatment of MSCs with 4-HPR		
Concentration	Time	μmol 4-HPR per EV
10 μM	48 h	1.68±0.43 E-14
20 μM	48 h	5.93±0.32 E-14
25 μM	48 h	6.48±0.51 E-14

Conclusions

- ✓ A method for the extraction of Fenretinide from Extracellular Vesicles and its detection by HPLC was optimized

Future Perspectives

- ✓ Experiments for the evaluation of uptake, apoptosis, cell cycle are on going
- ✓ In vivo experiments in metastatic mouse model to evaluate the effectiveness against minimal residual disease
- ✓ Use of bioreactors to scale up the production



Department of Pharmacy:

Prof. Eleonora Russo

Prof. Carla Villa

Prof. Silvana Alfei

ISTITUTO GIANNINA GASLINI

ISTITUTO PEDIATRICO
DI RICOVERO E CURA
A CARATTERE SCIENTIFICO

Cell Factory:

Dr. Danilo Marimpietri

Dr. Alessia Zorzoli

ECMC 2022

The 8th International Electronic Conference on Medicinal Chemistry

01–30 NOVEMBER 2022 | ONLINE