



LIPOSOMAL FORMULATIONS OF NEW DECORPORATION MOLECULES FOR THE TREATMENT OF INTERNAL STRONTIUM/COBALT CONTAMINATIONS

Krakow, 28 April 2022

Céline Bouvier-Capely
Elias Fattal
François Fay
Géraldine Landon
Guillaume Phan

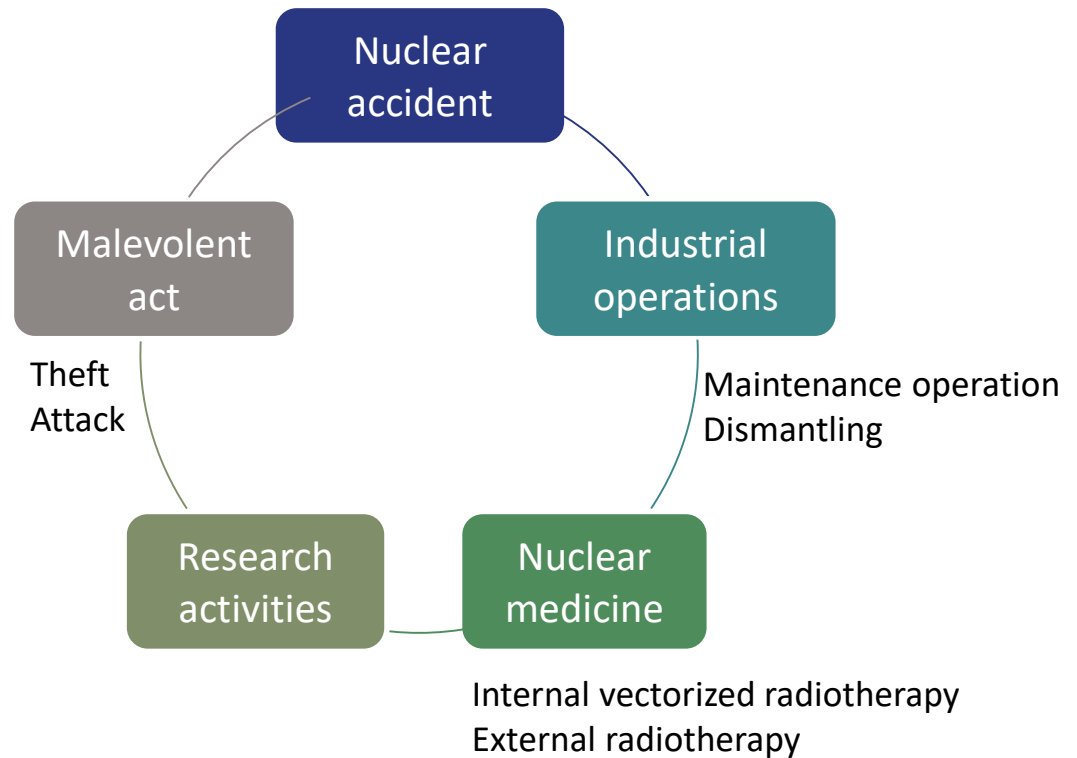
WHAT ARE THE EXPOSURE SITUATIONS?

COBALT-60

- Metal
- Activation product
- β^- emitter
- $T_{1/2} = 5.27$ years

STRONTIUM-90

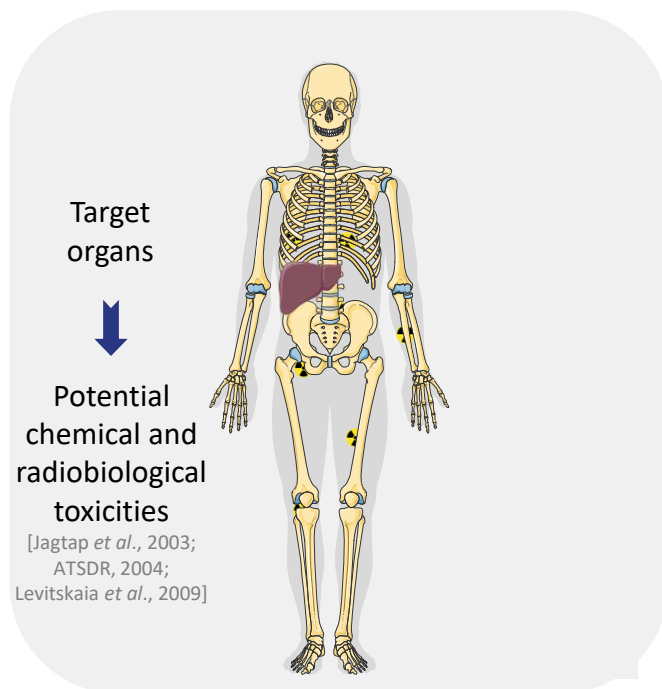
- Alkaline earth metal
- Fission product
- β^- emitter
- $T_{1/2} = 29.14$ years



- ➔ Civilian population
- ➔ Military responders
- ➔ Nuclear industry workers

WHAT ARE THE HEALTH RISKS?

INTERNAL CONTAMINATION



Recommended emergency treatment

[Guide ASN. 2008]

^{60}Co

- 1/ DTPA calcium salt
- 2/ Cobalt gluconate

^{90}Sr

- 1/ Ammonium chloride
- 2/ Calcium gluconate
- 3/ Sodium alginate

➔ Moderate efficacy and lack of specificity

[Fisher *et al.*, 1978; Sharma *et al.*, 2011]

To develop a more effective pharmacological treatment for decorporation

WHAT IS A DECORPORATING AGENT?

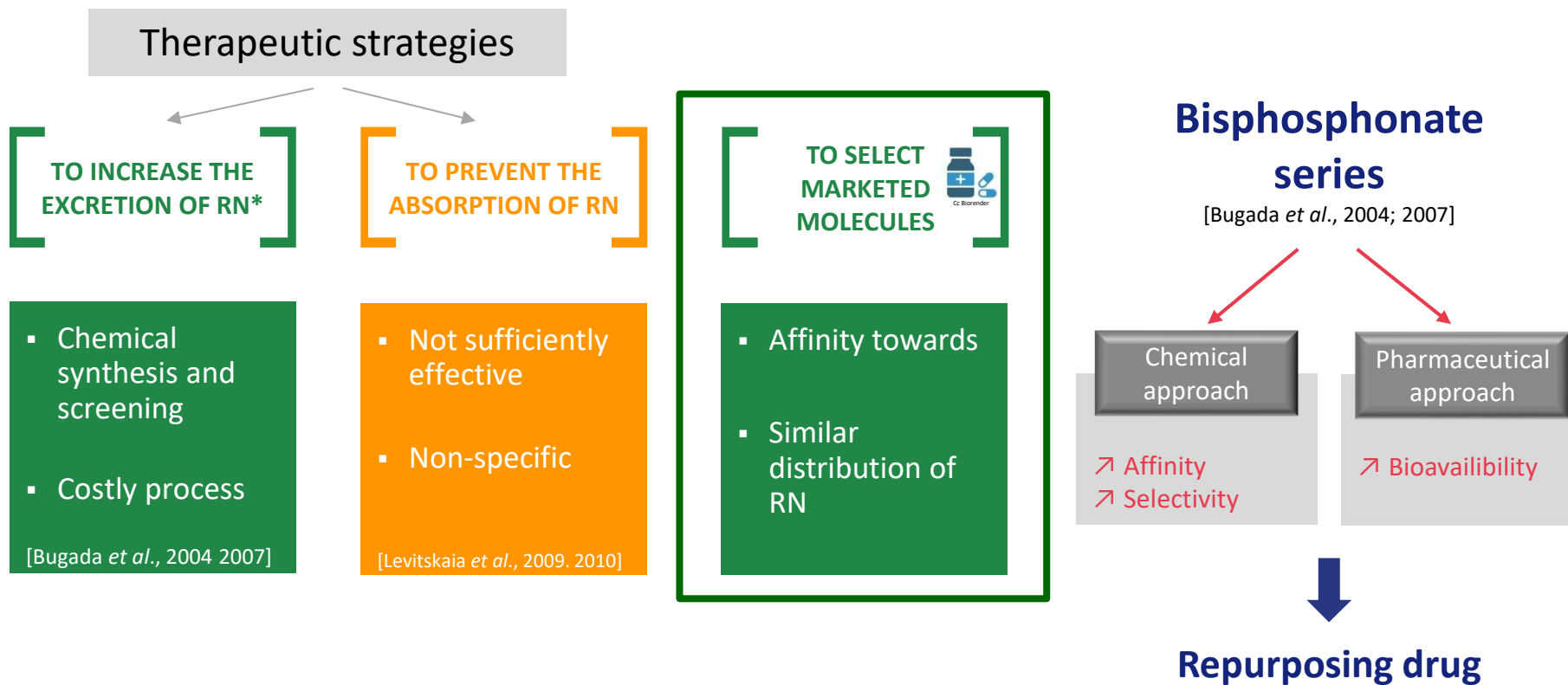
Definition of « decorporate » [Fisher, 2000]

To remove radioactive metals from the body using chelating agents or other administered pharmaceutical agents.

Main features required of a decorporating agent [Crisponi, 2013]

- ✓ Non-toxic chelating agent
- ✓ High affinity of the agent for the molecule of interest
- ✓ Formation of a stable ligand-metal complex
- ✓ Formation of a urinary soluble and excretable ligand-metal complex

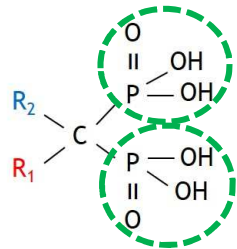
WHAT ARE THE POTENTIAL THERAPEUTIC STRATEGIES?



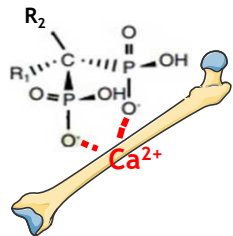
*radionuclide

BISPHOSPHONATES SERIES

Chemical structure



Mechanism of action

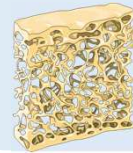


- Chelation of calcium
- Internalisation into macrophages
 - Apoptosis

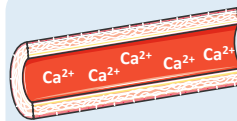
Inhibition of bone resorption

[Ebetino, 2011]

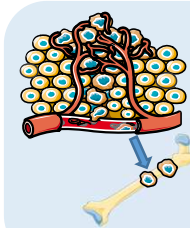
Therapeutic indications



Metabolic bone disease



Hypercalcemia

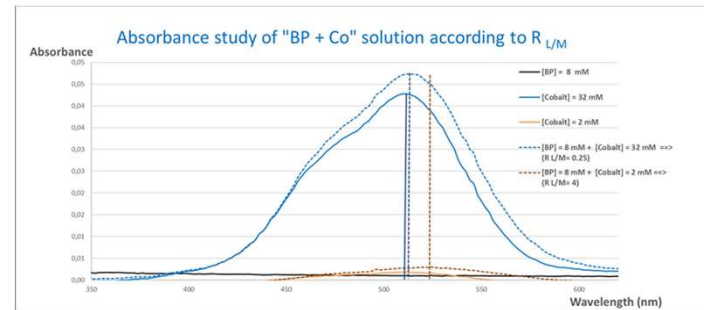


Adjuvant treatment of bone metastasis

[Cole *et al.*, 2016]

Chemical approach

Preliminary results on complexation studies



- Known to complex with BP on other divalent cations (Fe^{2+} , Mg^{2+} , Cu^{2+} ...)

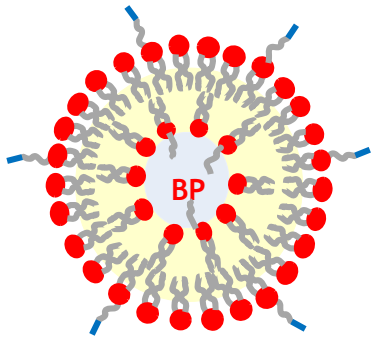
[Lamson, 1984; Barja, 2001; Foti, 2013]

- Identification of the complexation Co-L in the scientific literature

Cc Biorender

PHARMACEUTICAL APPROACH

→ To vectorize BP molecules inside liposomes



Schematic of a liposome

OBJECTIVES

1- To delay their elimination from the body

[Phan *et al.*, 2005]

2- To target the major retention organs

(skeleton and liver)

Use of liposomes in decorporation

LIPOSOMES OF DTPA

²³²Th contamination

[Kumar *et al.*, 2012]

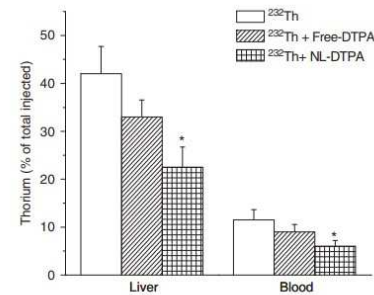


Figure 4. Effect of neutral liposomes encapsulated with DTPA (NL-DTPA) on ²³²Th decorporation from liver and blood of rats (*n* = 7) administered with ²³²Th. Free-DTPA was taken to compare the efficacy of neutral liposomal-DTPA. Results are expressed as percentage of injected ²³²Th (600 µg/kg, i.m. for 24 h). Data shown are mean ± SD. * indicates significantly higher decorporation of ²³²Th from liver and blood of animals treated with NL-DTPA compared to animals treated with free-DTPA therapy at *p* < 0.001.

²³⁸Pu contamination

[Phan *et al.*, 2006; Almaki *et al.*, 2021]

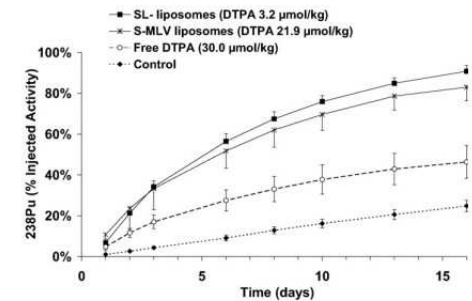


Fig. 1. Plutonium-238 cumulative excretion in urine + feces 16 days after i.v. injection of ²³⁸Pu-phytate followed by decorporation treatment (protocol 1). Treatments were injected 1 h after i.v. injection of 11.8 kBq ²³⁸Pu-phytate (phytate concentration 0.2 mM). Each value is the mean % of ²³⁸Pu-phytate injected activity (± S.D.) for five animals.

Encapsulation inside liposomes → Efficient strategy to decorporate RN

GALENIC FORMULATION PROTOCOL – METHODS TESTED

Method 1

1- Encapsulation of the molecule

- Introduction of the lipid solutions into the flask
- Evaporation of the organic solvent
- Rehydration of the lipid film with the solution containing the molecules

2- Reduction in liposome size

Extrusion or sonication

3- Purification

Ultracentrifugation

*Multilamellar vesicles

**Small unilamellar vesicles



Suspension of
MLV* liposomes ~ μm

Cc Biorender



Suspension of
SUV liposomes** ~ nm

Method 2

1- Preparation of blank liposomes

- Introduction of the lipid solutions into the flask
- Evaporation of the organic solvent
- Rehydration of the lipid film with a buffer solution

2- Reduction in liposome size

Extrusion or sonication

3- BP molecule encapsulation

Several freeze / thaw cycles

4- Purification

Ultracentrifugation

GALENIC FORMULATION PROTOCOL

Characterization of the galenic formulation

	Date	Method for liposomes ~ nm	Size (nm)	Polydispersity index	Zeta potential (mV)
METHOD 1	D ₇	Sonication	125.7	0.095	- 44.3
		Extrusion	147.1	0.067	- 42.1
	D ₃₁	Sonication	128.0	0.094	- 42.3
		Extrusion	147.3	0.043	- 40.7
	D ₇₀	Sonication	125.5	0.095	-43.6
		Extrusion	146.6	0.053	-42.1
D ₉₂	Sonication	127.9	0.127	-42.1	
	Extrusion	146.2	0.064	-46.8	
METHOD 2	D ₁₀	Sonication	133.3	0.103	- 42.9
		Extrusion	154.7	0.065	- 42.8
	D ₅₄	Sonication	128.4	0.080	NA
		Extrusion	150.7	0.026	-41.2

Determination of the encapsulation rate (RP-HPLC)

	Date	Method for Liposomes ~ nm	Encapsulation rate (%)
METHOD 1	D ₉	Sonication	40.90
		Extrusion	38.06
	D ₃₀	Sonication	38.60
		Extrusion	38.45
	D ₇₀	Sonication	40.71
		Extrusion	40.77
D ₉₂	Sonication	39.86	
	Extrusion	37.25	
METHOD 2	D ₁₀	Sonication	47.07
		Extrusion	50.77
	D ₅₄	Sonication	44.40
		Extrusion	51.14

Size and encapsulation rate of liposomes stable over time



Choice of method 1 - sonication

GALENIC FORMULATION PROTOCOL

Method 1
Sonication

Characterization of the galenic formulation (N=3)

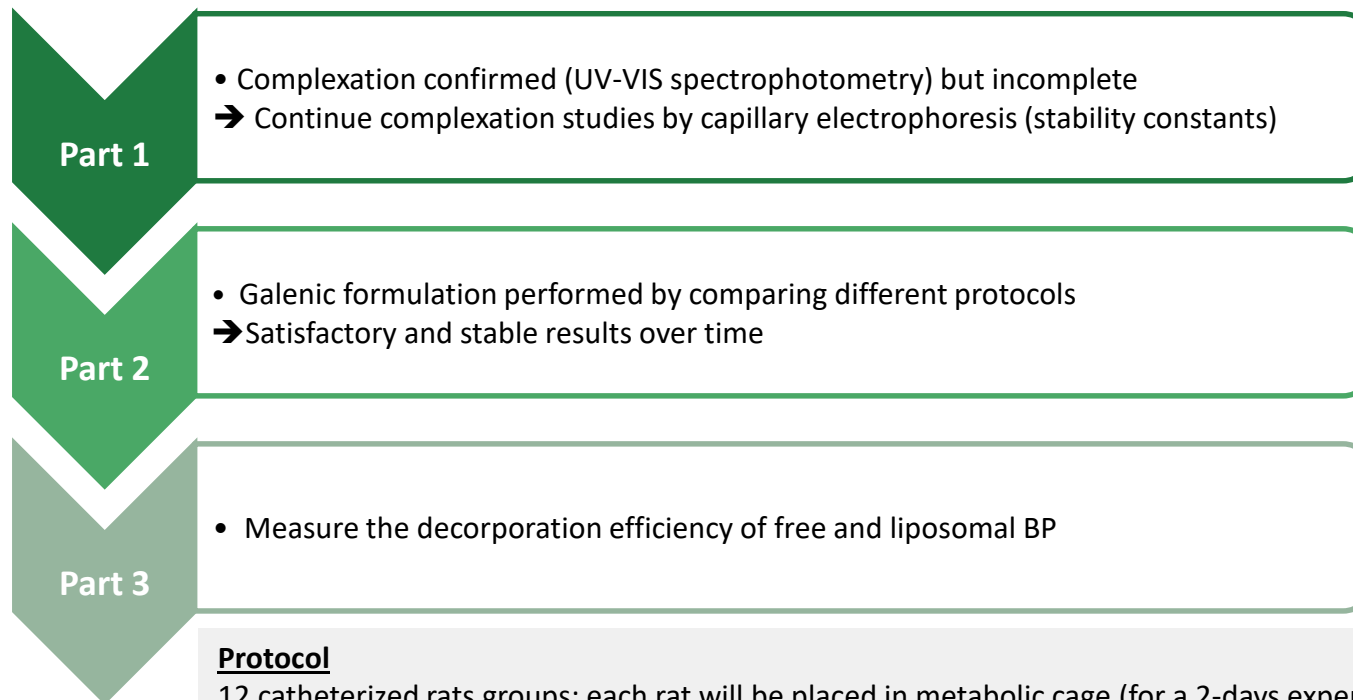
Date	Size (nm)	Polydispersity index	Zeta potential (mV)
D ₇	125.7	0.095	- 44.30
D ₉	126.8	0.098	-44.16
D ₉	122.8	0.115	-46.55
Mean	125.1		-45.00
SD	2.07		1.34

Determination of the encapsulation rate (N=3)

Date	Encapsulation rate (%)
D ₉	40.90
D ₁₀	40.18
D ₁₀	39.59
Mean	40.22
SD	0.007

→ Reproducible results

CONCLUSION - PERSPECTIVE



Protocol

- 12 catheterized rats groups; each rat will be placed in metabolic cage (for a 2-days experiment)
- RN and treatment injections after an early interval
 - Collect urine/feces/blood and organs (liver, femur, spleen and kidneys)
 - gamma counting measurement

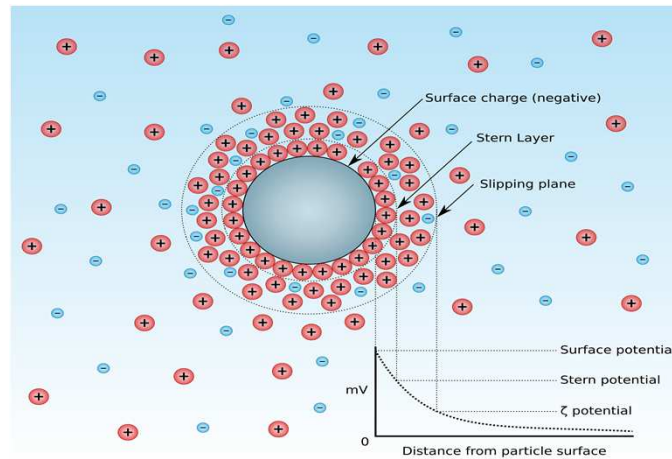


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ZETA POTENTIAL



Potentiel ζ

EXPERIMENTAL PROTOCOL

