I.N.S.C.B. Institut Nord-Sud de Cooperation Biopharmaceutique

# Bone Targeting

## Bisphosphonate based Osteotropic Drug Delivery System for metastatic bone treatment by oral route


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# I INTRODUCTION

***Bisphosphonate*** **based Osteotropic Drug Delivery System for** ***metastatic bone treatment by oral route***

**Many oncology patients with solid tumors get secondary metastases to bone.**

**Current metastatic bone treatments are *bisphosphonates by intravenous route***

***(WorldWide sales > $ 1 billion)***

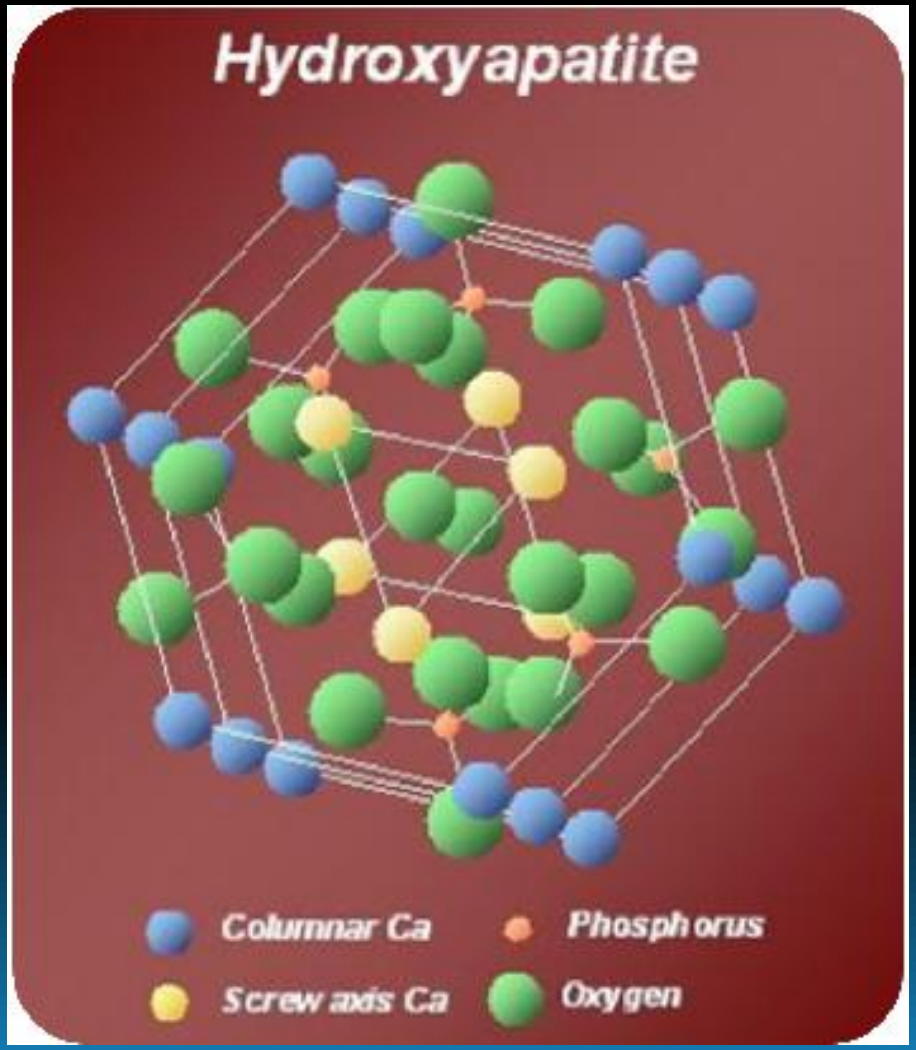
→ ***Unmet medical need*** **for patients treated by regular infusions at hospitals / clinics**

***as no oral formulation is available***

Bone : a complex organ responsible for structure, calcium storage and hematopoïesis

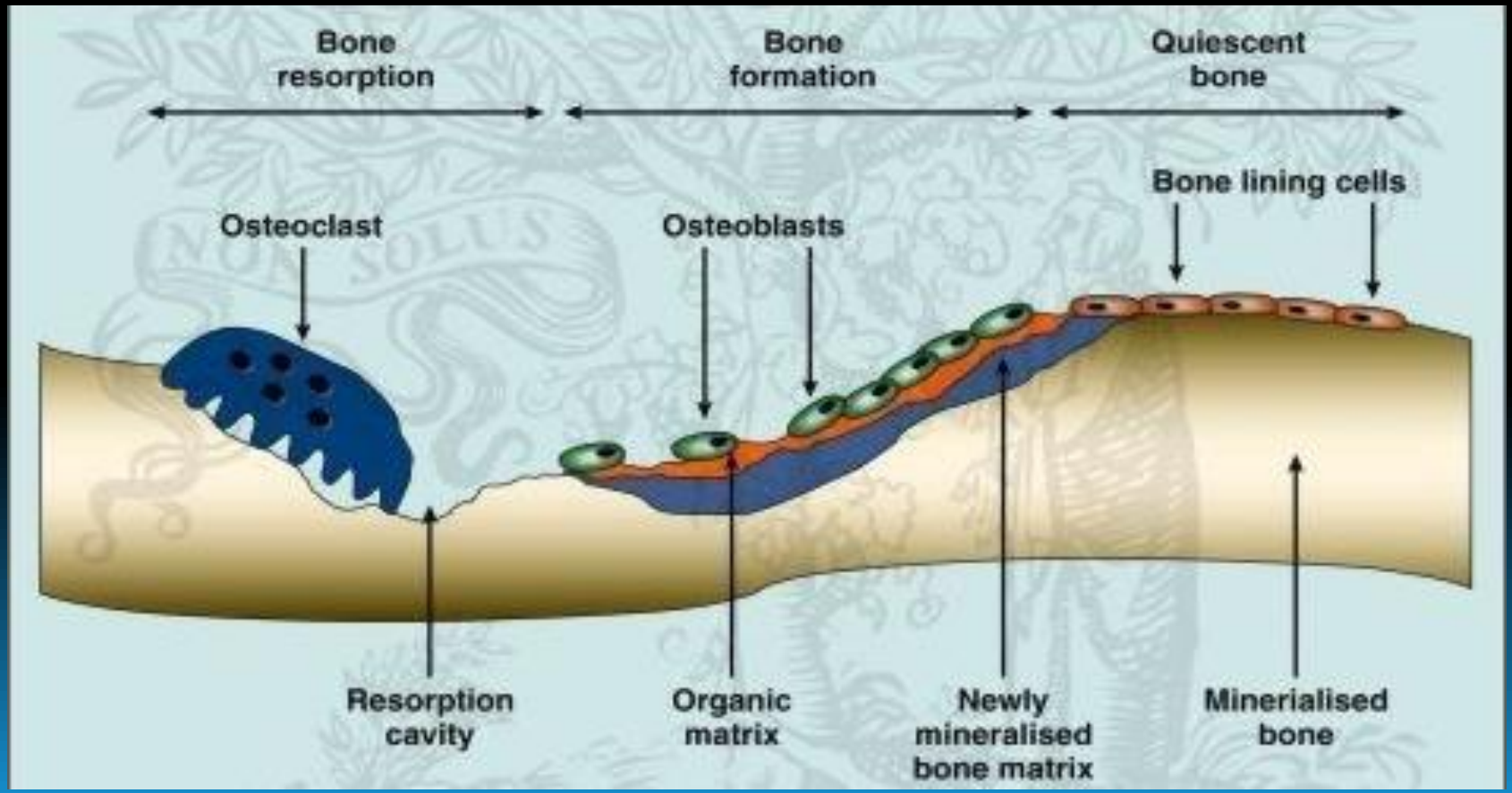
**Structure**:
50-70% mineral
20-40 % organic matrix
5-10% water
1-5% lipids

Main component : hydroxyapatite crystals (HAP)



# Bone turn-over : a bone remodeling cycle :

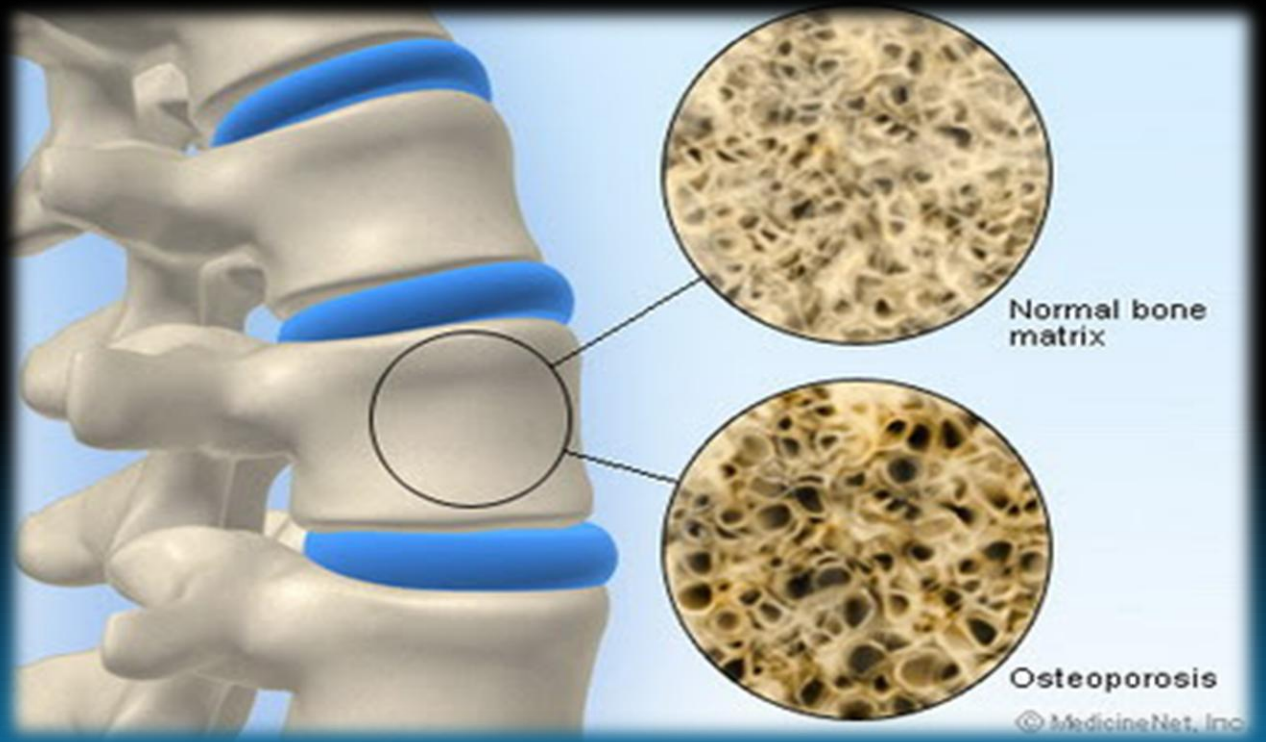
Osteoblastic bone formation
Osteoclastic bone resorption
Mineralization



# Alteration of bone's catabolism and/or anabolism of bone diseases

**Osteoporosis :**

bone resorption
> bone formation




**Other pathologies**:

osteosarcoma,

bone metastasis,

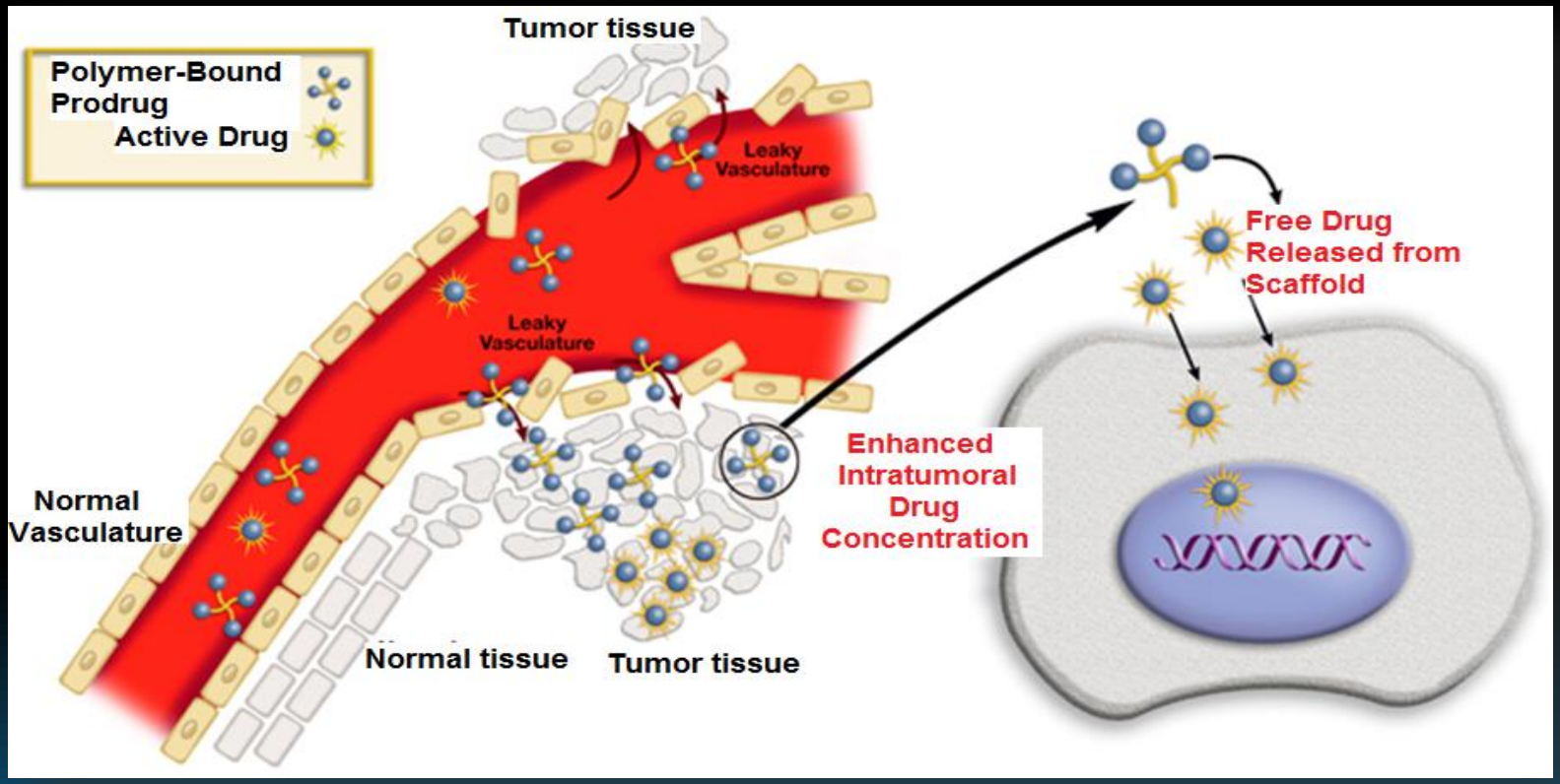
osteoarthritis,

osteomyelitis…

# II Bone Targeting

Characteristics of Bone disease state : INFLAMATION

Enhanced Permeability & Retention (EPR)

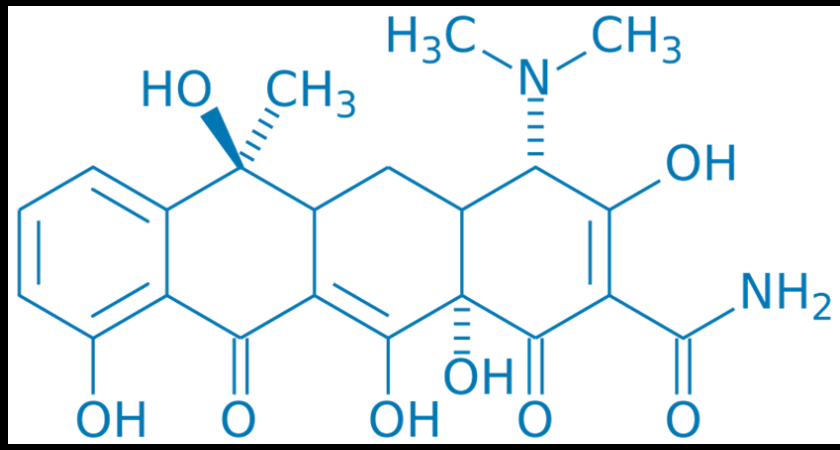



**Exposure of HAP to blood used to deliver drugs to diseased tissue**

# Bone seeking agents

## Tetracyclines

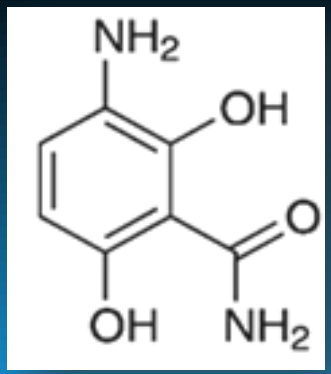
Mode of action :
Stop Protein elongation
via inhibition of aminoacyl-tRNA




Correct orientation of Tetracycline required to bind to HAP

Research of a minimalized tetracycline structure to reduce side effects and retain capacity to bind to HAP

3-amino-2,6 dihydroxybenzamide
( 50 % ability to bind to HAP vs tetracycline)

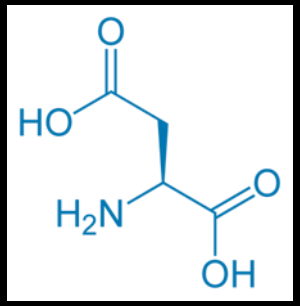


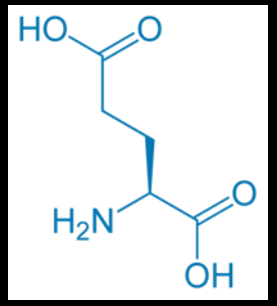
# Bone seeking agents

## Acidic Oligopeptides

Bone SialoProtein (BSP) : Bone protein with high affinity to HAP

From BSP Acidic, Oligopeptides
with 4 to 10 AminoAcids
have been designed for
enhanced Biocompatibility




*Based on Glutamic and Aspartic acids*

***In vivo* trials :**

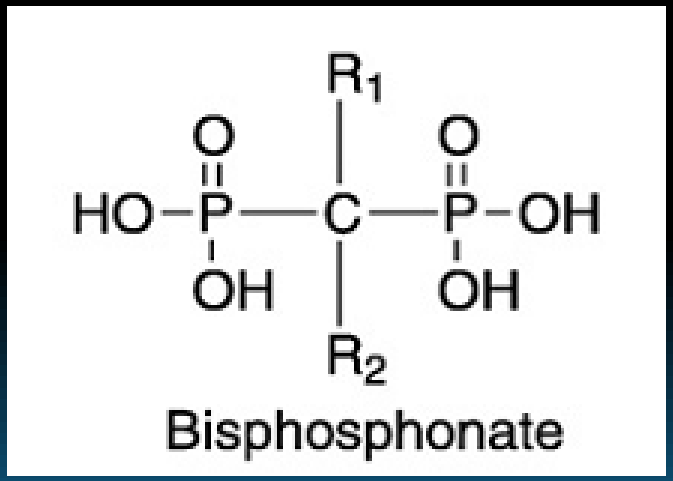
i.v injection into mice of estradiol-17β-succinate-$(L\text{-}Asp)_6$

→ ***affinities of AO depend only the number of AA, not on their L or D characters or their species***

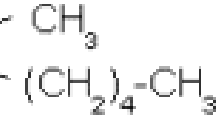
# Bone seeking agents :

# *BISPHOSPHONATES*

***The most studied bone targeting molecules***



Bisphosphonate

| Molécule | $R_1$ | $R_2$ |
|---|---|---|
| Etidronate | -OH | $-CH_3$ |
| Clodronate | -Cl | -Cl |
| Tiludronate | -H | -S-  -Cl |
| Pamidronate | -OH | $-CH_2-CH_2-NH_2$ |
| Neridronate | -OH | $-(CH_2)_5-NH_2$ |
| Olpadronate | -OH | $-(CH_2)_2N(CH_3)_2$ |
| Alendronate | -OH | $-(CH_2)_3-NH_2$ |
| Ibandronate | -OH | $-CH_2-CH_2N$  |
| Risedronate | -OH |  |
| Zoledronate | -OH |  |

***<u>Mode of action :</u> binding to the inorganic part of HAP***

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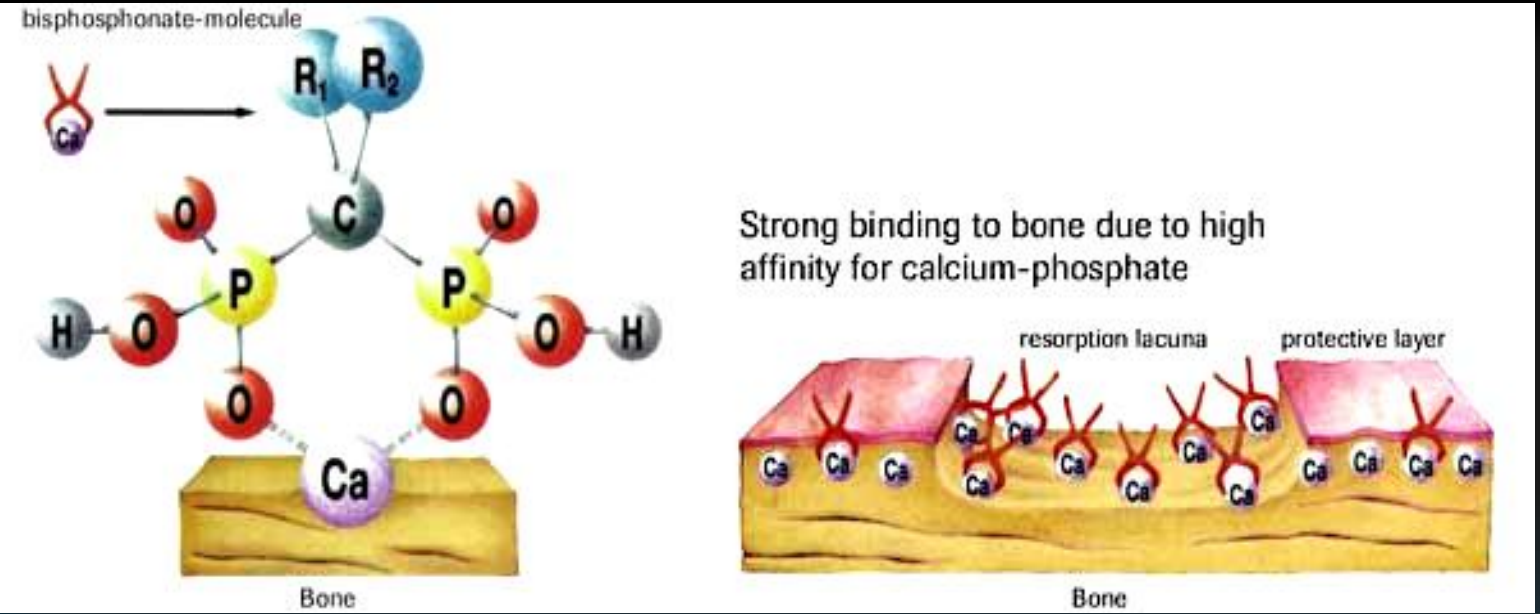
# BISPHOSPHONATES

First biological activity discovered in 1968

⇨ BPs' strong affinity to HAP :




- Retained even **conjugated** to other molecules
- Relevant in **bone scintigraphie**

Use of Tc99 labeled methylene diphosphate (MDP)
or hydroxy methylene diphosphate (HDMP)

# Advantages of using Bisphosphonates as polymer/nanoparticle targeting moieties

- **Amino BP** :
  - primary amine
  - Can be conjugated to carboxylic acids

- **Conjugaison to nanomedecines** via a **degradable linker**
  - Product of synergic effects when coupled with appropriate drugs

**+ Soon expiration of BPs' patent protection**

**→ economic option**

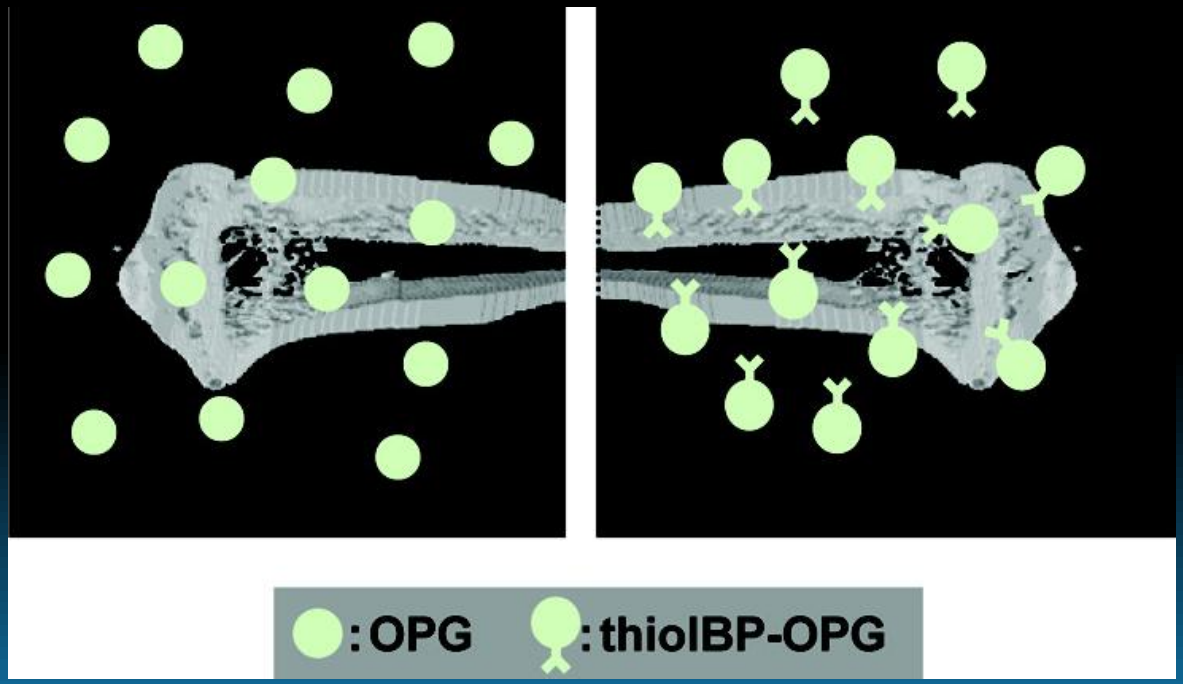
# Bone Targeting using Bisphosphonates

Efficiency demonstrated *in vivo* with targeted osteoprotegerin (OPG) as a model therapeutic protein

*(M.R. Doshak, Mol. Pharmaceutics 6, (2009) 634-640)*

Method :

Conjugaison of OPG with a « Bone seeking » Thiol Bisphosphonate



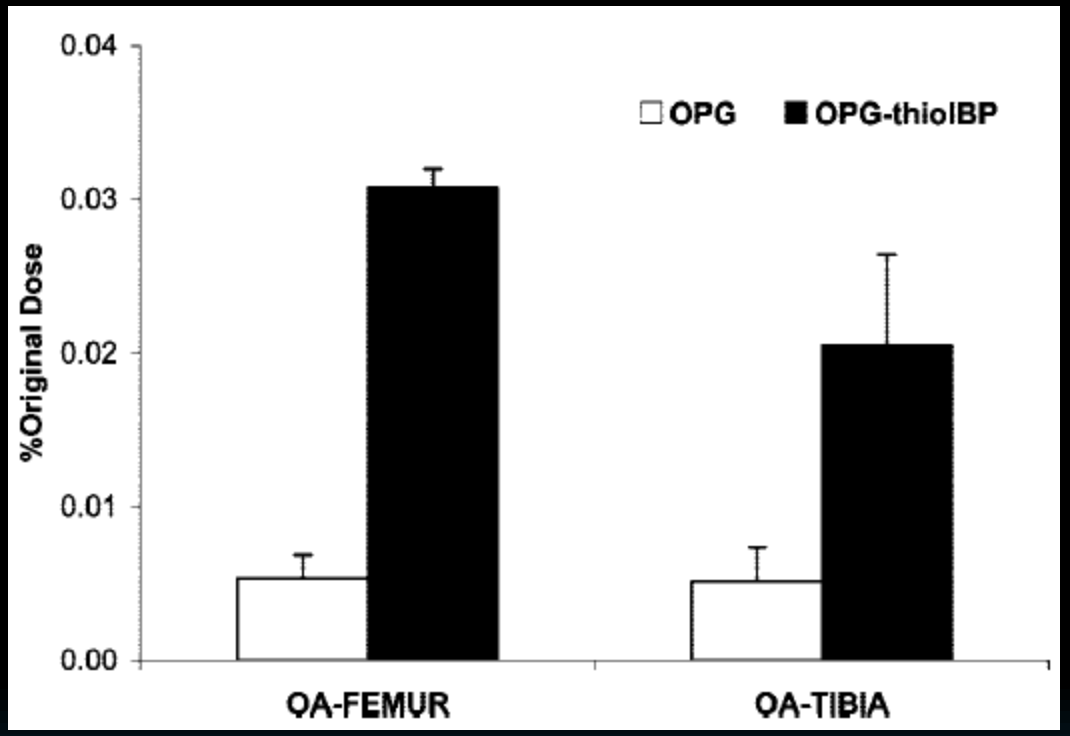

*Intravenous administration* in a rat model of osteoarthritis

# Bone Targeting using Bisphosphonates

Results:

**Delivery** of OPG-thiol-BP to bone :

**4 fold / control OPG** in osteoarthritis rats




Targeting of control OPG and OPG-thiolBP conjugates to femur and tibia in rats with osteoarthritis

Significant **advantage of BP conjugation** as **strategy** in **osteopenic bone diseases**

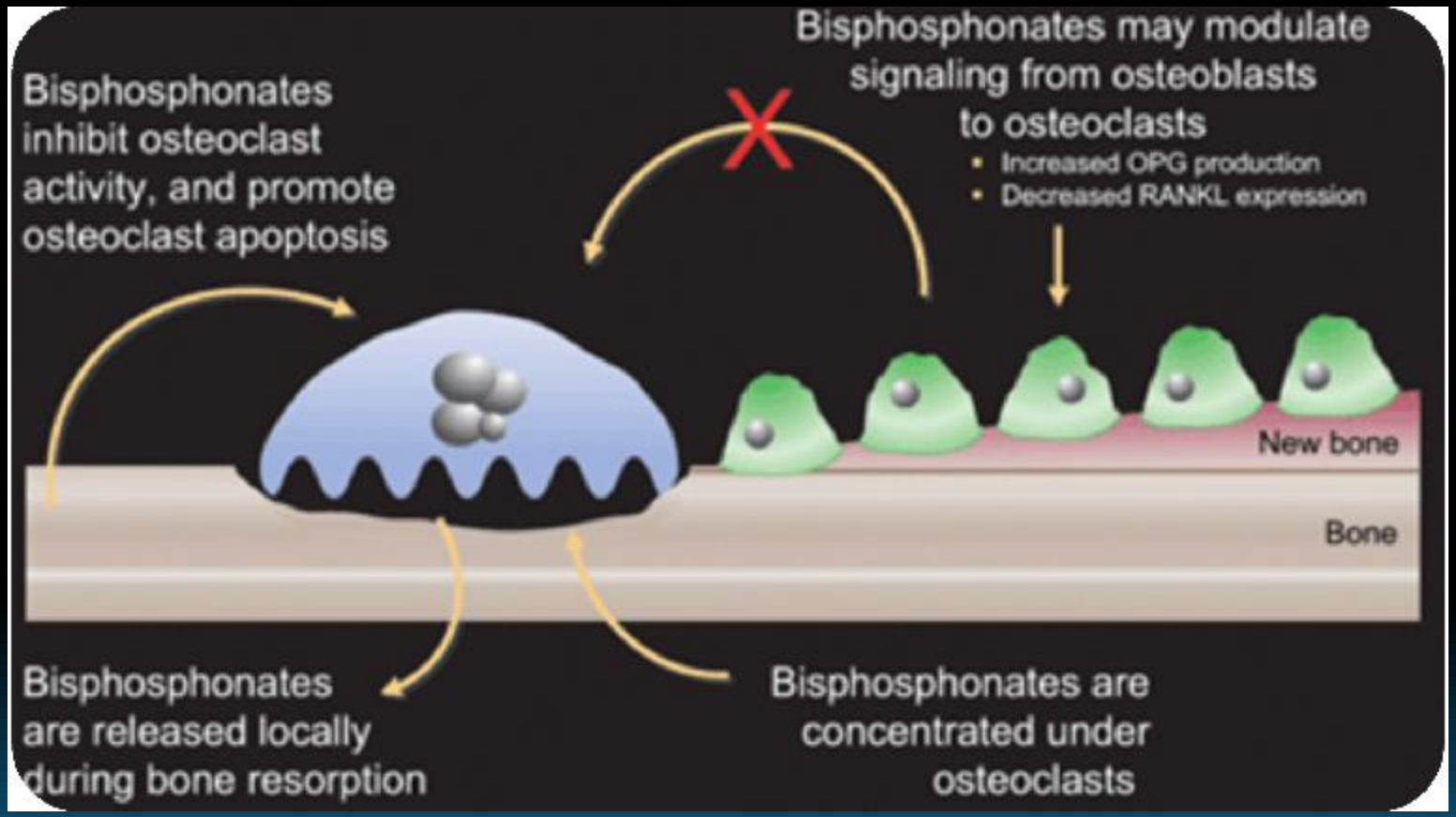
# Bisphosphonates anti-angiogenic properties

most bone metastases are characterized by excess osteoclast number and activity.

Some bisphosphonates, potent inhibitors of osteoclast activity, are widely used for prevention of bone metastases.

*Bisphosphonates are also used to treat hypercalcemia of malignancy, osteosarcoma and multiple myeloma.*

# Bisphosphonates anti-angiogenic properties




***Effects: apoptosis, inhibition of migration, reduction of angiogenic sprouts of endothelial tissue***

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# OSTEOTROPIC DRUG DELIVERY SYSTEMS

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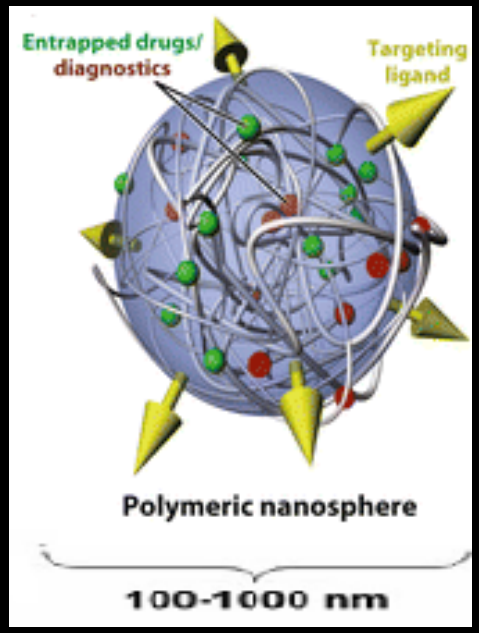
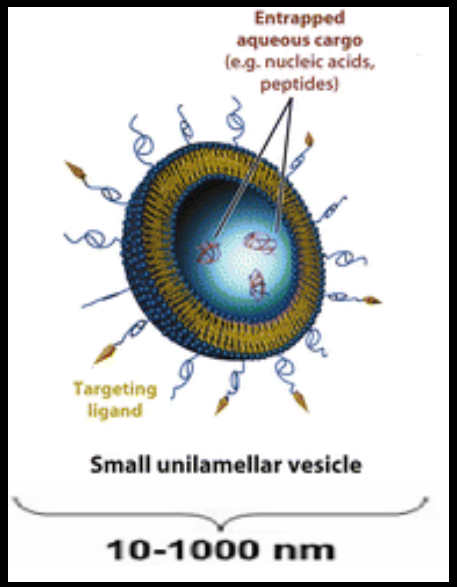
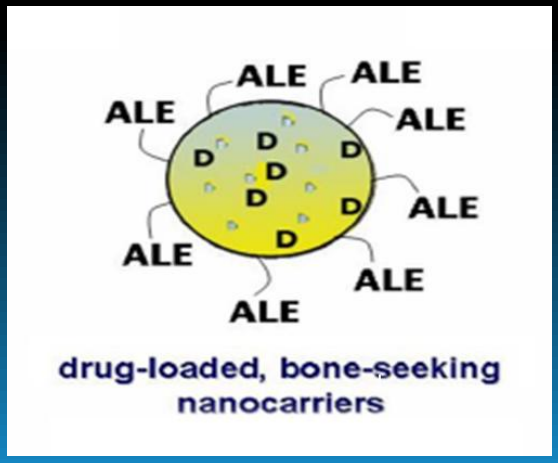
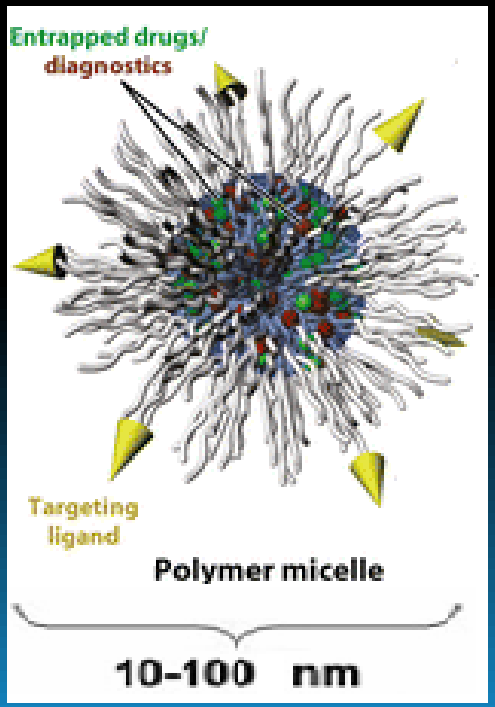
# OSTEOTROPIC DRUG DELIVERY SYSTEMS

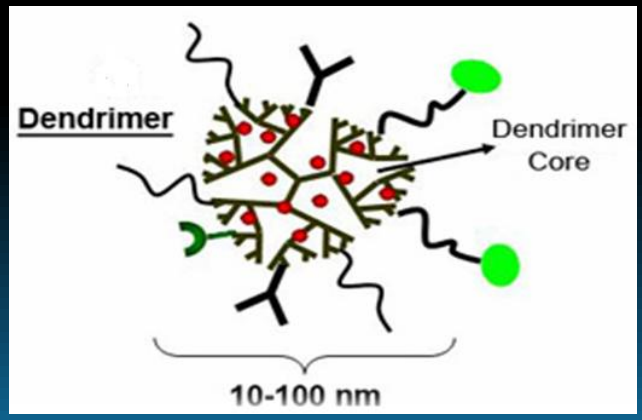
## A lot have been studied !



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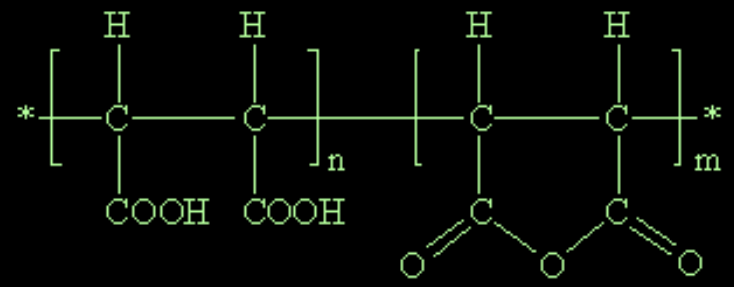
# OSTEOTROPIC DRUG DELIVERY SYSTEMS

## Poly[N-(2-hydroxypropyl)methacrylamide]: HPMA

The most studied polymer therapeutics to bone
*Used in the design of micelles and dendrimers*

Properties :
Biodistribution to bone
Bone targeting abilities
Low toxicity profile




## In vivo data

*HPMA copolymer – D-Asp8 conjugate: Administration i.v of bone-targeted and non-targeted HPMA copolymers into mice*

*HPMA copolymer−alendronate (ALN) conjugate*
*I.V injection of conjugates with different Mw and ALN content into mice*

**_All_ Osteotropic Drug Delivery Systems Evaluated _in vivo_ to date have been administered _intravenously_**

This is why a novel Bisphosphonate Osteotropic Drug Delivery System ***(BP-ODDS)*** has been developed by INSCB ***for ORAL ROUTE***

**to improve**

**Bisphosphonates oral bioavailability**

***…thereby enabling the development of oral dosage forms for oncology indications.***

Bisphophonates for cancer therapies are administered *intravenously by slow infusion*.

*Infusion of bisphosphonates is however associated with dose and infusion rate dependent effects on renal function.*

Oral administration, is complicated by poor bioavailability and poor gastrointestinal tolerability.

This limits their use in oncology to intravenous infusion to achieve the doses required for efficacy.

Bisphosphonates have very poor oral bioavailabilities :

***0.6% in average*** !


→


***Design of drug delivery systems enhancing oral absorption is required for treatment efficiency !***

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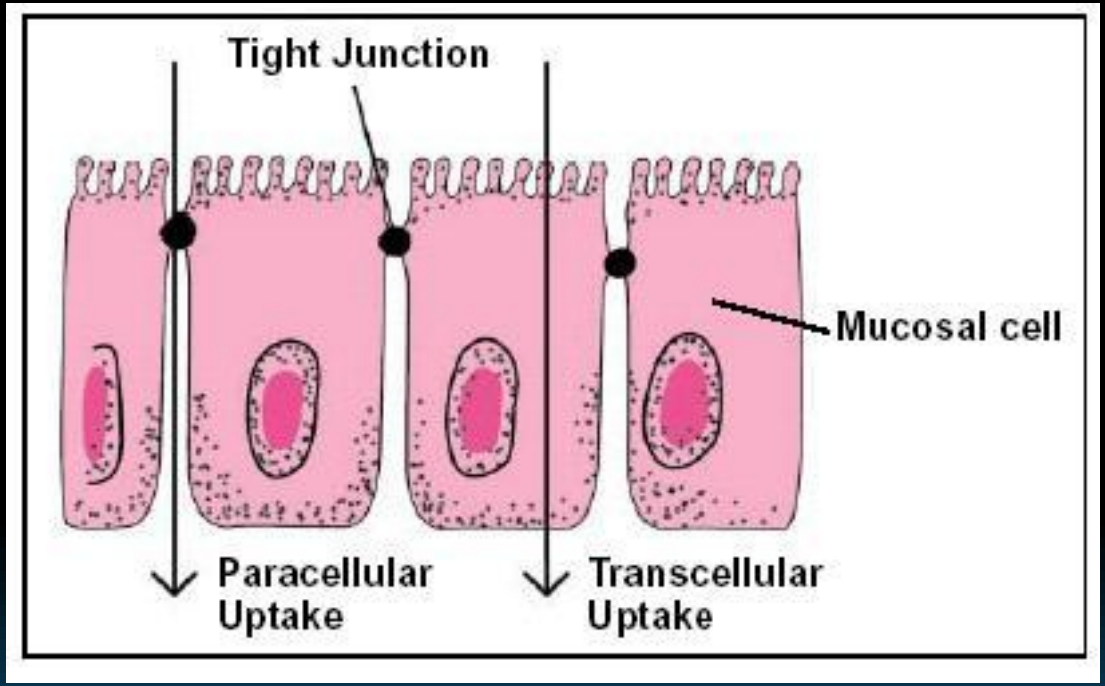
# INSCB proprietary technology combines two factors affecting Bisphosphonates physico-chemical and pharmacological behaviour *in vivo*

**Their specific transport through intestinal membrane by paracellular pathway**




**&… Their ability to form insoluble calcium complexes *in vivo* in the GI tract**

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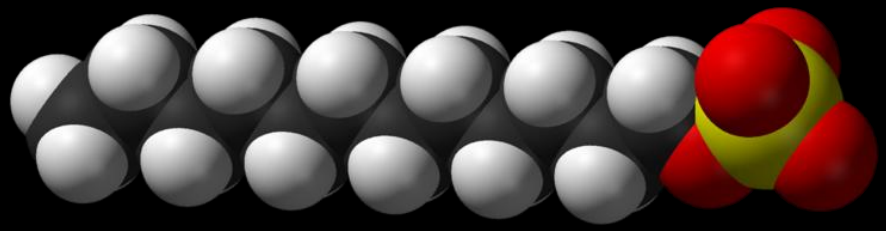
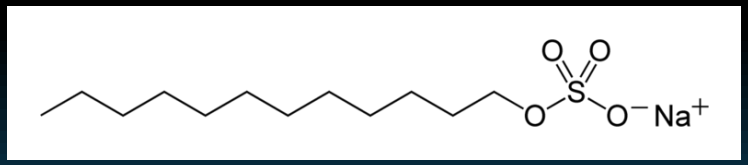
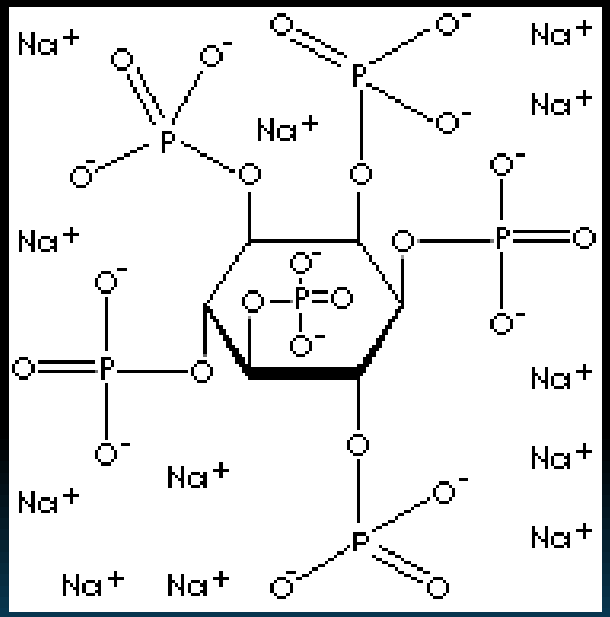
# INSCB Bisphosphonate Osteotropic Drug Delivery System : (BP-ODDS)

Two excipients, both registered at pharmacopeias and authorized for oral administration were used to modulate these bisphosphonates properties






**one intestinal penetration enhancer :**

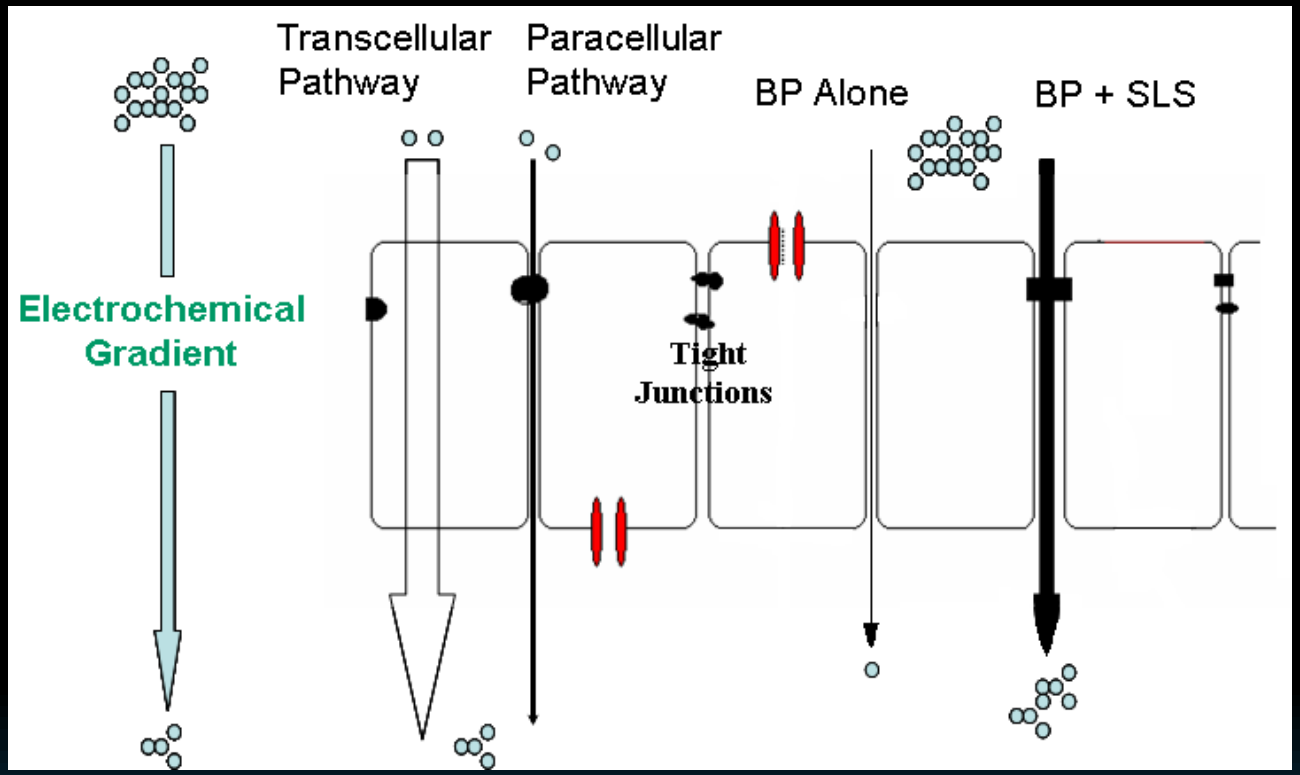
Sodium Dodecyl Sulfate

**one calcium chelatant agent :**

Myo-Inositol hexakis dihydrogen phosphate dodecasodium

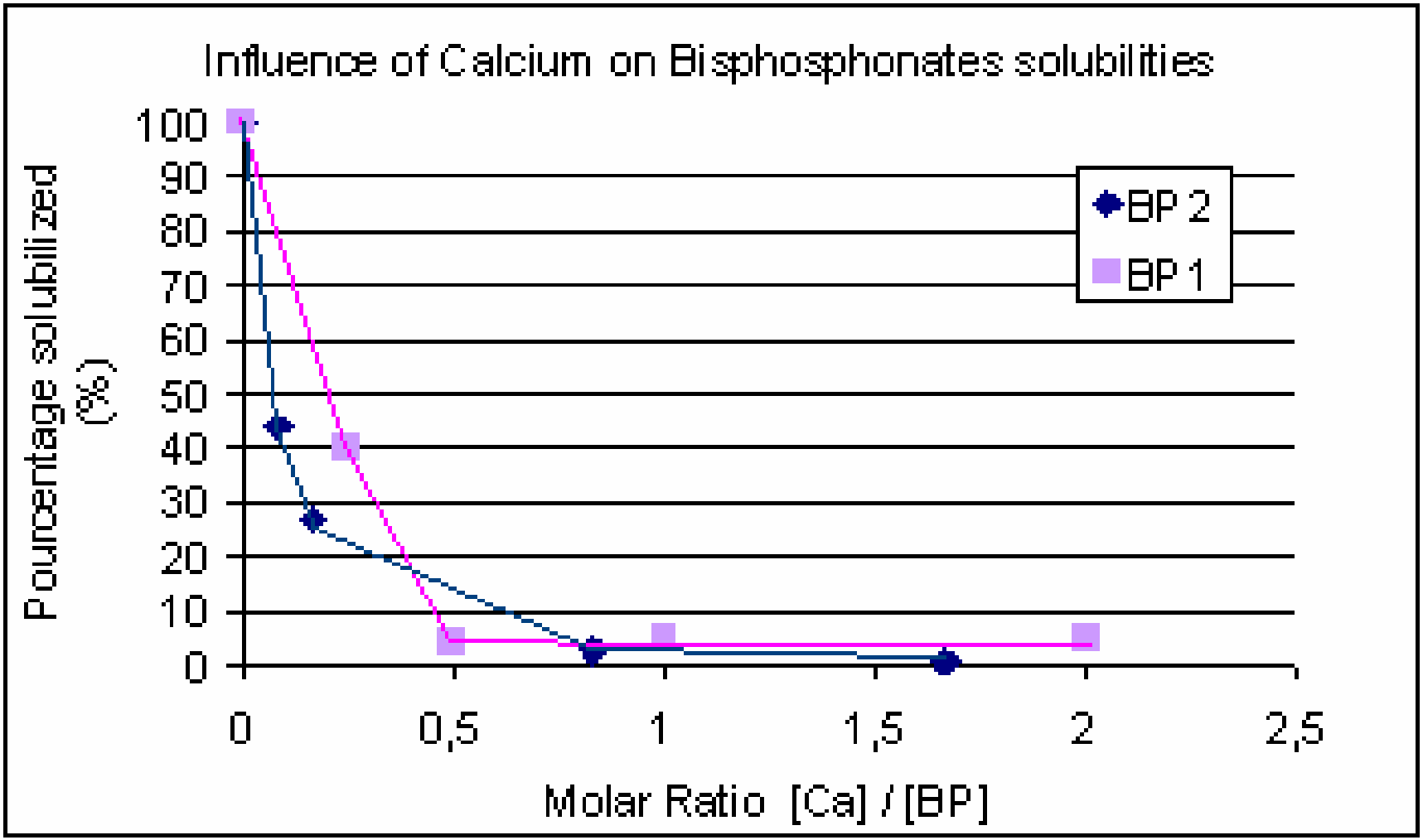
# Intestinal penetration enhancement :

Bisphosphonates are highly hydrophilic, they can cross the intestinal membrane only by using the paracellular pathway of intestinal tight junctions




**Sodium Dodecyl sulfate increases intestinal permeability by opening tight junctions, thus enhancing paracellular absorption of Bisphosphonates**

# Physiological Calcium chelation



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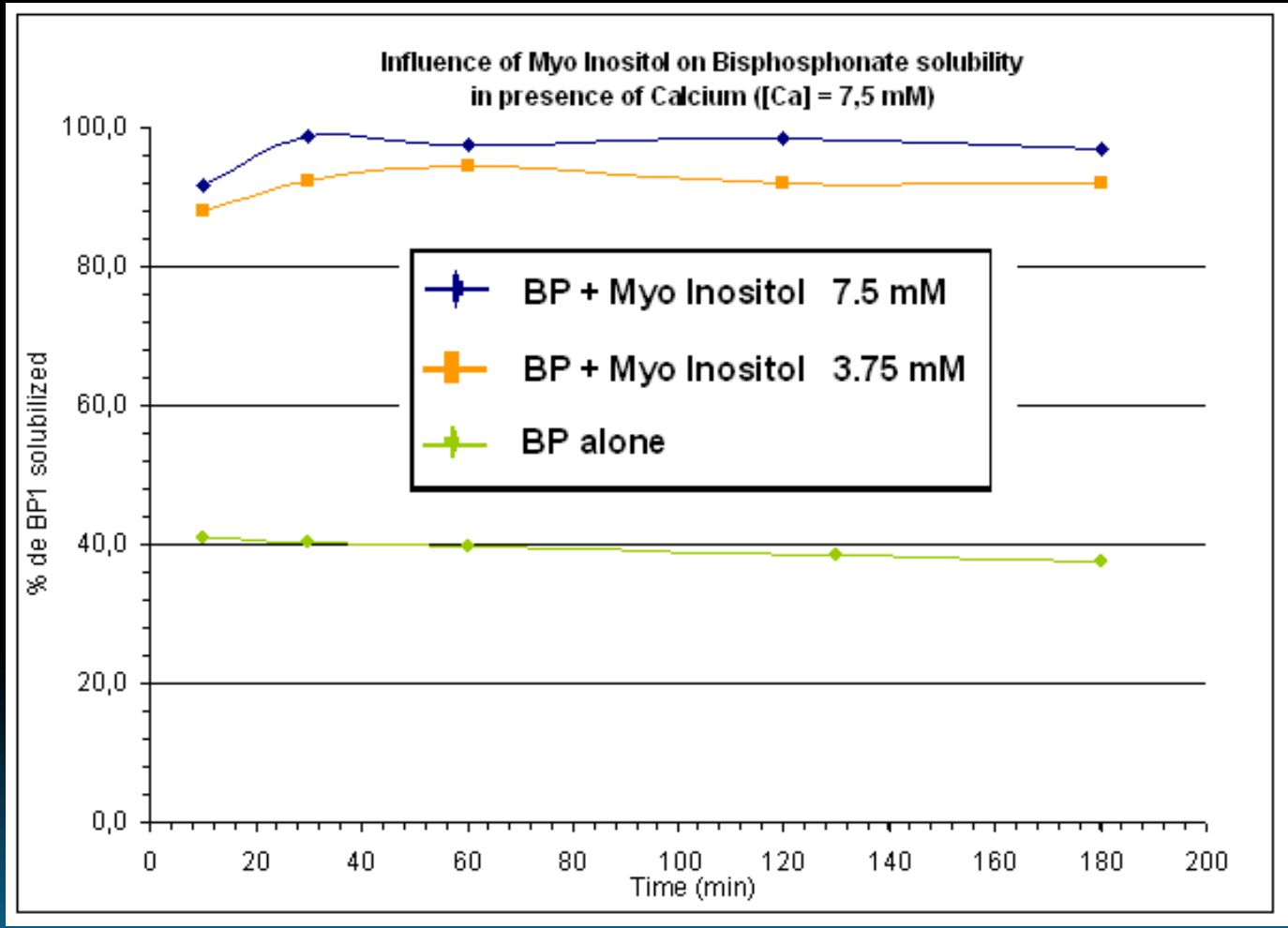
# Physiological Calcium chelation

Myo-Inositol is a stronger calcium chelatant than bisphosphonates

Its presence ***increases Bisphosphonate solubility in GI Tract***




→ ***Bisphosphonates intestinal absorption Is enhanced***

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## INSCB BP-ODDS

# Manufacturing process

Classical physical dry mixture of excipients and drug substance

Can be processed in a film coated tablet or in hard gelatine capsules.

Technology does not increase manufacturing costs compared to classical tablet or capsule production.

***The new formulation shows no degradation of the active drug after one year stability, either in film coated tablet or hard gelatine capsule form***

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# Comparative Bioavailability study

**Purpose : To assess relative bioavailability of BP-ODDS, vs reference formulation, a marketed film coated tablet of a bisphosphonate (Actonel 35 mg ®),**

Dose : 35 mg single dose, administered *per os,* one film-coated tablet of 35 mg with 200 ml of low carbonated water.

Subjects : 12 healthy volunteers in fasting conditions

Selection criteria : Male & female, aged 18-45, body mass index : 19 - 27.5

Methodology: *Two period, two sequence, cross-over, block randomized*

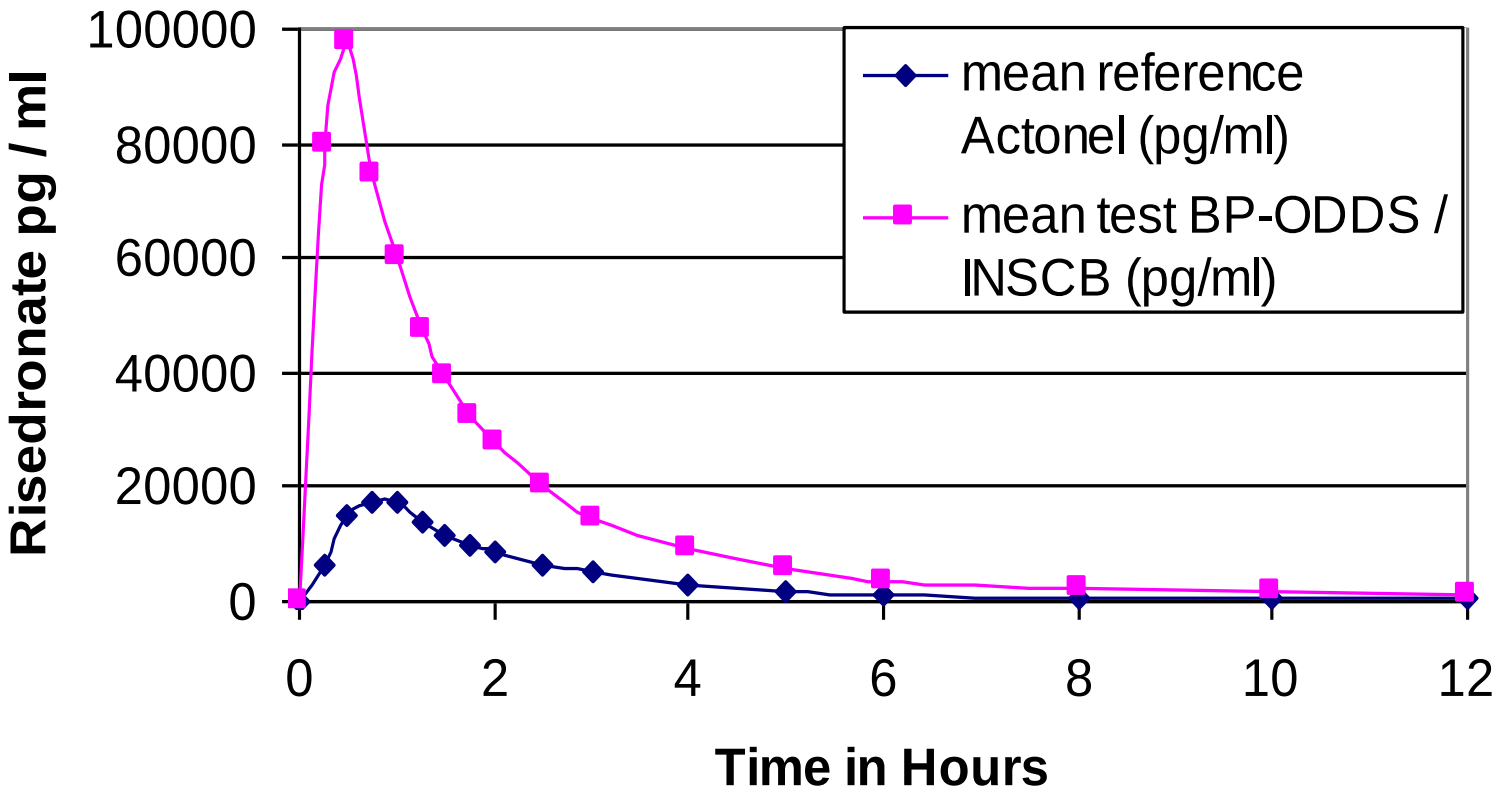
Duration of treatment : One day per period

## Pharmacokinetic criteria for evaluation :

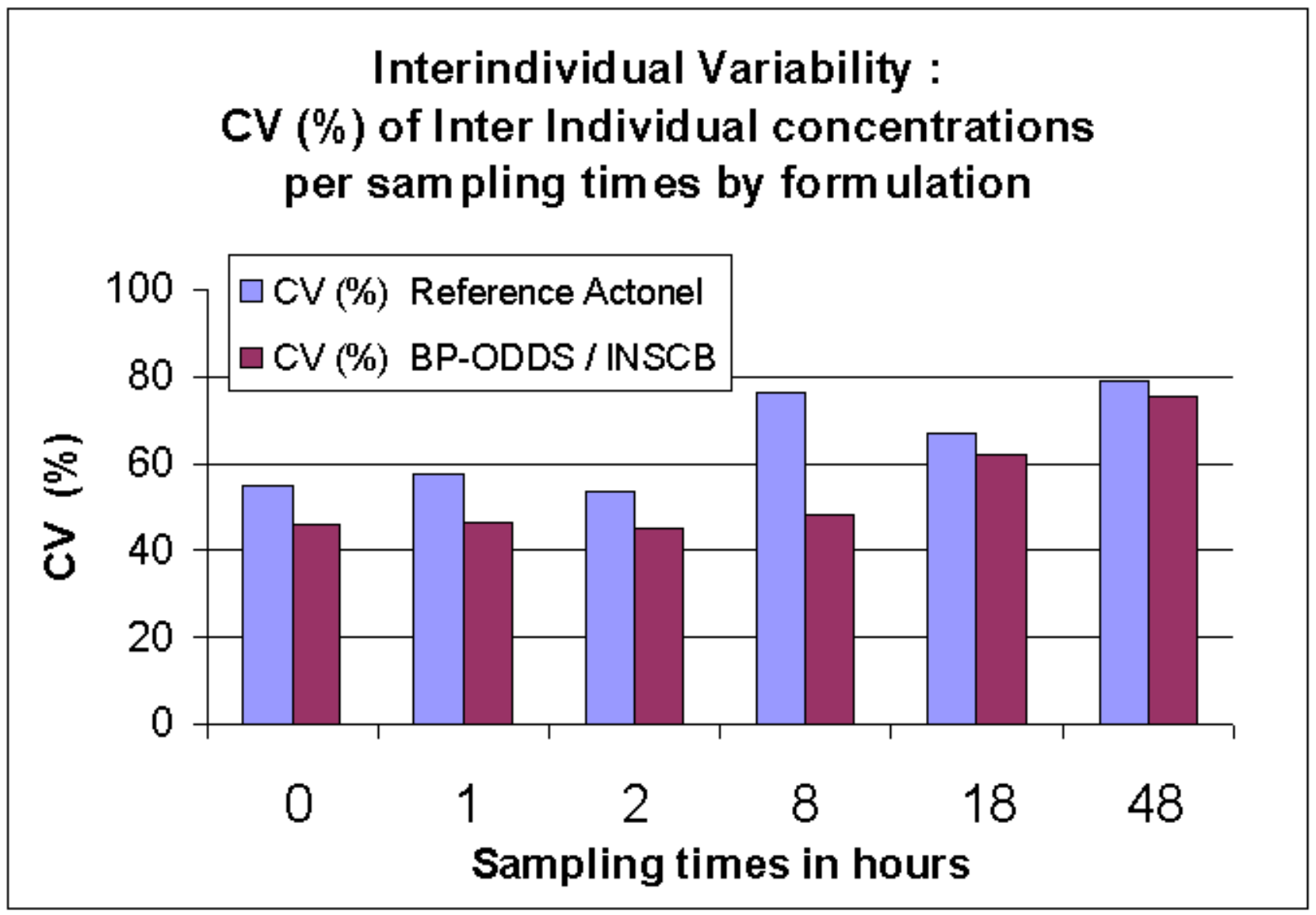
Risedronate AUC0-t , Cmax, AUC O-inf , Tmax + (T1/2 , MRT).

# Comparative Bioavailability study

## Comparative Bioavailability of BP-ODDS / INSCB risedronate 35 mg vs marketed Actonel



# Comparative Bioavailability study



# CONCLUSIONS

Statistical analysis of pharmacokinetic data obtained proves that :

***Test product : BP-ODDS / INSCB Sodium Risedronate 35 mg, is relevantly better absorbed than reference ACTONEL® 35 mg***

**AUC of BP-ODDS is almost 300 % higher than reference Actonel®**

**Cmax of BP-ODDS is almost 500 % higher than reference Actonel®.**

***The Bisphosphonate Osteotropic Drug Delivery System (BP-ODDS) developed, is supra-bioavailable when compared to its reference marketed product***

# CONCLUSIONS

**$T_{max}$ of BP-ODDS is half of $T_{max}$ of reference Actonel®**

→ ***twice quicker efficiency for BP-ODDS*.**

**Variability of primary pharmacokinetic parameters of BP-ODDS are lower than variability of reference Actonel®**

***(20 % lower for C max and 60% lower for AUC 0-inf)***

***+ Safety :***

***BP-ODDS administered in single dose, orally, was very well tolerated by the participant subjects.***

BP-ODDS tablets or capsules offer a new paradigm for

**metastatic bone cancer treatment**
**through the *oral route***

*Their ability to better target growing metastatic tissues, cover an unmet market need for oral bisphosphonates in oncology.*

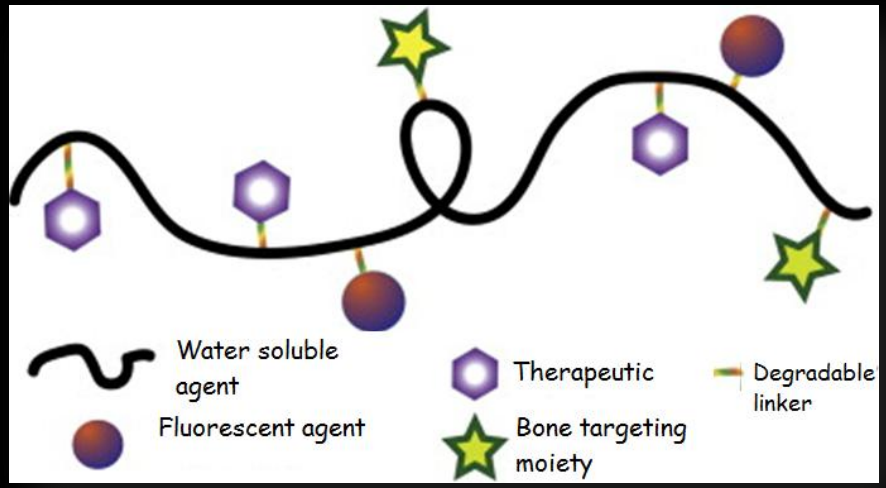
BP-ODDS is an effective and potentially safer alternative
to bisphosphonate intravenous infusions
which could offer the following advantages :

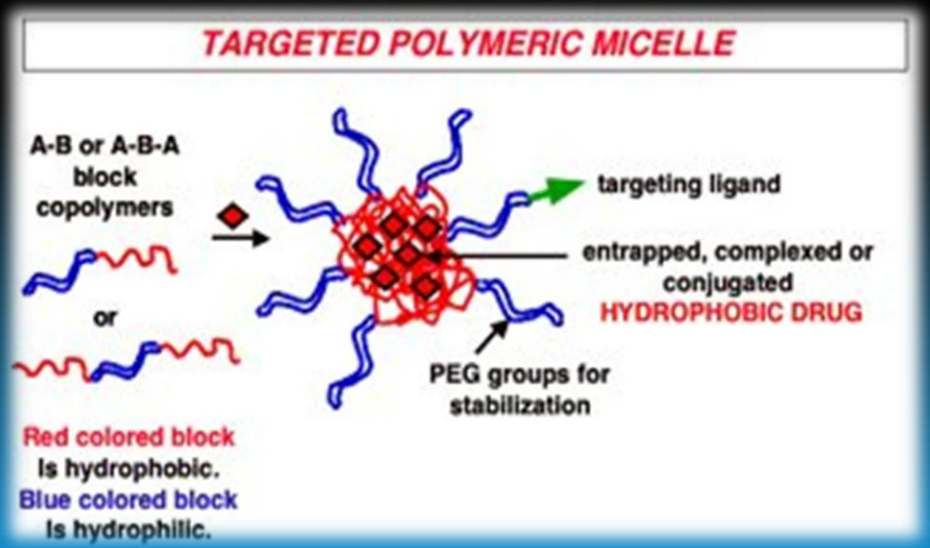
***Improved quality of life for the patient***
***Cheaper treatment***
***Flexibility in the dosing regimen***

BP-ODDS could also be used as a

## *carrier for drugs needing vectorization to bone*

Either using the **Micelle forming ability of some bisphosphonates** to carry the drug candidate




Or by use of a **biodegradable linker to be conjugated to a bisphosphonate** or a bisphosphonic group

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# This Osteotropic Drug Delivery System has been patented by INSCB in 2011 and is available for license

## Contact : contact@inscb.org

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# Thanks for your Attention !

# APPENDICES

# Bone seeking agents

## Estradiol analogs

Localisation in bone tissue + lack of estrogenic properties

Methods : Attaching calcium chelators to an estradiol moiety via succinoyl or carboxyethyl linkers

***Improvement of targeting potential***

# 2 sorts of Bisphosphonates

**Amino BP**: disturbance of mevalonate pathway

More potent

inhibition of protein prenylation

and osteoclasts' loss of function

**Non amino BP**: creation of a modified ATP

inability to be hydrolysed

***Osteoclasts' apoptosis and reduced bone turn-over***

# Comparative bone seeking properties of Bisphosphonates (BP) Acidic Oligopeptides (AO) and Tetracycline

**Rate of binding to HAP :** *Faster for AO than BP due to a larger binding area of AO*

**Binding strength :** *Greater for BP than AO due to a higher specificity of BP for HAP*

**Binding sites :**

- *All bone for BP*
- *Higher cristalline HAP for AO*
- *Growing surfaces with low cristallinity for Tetracycline*

# Bone targeting principles

***Osteoclast targeting :***

Use of BP and AO because of their favored binding onto absorbing surfaces

***Osteoblast targeting :***

Use of Tetracyclines or analogs because of their favored binding on bone's growing surfaces

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# Comparative Bioavailability study

## Methodology:

***Two period, two sequence, cross-over, block randomized***

Hospitalization of subjects : until 24 hours post administration.

Washout period : 46 days.

Blood samplings collected : Before dose (0.0) & 20 sampling points
Post dose from 0.25, to 168.0 hours.

Analytical method : Determination of Risedronate in plasma
by HPLC- MS/MS.

*Comparative bioavailability assessment based on plasma drug levels of test bisphosphonate used, (sodium risedronate).*

**+ Safety : Laboratorv data / Vital signs / Adverse events**

# RESULTS: Pharmacokinetics parameters :

Mean pharmacokinetics characteristics of risedronic acid after treatment with REFERENCE : Actonel ® and TEST : BP-ODDS / INSCB products

Comparative bioavailability of the primary parameters (test name : Classic 90% CI) :

| Parameter | AUC 0-t | AUC 0-inf | C max | T max |
|---|---|---|---|---|
| Test Value (Test / Reference) | 295.854 | 266.363 | 480.004 | 0.530 |

Comparative coefficients of variation (CV) of the primary pharmacokinetic parameters

| Parameter | AUC 0-t | AUC 0-inf | C max |
|---|---|---|---|
| Test Value (CV Test / CV Reference) | 0.85 | 0.38 | 0.79 |

# Future of the field

Development of bone targeted anti-neoplastic agents
Because bone metastases = unsolved problem in oncology

Research about bone targeted therapeutics focusing on:

**EPR** effect , Delivery of **siRNA** to solid tumors
Combination therapies: (i.e : Paclitaxel / BP; Statins : BP…)
bone targeting of relevant drugs in order to reduce pain

**+ improve quality of life**

**Continous increase** in **prevalence**
of **bone diseases** with **aging population**

# V.1 Gaps in current research

- Many novel drug applications: yet to be explored
- Better development of disease specific targeting
- Research of bone conditions present in each disease + targeting mechanisms
- Discovery of specific biochemical pathways => disease states