



LIÈGE université

**Center for Interdisciplinary
Research on Medicines**

NANOTECHNOLOGY BASED SYSTEMS FOR THE DELIVERY OF ANTICANCER THERAPEUTICS

Ange ILANGALA

CENTER FOR INTERDISCIPLINARY
RESEARCH ON MEDICINES

PLAN

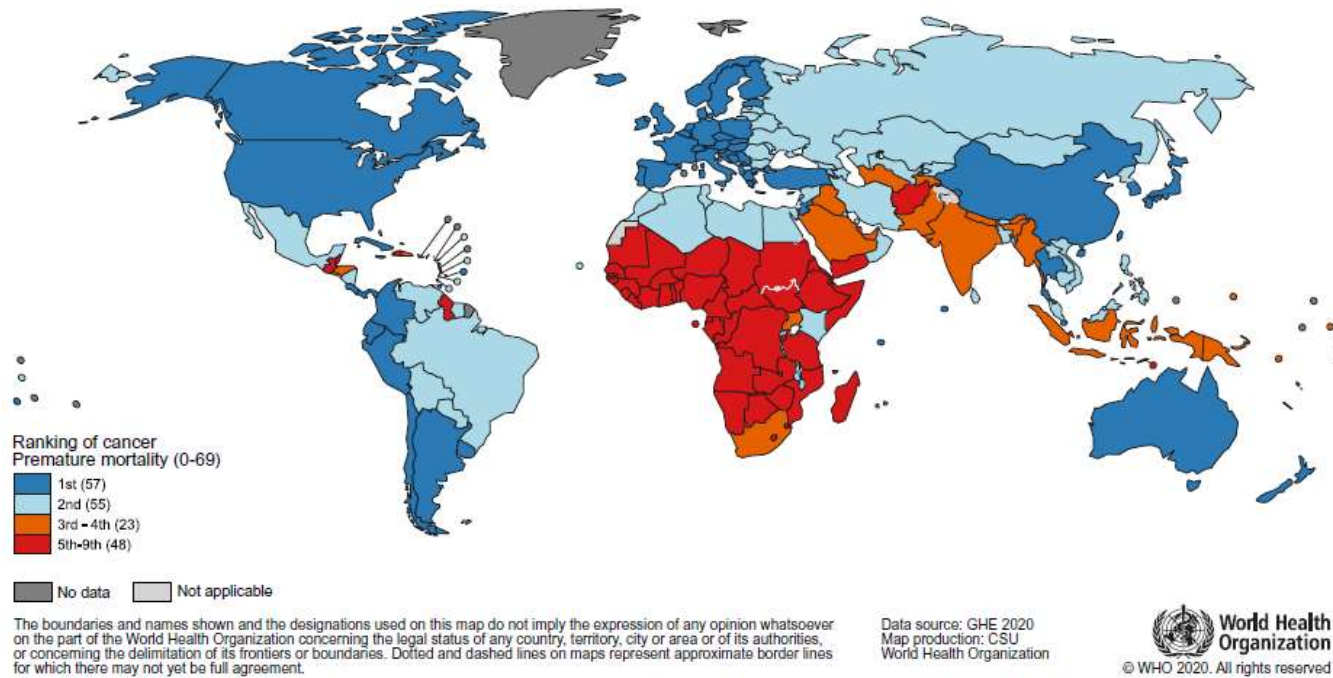
1. Definition, etiology, challenges for cancer management (Clinical and therapeutic aspects of cancers)

2. Applications of nanotechnology in cancer management (diagnostics and treatment):

➤ Improvement of PK profiles of existing or old anticancer drugs (conventional chemotherapy drugs)

➤ Innovative treatments for cancer (underdevelopment therapy)

3. Conclusion (Take home message)



Cancer - a growing problem

2021 -----> 2030

WORLDWIDE CANCER CASES ARE
PROJECTED TO INCREASE BY

↑ 50%

From 14 million To 21 million

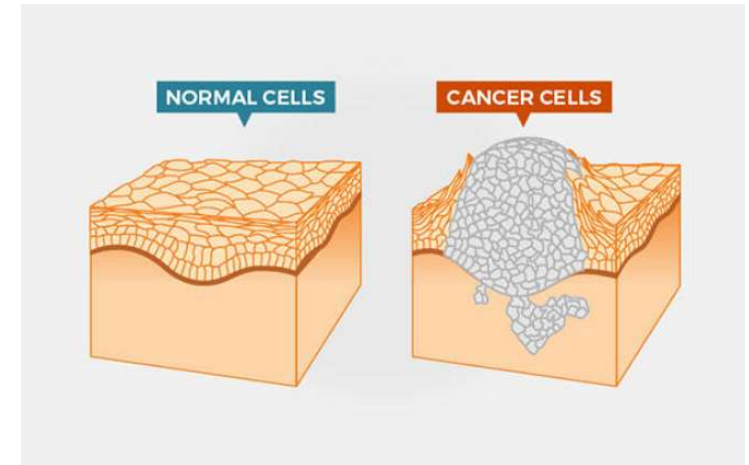
WORLDWIDE CANCER DEATHS
ARE PROJECTED TO INCREASE BY

↑ 60%

From 8 million To 13 million

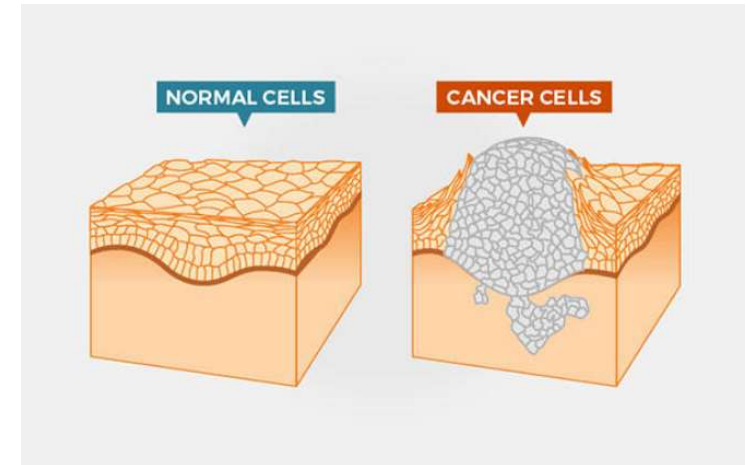
Cancer

- ❑ Cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body.
- ❑ Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumors.
- ❑ A tumor cell is part of tissue that is abnormally growing, it may either malignant or benign in nature.

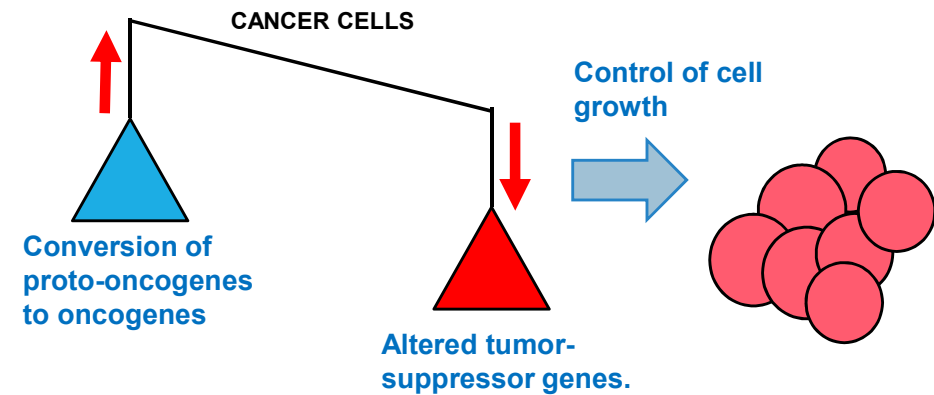
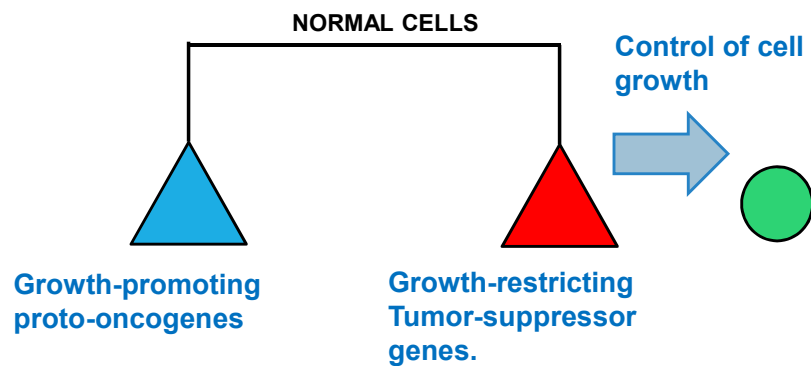


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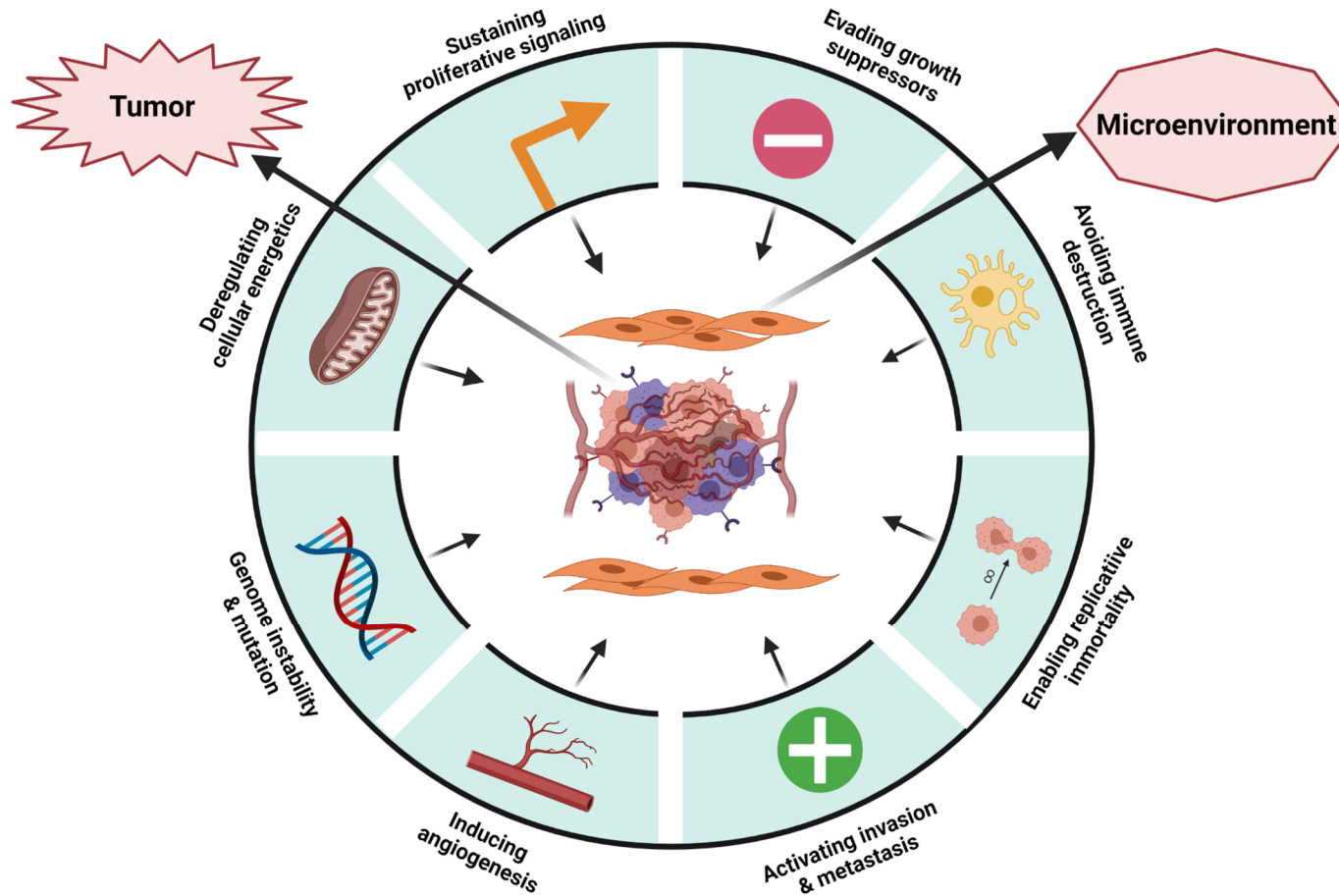


Etiology of cancer



Challenges

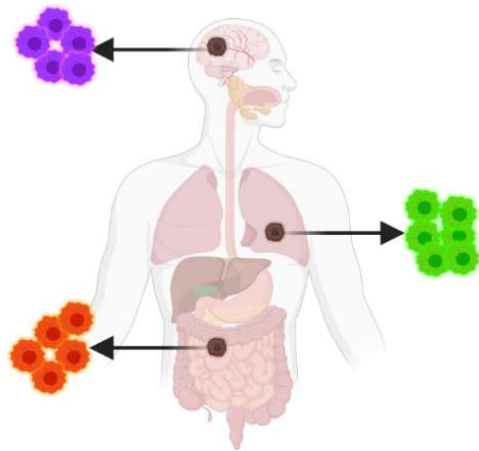
Hallmarks of Cancer



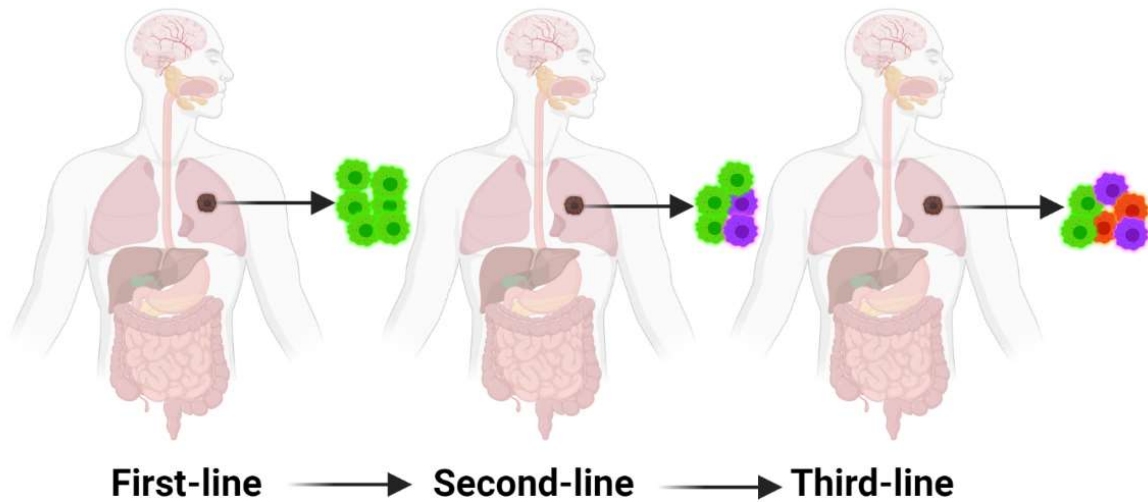
Challenges

Cancer is complex disease and heterogeneous disease
- Multiple therapeutic strategies are needed

a) Spatial heterogeneity



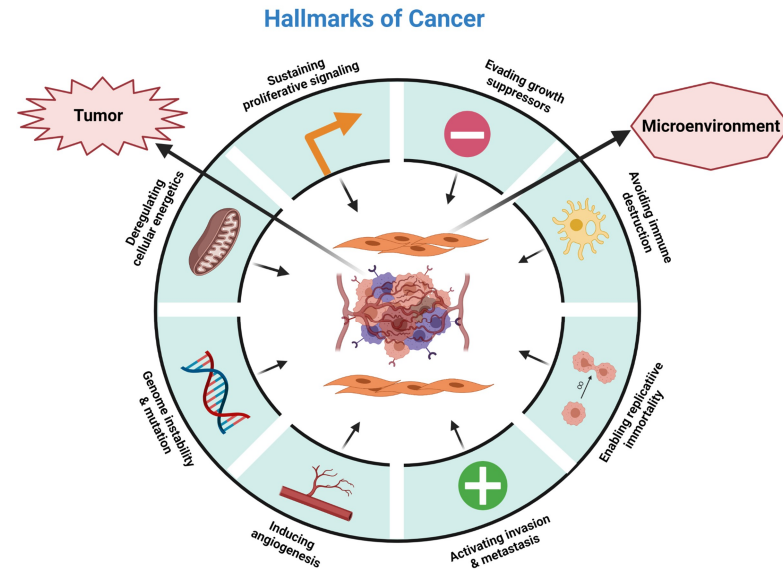
b) Temporal heterogeneity



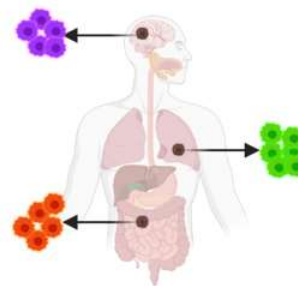
Challenges



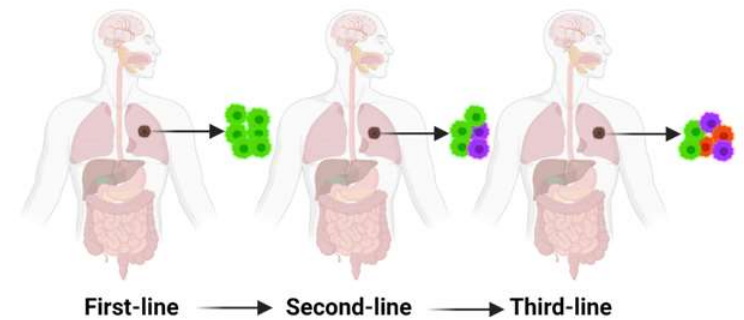
- High complexity
- Difficult to treat
- Resistance (high mortality)
- Poor drug penetration
(limited intratumoral bioavailability)



a) Spatial heterogeneity

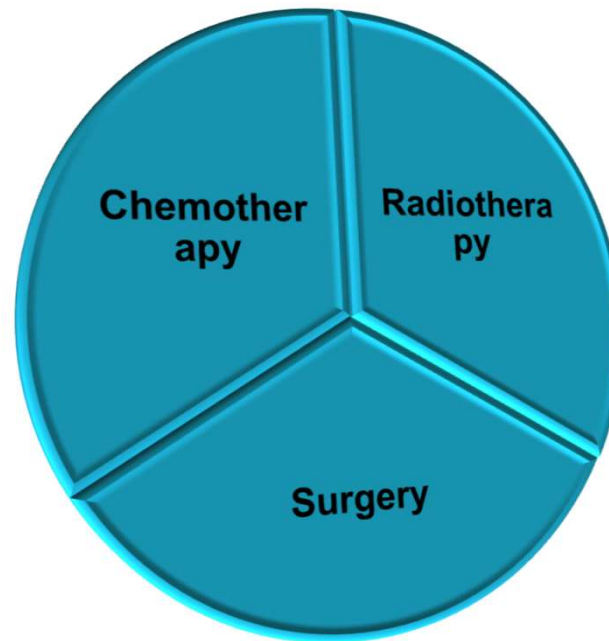


b) Temporal heterogeneity



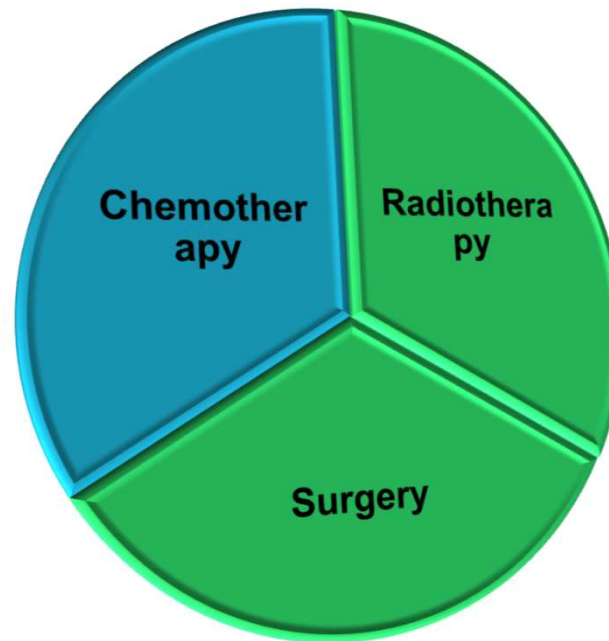
Treatment options

« Multimodal treatment plan »



Treatment options

« Multimodal treatment plan »

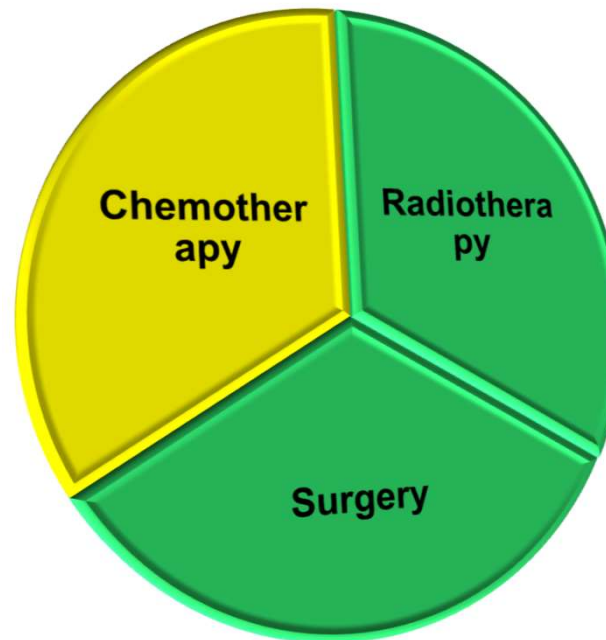


Localized tumors: used at about 60% of cases.

Treatment options

« Multimodal treatment plan »

- Complement to surgery
- Metastasis
- Combination therapy is required for most of the patients

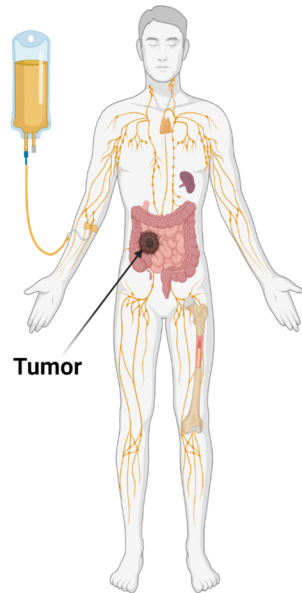


Localized tumors: used at about 60% of cases.

Treatment options

Short and long term side effects of conventional chemotherapy

- Dose limiting toxicity
- Infections
- Nausea and vomiting
- Cardiac problems
- Bleeding problems
- Digestive problems
- Lung problems
- Kidney problems
- Neuropathy
- Loss of appetite

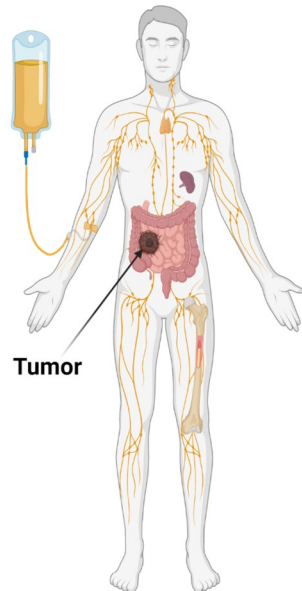


Side effects can often lead to hospitalisation to treat side effects of the treatment

Treatment options

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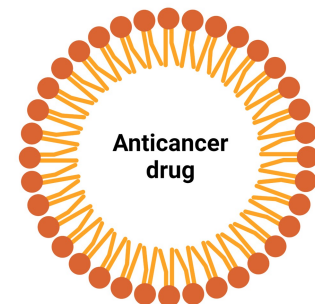


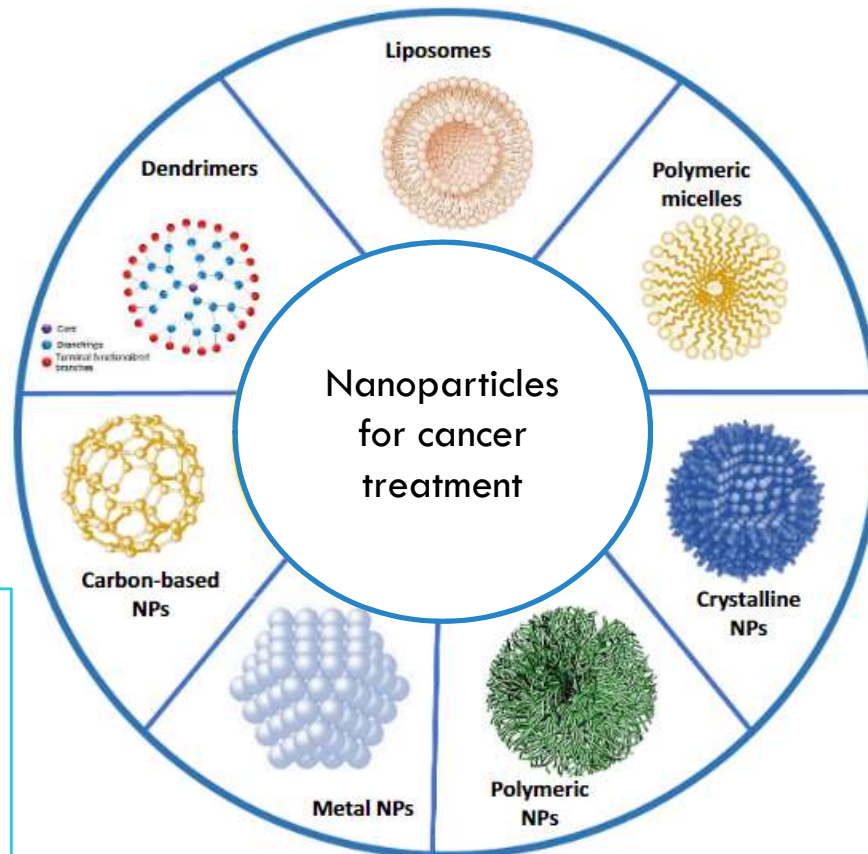
Side effects can often lead to hospitalisation to treat side effects of the treatment

There is a urgent need to develop effective and less toxic therapies:

- ✓ To reduce the short and long term side effects of therapy
- ✓ To enhance the efficacy of treatments

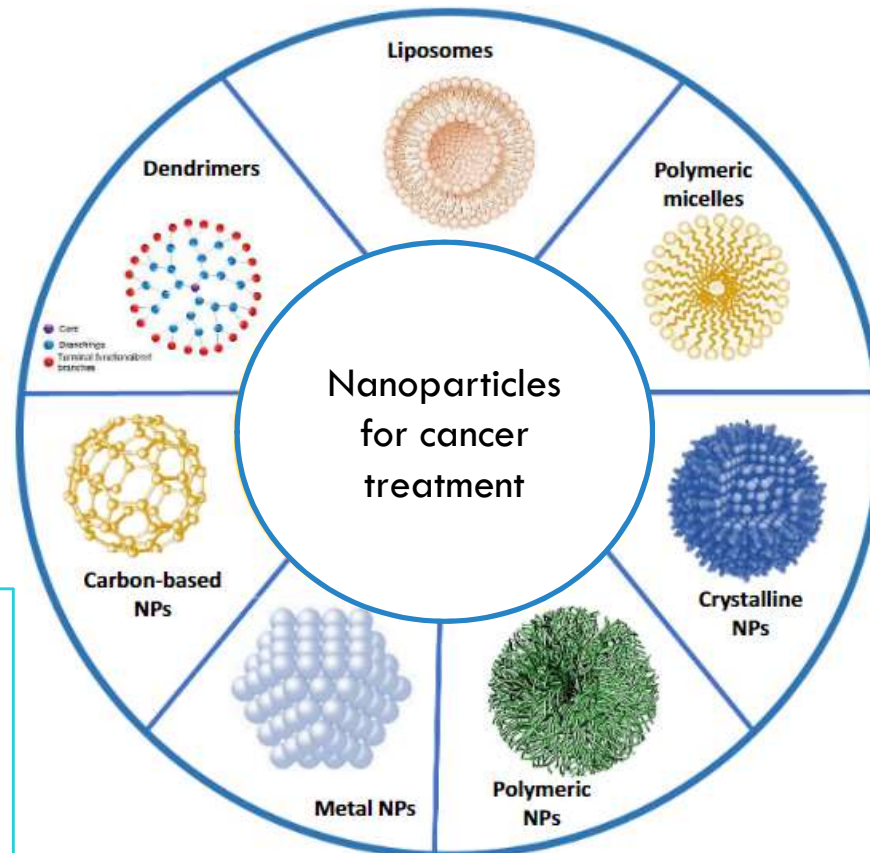
“nanotherapeutics”



Non-viral nanoparticles for delivery

- Polymeric Nanoparticles
- Lipid-based Nanoparticles
- Inorganic Nanoparticles

Non-viral nanoparticles for delivery

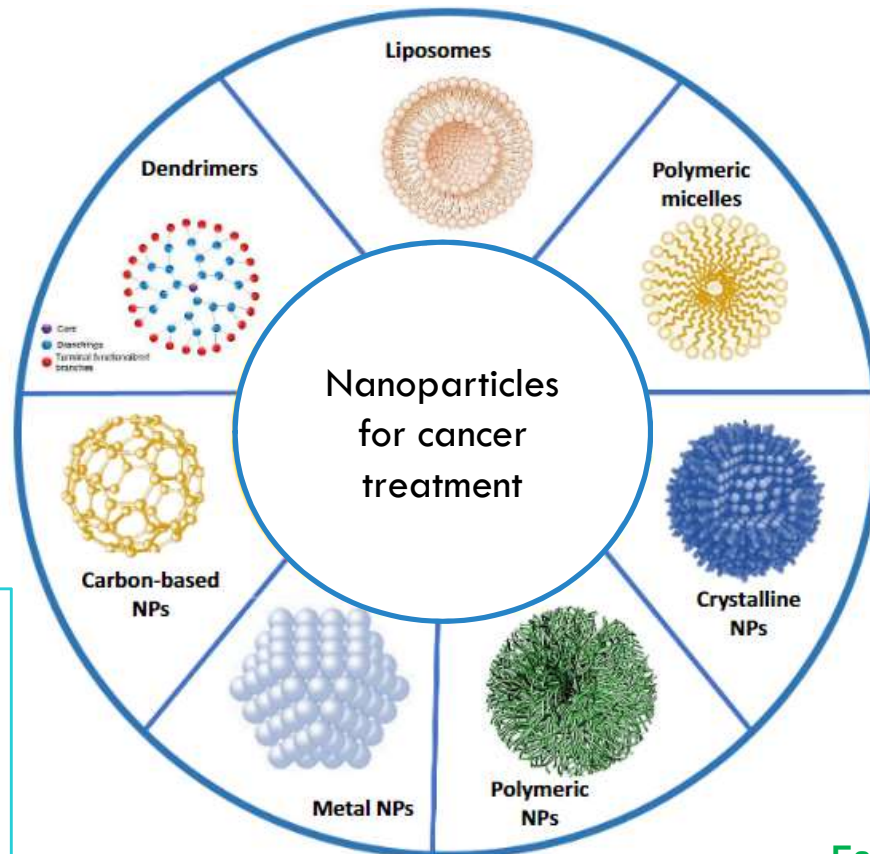


Can load different anticancer drugs

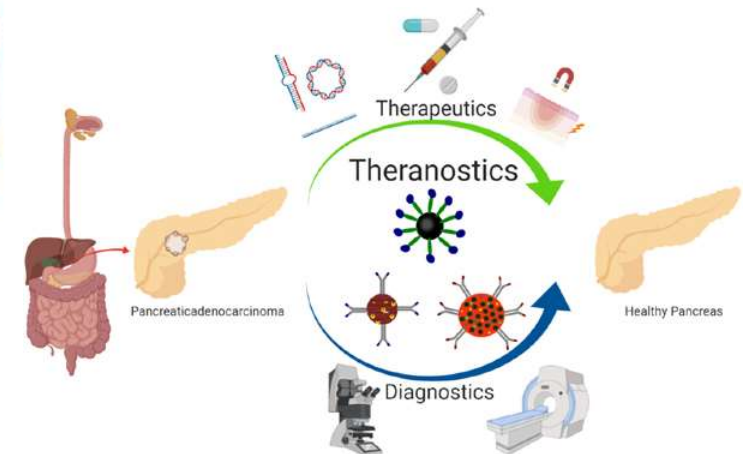
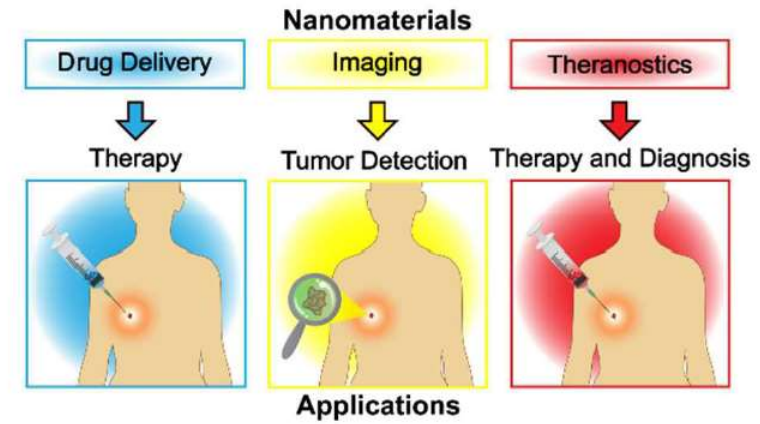
- **Small molecules : Paclitaxel, Doxorubicine, Topotecan etc.**
- **Nucleic acids: siRNA, mRNA**
- **Peptides/proteins**

- **Polymeric Nanoparticles**
- **Lipid-based Nanoparticles**
- **Inorganic Nanoparticles**

Non-viral nanoparticles for delivery



- Polymeric Nanoparticles
- Lipid-based Nanoparticles
- Inorganic Nanoparticles



Early detection of tumors: antigens and biomarkers.

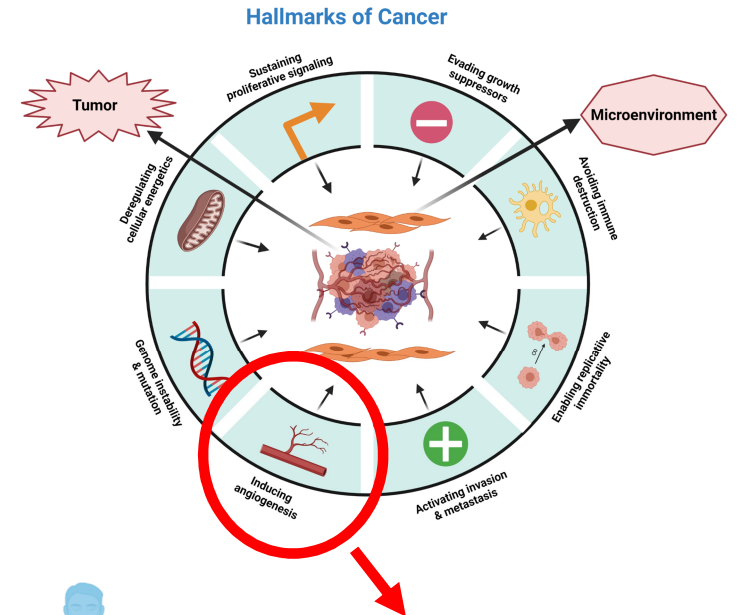
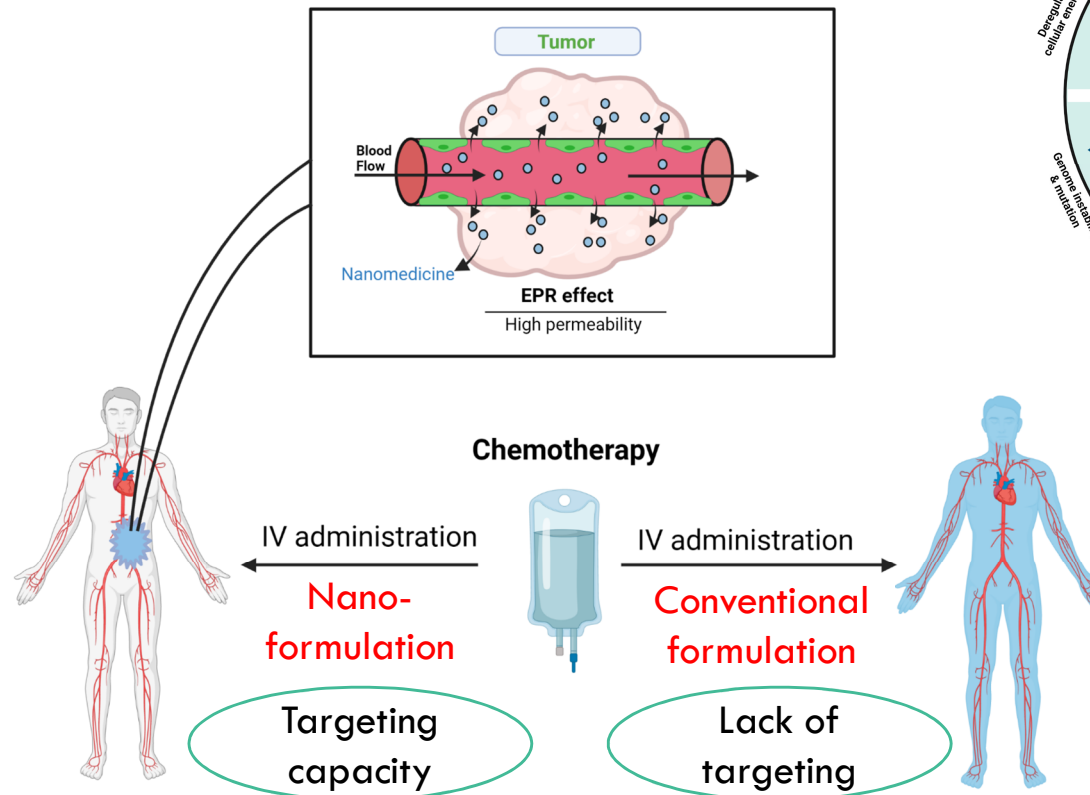
Non-viral nanoparticles for delivery of small molecules on the market

Trade name	Formulation	Indication	Delivery route	Size	Material
Abraxane	Paclitaxel	Various cancers	i.v	130	Liposomes
Doxil	Doxorubicin	Ovarian, breast, multiple myeloma	i.v	90	PEG liposomes
Marqibo	Vincristine	Acute lymphoblastic leukaemia	i.v	100	Liposome
Onivyde	Irinotecan	Metastatic pancreatic cancer	i.v	110	PEG liposomes
DepoCyt	Cytarabine	Malignant lymphomatous	i.v	20	Liposome
Eligard	Leuproline acetate	Advanced prostate	s.c	n/a	PLGA polymer

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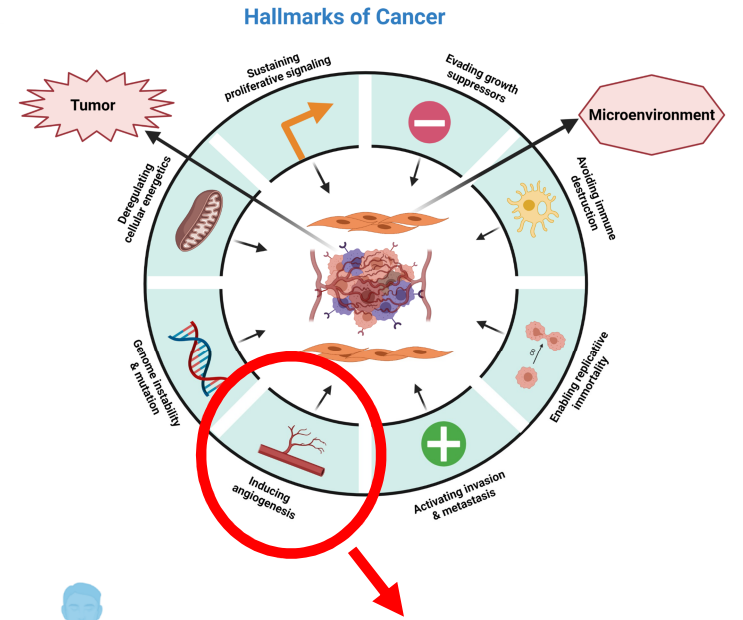
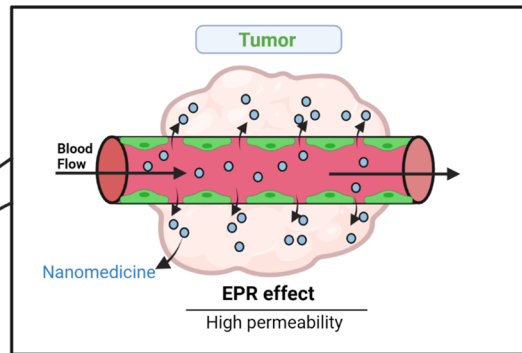
Passive targeting – controlled biodistribution



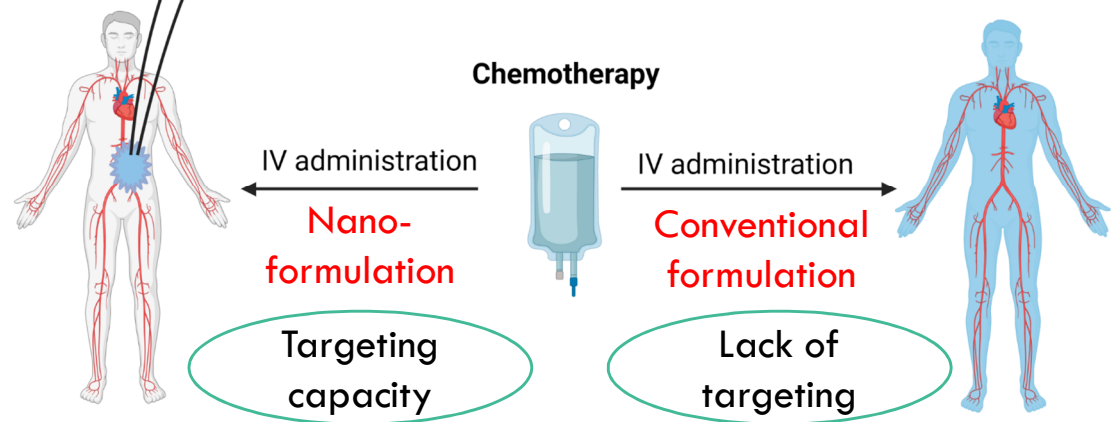
Discovery of Enhanced Permeability and Retention effect (EPR)

Passive targeting – controlled biodistribution

Improved safety profile than efficacy!



Discovery of Enhanced Permeability and Retention effect (EPR)



Limits of conventional chemotherapy

Conventional small molecules:

1. Long term toxicity (large biodistribution)
2. Emergence of Resistance
3. Lack selectivity (all rapidly growing cells)
4. Can't treat all types of cancers (limited targets)

Innovative cancer therapy under development

Conventional small molecules:

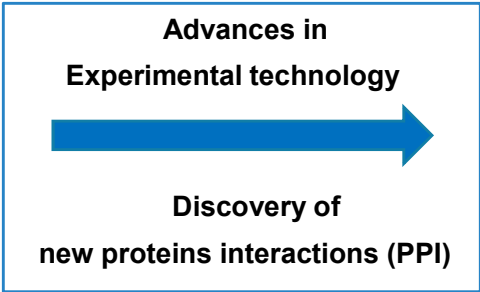
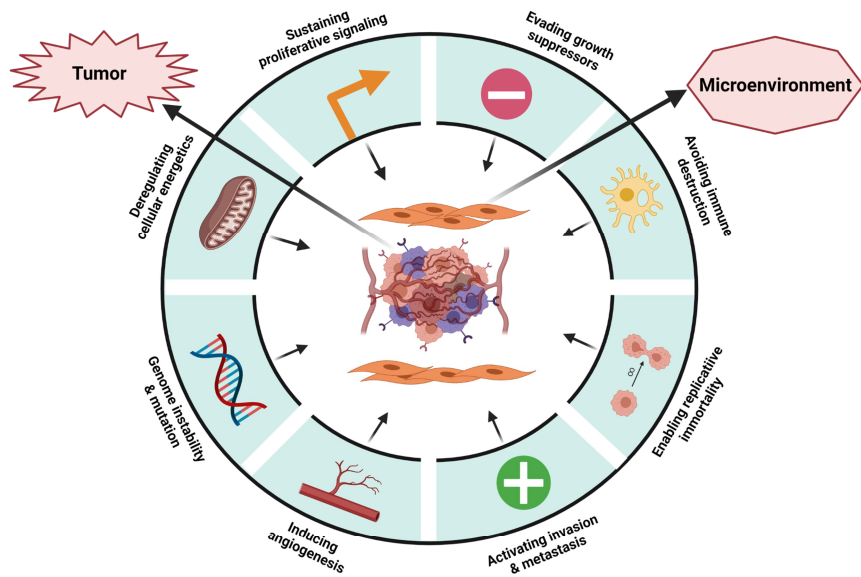
1. Long term toxicity (large biodistribution)
2. Emergence of Resistance
3. Lack selectivity (all rapidly growing cells)
4. Can't treat all types of cancers (limited targets)

Novel therapy: smart approach to tackle with biopharmaceuticals

1. Validate new targets (untreated cancers)
2. Better tumor selectivity « Targeted therapy » (less toxicity)
3. Enhanced efficacy « mechanism of action »
4. Reduce long term toxicity (children)

Innovative cancer therapy under development

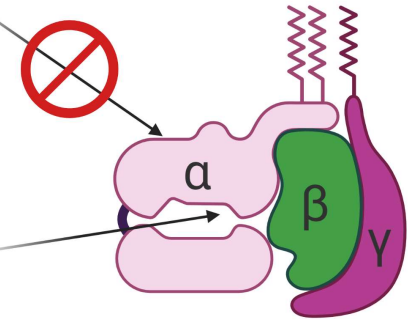
Hallmarks of Cancer



Silencing Gene that produces specific protein

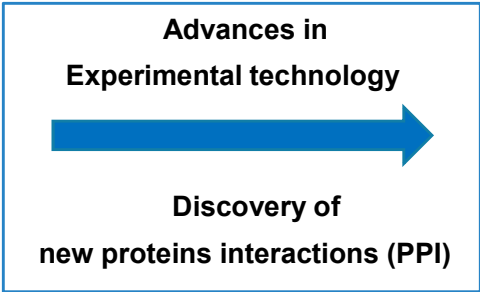
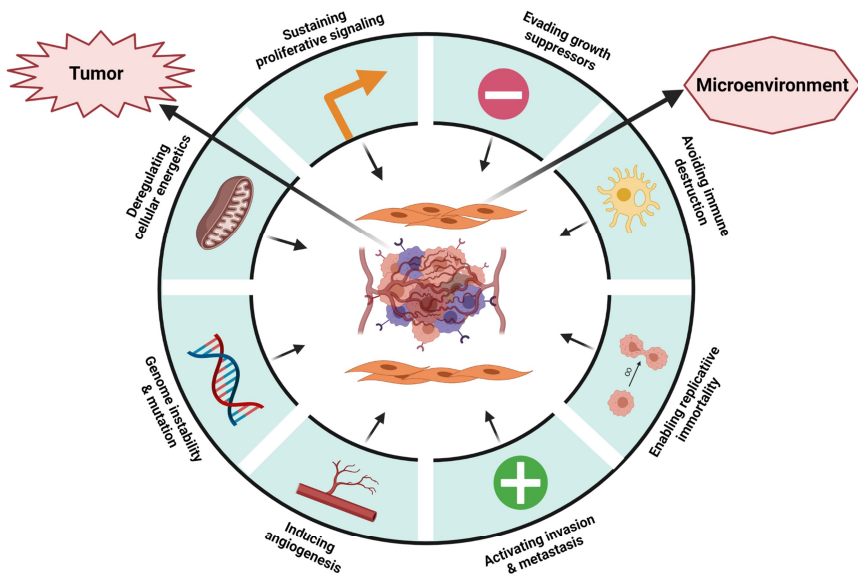
Oncogenic Protein

Inhibition of Protein-Protein interactions (PPI)



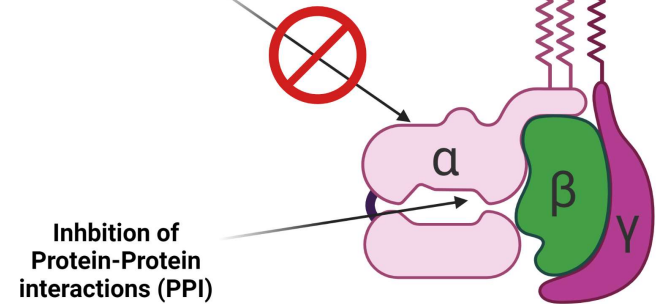
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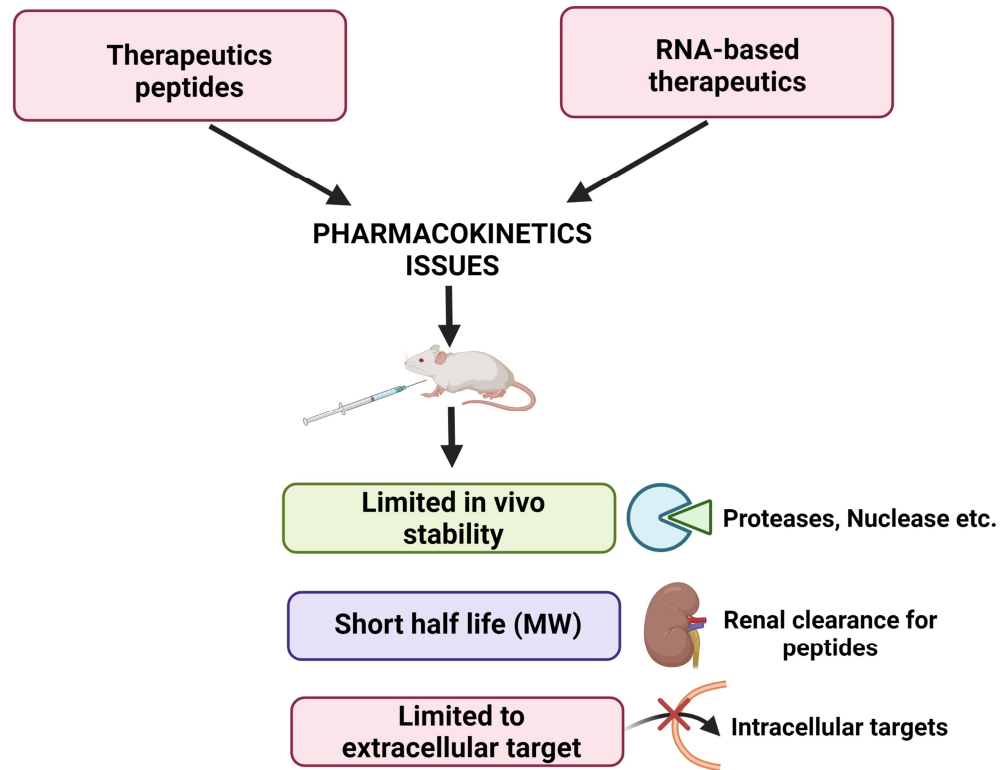


Novel therapy: smart approach to tackle with biopharmaceuticals

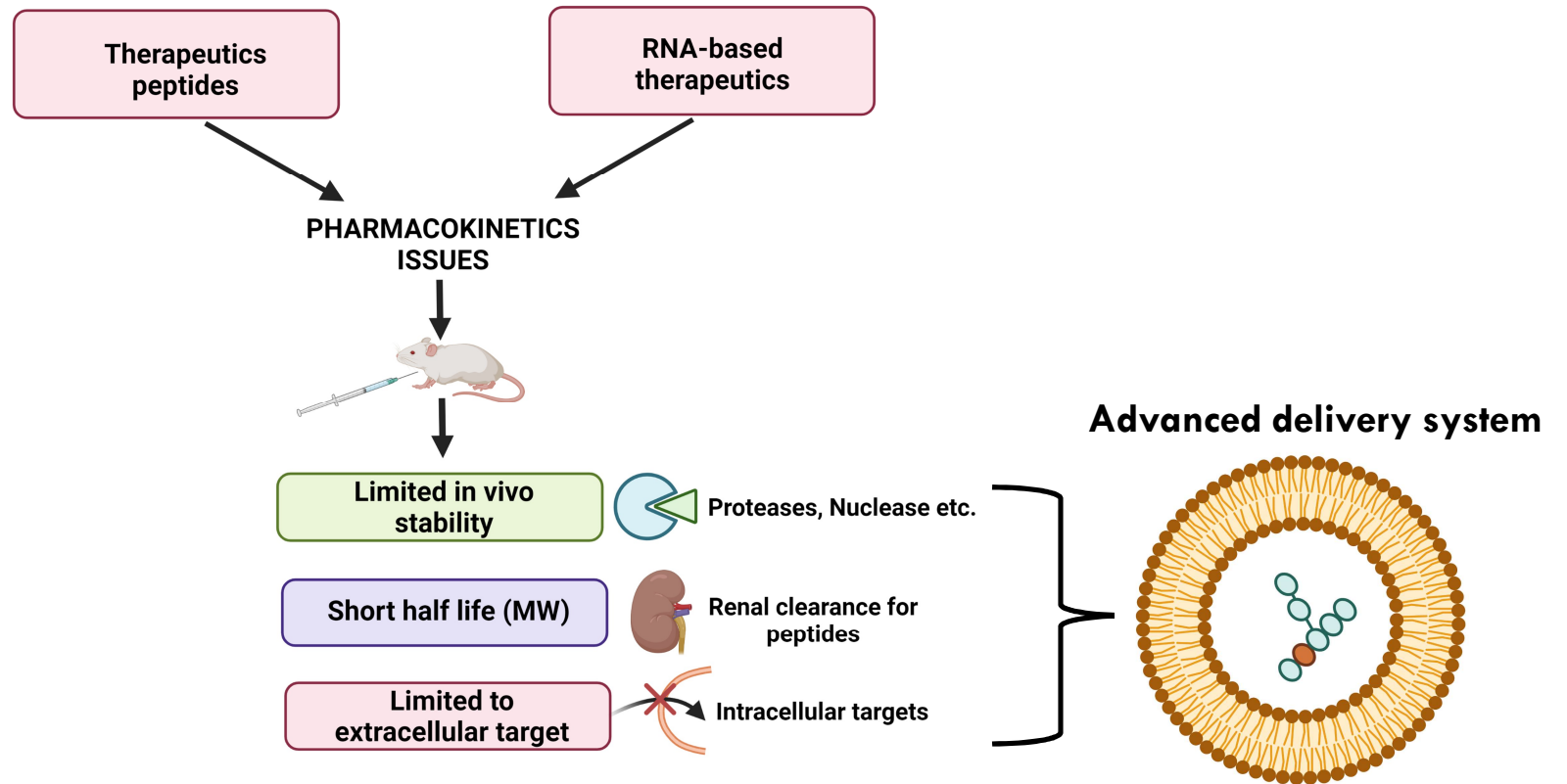
Validation of undruggable targets

« peptides, RNA based therapeutics (SiRNA, mRNA and antisense oligonucleotides) »

Innovative cancer therapy under development



Innovative cancer therapy under development



LDH Project

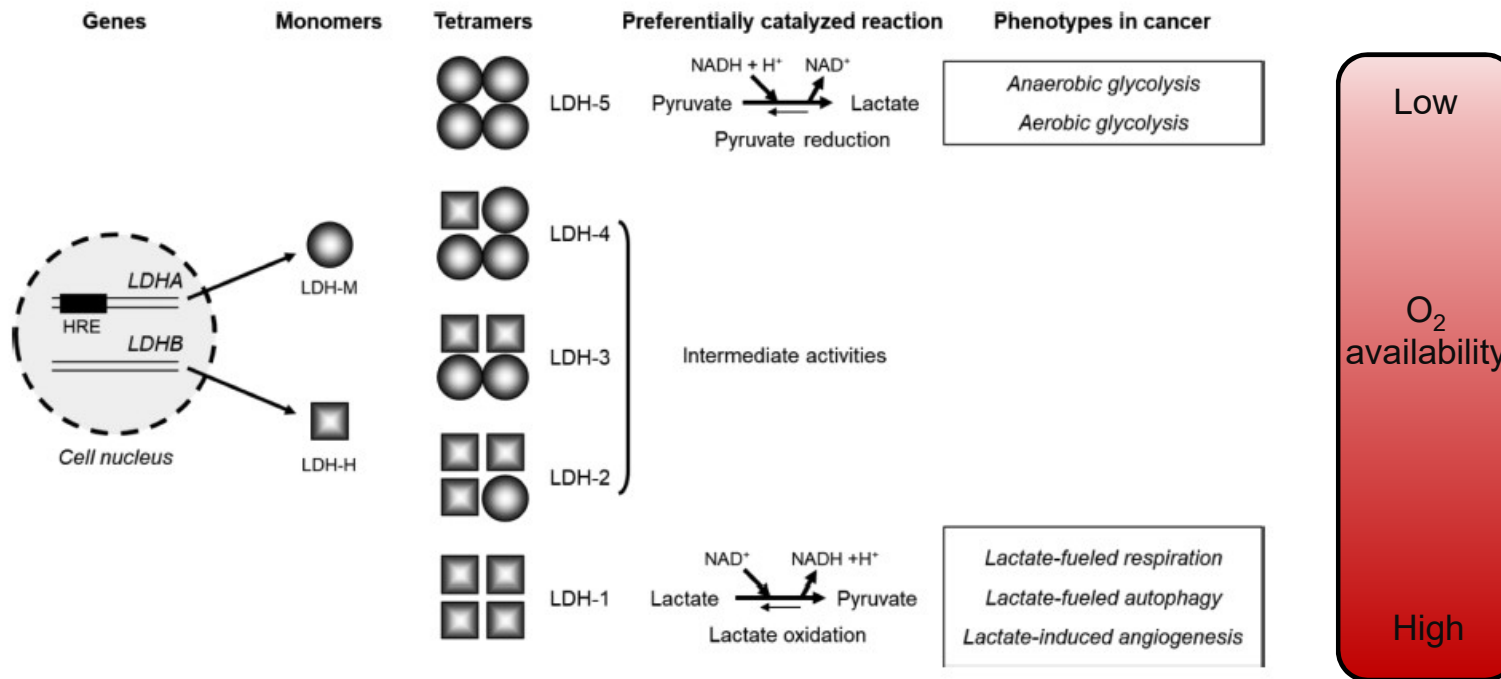
Journal of
**Medicinal
Chemistry**

pubs.acs.org/jmc

Article

Interrogating the Lactate Dehydrogenase Tetramerization Site Using (Stapled) Peptides

Léopold Thabault, Lucie Brisson, Chiara Brustenga, Santiago A. Martinez Gache, Julien R. C. Prévost, Arina Kozlova, Quentin Spillier, Maxime Liberelle, Zohra Benyahia, Joris Messens, Tamara Copetti, Pierre Sonveaux* and Raphaël Frédérick*



Peptides properties

LB19

ATLKEKLIAPVAEEEEATVP**C₉₀H₁₅₃N₂₁O₃₀ MW: 2009,33 g/mol**NH₂- Ala - Thr - Leu - Lys - Glu - Lys - Leu - Ile - Ala - Pro - Val - Ala - Glu -
Glu - Glu - Ala - Thr - Val - Pro -COOH**Kd = 200 μM – 1 mM**

Theoretical pI: 4.49

Net charge at pH 7: - 2

Average hydrophilicity: 0.4

Ratio of hydrophilic residues / total number of residues: 32 %

MC-7

CTLKCKLI: “p-tetrafluorophenyl analogue”**C₄₀H₇₆N₁₀O₁₀S₂ MW: 921.22 g/mol**NH₂- Cys - Thr - Leu - Lys - Cys - Lys - Leu - Ile -COOH**Kd = 11 μM**

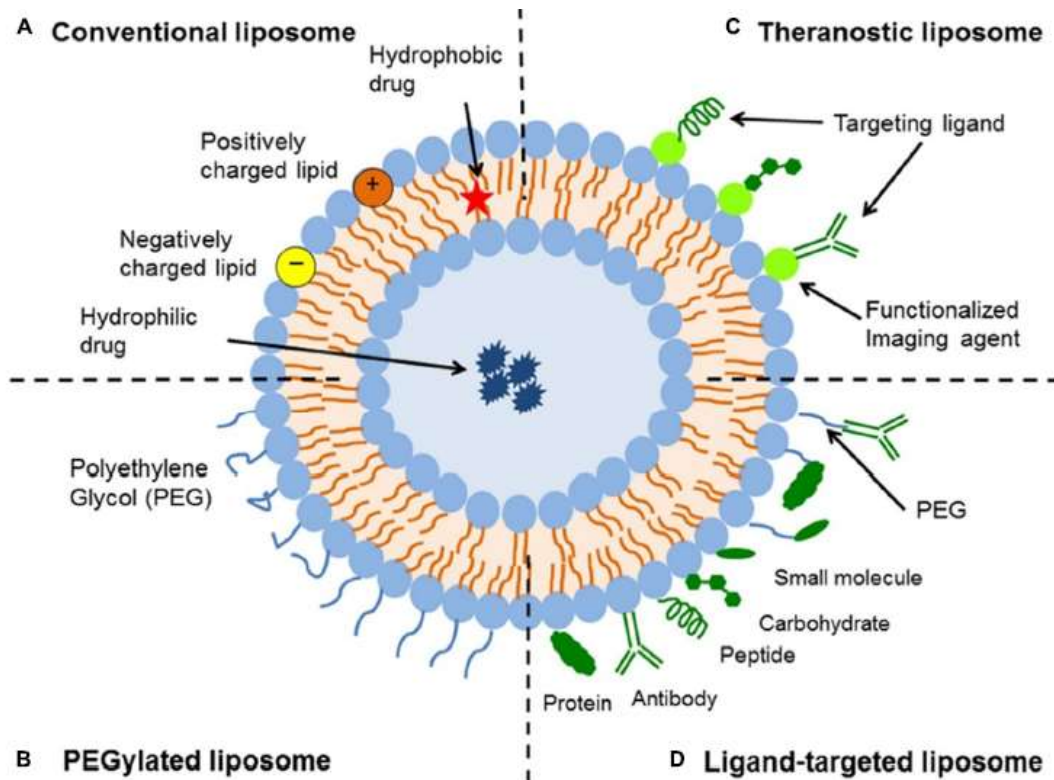
Theoretical pI: 8,90

Net charge at pH 7: + 1.9

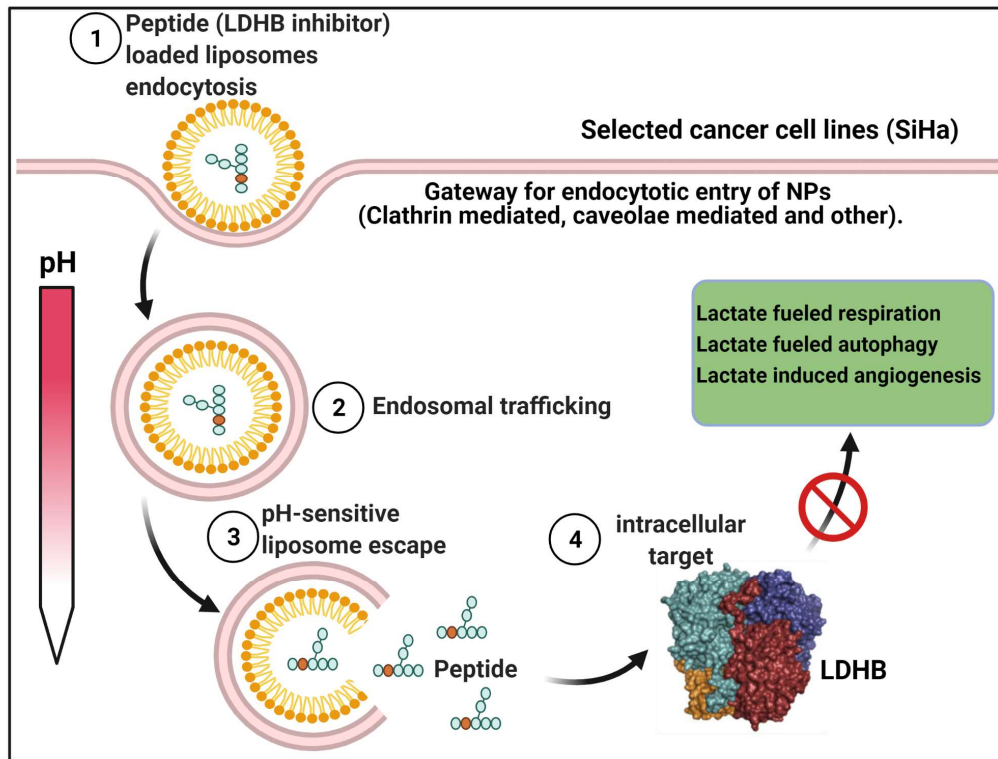
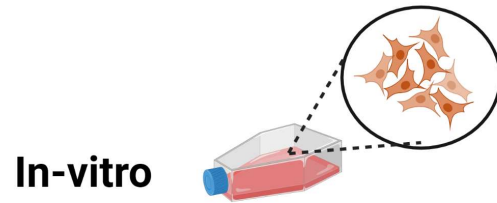
Average hydrophilicity: - 0.2

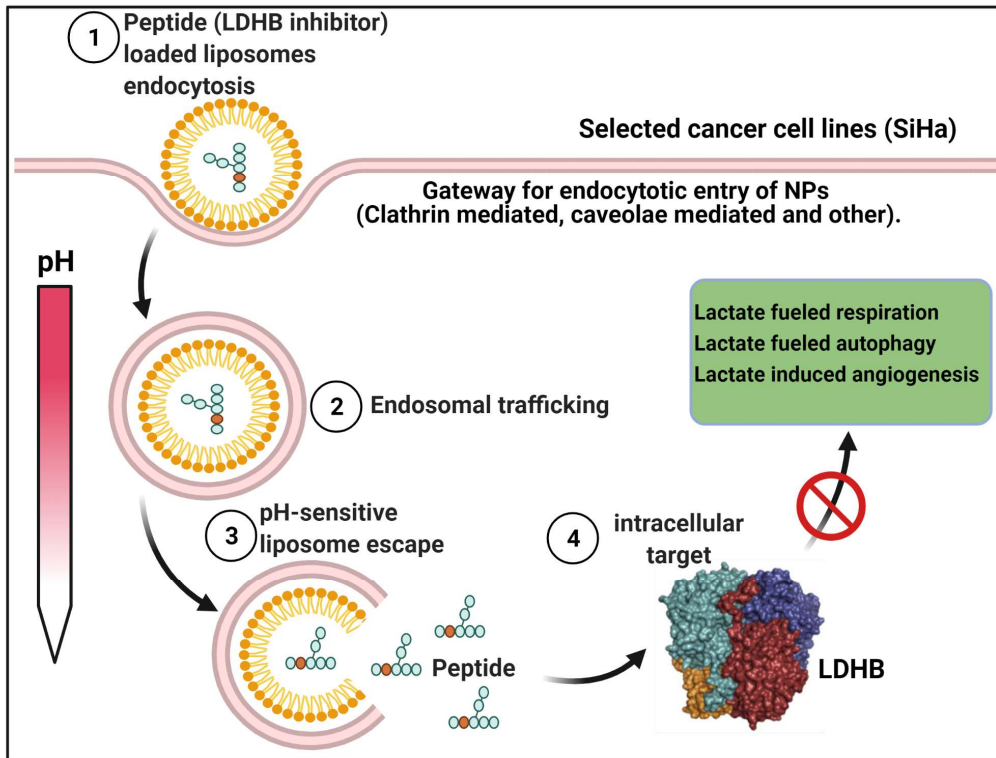
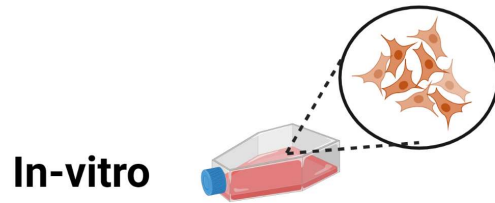
Ratio of hydrophilic residues / total number of residues: 25 %

Liposomes as drug carrier – a versatile delivery platform



- ✓ Biocompatible and biodegradable excipients
- ✓ Encapsulated both hydrophilic and hydrophobic drugs.
- ✓ Protection of the encapsulated drugs
- ✓ Versatility when chemically modified (**stimuli responsive, tunable surface chemistry etc**)





pH sensitive lipids for the design of liposomes

Fusogenic lipids

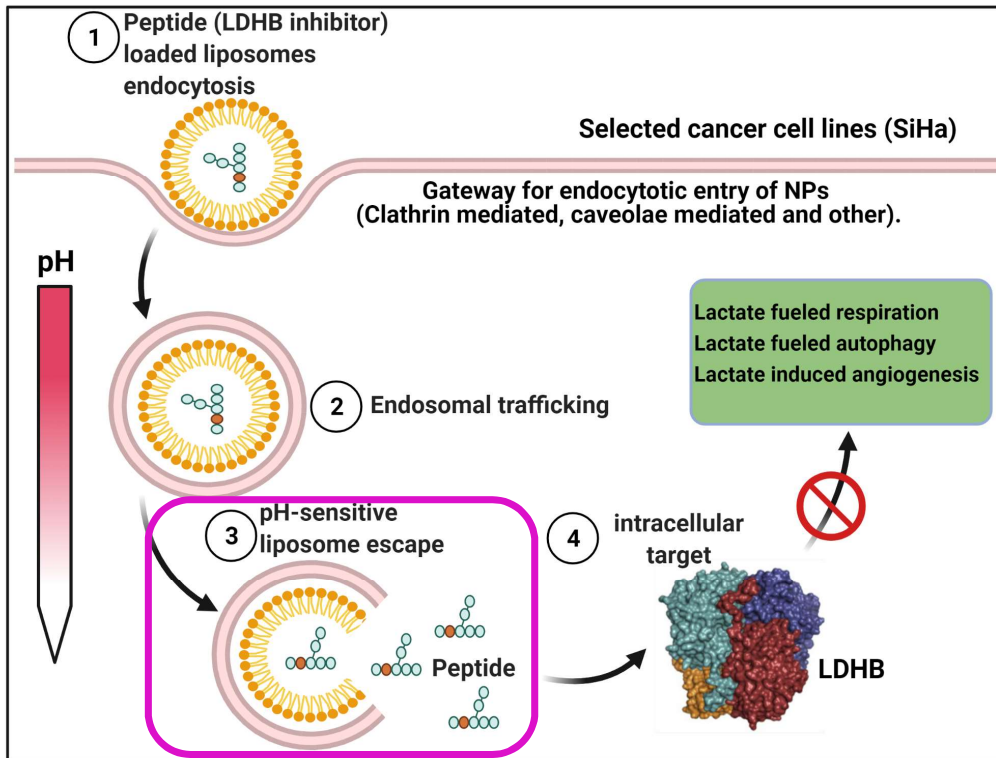
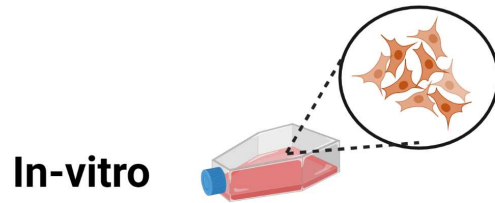
- DOPE
- CHEMS

Ionisable lipid in endosomal pH

- DODAP
- D-Lin-MC3-DMA
- ALC-0315, SM-102

Switchable lipids in endosomal pH

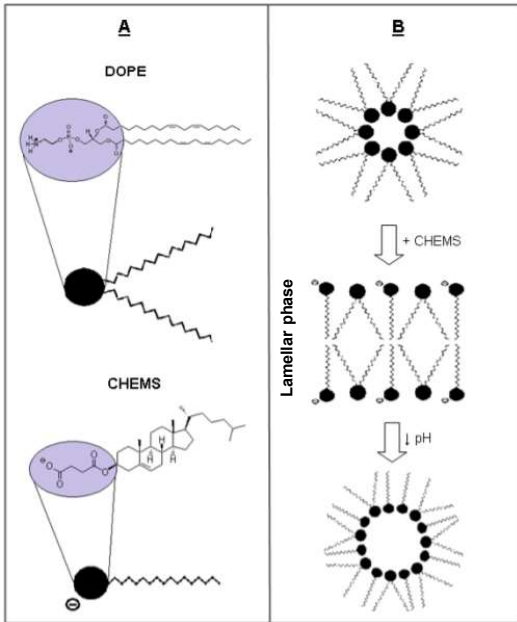
- CSL3



pH sensitive lipids for the design of liposomes

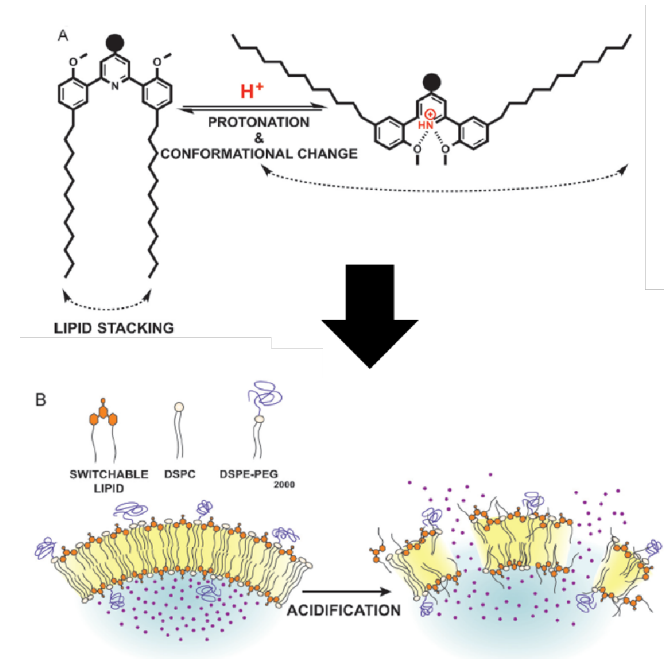
- Fusogenic lipids**
 - DOPE
 - CHEMS
- Ionisable lipid in endosomal pH**
 - DODAP
 - D-Lin-MC3-DMA
 - ALC-0315, SM-102
- Switchable lipids in endosomal pH**
 - CSL3

1. Fusogenic lipids

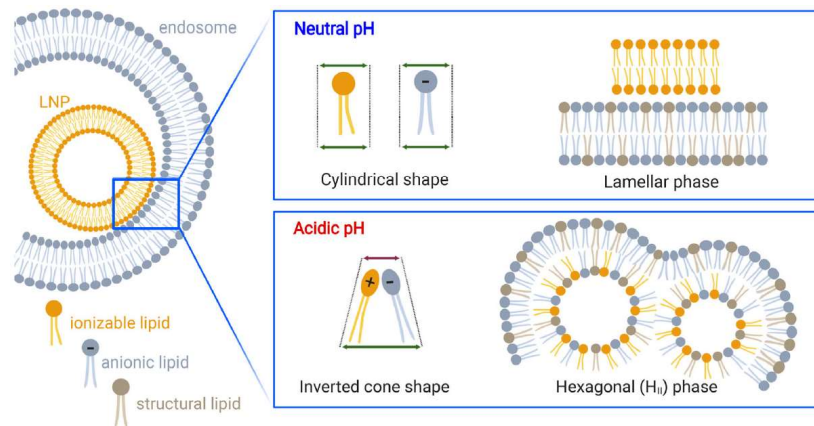
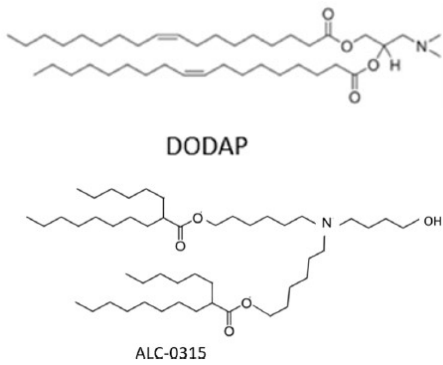


pH responsiveness of lipids as trigger for endosomal escape
 « Cone shape requirement »

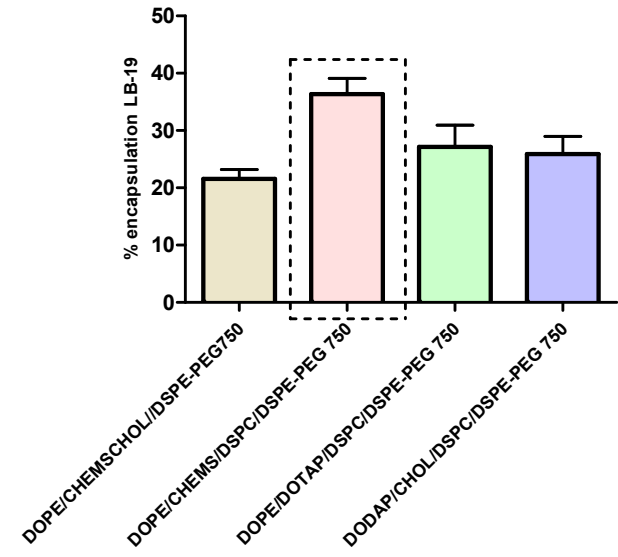
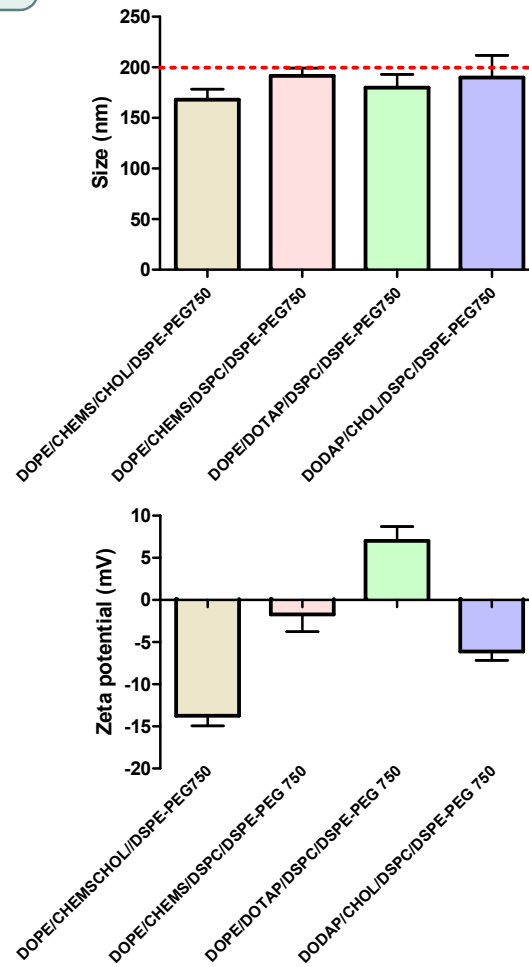
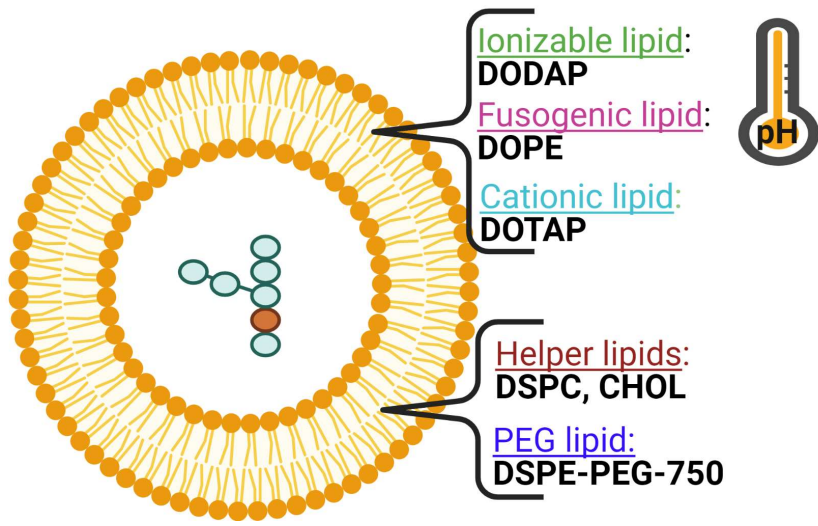
3. Switchable lipids



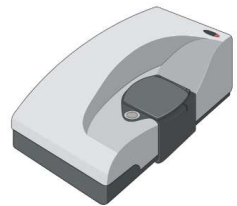
2. Ionizable lipids



Liposomes formulation design and physicochemical characterization at physiological pH

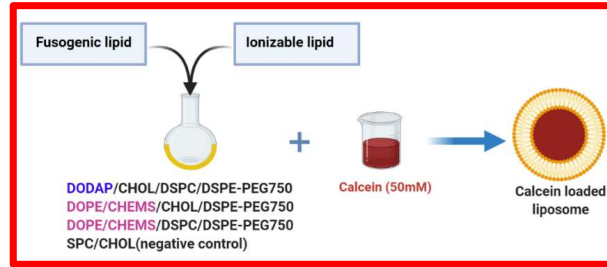


Physicochemical characterization at endosomal mimicking pH



Zetasizer (Malvern)

Size analysis at endosomal pH

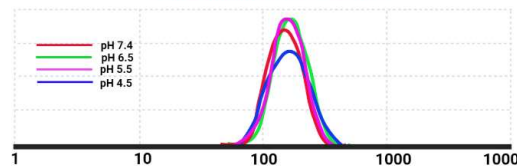


Calcein release assay at endosomal pH

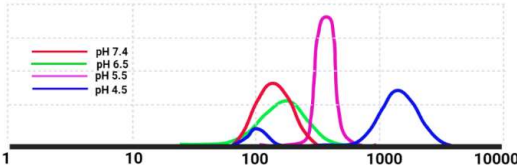


Flexstation reader

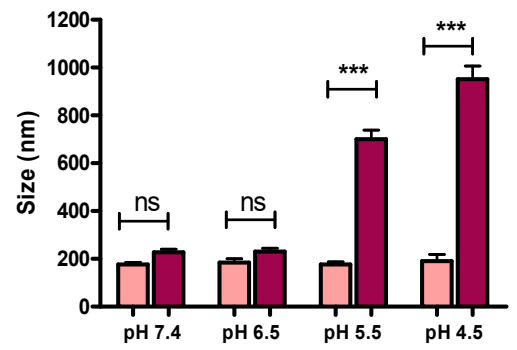
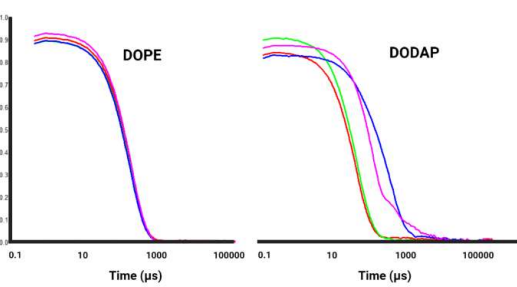
Size Distribution by intensity
DOPE/CHEMS/CHOL-PEG-750



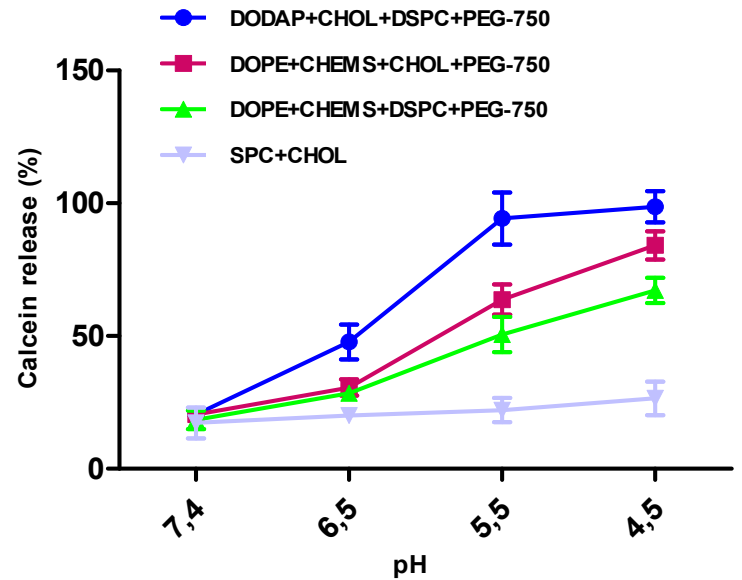
Size Distribution by intensity
DODAP/CHOL/DSPE-PEG-750



Correlation coefficient



Legend for size chart:
 DOPE/CHEMS/CHOL/DSPE-PEG-750 (light pink)
 DODAP/CHOL/DSPC/DSPE-PEG-750 (dark pink)



Release after 20 min of incubation at 37°C

Confocal analysis

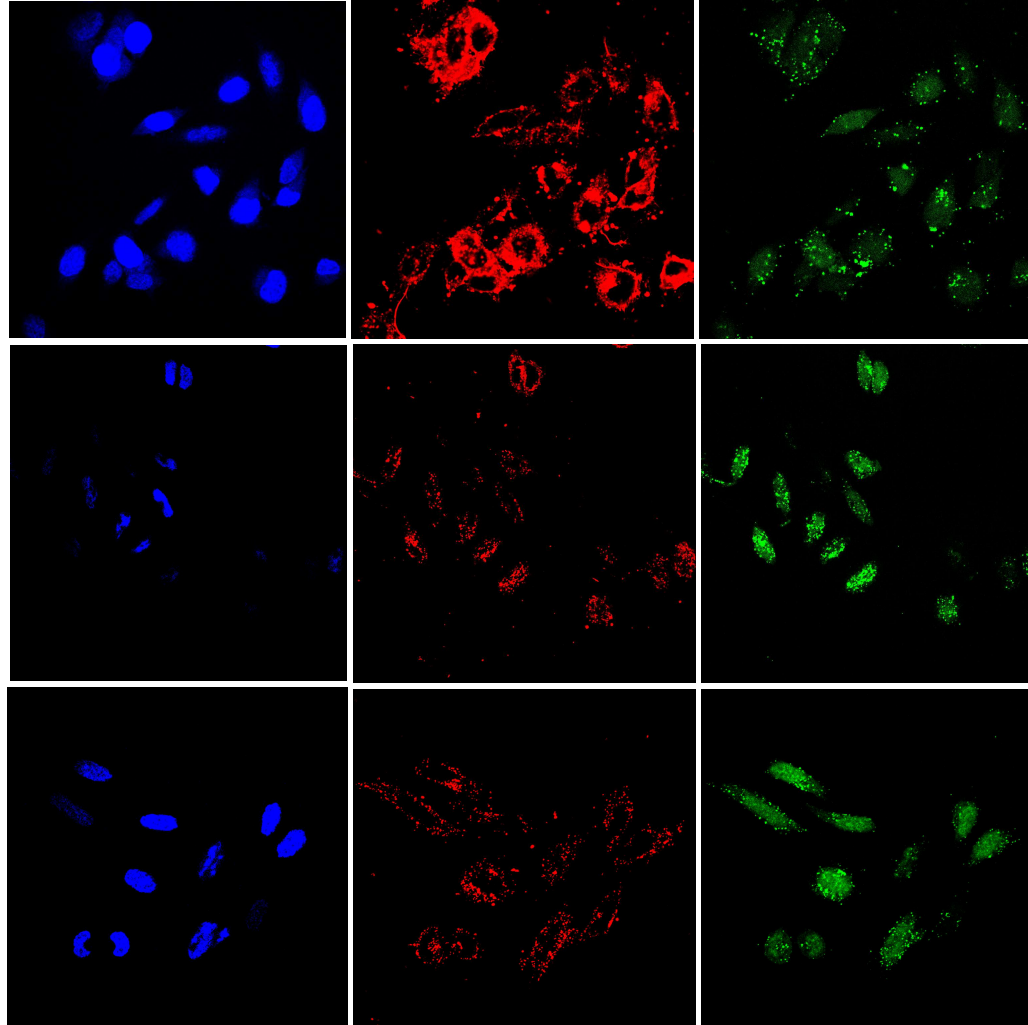


1. Cell uptake and subcellular localization distribution of liposomes (SiHa):

- Nuclei staining with DAPI
- Rhodamine B -PE labelled liposomes

2. Intracellular release of the cargo (hydrophile)

- Calcein green fluorescence



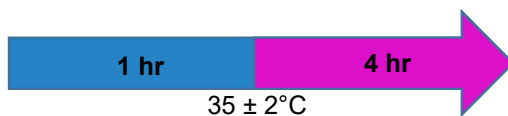
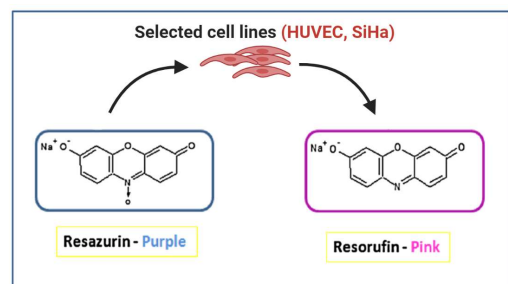
DODAP/CHOL/DPSC/PEG-750: (45/20/30/5)

DOPE/CHEMS/DPSC/PEG-750: (45/25/30/5)

DOPE/CHEMS/CHOL/PEG-750: (60/25/10/5)

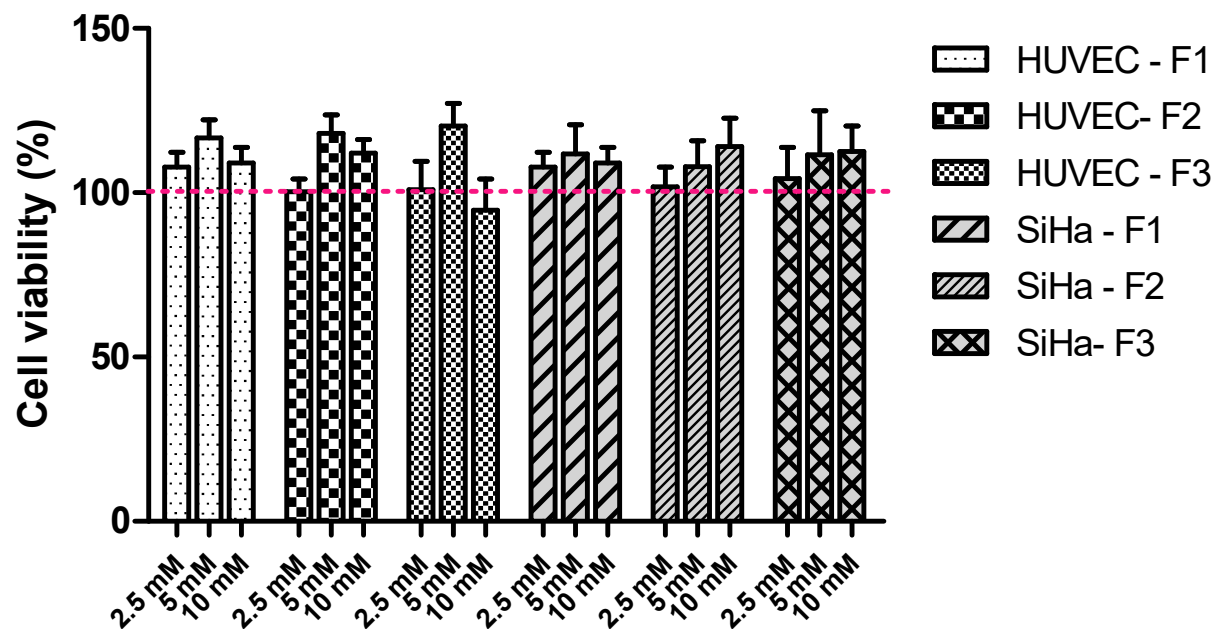
Cytotoxicity against primary and tumor cell lines

Toxicity against Human umbilical vein endothelial cells (HUVEC) and Cervical carcinoma cell lines (SiHa)

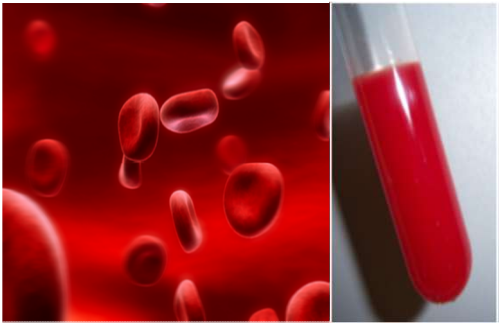


Resazurin viability assay

Composition	Proportion (%Mol)
F1: DOPE/CHEMS/CHOL/DSPE-PEG750	60/25/10/5
F2: DOPE/CHEMS/DSPC/DSPE-PEG750	45/20/30/5
F3: DODAP/CHOL/DSPC/DSPE-PEG750	45/20/30/5

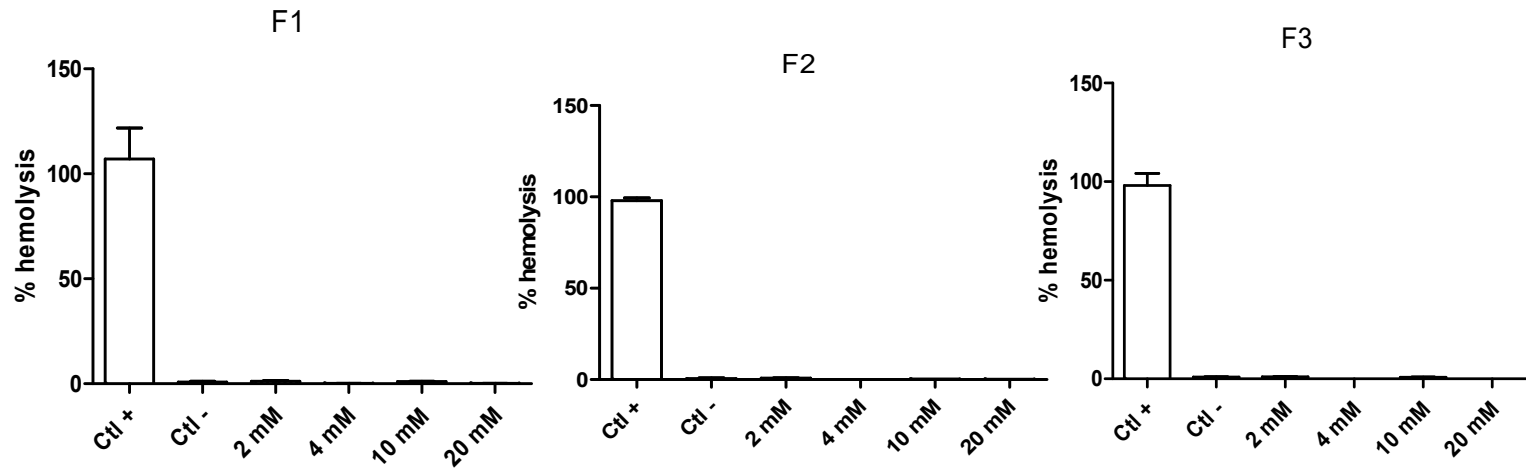


Hemocompatibility tests

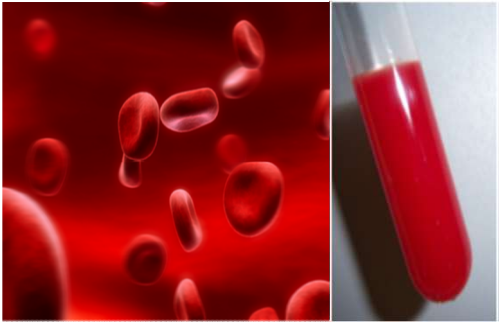


• Hemolysis

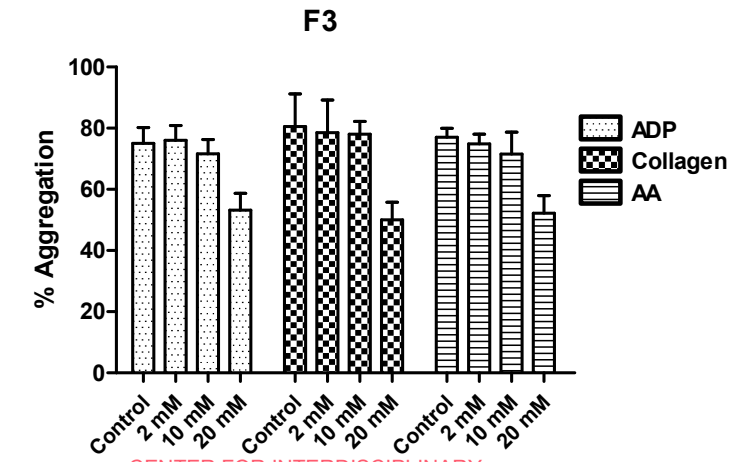
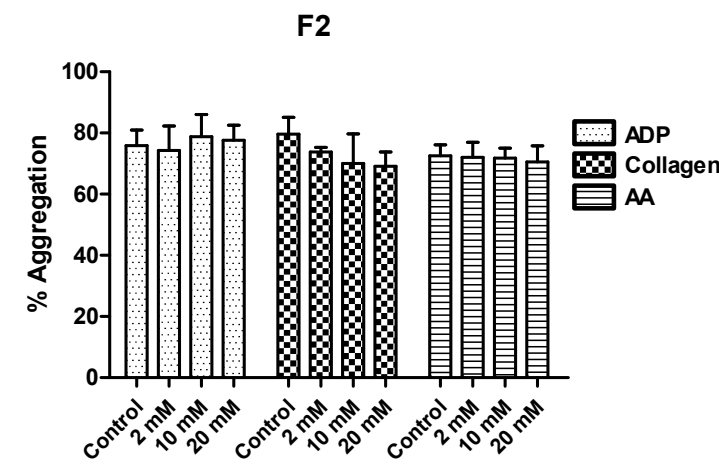
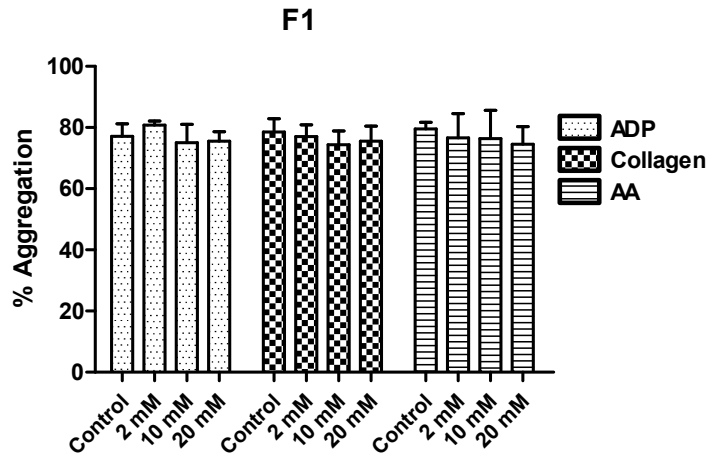
Composition	Proportion (%Mol)
F1: DOPE/CHEMS/CHOL/DSPE-PEG750	60/25/10/5
F2: DOPE/CHEMS/DSPC/DSPE-PEG750	45/20/30/5
F3: DODAP/CHOL/DSPC/DSPE-PEG750	45/20/30/5



Hemocompatibility tests

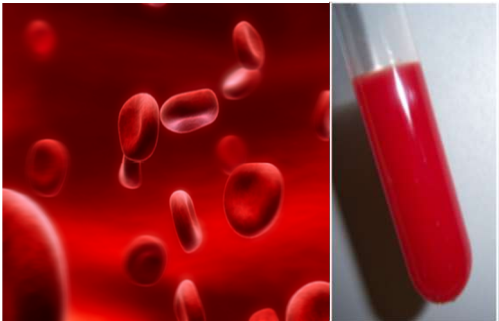


• Platelet aggregation

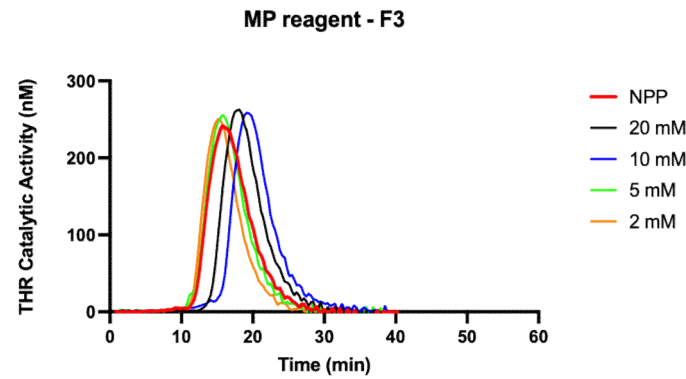
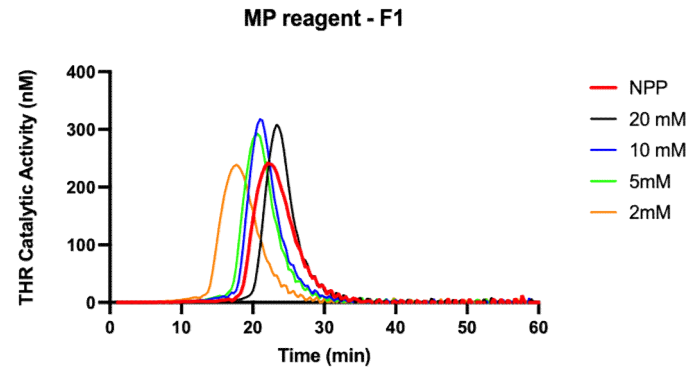


Composition	Proportion (%Mol)
F1: DOPE/CHEMS/CHOL/DSPE-PEG750	60/25/10/5
F2: DOPE/CHEMS/DSPC/DSPE-PEG750	45/20/30/5
F3: DODAP/CHOL/DSPC/DSPE-PEG750	45/20/30/5

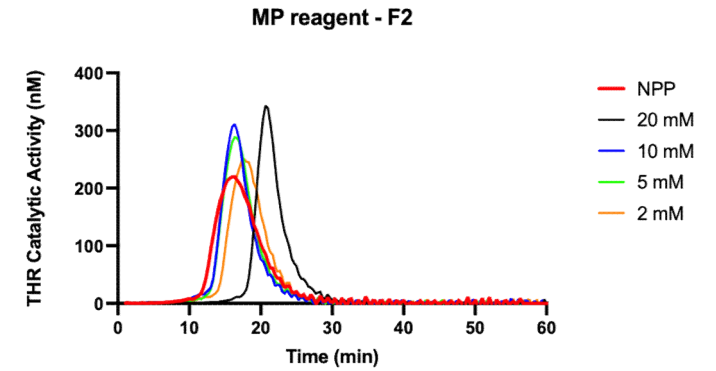
Hemocompatibility tests



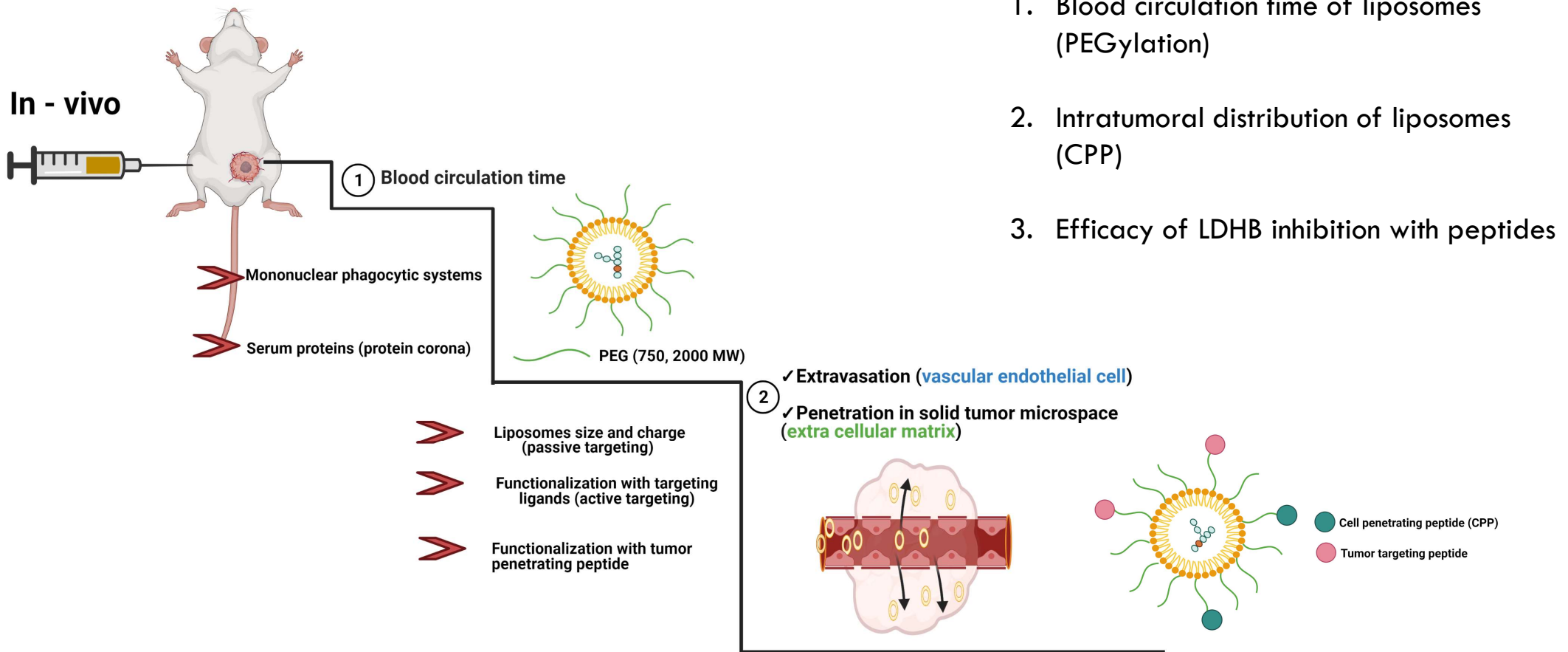
• Generation of thrombine



Composition	Proportion (%Mol)
F1: DOPE/CHEMS/CHOL/DSPE-PEG750	60/25/10/5
F2: DOPE/CHEMS/DSPC/DSPE-PEG750	45/20/30/5
F3: DODAP/CHOL/DSPC/DSPE-PEG750	45/20/30/5



Efficacy Test in vivo in perspectives



TAKE HOME MESSAGE FOR NANOMEDICINE AND CANCERS

- Improved toxicity and efficacy profiles of old anticancer drugs (therapeutic index)
- Evaluation and validation of new targets with innovative drugs (biopharmaceuticals with great therapeutic potential)
- Tunable composition for tackling the pathophysiological complexity of cancer



- Need in-vitro and in-vivo models = tumor environment in human for clinical translation
- Complexity of NPs = challenges regarding scale up production and QC of nanomedicines



Acknowledgements



LIÈGE université

Center for Interdisciplinary
Research on Medicines

FEDER



UNION EUROPEENNE



Wallonie

LE FONDS EUROPÉEN DE DÉVELOPPEMENT RÉGIONAL
ET LA WALLONIE INVESTISSENT DANS VOTRE AVENIR

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