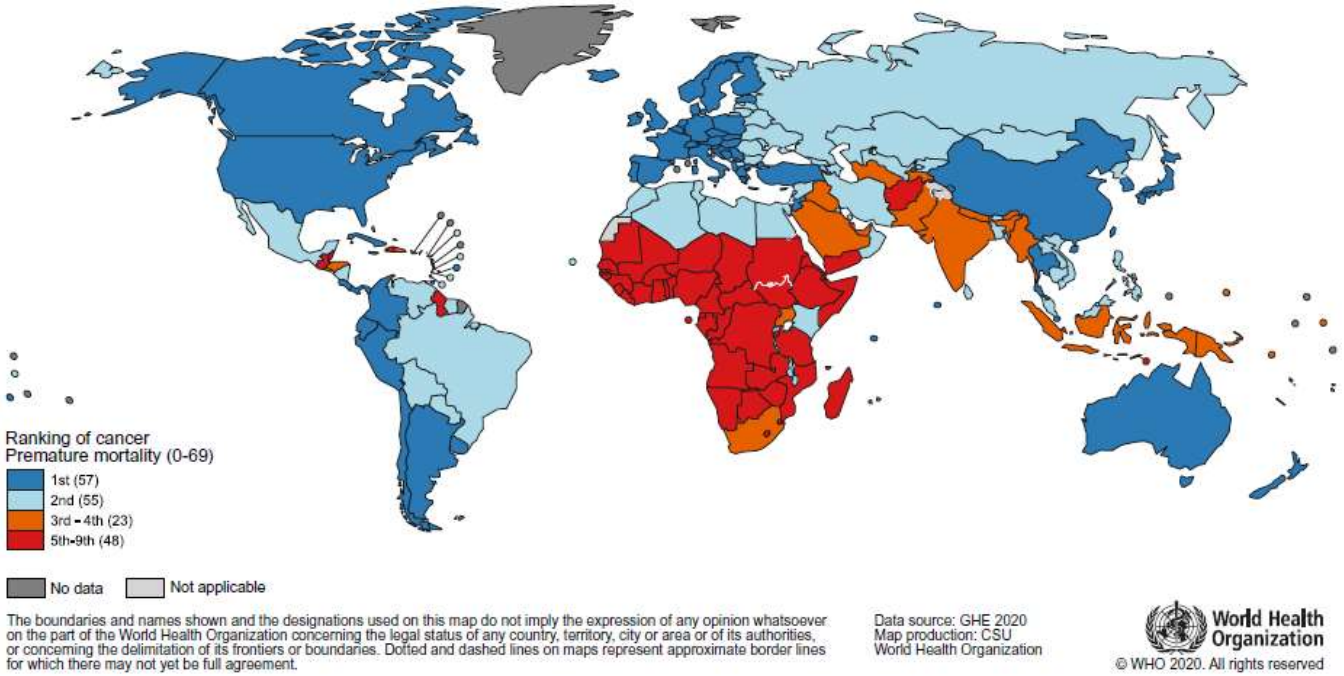
CIRM

LIÈGE université
Center for Interdisciplinary Research on Medicines

ADDRESSING THE CURRENT MEDICAL NEEDS FOR BETTER MANAGEMENT OF CANCER USING NANOTECHNOLOGY-BASED DRUG DELIVERY SYSTEMS

Ange ILANGALA

CENTER FOR INTERDISCIPLINARY
RESEARCH ON MEDICINES



Cancer = growing and global public health issue

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**

CA CANCER J CLIN 2021;71:209–249

PLAN

1. What causes cancer (molecular prospective) and challenges for cancer management (Clinical and therapeutic aspects of cancers)

2. Applications of nanotechnology in cancer management

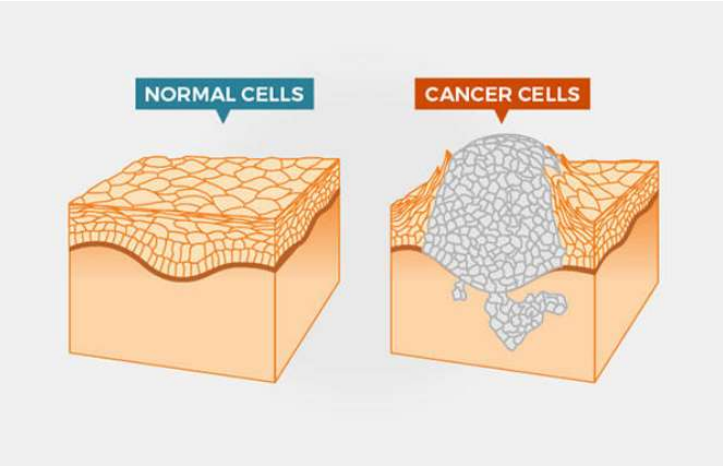
- Improvement of PK profiles of existing anticancer drugs **"conventional chemotherapy drugs"**
- Innovative anticancer therapy (underdevelopment therapy) **"targeted anticancer drugs"** **Part of my PhD Research = some results**

3. Conclusion (Take home message)

Cancer at glance

- Cancer is a disease in which some of the body's cells proliferate uncontrollably to **form masses or a collection of cells (tissue) called tumors.**
- A tumor cell is part of tissue that is abnormally growing, it may either:

- Benign: grow slowly and do not spread.
- Malignant: grow rapidity, invade and destroy nearby normal tissues, and spread throughout the body.



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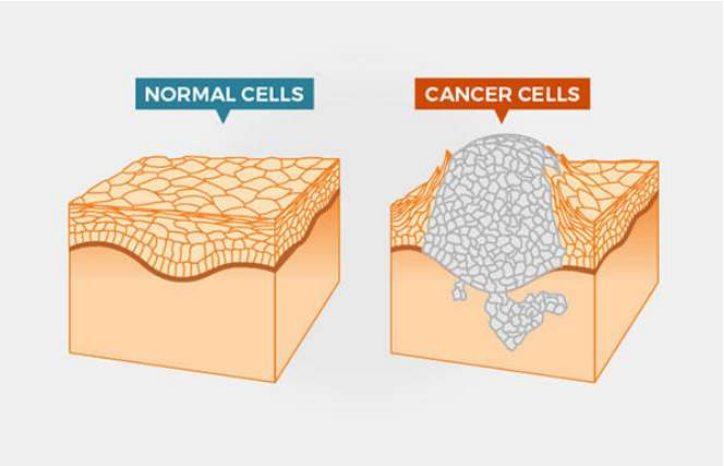

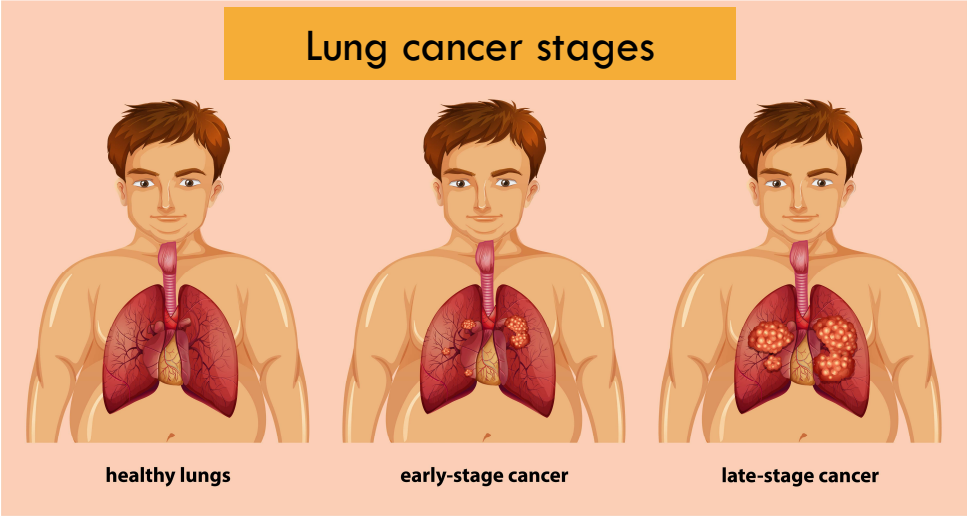

- **Cancers can arise from various tissues**

1. **Carcinoma:** 80 – 90% of cancer, derived from epithelial tissue such as Skin, GIT etc.

2. Blood cancer: Blood-forming tissues including bone marrow

3. Sarcoma: Connective and soft tissues such as bones, cartilage, fat cells, etc.

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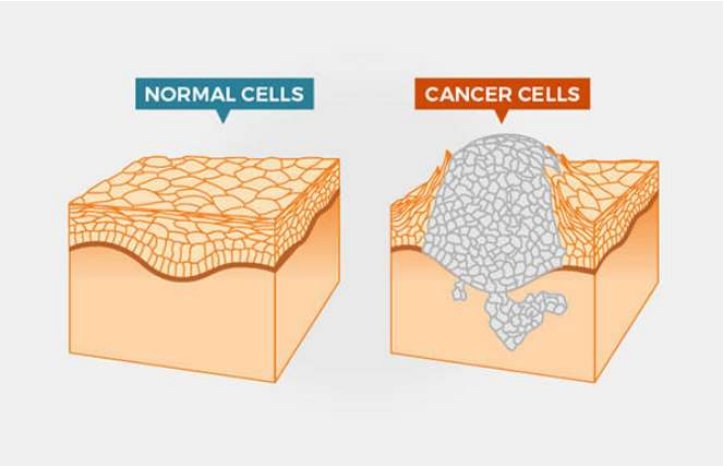
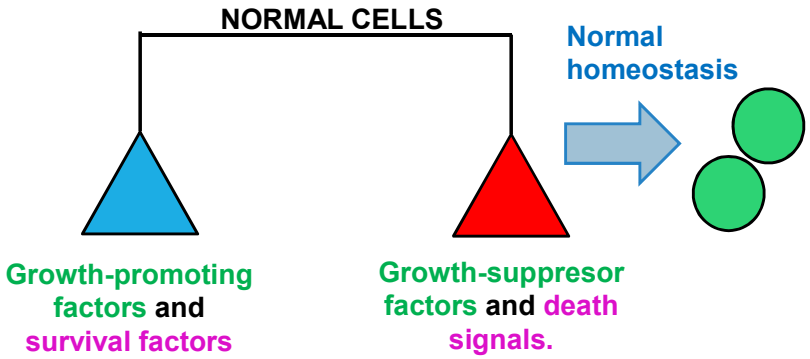


What causes cancer?



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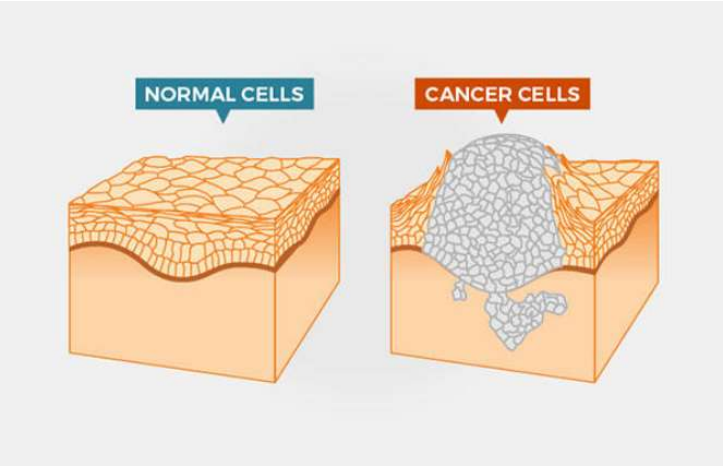

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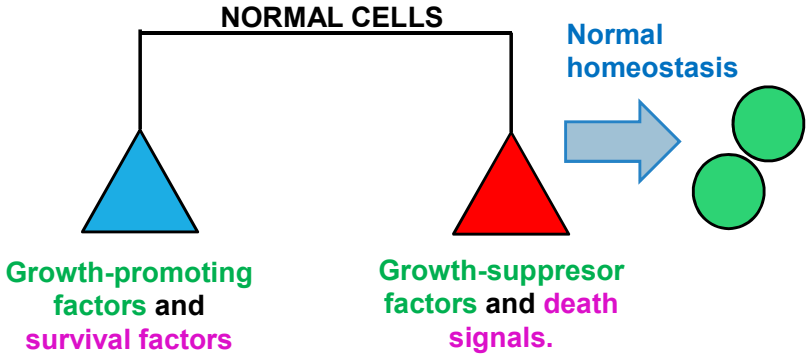
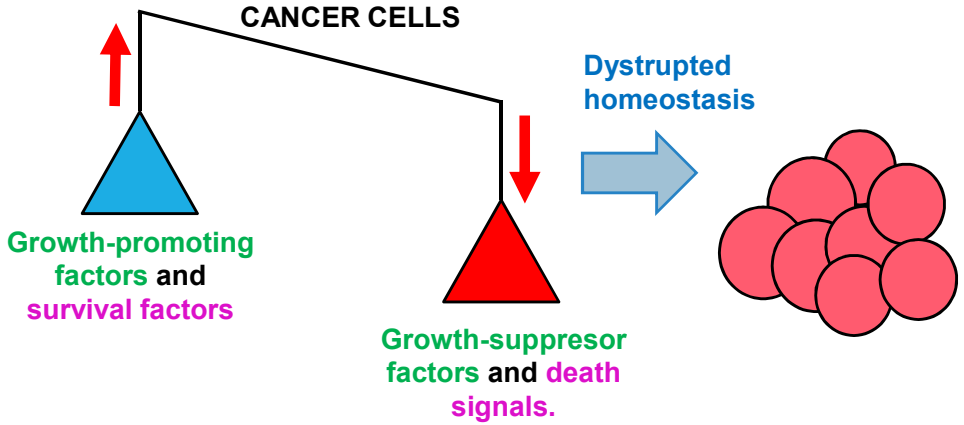
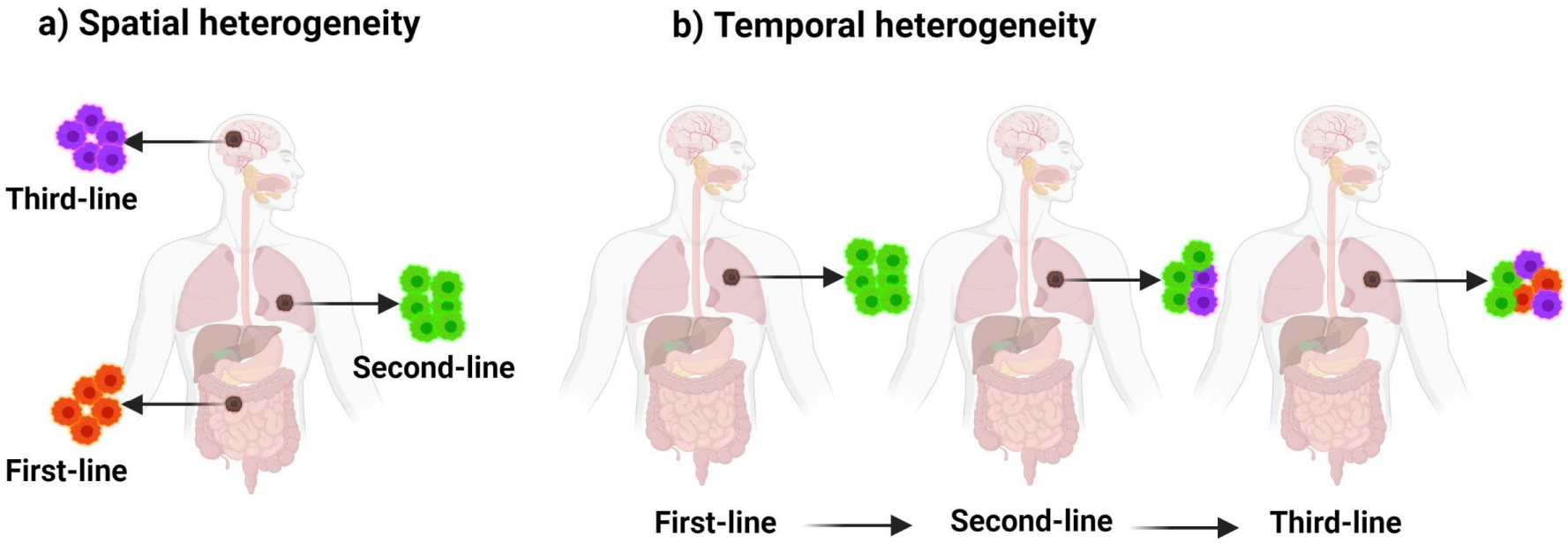




Challenges

Cancer is complex disease and heterogeneaous disease

"Multiple therapeutic strategies are needed"



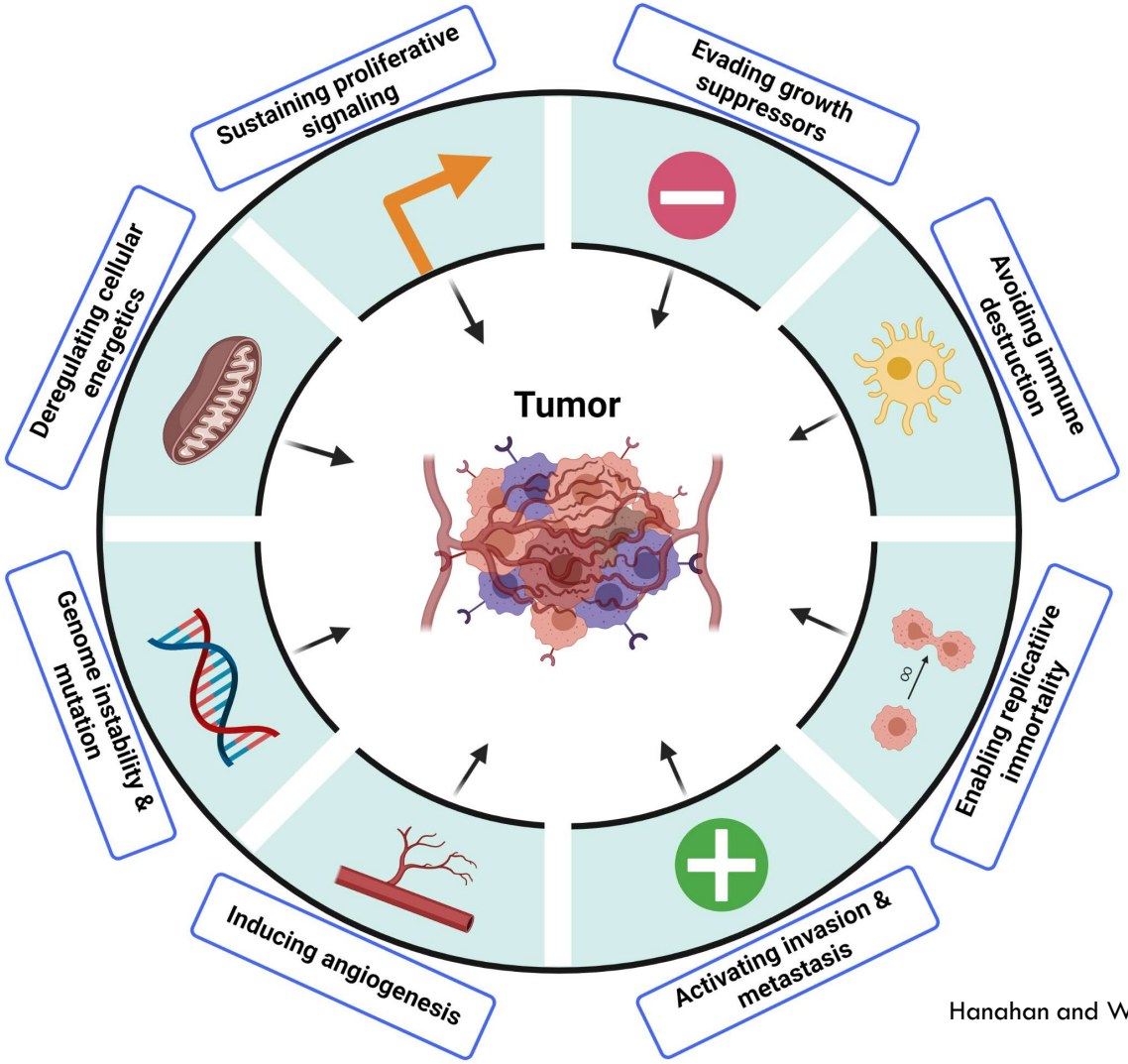
More than 200 different histologic cell types have been identified in human
(Cancer biological feautures)

Challenges

Simplify and unify the **key (common) characteristic and properties of human cancer**

Understanding of molecular profiles of cancer provides information about the prognosis, exact diagnosis, and more importantly the treatment options.

Hallmarks of cancer



Hanahan and Weinberg (2000, 2011, 2022)

Treatment options

Multimodal treatment plan

Chemotherapy

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

Radiotherapy

- Early stage of cancer
- Metastasis
- Combination with **chemotherapy**

Surgery

- Solid tumors contained in one are
- Sometimes only needed
- Combination with **chemotherapy**

Immunotherapy

- Can treat many types of cancer
- Combination with **chemotherapy**

Classification of chemotherapy

Conventional chemotherapy

Act by inhibiting cellular proliferation which often kills cells that grow and divide quickly.

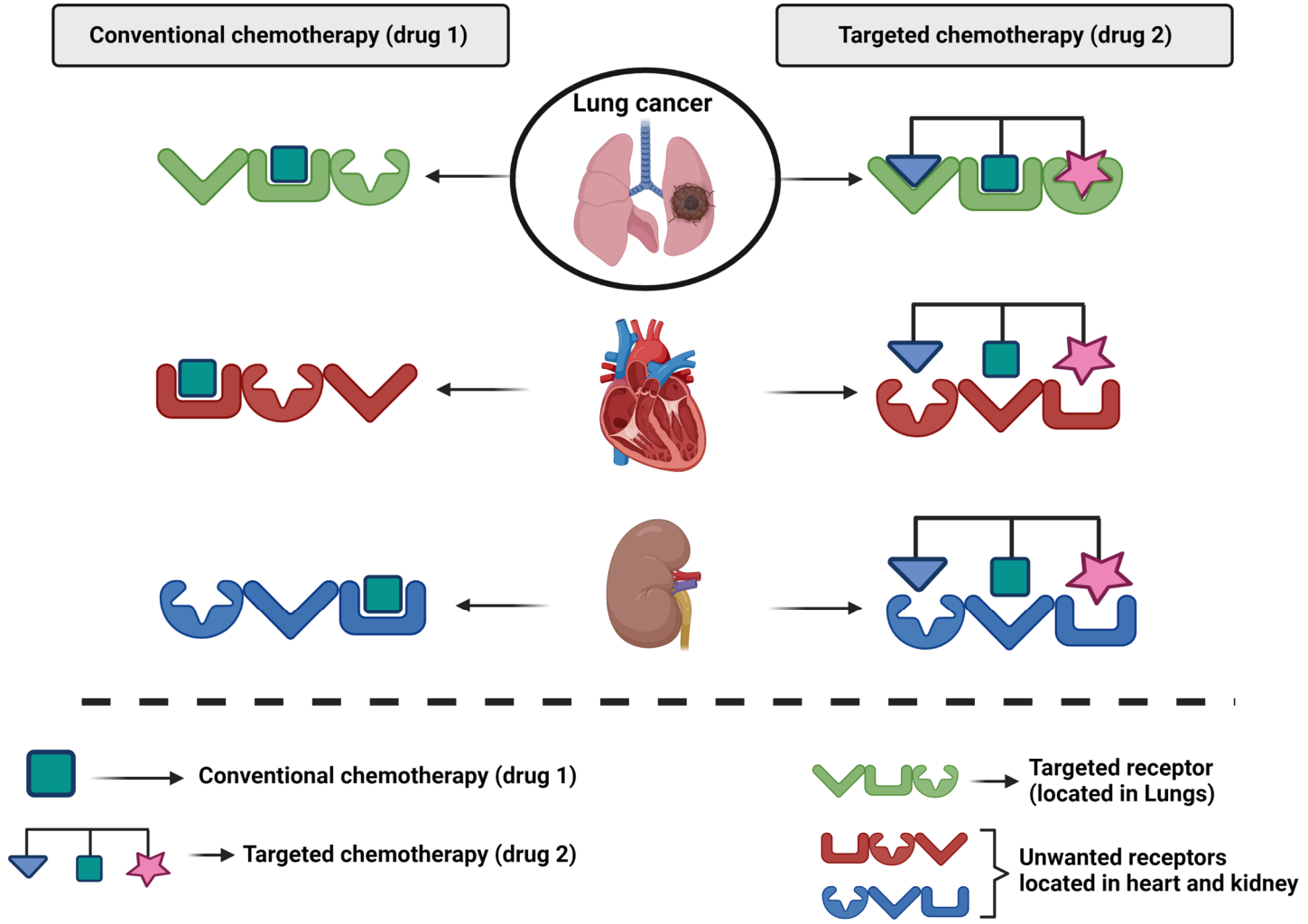
- Kill cancer cells
- Kill healthy rapidly growing cells

Targeted chemotherapy

Act by interfering with specific proteins that help tumors grow and spread throughout the body.

- Specific to cancer cells
- Often don't kill the cell, but slow down cancer growth.

Conventional vs Targeted chemotherapy



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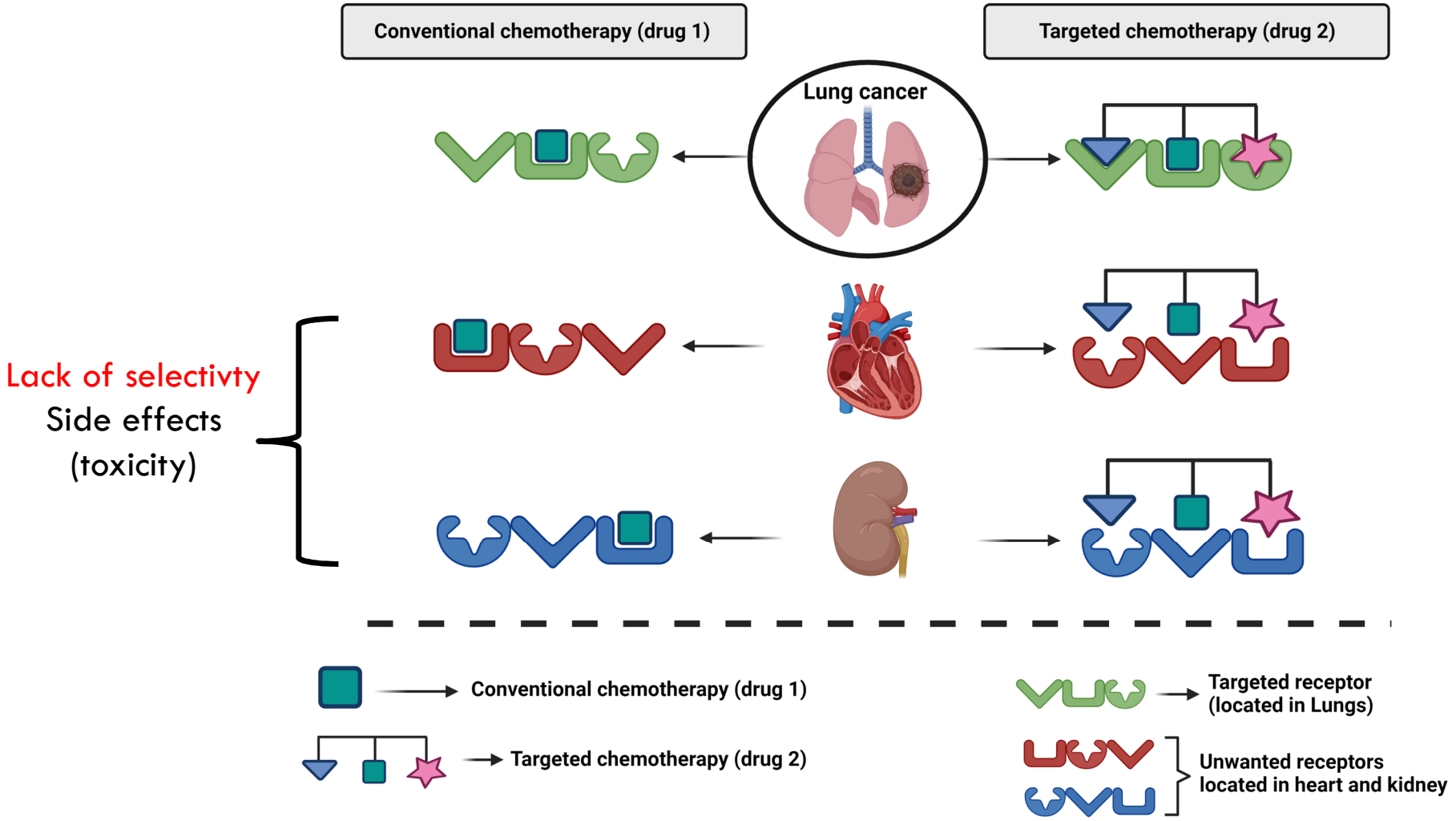
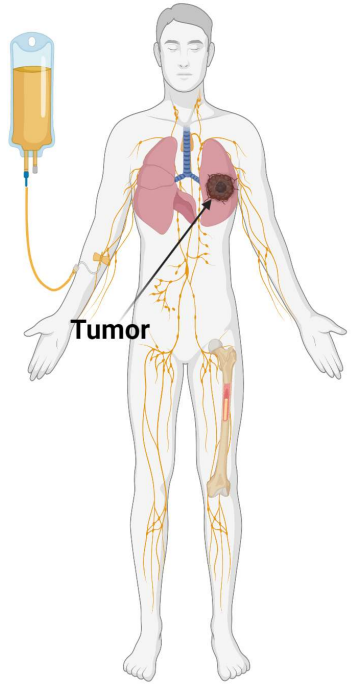


Conventional vs Targeted chemotherapy

Short-term side effects (toxicity) of conventional chemotherapy

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



Side effects can often lead to hospitalisation and even treatment discontinuation

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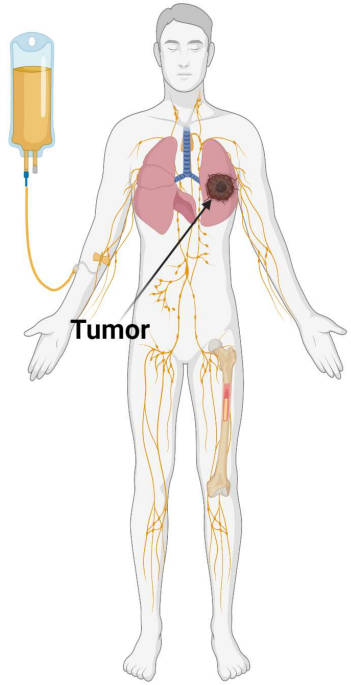
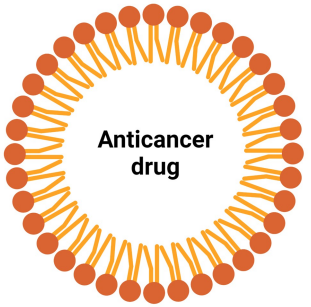


Side effects can often lead to hospitalisation and even treatment discontinuation

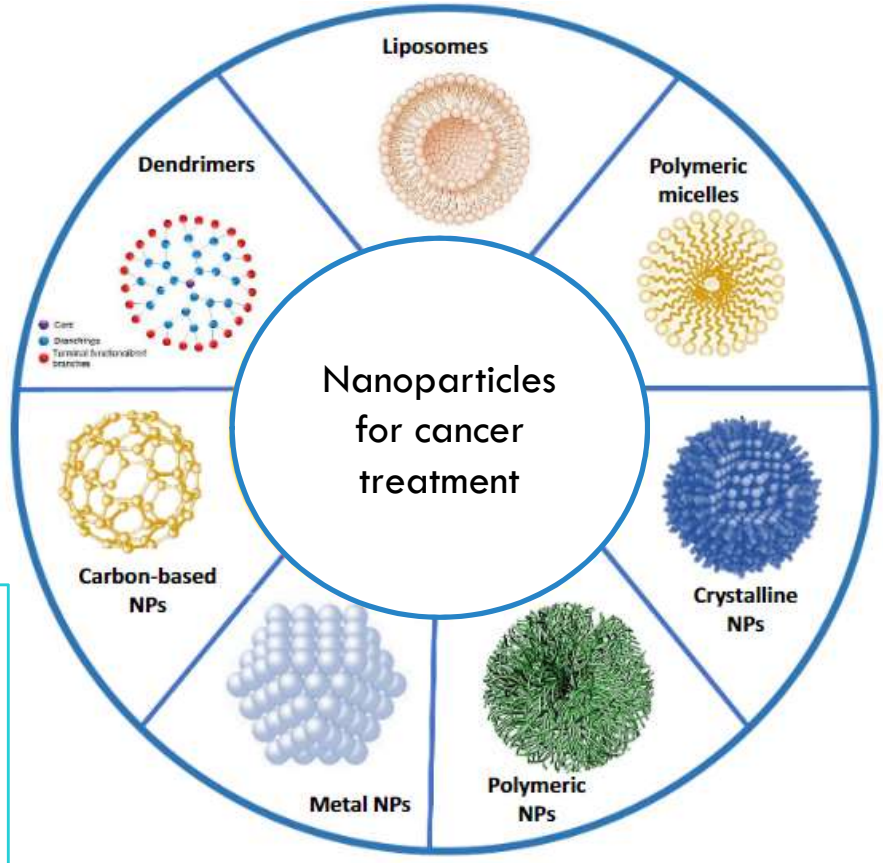
There is a urgent need to develop effective and less toxic therapies (targeted anticancer drugs or smart treatment):

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

"nanotherapeutics"



Nanoparticles for anticancer drug delivery

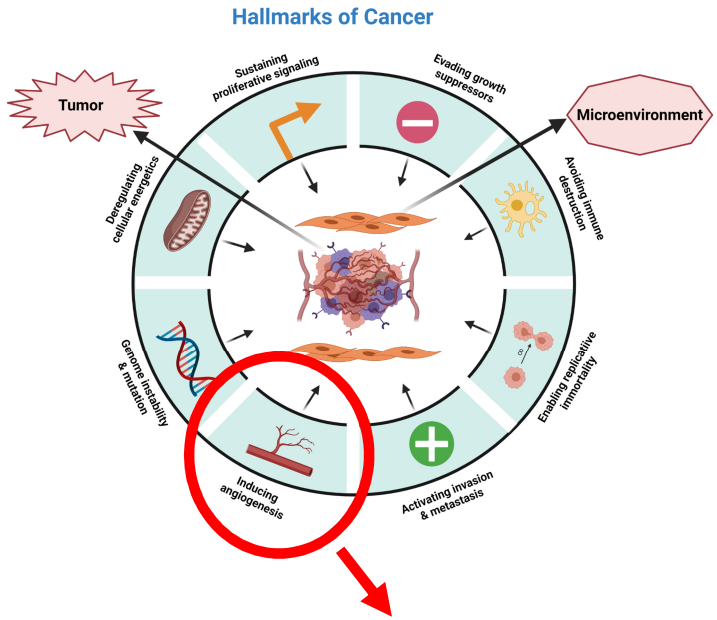


Can load different anticancer drugs

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

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

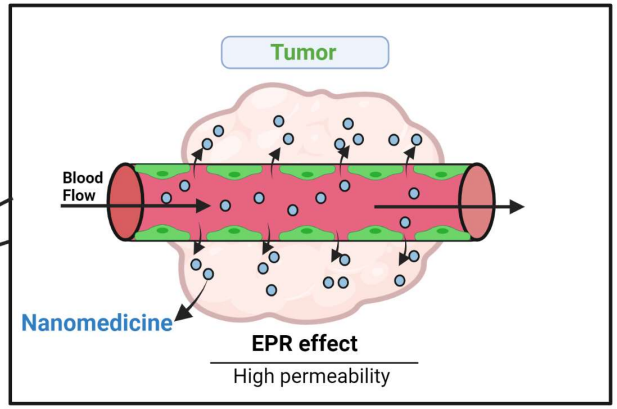
Why Nanoparticles for conventional chemotherapy

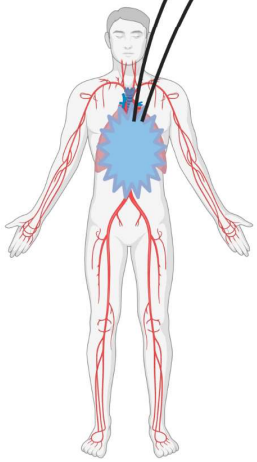


Discovery of Enhanced Permeability and Retention effect (EPR) = Blood vessels with leaky struture (~ 200nm)

Why Nanoparticles for conventional chemotherapy

enhanced accumulation of administered drug in tumor sites





IV administration

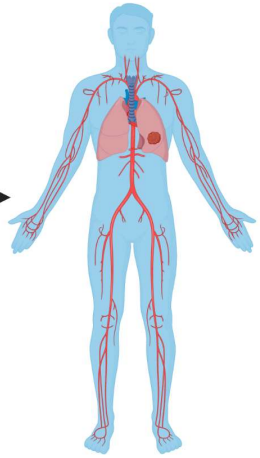
Nanoformulation

~ 10% of the administered drug reach the target (disase sites)

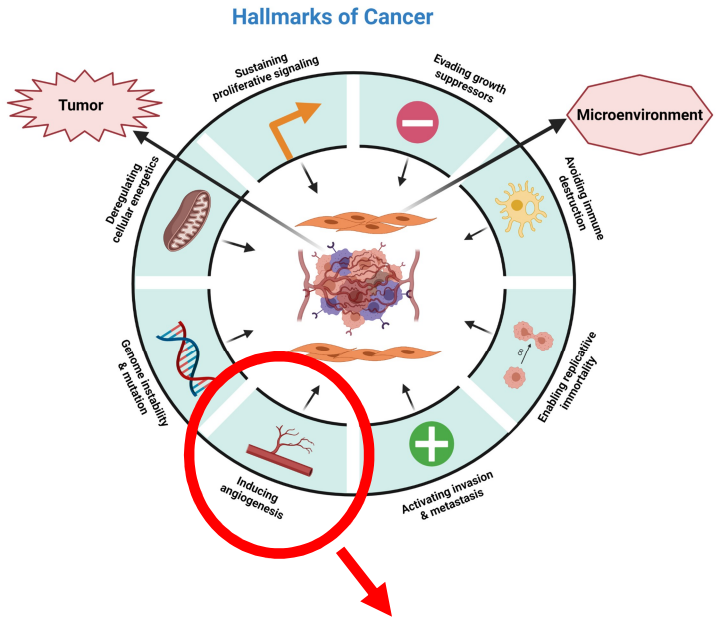
Chemotherapy

IV administration

Conventional dosage form



~ 0.1 % of the administered drug reach the target



Discovery of Enhanced Permeability and Retention effect
(EPR) = Blood vessels with leaky struture (~ 200nm)

Marketed Nanoparticles chemotherapy

Trade name	Formulation	Indication	Delivery route	Size (nm)	Material
Abraxane	Paclitaxel	Various cancers	i.v	130	Liposomes
Doxil	Doxorubicin	Ovarian, breast, multiple myoloma	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

Limitations of conventional chemotherapy

Existing anticancer drugs:

1. **Long term toxicity**

2. **Lack of intrinsic selectivity (attack healthy cells)**

3. **Can't manipulate all relevant targets of cancer (limited mechanism of action)**

Limitations of conventional chemotherapy

Existing anticancer drugs:

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3. Can't manipulate all relevant targets of cancer (limited mechanism of action)

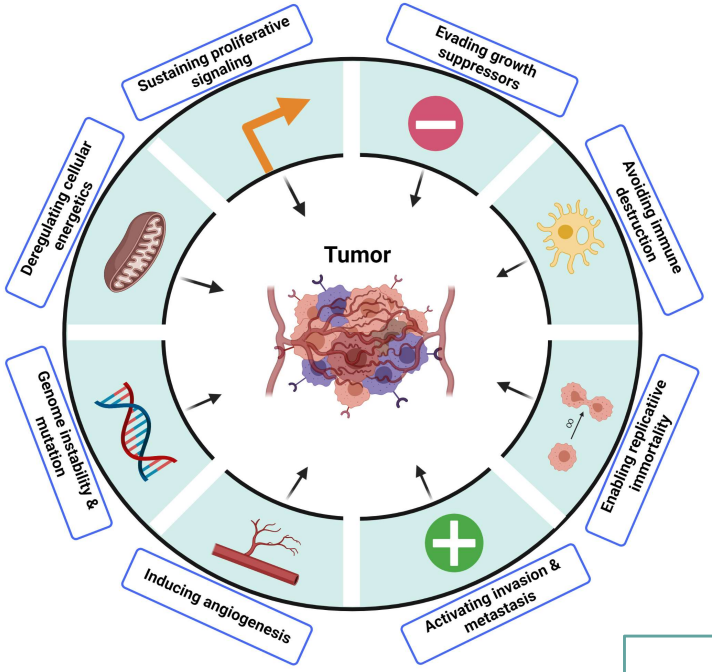
Targeted therapy: smart approach to tackle with « biopharmaceuticals »

1. Validate new targets for poorly managed cancers

2. Better tumor selectivity « smart mechanism of action »

3. Reduce long term toxicity (children)

Innovative targeted chemotherapy drugs



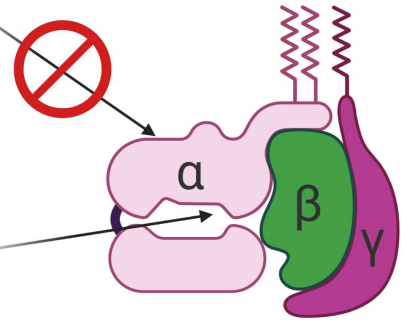
Advances in Molecular technology

Discovery of new proteins-proteins interactions (PPI)

Silencing Gene that produces specific protein

Inhbition of Protein-Protein interactions (PPI)

Oncogenic Proteins

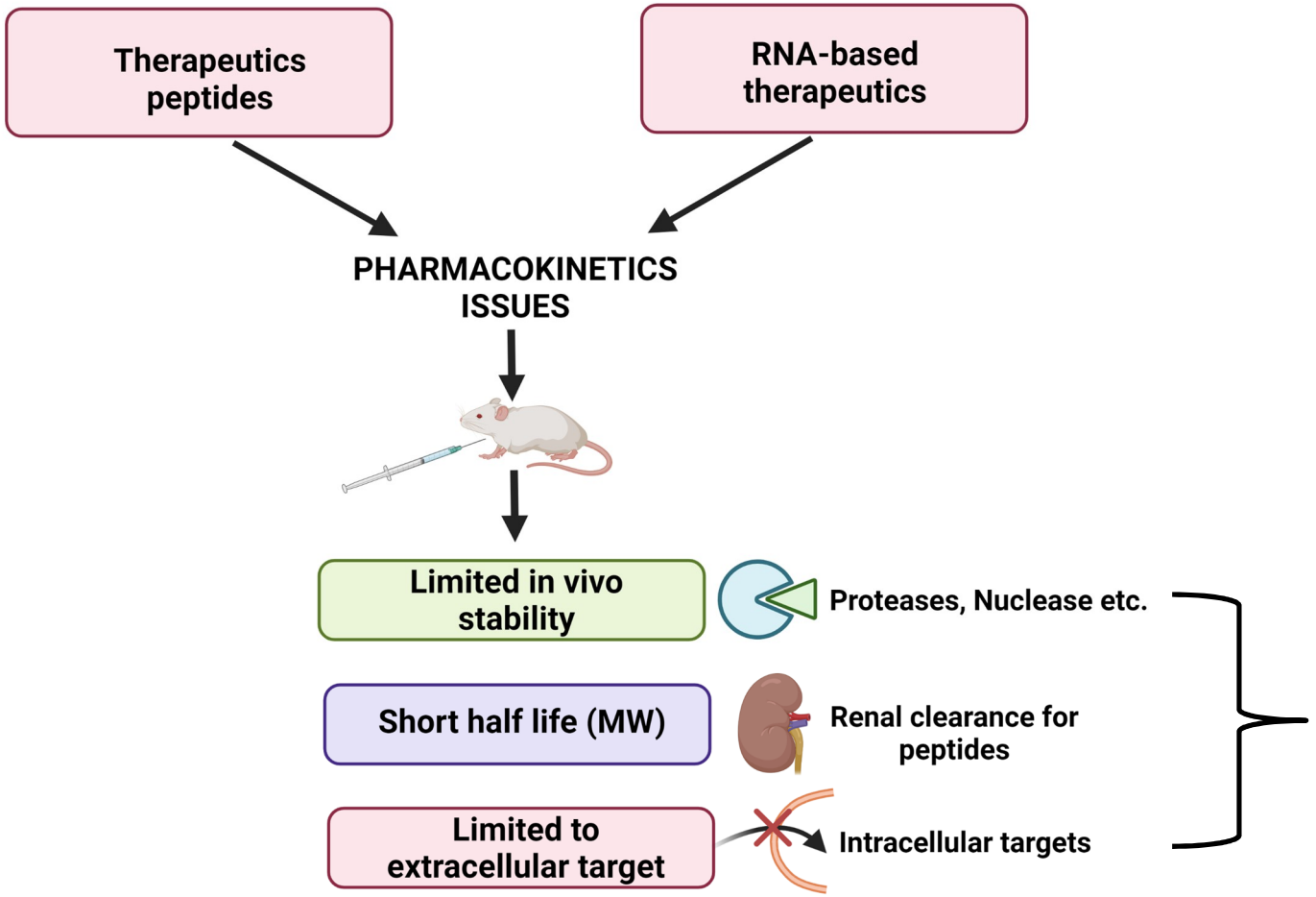


Smart approach to tackle cancer with biopharmaceuticals

Validation of undruggable targets

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

Innovative targeted chemotherapy drugs



Innovative targeted chemotherapy drugs

Therapeutics peptides

RNA-based therapeutics

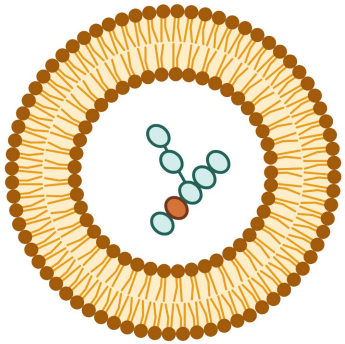
PHARMACOKINETICS ISSUES

Limited in vivo stability — Proteases, Nuclease etc.

Short half life (MW) — Renal clearance for peptides

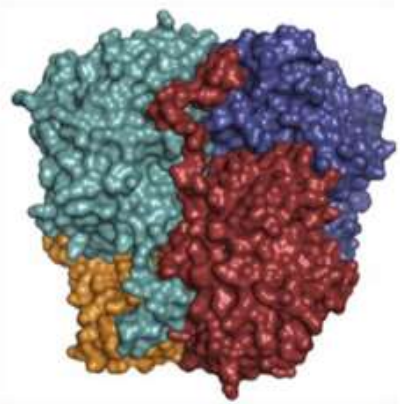
Limited to extracellular target — Intracellular targets

Advanced delivery system



My PhD Project – Interdisciplinary research

Targeting LDHB Tetramerization site

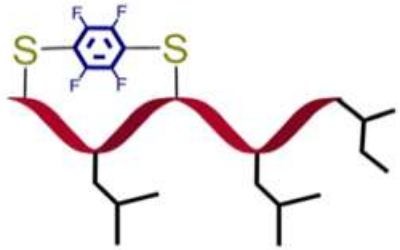


Attractive target for cancer therapy

1. SiHa: Cervical cancer
2. MCF-7: Breast cancer
3. HCT116: Colon cancer

1. **Lactate fueled respiration**
2. **Lactate fueled autophagy**
3. **Lactate induced angiogenesis**

Designing new ligands
« Therapeutic peptides »



LDH disruptor

Pierre Sonveaux and Raphael Frederich
University of Louvain (UCLouvain) Medical School
Brussels, Belgium

LIÈGE université
IREC UCLouvain
UNIVERSITÉ DE NAMUR

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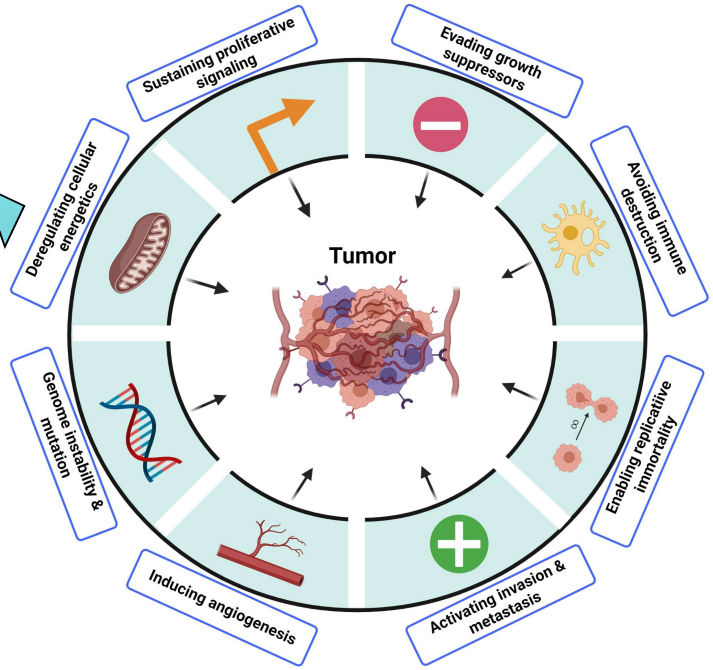

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Anticancer peptides properties

LB19

ATLKEKLIAPVAEEEATVP

$C_{90}H_{153}N_{21}O_{30}$ **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"

$C_{40}H_{76}N_{10}O_{10}S_2$ **MW: 921.22 g/mol**

NH2- Cys - Thr - Leu - Lys - Cys - Lys - Leu - Ile -COOH

Kd = 11 µM

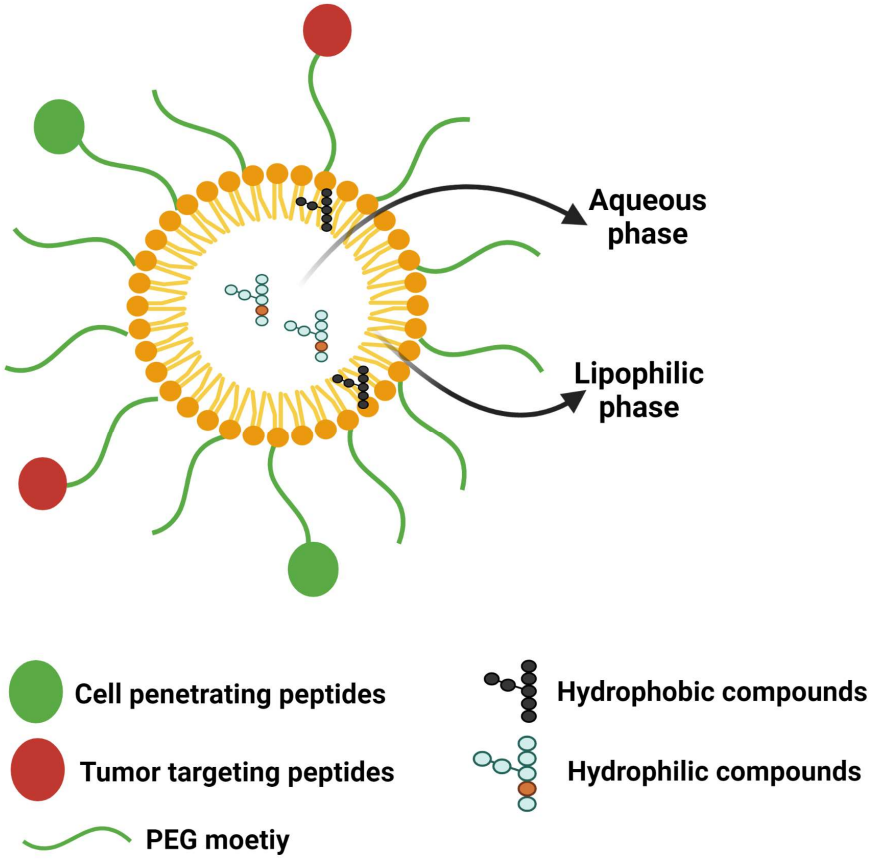
Theoretical pI: 8,90

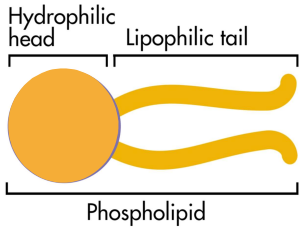
Net charge at pH 7: + 1.9

Average hydrophilicity: - 0.2

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

Liposomes as drug carrier – A versatile delivery Platform.

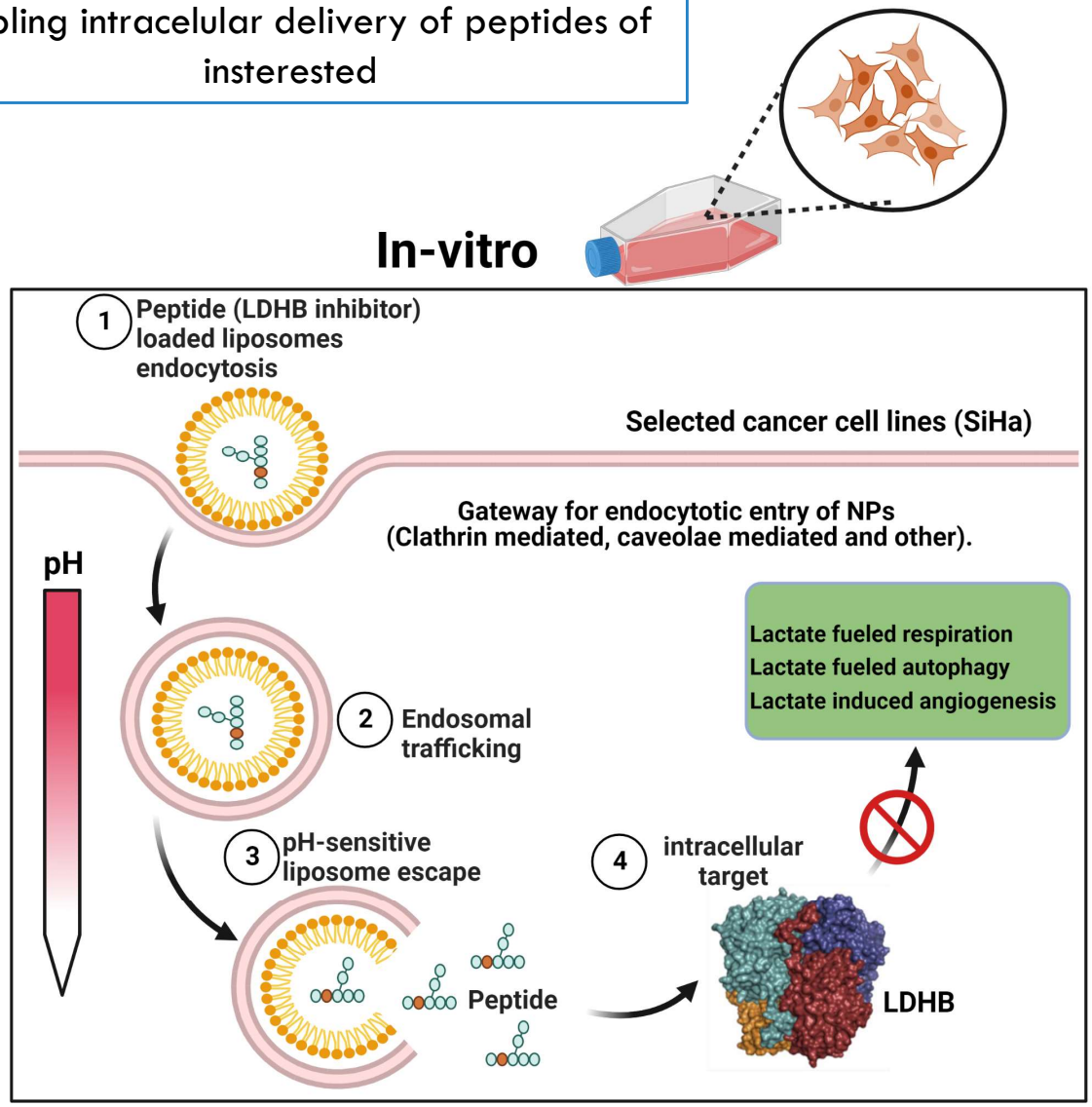




- ✓ Biocompatible and biodegradable excipients
- ✓ Encapsulated both hydrophilic and hydrophobic drugs.
- ✓ Protection of the encapsulated drugs
- ✓ Versatility when chemically modified **(stimuli responsive, tunable surface chemistry etc)**

Robson A-L et al, ***Front. Pharmacol. 2018***

Enabling intracelular delivery of peptides of insterested



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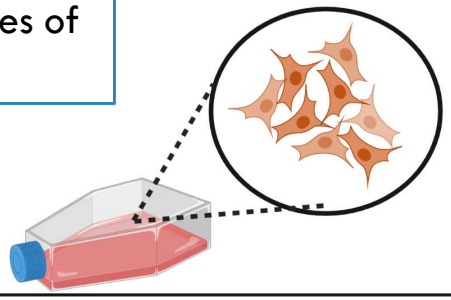
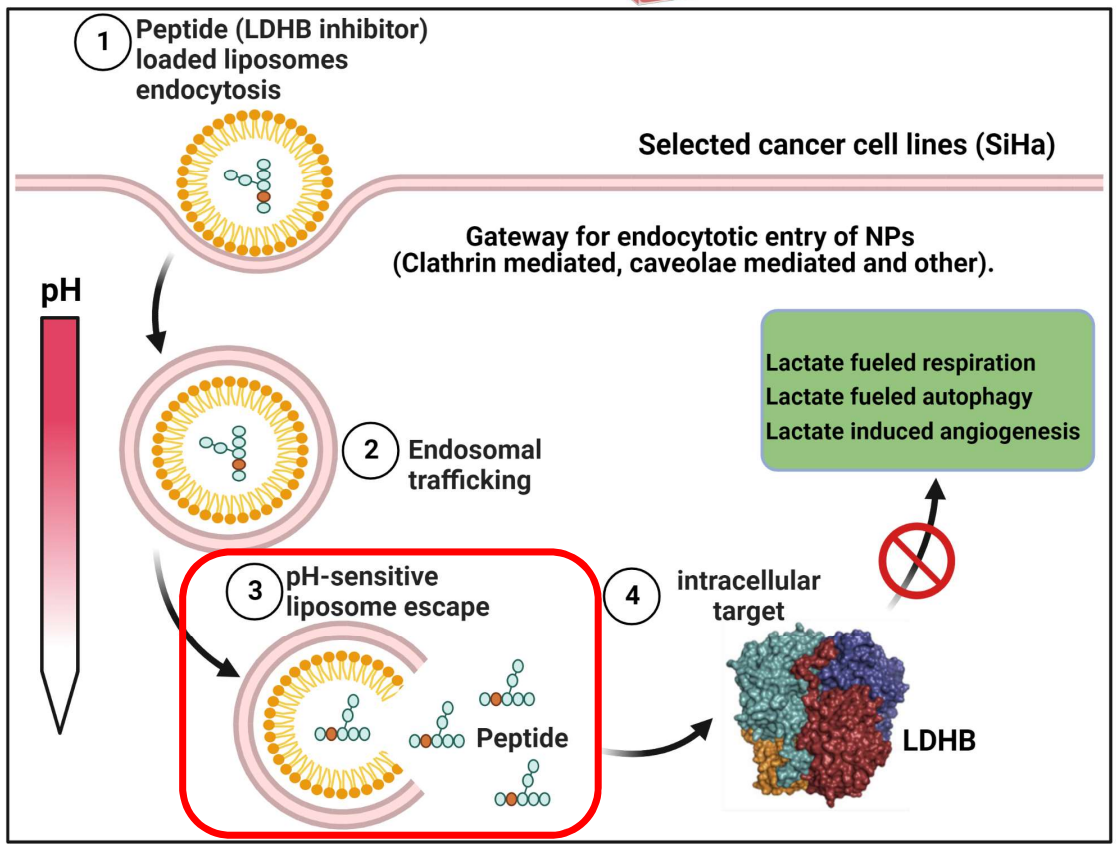


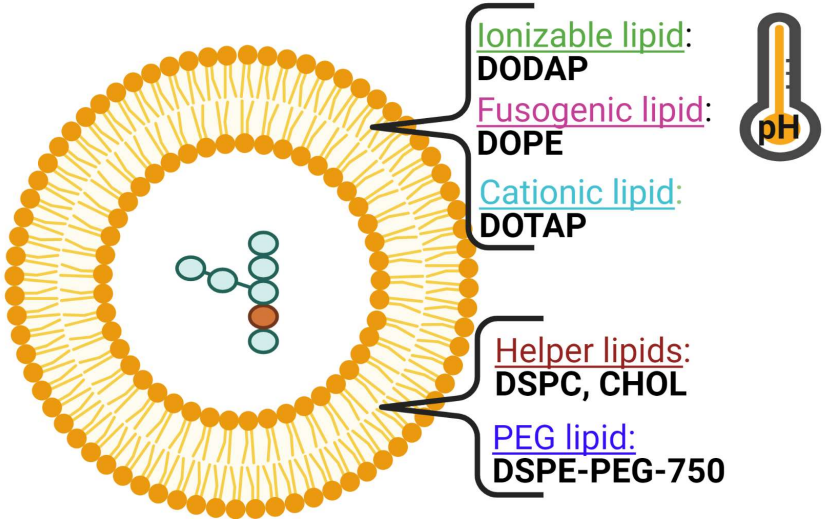
In-vitro



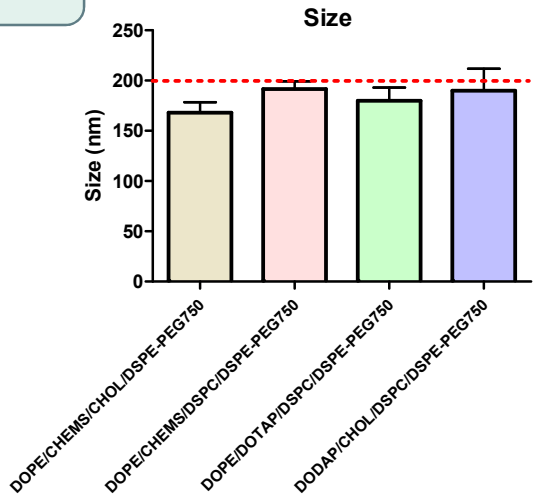
pH sensitive lipids for the design of liposomes

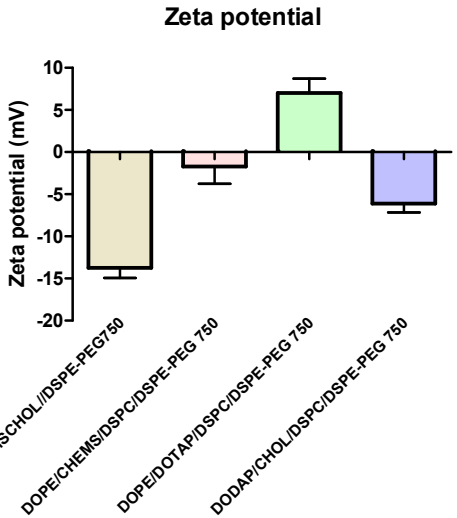
Fusogenic lipids	❑ DOPE ❑ CHEMS
Ionisable lipid in endosomal pH	❑ DODAP ❑ D-Lin-MC3-DMA ❑ ALC-0315, SM-102
Switchable lipids in endosomal pH	❑ CSL3

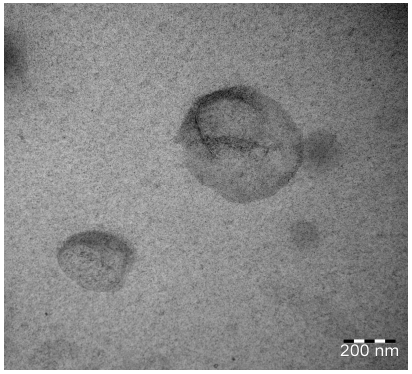
Liposomes formulation design and physicochemical characterization at physiological pH

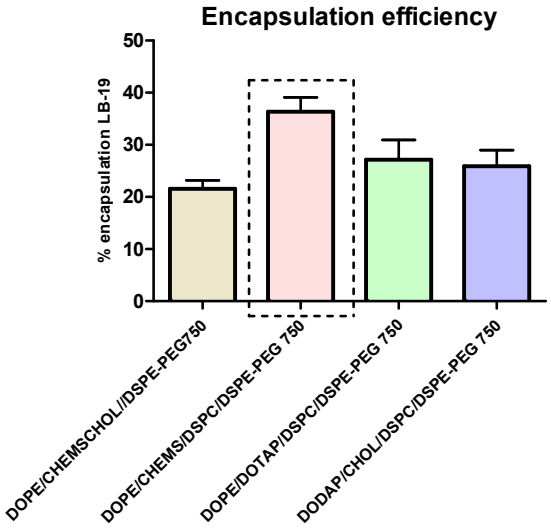


Liposomes extruder







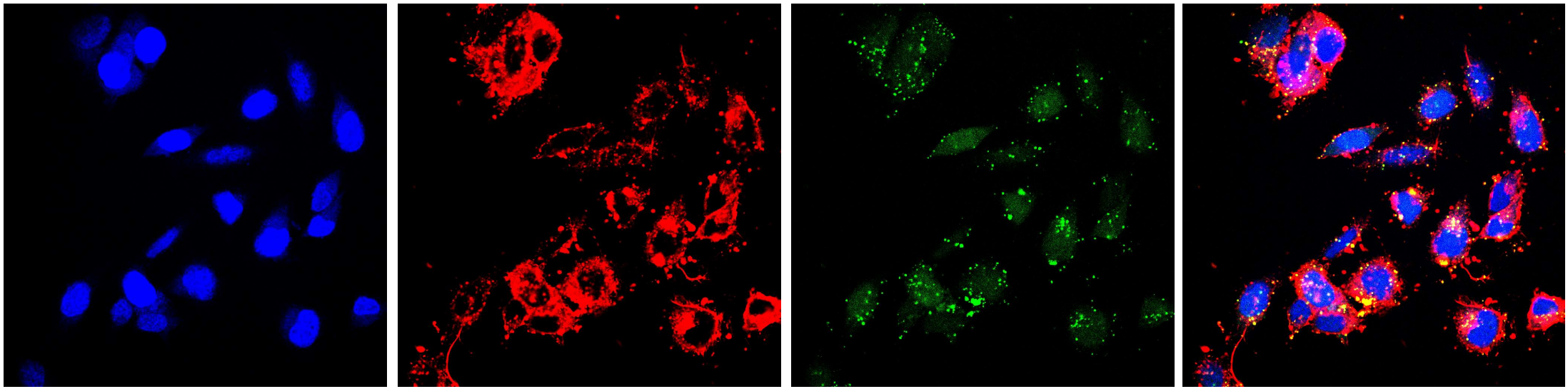


Confocal analysis

Nuclei staining with DAPI

Rhodamine B labelled liposomes (red fluo)

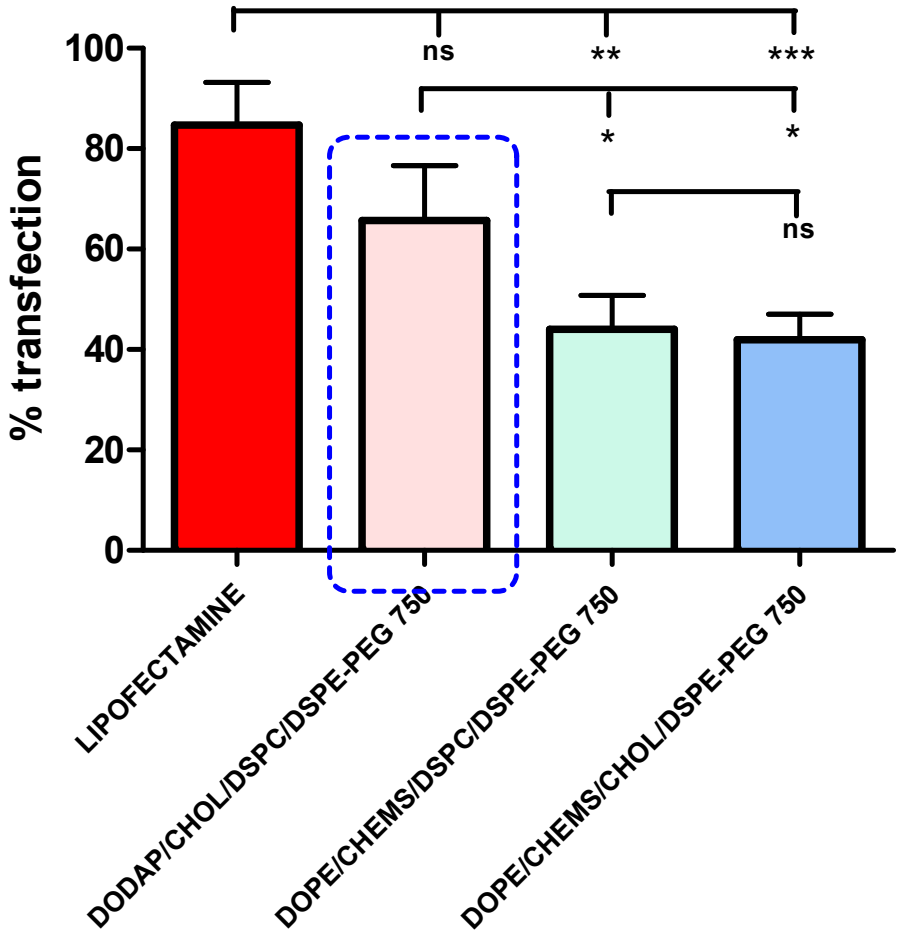
Dextran 1kD-FTIC (green fluorescence)



Conclusion: The liposomes are well internalized inside cell (SiHa) as it can be seen in the area around the nucleus (blue). And achieved the intracellular release of the cargo. Incubation time: 5 hr

Flow cytometry assay

Transfection efficiency of pH sensitive liposomes on SiHa

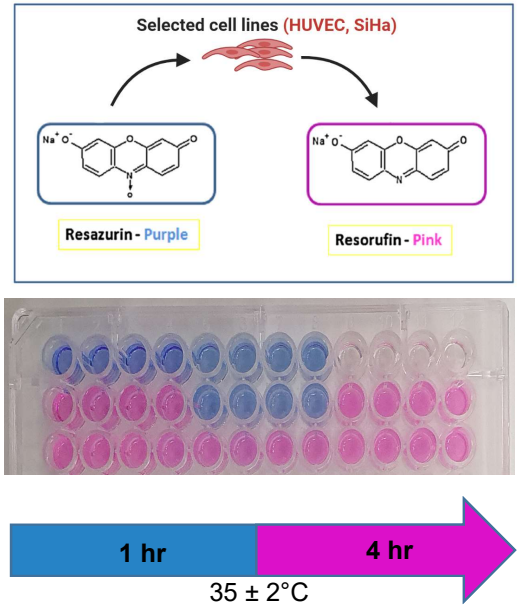


((*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$). n=3.

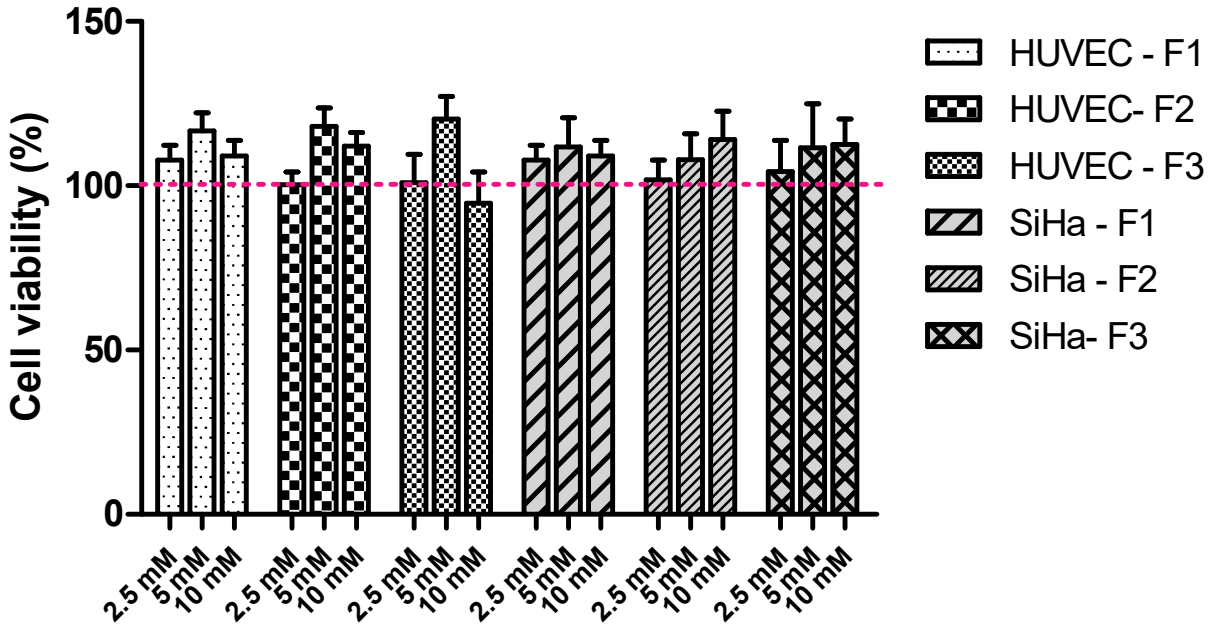
Biocompatibility towards primary and tumor cell lines

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

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

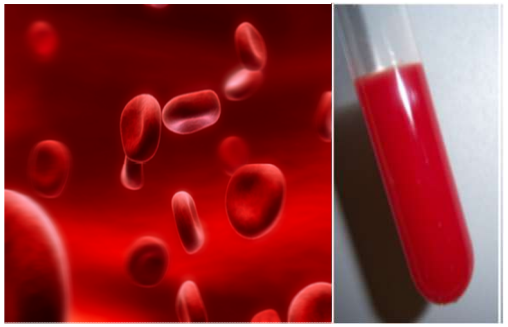


Resazurin vialibility assay

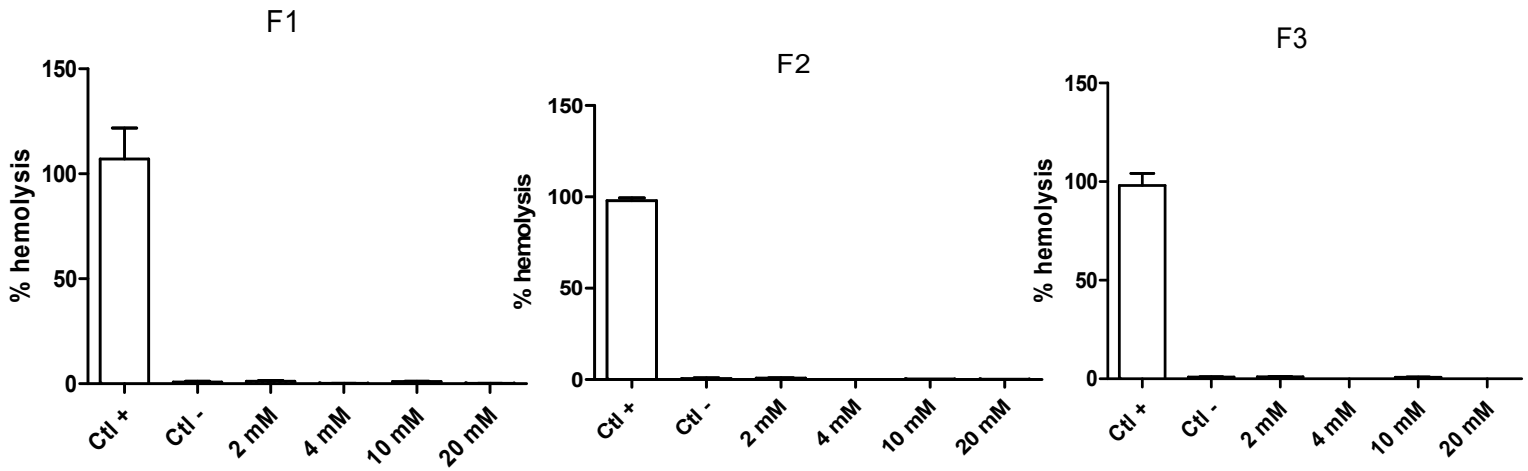


Hemocompatibility tests

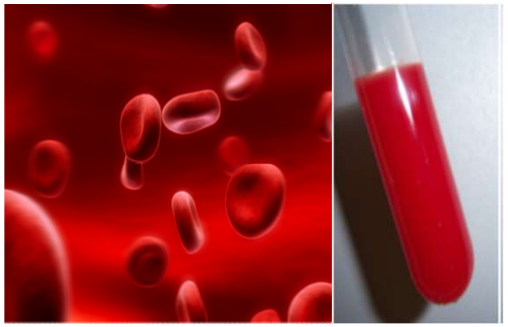
Composition	Proportion (%Mol)
F1: DOPE/CHEMS/CHOL/DSPE-PEG750	60/25/10/5
F2: DOPE/CHEMS/DSPC/DSPE-PEG750	45/20/30/5
F3: DODAP/CHOL/DSPC/DSPE-PEG750	45/20/30/5



- **Hemolysis (red blood cell)**

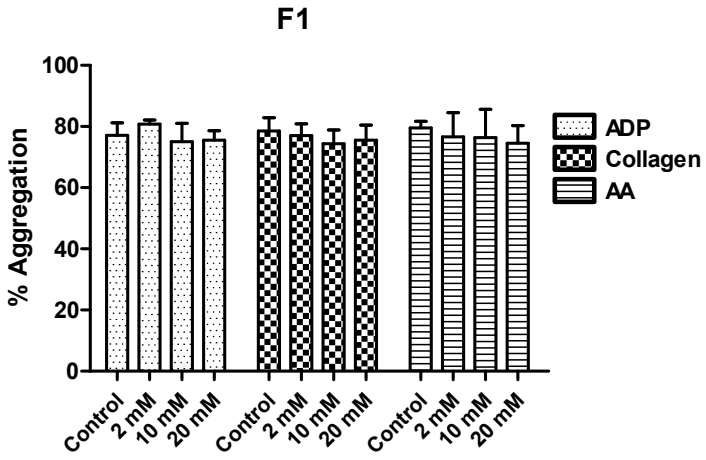


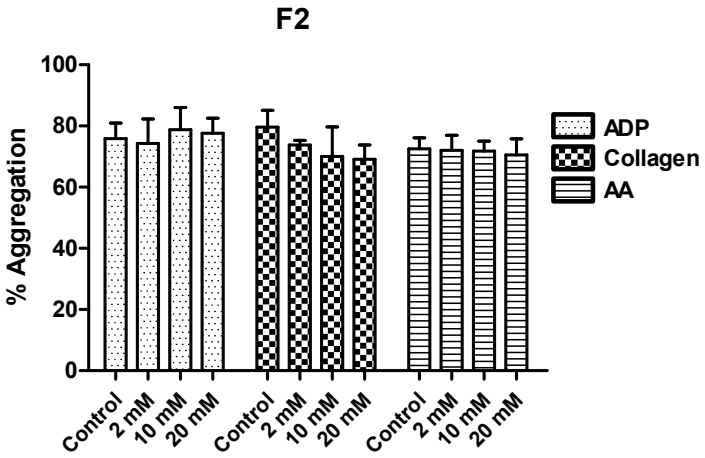
Hemocompatibility tests

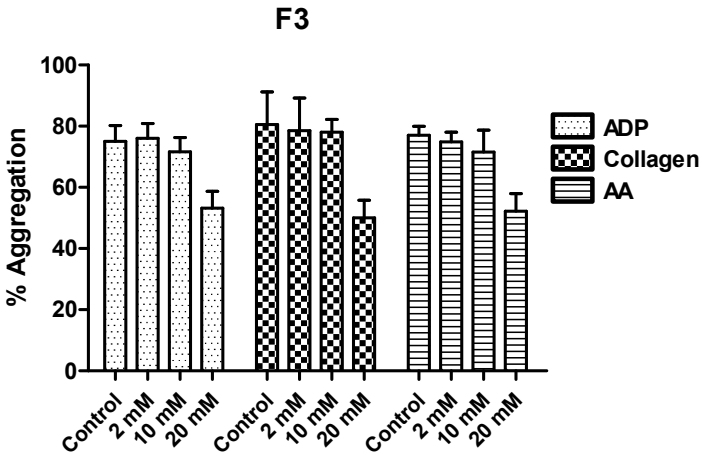


- Platelet aggregation

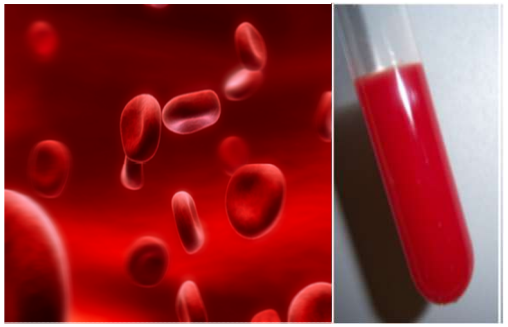
Composition	Proportion (%Mol)
F1: DOPE/CHEMS/CHOL/DSPE-PEG750	60/25/10/5
F2: DOPE/CHEMS/DSPC/DSPE-PEG750	45/20/30/5
F3: DODAP/CHOL/DSPC/DSPE-PEG750	45/20/30/5





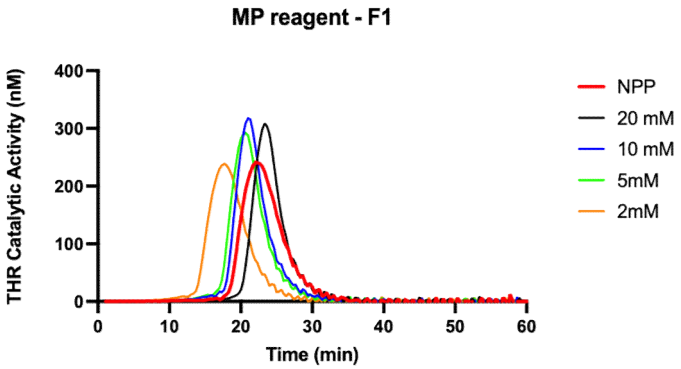


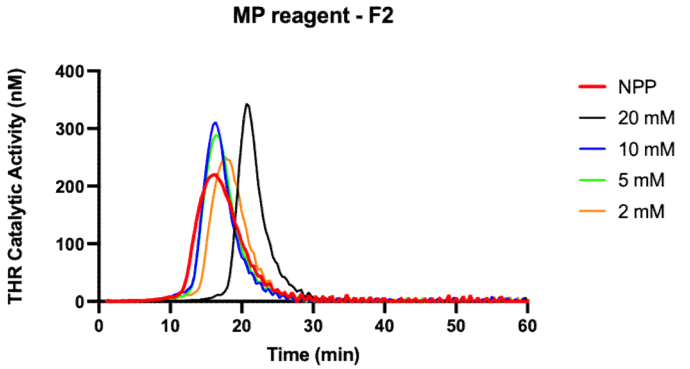
Hemocompatibility tests

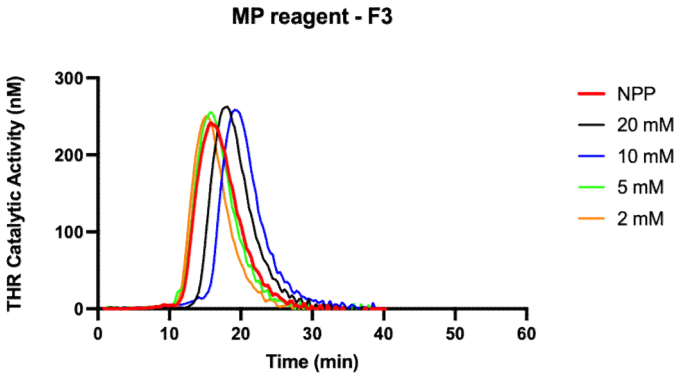


- Generation of thrombine

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







In-vivo studies

1. Mice xenograph model that mimic cancer in human (SiHa over-expressing LDHB).

2. Intratumoral distribution of liposomes (in-vivo imaging systems)

3. Efficacy of LDHB inhibition with peptides (biomarkers of autophagy inhibition, tumor volume, etc.)

I am not allowed to share about this part as it is confidential!

TAKE HOME MESSAGE FOR NANOMEDICINE AND CANCERS

- Cancer is still a major health problem with unmet medical needs
- Improved toxicity and efficacy profiles of conventional anticancer drugs **(better therapeutic index)**
- Evaluation and validation of novel targets using innovative drugs **(biopharmaceuticals with great therapeutic potentials)**
- Flexibility of their composition could be the key to enhance and facilitate the clinical translation of innovative treatment **(improve biostability and active targeting therapy)**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Acknowledgments

LIÈGE université
Center for Interdisciplinary Research on Medicines

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