Liposomes for targeted cancer multi-therapy and imaging

joão carvalho

Departamento de Física

LIP-Lisboa, 09 maio 2013

Encapsula Nano Sciences

· U C ·

FCTUC FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

INTRODUCTION

Cancer is the most common human **GENETIC DISEASE** (a large group of diseases characterized by uncontrolled growth and spread of abnormal cells)

Approximately **one in three** individuals in Europe and North America develops one of the approximately 200 different types of cancer, and is the cause of death of **one in five**

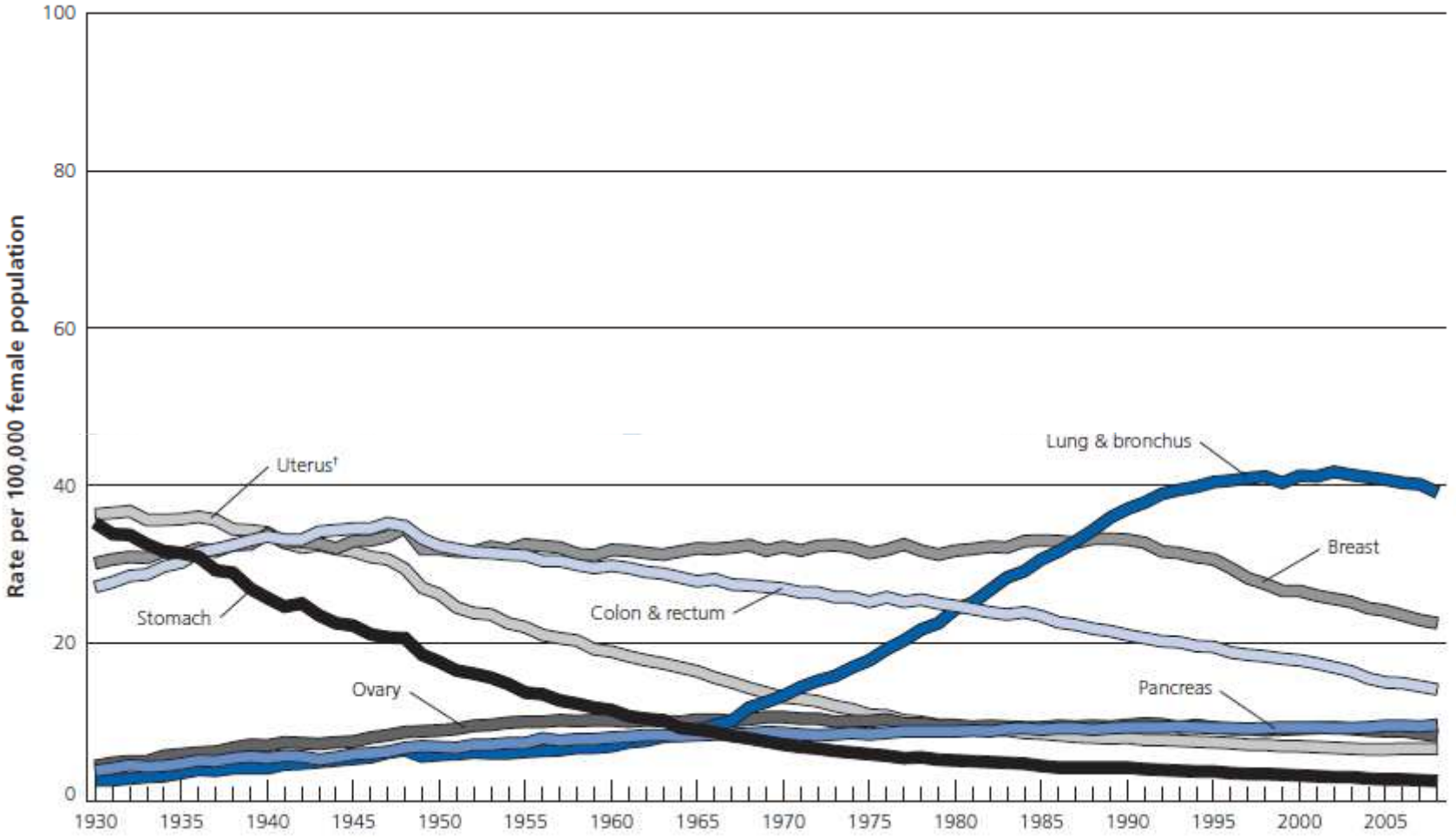
Is believed that cancer is caused by **genetic mutations** – most often, by a **series of mutations**, some of which may be inherited

Cancer incidence rate has been increasing and **death rate** is more or less stable even with improved diagnosis tools (other death causes were reduced and life expectancy raised, increasing the chances of developing cancer)

Opinion: therapies didn't improved much in the last decades and the **CANCER WAR** is not being won!

The death rate for cancer in the U.S., adjusted for population size and age, **dropped only 5 percent from 1950 to 2005**

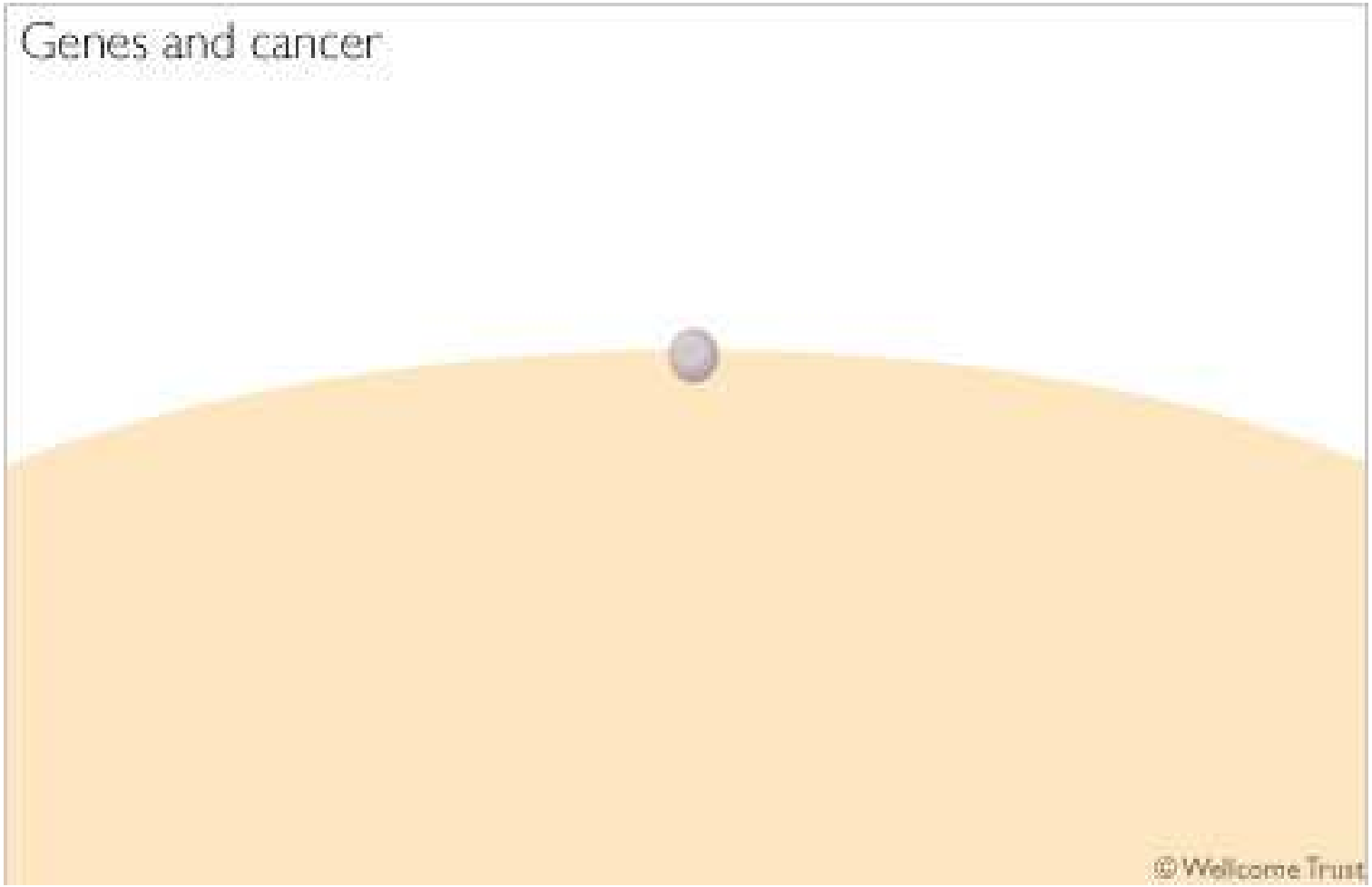
Kolata, Gina (April 24, 2009). "As Other Death Rates Fall, Cancer's Scarcely Moves". The New York Times.



* Per 100,000, age adjusted to the 2000 US standard population (American Cancer Society)

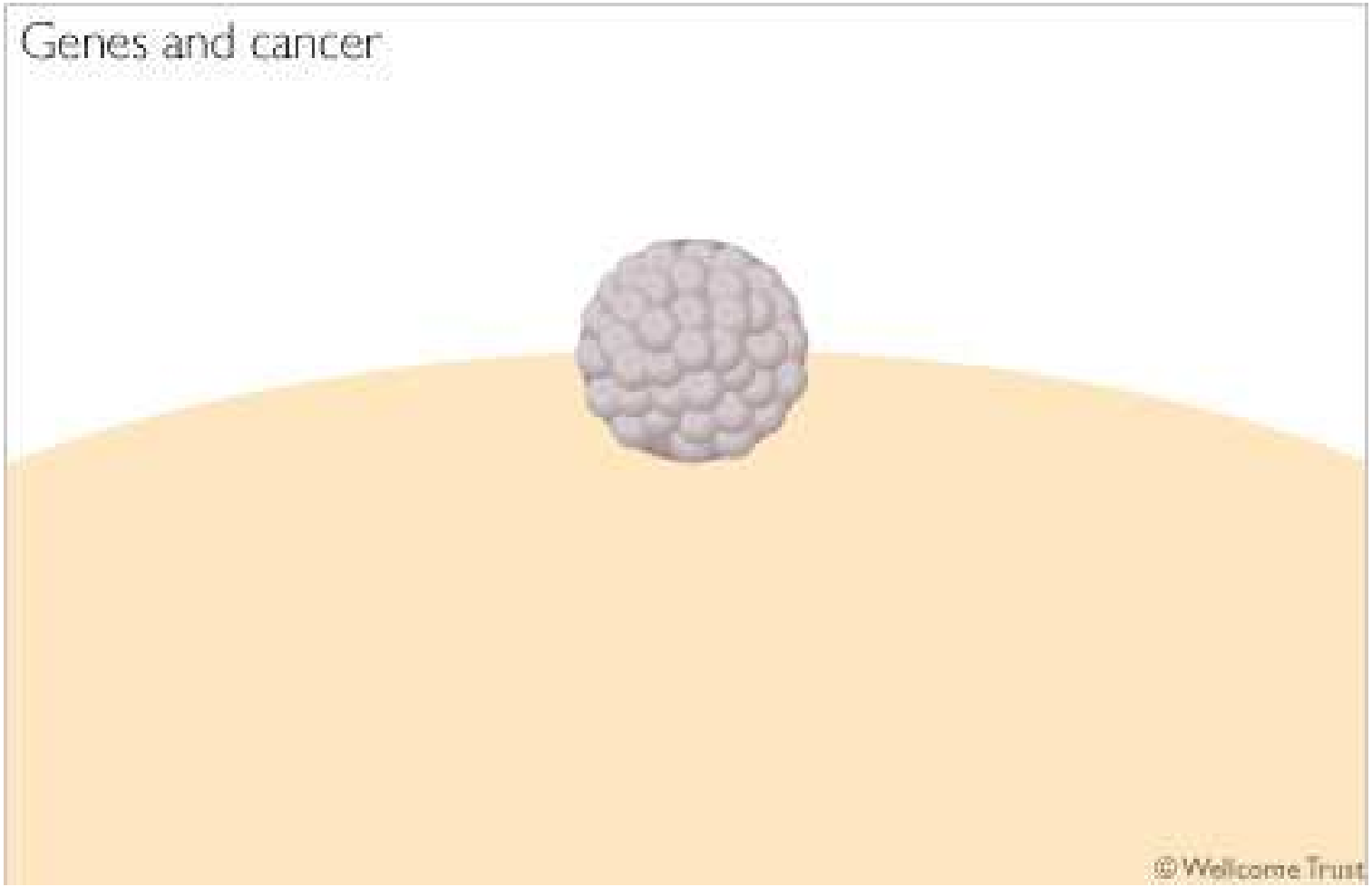
MAIN CANCER CHARACTERISTICS

- self-sufficiency in growth signaling
- insensitivity to growth inhibitors
- evasion of apoptosis
- limitless replicative potential
- sustained angiogenesis
- tumor invasion and metastasis



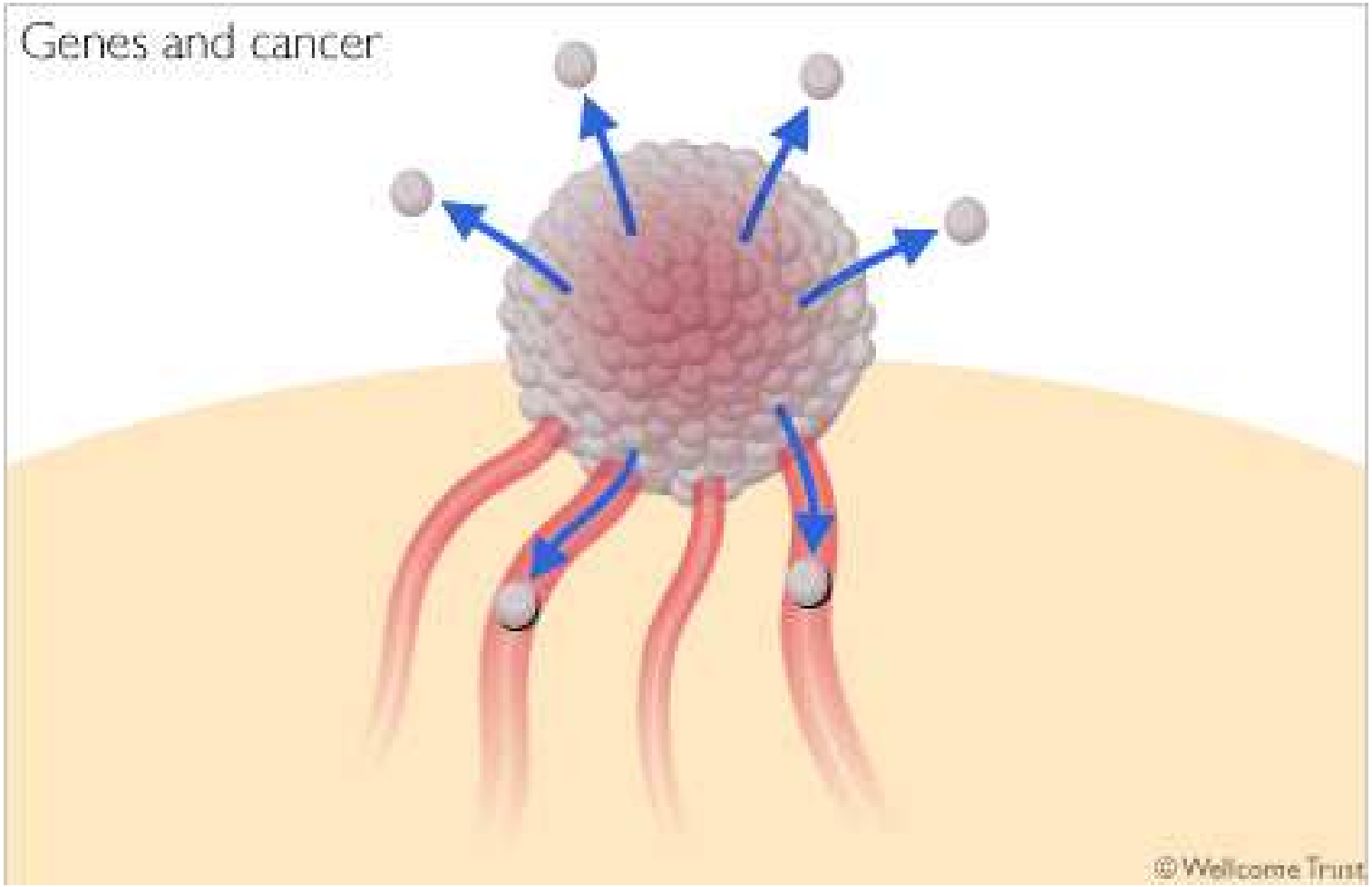
A **growth control gene** is mutated in a single cell and excessive growth begins

Further mutations in **tumor suppressor genes** allow the tumor to resist signals to undergo programmed cell death (apoptosis)



The tumor reaches a limit in its size, where cannot acquire sufficient **nutrients** to grow larger

Other mutations promote new blood vessels to grow to the tumor, providing nutrients and allowing further growth (**angiogenesis**)



Mutations allow the cancer cells to colonize other parts of the body (**metastasis**)

CANCER THERAPIES (U.S. National Cancer Institute)

Adjuvant and Neoadjuvant Therapy for Breast Cancer
Angiogenesis Inhibitors
Biological Therapies for Ca
Bone Marrow Transplantat
Cancer Vaccines
Cryosurgery in Cancer Tre
Drug Discovery, Investigat
Gene Therapy for Cancer
Herceptin® (Trastuzumab)
Hormone Therapy for Bre
Hyperthermia in Cancer T
Lasers in Cancer Treatmer
Photodynamic Therapy for
Preventive Mastectomy
Radiation Therapy for Can
Sentinel Lymph Node Biop
Targeted Cancer Therapies, etc.

RESUME

Mainly **Radiotherapy**, **Chemotherapy** and **Surgery**

Very **aggressive** treatments, with serious side effects and high probability of relapses

Non specific (targeted) drugs

Metastases?

GENOMICS

The **Human Genome Project** was able to sequence the full human genome

Genome contains 3167.8 M nucleotide bases in 23 chromosome pairs, but only ~2% encode genes

Consortium researchers confirmed the existence of **19,599 protein-coding genes**

genomics.energy.org

Genes are like computing routines for protein coding, for cancer development is fundamental to discover the **control sequences** for their expression (the routine calling **main program**), a very active research area

CANCER GENOME

Finally there is technology to sequence the Human Genome in a (relatively) fast and cheap way

The **genetic defects** that lead to cancer can be studied in a detailed way

Many projects and laboratories dedicated to this research

Genomic studies can lead to new **THERAPIES**, but **gene therapy** is still a long way to be feasible for cancer (multigene disease, gene expression control)

Tumor cells have origin in normal cells, with mainly similar genetic signatures and gene expression (hard to target by the **immune** system)

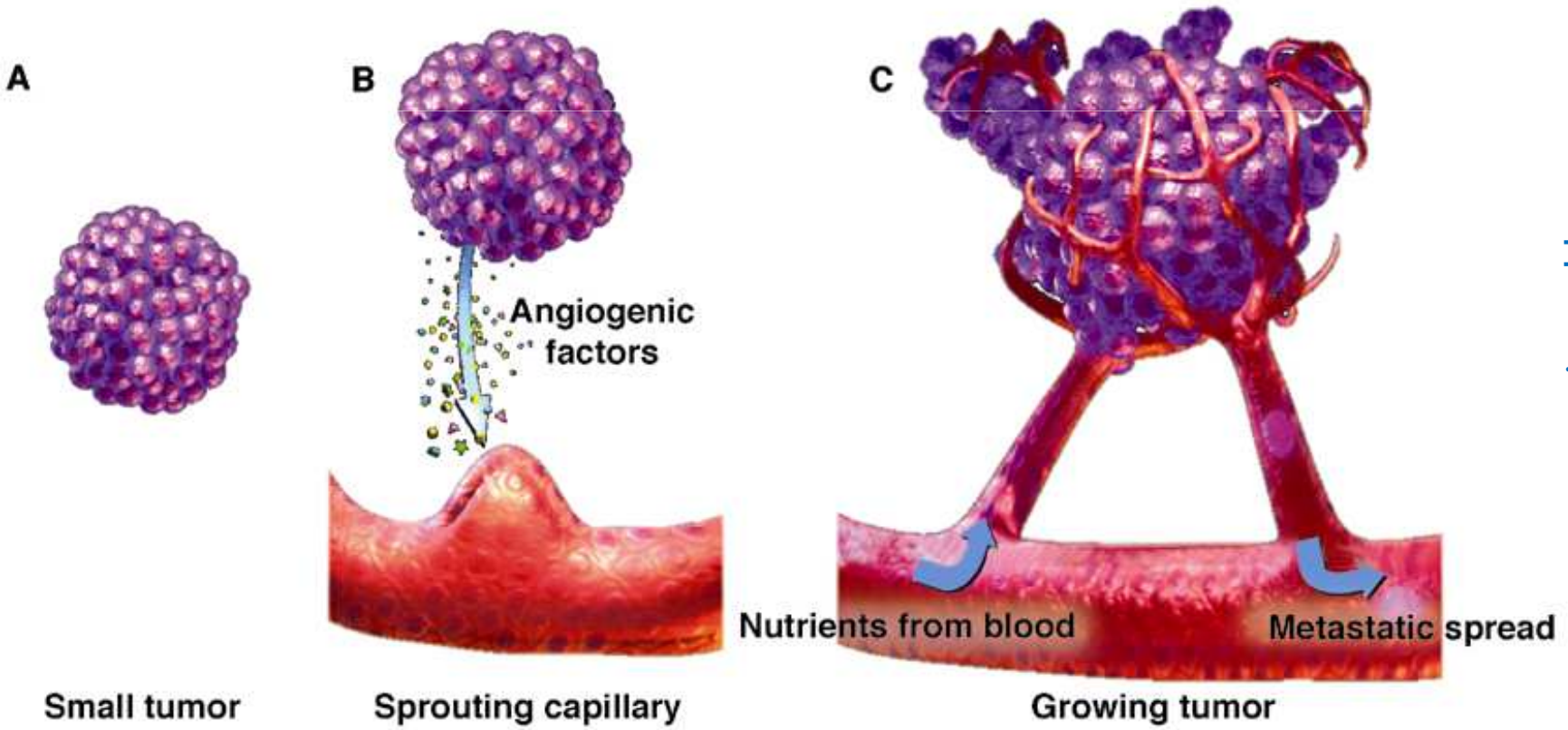
Search for overexpressed or tumor exclusive receptors due to mutant gene expression is going on

Looks like there is no "**magic bullet**" able to selectively target only cancer cells (many different research roads)

Here is presented one of the strategies, being pursued in the University of Coimbra and elsewhere, using **liposomes** as drug delivery vehicles

ANGIOGENESIS

Physiological process involving the growth of **new blood vessels** from pre-existing vessels (result of a cascade of events)



angioworld.com

Tumors cannot grow beyond a certain size, generally 1–2 mm^3, due to lack of oxygen and other essential nutrients

Tumors induce **angiogenesis** by secreting various growth factors

Angiogenesis is also required for the spread of a tumor (metastasis)

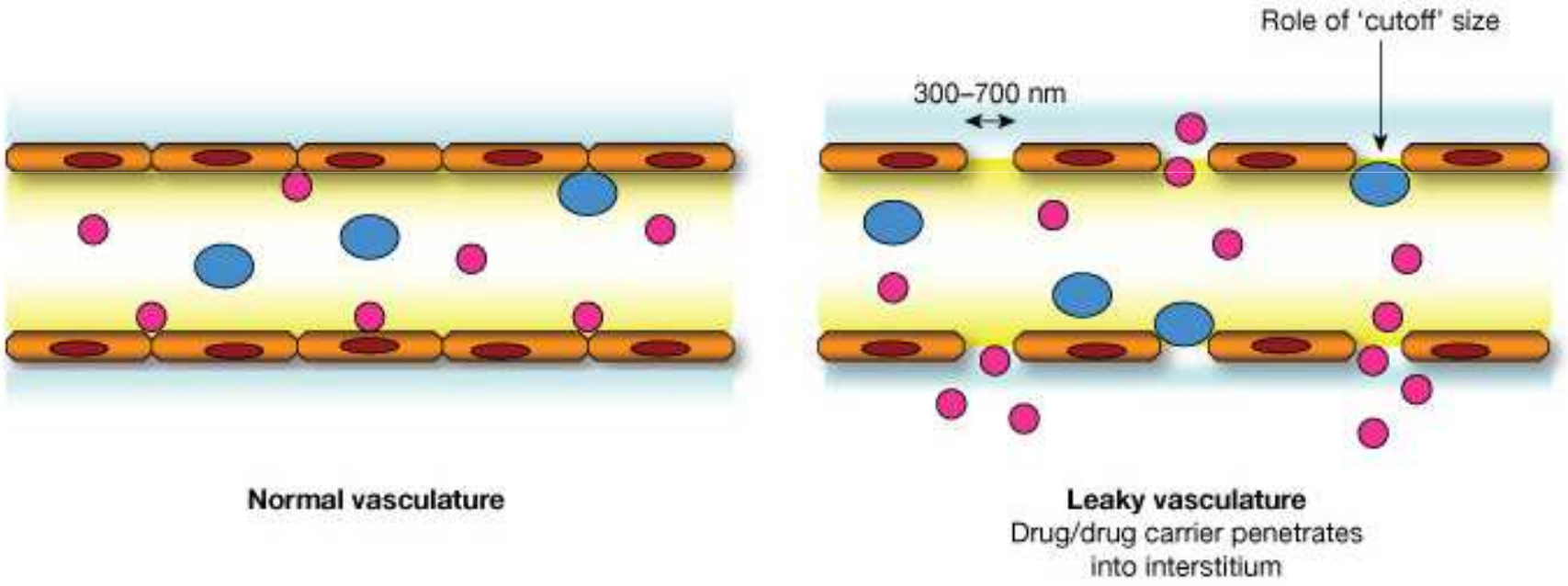
The blood vessels in tumors are generally **disorganized** and **leaky** (**EPR**)

ENHANCED PERMEABILITY AND RETENTION

Molecules of certain sizes (typically liposomes, nanoparticles, and macromolecular drugs) tend to **ACCUMULATE in tumor tissue** much more than in normal tissues (Maeda e Matsumura, 1986)

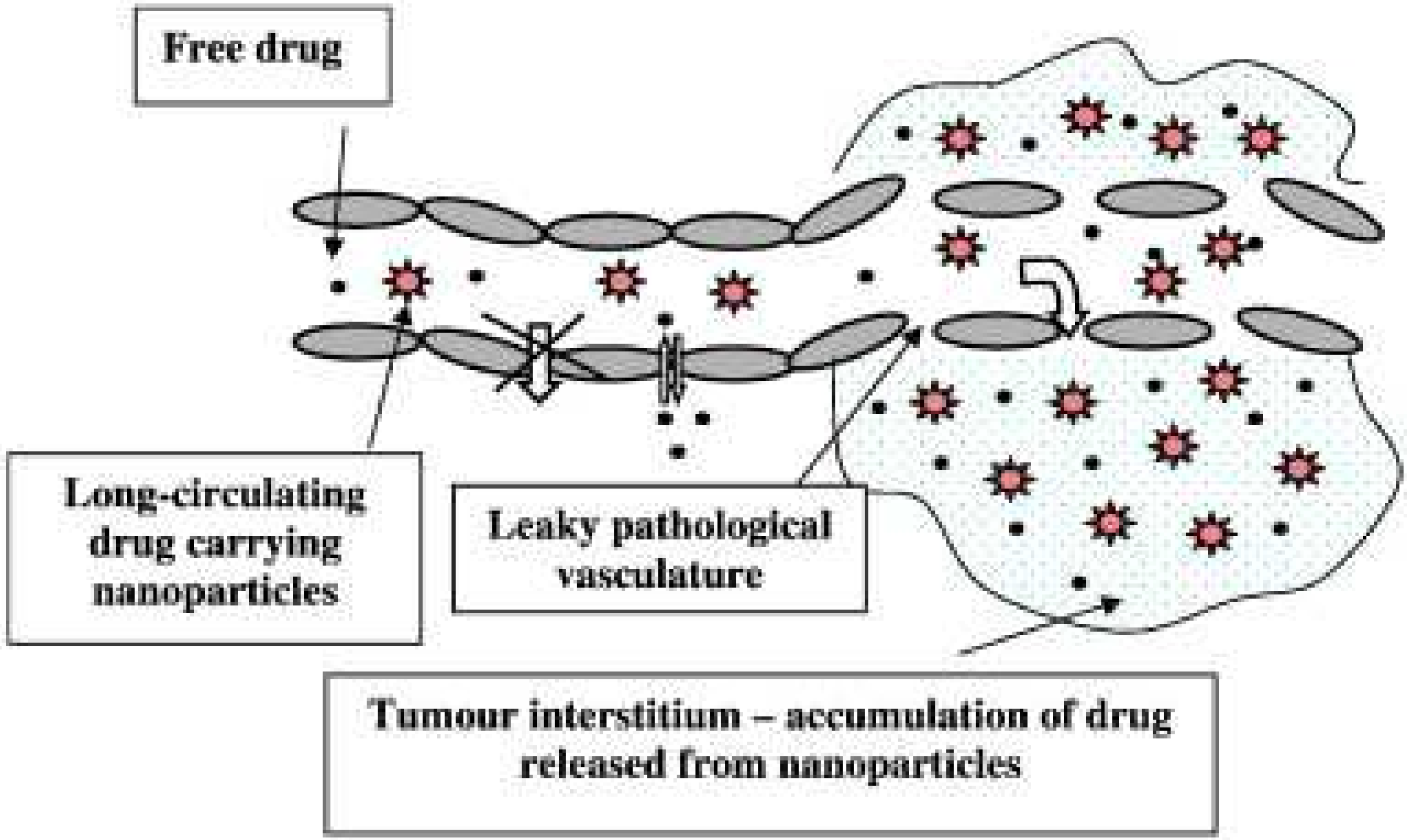
Matsumura Y, Maeda H "A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs". Cancer Research 46, 1986

In tumor angiogenesis the newly formed tumor vessels are usually **abnormal** in form and architecture



"*Nanoparticulate delivery systems for antiviral drugs*" Antiviral Chemistry et Chemotherapy 2010; **21**:53-70

Wide fenestrations (100 to 800 nm) lead to abnormal molecular and fluid transport dynamics, especially for **macromolecular drugs**, and are used for drug delivery in tumor tissues by, e.g. **LIPOSOMES** (passive targeting)



"*Molecular and magnetic resonance imaging:The value of immunoliposomes*" Advanced Drug Delivery Reviews 61 (2009) 1402–1411

DRUG CARRIERS

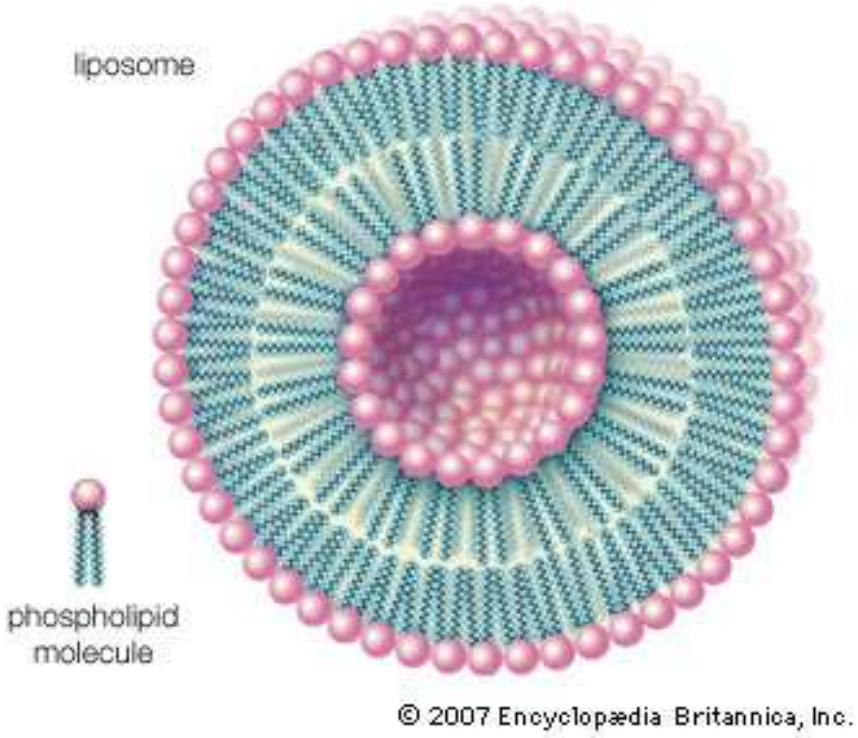
The main requirements of a drug vehicle for an efficient therapy are to "**Retain, Evade, Target, Release**"

Needs to **retain** the therapeutic agent, **evade** the body's defenses while in circulation, **target** the specific tissue and **release** the therapeutic agent in the diseased site

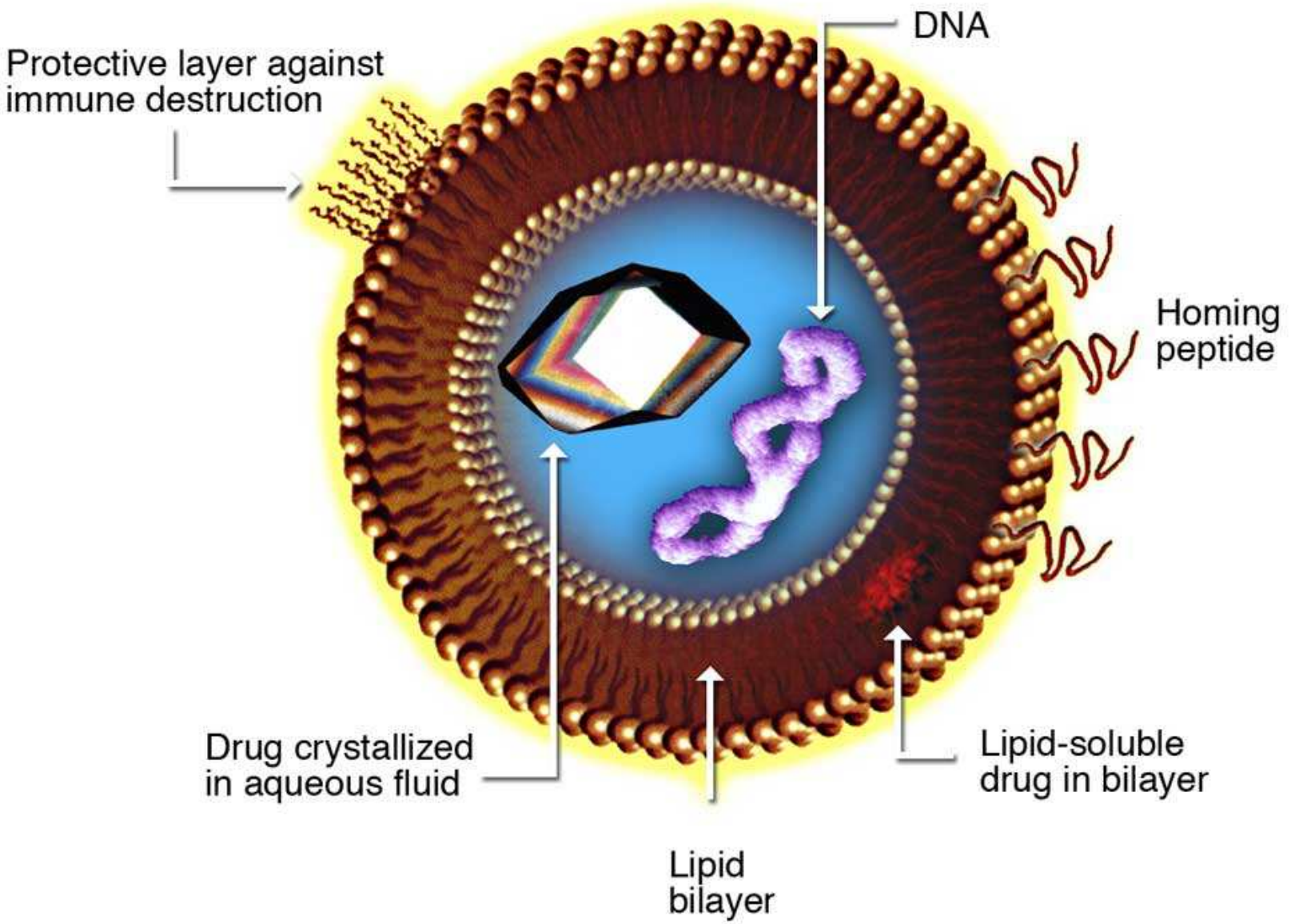
LIPOSOMES are an example of a drug carrier

LIPOSOMES

A liposome is an artificially-prepared vesicle composed of a **lipid bilayer** (cell membrane), which can be used as a **vehicle** for administration of pharmaceutical drugs



LIPOSOME FOR DRUG DELIVERY



Torchilin,V "Multifunctional nanocarriers".Advanced Drug Delivery Reviews 58 (14): 1532–55, 2006.

Liposomes size varies from ~50 nm to ~1000 nm

Typical dimension used in drug delivery is **~100 nm** (the larger the liposome, the faster the clearance by the reticuloendothelial system but also the higher encapsulating capacity)

Allow to deliver both **hydrophilic** and **hydrophobic** drugs

Are being used and in clinical trial with drugs for many different diseases, from fungal infections to metastatic breast cancer

LIPOSOMES PROPERTIES

The therapeutic advantages of liposomes range from the ability of long-circulating to preferentially **accumulate** at disease sites such as tumors, sites of infection and sites of inflammation

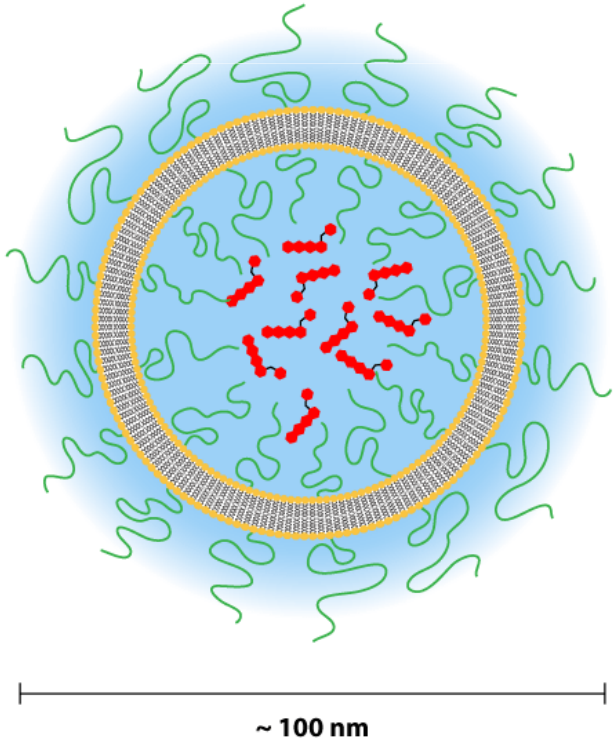
Some of these properties are exploited in **cancer therapy**

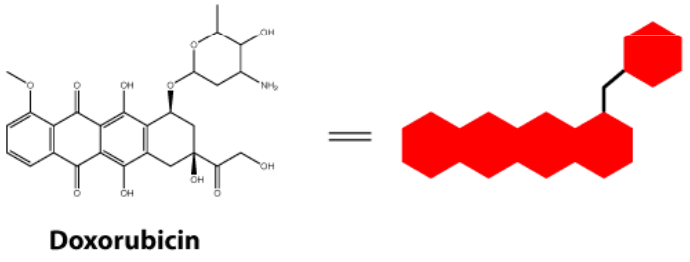
- **TARGETING**: cancer cells that overexpress some receptors can be targeted by peptides, monoclonal antibodies, aptamers, etc. that can be attached to the liposome surface, leading to **specific accumulation** in tumor tissues (active targeting)

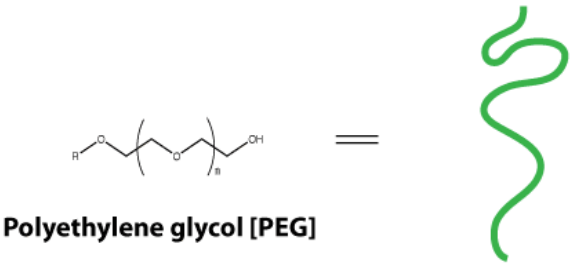
Passive (EPR, pH, cationic, etc.) methods can also be used

- **ENCAPSULATION**: many drugs or particles can be encapsulated, like chemotherapeutic drugs, radioactive isotopes, nanoparticles,…

- **STEALTH**: the covalent attachment of polyethylene glycol (PEG) allows to avoid detection by the body's immune system and leads to longer circulation time







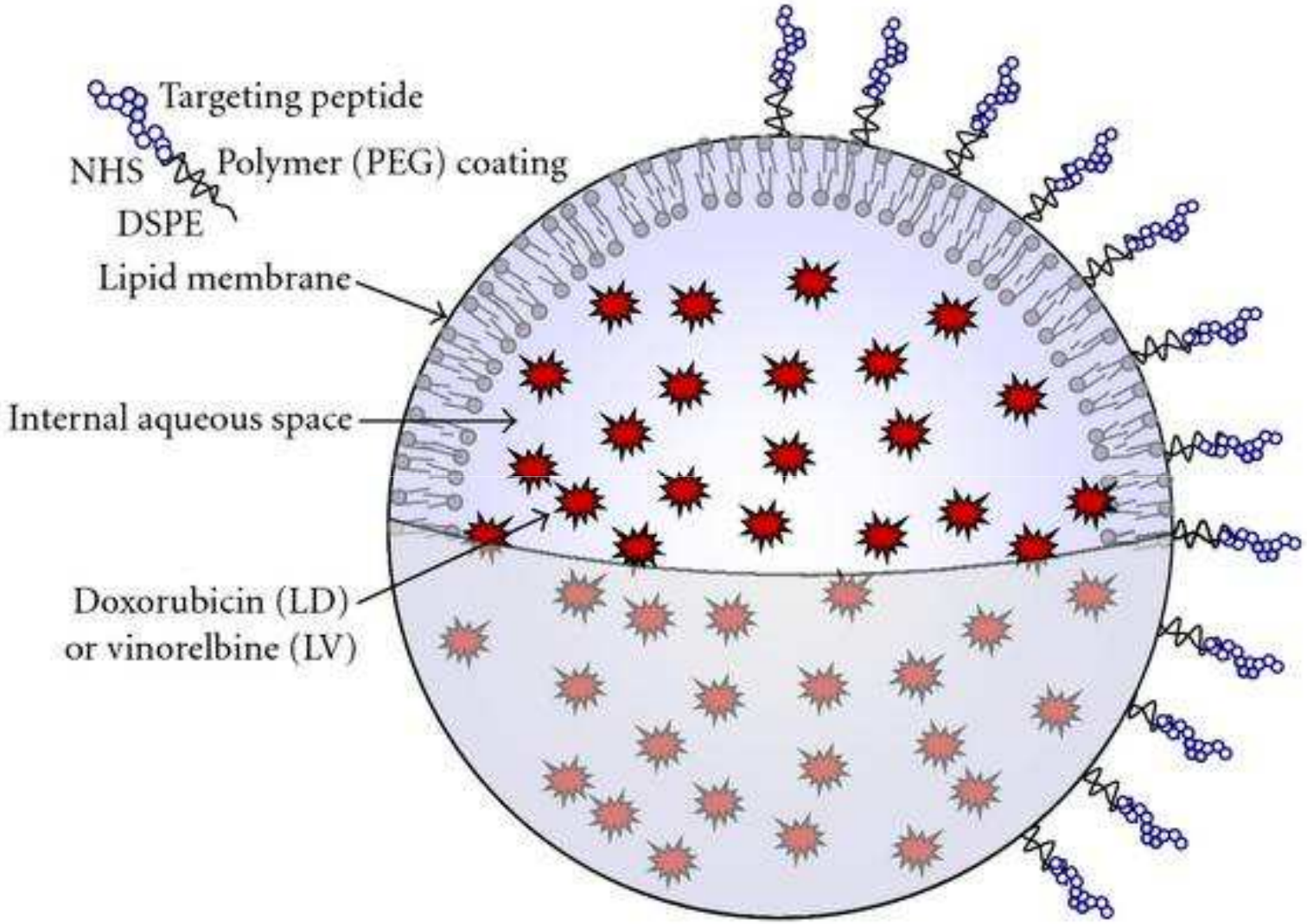
Bio Imaging, *http://www.scienceinyoureyes.com*

- **TRIGGERING**: e.g. a temperature sensitive formulation is a possible solution for **external triggering**

MULTIFUNCTIONAL LIPOSOMES

Grafting the **adequate molecules** to the surface and filling with therapeutic agents, liposomes properties can se used for different functions

- PEGylated to extend blood circulation half life
- Peptides and/or monoclonal antibodies to **target** overexpressed receptors in tumor tissues
- Encapsulation of chemotherapeutic drugs and/or radioisotopes (α and/or β emitters) for **therapy**
- γ emitting **radioisotopes** for **imaging** and monitoring (and/or fluorescent dye)
- **Triggered** release on tumor environment (pH or temperature sensitive formulation)



ARTICLE IN ANALYSIS

Breast Cancer Res. Treat. DOI 10.1007/s10549-011-1688-7

"Targeted and intracellular triggered delivery of therapeutics to cancer cells and the tumor microenvironment: impact on the treatment of breast cancer"

Vera Moura, Manuela Lacerda, Paulo Figueiredo, Maria L. Corvo, Maria E. M. Cruz, Raquel Soares, Maria C. Pedroso de Lima, Sérgio Simões, João N. Moreira

(researchers from Center for Neuroscience and Cell Biology, Faculty of Pharmacy of the University of Coimbra, Portuguese Institute of Oncology FG Coimbra, Research Institute for Medicines and Pharmaceutical Sciences, Faculty of Pharmacy of the University of Lisbon, Department of Biochemistry, Faculty of Medicine of the University of Porto, Department of Life Sciences of the University of Coimbra)

STUDY OF 30 CLINICAL CASES OF BREAST CANCER

The **nucleolin receptor** is overexpressed on cancer and endothelial cells of tumor blood vessels, providing an opportunity to develop **multi-targeting** strategies toward the tumor microenvironment

A possible ligand for this receptor is the **F3 peptide**

Tested **liposomes** are non-targeted (SL), targeted to non-specific receptors (SLNS), or **TARGETED** with F3 (SLF3)

The liposome formulation can be **pH sensitive** (the tumor environment is slightly more acidic, pH~6, than normal tissues, pH~7.4)

Filling the liposomes with **Doxorubicin** (DXR or DOX, chemotherapy drug) allows to study the effectiveness in causing cancer cells death [**IC_{50}** is the half maximal (50%) **inhibitory concentration** (IC)]

Table 1 Cytotoxicity of several formulations of DXR against MDA-MB-435S or HMEC-1 cell lines

DXR $IC_{50}(\mu M) \pm SD$		SL[DXR] $IC_{50}(\mu M) \pm SD$	SLF3[DXR] $IC_{50}(\mu M) \pm SD$	pSL[DXR] $IC_{50}(\mu M) \pm SD$	pSLF3[DXR] $IC_{50}(\mu M) \pm SD$
HMEC-1					
Time (h)					
1	0.434 ± 0.030	614.6 ± 0.029	>300	87.66 ± 0.062	47.69 ± 0.064
3	0.401 ± 0.059	89.16 ± 0.0108	11.54 ± 7.54	12.07 ± 2.541	6.688 ± 0.119
24	0.072 ± 0.010	31.6 ± 0.661	7.2 ± 0.093	3.57 ± 0.200	0.195 ± 0.049
48	0.058 ± 0.008	3.575 ± 0.052	0.856 ± 0.091	0.03 ± 0.026	ND
MDA-MB-435S					
Time (h)					
1	1.608 ± 0.460	>800	>300	>500	>600
3	1.449 ± 0.270	>700	230.5 ± 0.042	>400	159.0 ± 4.950
24	0.232 ± 0.045	>700	41.24 ± 0.069	87.33 ± 0.139	3.953 ± 0.263
48	0.113 ± 0.078	>700	25.5 ± 0.049	55.2 ± 0.049	3.366 ± 0.124

DXR-containing F3-targeted pH-sensitive (pSLF3 [DXR]) liposomes were more effective at causing cancer (MDA-MB-435S) and endothelial (HMEC-1) cell death than F3-targeted non-pH-sensitive (SLF3 [DXR]) or non-targeted, either non-pH-sensitive (SL [DXR]) or pH-sensitive (pSL [DXR]) liposomes, for 1, 3, 24, and 48 h exposure to DXR. Data are means ± SD of three independent experiments, each done in triplicate

MAIN PAPER RESULTS

Within a solid tumor, is possible to **limit tumor invasion** with the same nanoparticle by **triggered targeted drug delivery** to more than one cell population

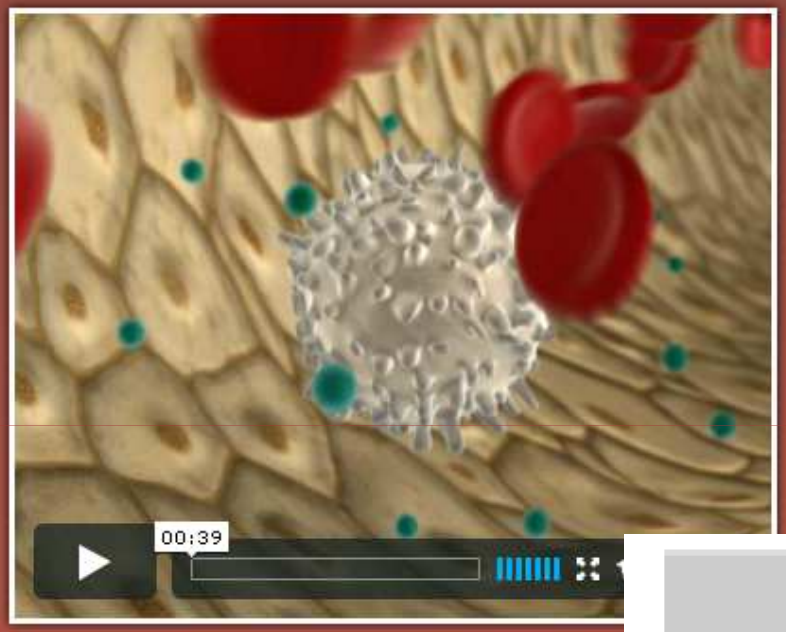
The **nucleolin receptor** was identified in endothelial and cancer cells from breast cancer patients, enabling the design of a **F3-peptide-targeted pH-sensitive liposome**

In vitro, the nanoparticle targeted the nucleolin receptor and improved **cytotoxicity** of DXR towards **breast cancer** (tumor tissue) and **endothelial cells** (neovasculature) by **177−** and **162−fold**, respectively, relative to non-targeted non-pH-sensitive liposomes

In vivo, the **accumulation** of the dual-targeted pH-sensitive nanoparticle in the tumor tissue was **33−fold** higher than the non-targeted non-pH-sensitive counterpart

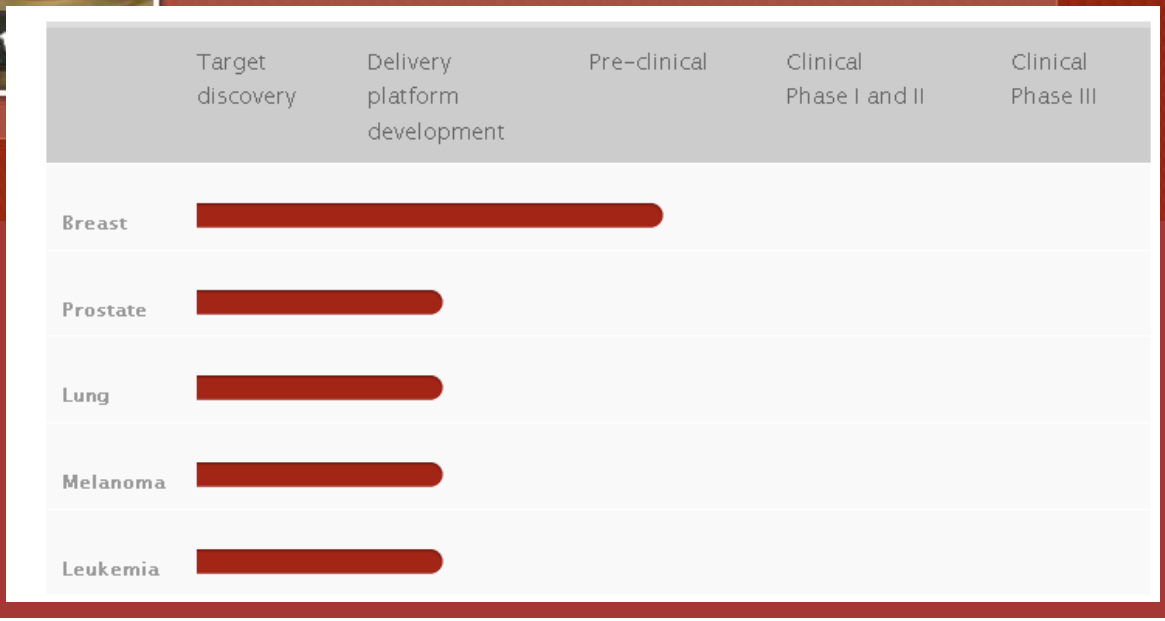
TREAT-U

ABOUT | R&D | PIPELINE | NEWSROOM | CONTACT US



PEGASEMP®

PEGASEMP is a platform conceived for delivering a cytostatic agent specifically to the tumor microenvironment, targeting two distinct cell populations: the cancer cell and the endothelial cells from the tumor blood vessels.



http://treatu.pt/products/

THERANOSTICS

Liposomes can be used in a **multifunctional** mode, for diagnosis, multi-therapy and monitoring (using the same or different liposomes)

RADIOISOTOPES

α and β emitters, encapsulated in **targeted liposomes**, can be used for internal **micro radiotherapy**

γ emitters can be used for **imaging** and **monitoring** (γ-camera and SPECT), a β^+ emitter can be used for PET scan

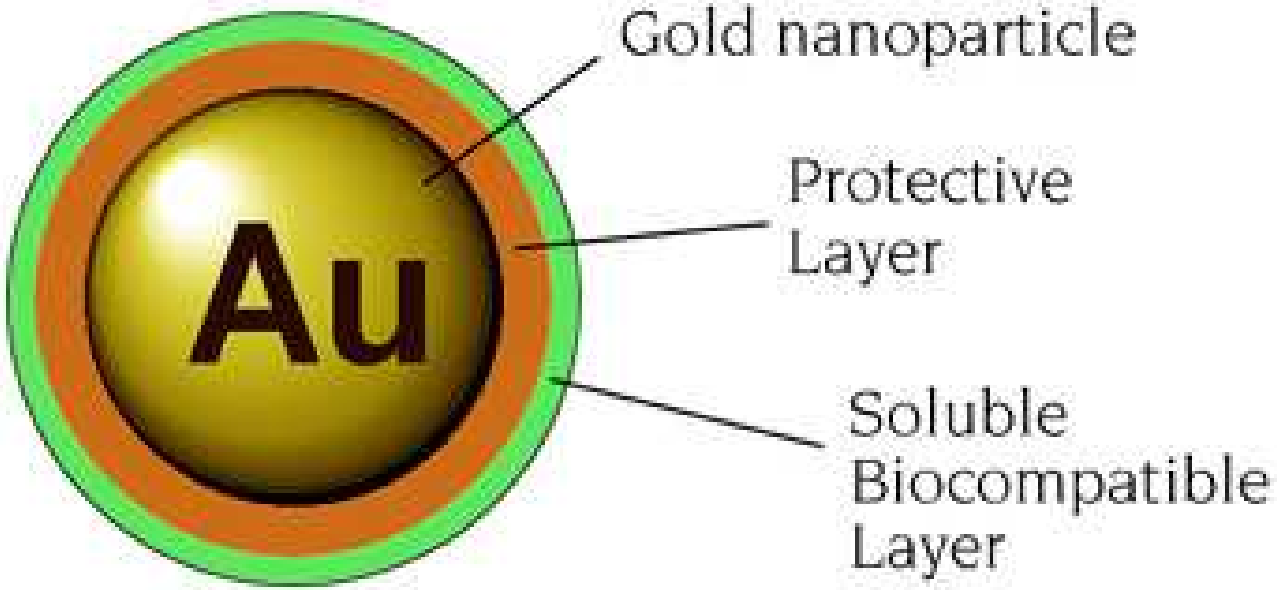
The chosen radioisotope will depend on the decay chain, emitted particles or radiation, half life, radiation energy, production process, availability, daughter nuclei toxicity, …

For small tumors, micro metastases and post-surgery an **α source** is more adequate, with a path length of ~50-100 μm (a few cells deep, with a high linear energy transfer – quality factor = 20), for larger tumors a **β source** is preferable (longer path length ~1–20 mm)

The radioisotope doesn't need to be internalized, isotropic effect also from the cell membrane or vasculature

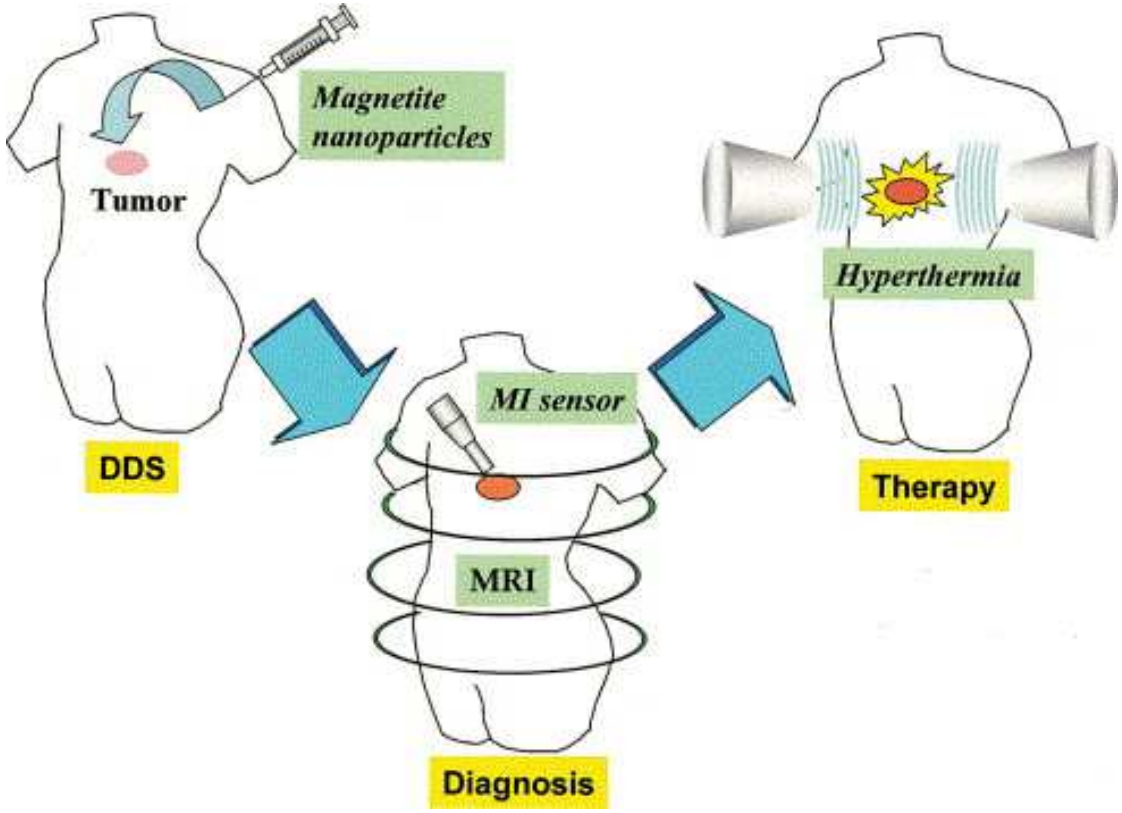
ALTERNATIVES

- nanostructures, as hollow gold particles, can be encapsulated in liposomes for **hyperthermal therapy** with heating by an **external** electromagnetic field



nanoprobes.com

- superparamagnetic iron oxide nanoparticles can be used as contrast agents for **magnetic resonance imaging**, as controllable carriers and for hyperthermia



"*Medical application of functionalized magnetic nanoparticles*" Akira Itoa, Masashige Shinkaib, Hiroyuki Hondaa, Takeshi Kobayashic, Journal of Bioscience and Bioengineering, Volume 100, Issue 1, July 2005, 1–11

PROJECT

Liposomes for targeted internal micro radio and chemotherapy with imaging
(not all the functions in a single carrier before, as far as we are aware)

Needs a **multidisciplinary team**
(pharmacy, biology, medicine, biochemistry, engineering, physics, …)

CONCLUSIONS

New technologies are needed for **cancer therapy** otherwise will be the major cause of death in the 21st century…

Genomic technology is evolving fast but hard to convert this knowledge into cancer therapies (but important to discover receptor overexpression in tumor cells)

Nanotechnology is being used and has a big potential for cancer imaging, diagnosis and therapy

LIPOSOMES have many advantages for drug delivery, and are being used in many applications: is a platform for integration of different diagnoses and therapies (**multifunctional**, with *in vivo* real time monitoring capability)

A multidisciplinary project in Coimbra (with national and international **collaboration**) with liposomes can be expanded

THANK you

All questions, comments and suggestions are welcome!

"Of course, beyond the pure intellectual challenge of cancer there is the technological aspect of physics that can be applied to **cancer treatment**. Physicists have made extraordinary contributions to biology over the past 100 years that have transformed the field. But we can't stop with genome sequencing; there are far deeper problems in cancer that will hopefully yield to new technologies that are still in the formative stages. Another aspect of physics which has not fully moved into the cancer world is the highly **quantitative** and **predictive** growth of our ability to create sophisticated **models** to understand the growth and dynamics of mutating cancer cells under stress, both mechanical and metabolic. "

"*Preface: Physics of Cancer*" Robert H. Austin and Bernard S. Gerstman, AIP Advances 2, 010901 (2012); doi: 10.1063/1.3699622