INTRODUCTION TO NANOMEDICINES

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REPÚBLICA PORTUGUESA
SAÚDE

Infarmed
Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.

OUTLINE

- **Introduction to controlled drug delivery systems**
- **Nanomedicines**
  - Physical Features
  - Advantages & Applications
  - In Clinical Development
  - In the Market
- **Nanomedicines Challenges to Clinical Translation**
  - Materials Science
  - Manufacturing Process Control and Scale-Up
  - Translational Research
  - Clinical Development
- **Regulatory Framework on Nanomedicines**

ACTIVE PHARMACEUTICAL INGREDIENTS (API)

- Reach the site of action at the right time and with the pharmacological concentration
- Be available during the treatment
- Be completely eliminated when not needed
- Therapeutic efficacy
- Few adverse effects

- **Extracellular action**
- **Site-specific targeting**
  - Organ
  - Tissue
  - Cellular surface – receptor-mediated action
  - Intracellular action
- **Cytosolic delivery**

ACTIVE PHARMACEUTICAL INGREDIENTS (API)

Drug availability at the site of action depends on:

- Drug physicochemical properties
- Lipid-water partition coefficient
- Degree of ionization (pKa and pH)
- Molecular weight and volume
- Chemical stability
- Aqueous and lipid solubility (DDS)
- Drug concentration
- Site of absorption vascularization
- Surface area

Weak acids functional groups
- Carboxilic acids
- Phenols
- Sulfonic acids
- Thiols
- Imides

Weak base functional groups
- Amines
- Pyridines
- Imidazoles
- Quaternary ammonium salts

Functional groups non ionizable
- Ethers
- Esters
- Ketones
- Aldehydes
- Most amides

Macromolecular drugs (recombinant proteins and plasmid DNA) – action in the cytoplasm and nucleus

- Highly susceptible to enzymatic degradation
- Delivery systems should be used to ensure protection from proteases and nucleases.
- Liposomes, cell penetrating peptides, cationic polymer conjugates and polymeric nanoparticles

PHARMACEUTICAL STRATEGIES

- Drug release at the site of action
- Controlled drug release rate
- Not affected by physiological conditions
- Easy to be administered
- Extended circulation in blood
- Cost effective
- Compliance

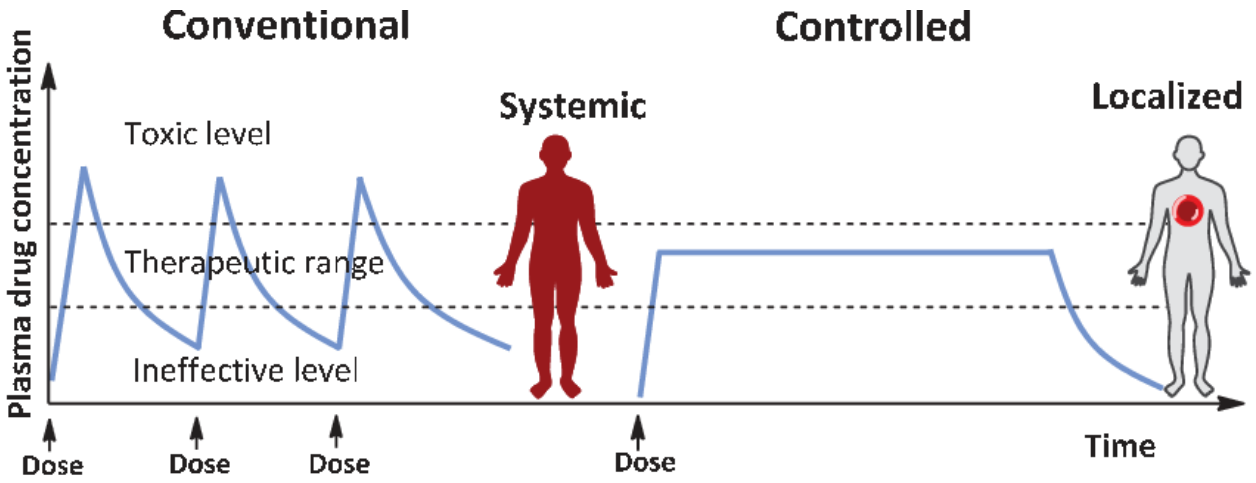
- Conventional dosage form - simple oral, topical or injection formulations.
- Drug delivery systems – association of advanced technologies such as rate-control, pulsatile release or bioresponsive release.
- Administration route

Ideal DDS

- specifically target the drug to target cells or target tissue;
- keep the drug out of non-target organs, cells, or tissue;
- ensure minimal drug leakage during transit to target;
- protect the associated drug from metabolism;
- protect the associated drug from premature clearance;
- retain the drug at the target site for the desired time;
- facilitate the transport of the drug into the cell;
- deliver the drug to the appropriate intracellular target site;
- be biocompatible, biodegradable, and non-antigenic.

CONTROLLED DRUG DELIVERY: AIMS

- Sustain drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body resulting in lower undesirable side effects
- Localize drug action by using a controlled release adjacent to or in the diseased tissue or organ
- Target drug delivery/action by using carriers or functionalization to deliver drugs to a particular “target” cell
- Control of PK/PD:
  - Drug structure modification
  - Modify physiology
  - Design of drug delivery system
- The duration of drug action depends on the rate of the controlled delivery system and not on the physicochemical properties of the API molecule.



https://www.semanticscholar.org/paper/Crosslinked-poly(ester-anhydrides)-for-controlled-Hakala/790fa8bcf1fe304cc0832b9cdcb6cf5a4459c2a6

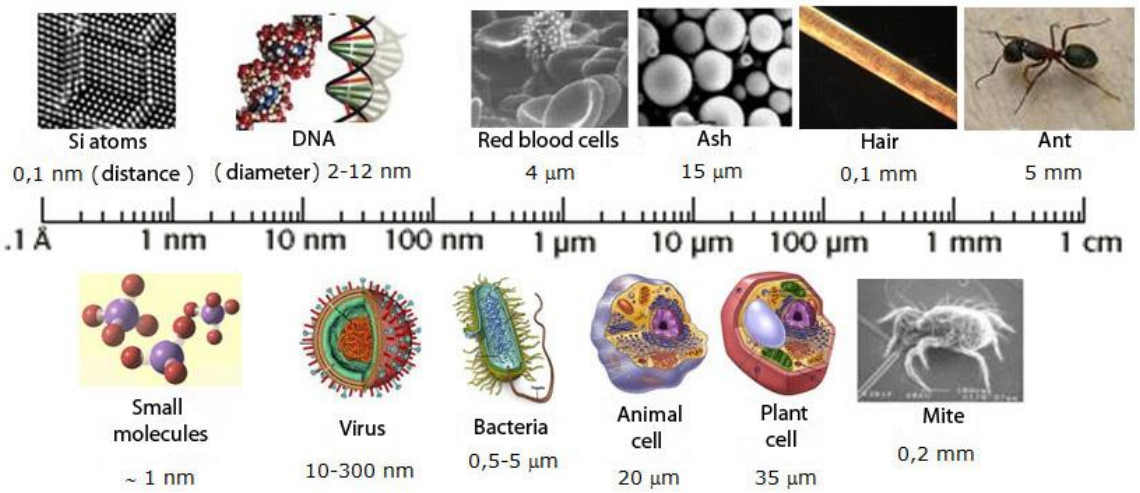
NANOMATERIALS (EUROPEAN COMMISSION IN 2022)

"Nanomaterial' means a natural, incidental, or manufactured material consisting of solid particles that are present, either on their own or as identifiable constituent particles in aggregates or agglomerates, and where 50% or more of these particles in the number-based size distribution fulfil at least one of the following conditions:

(a) one or more external dimensions of the particle are in the size range 1 nm to 100 nm;

(b) the particle has an elongated shape, such as a rod, fiber, or tube, where two external dimensions are smaller than 1 nm and the other dimension is larger than 100 nm;

(c) the particle has a plate-like shape, where one external dimension is smaller than 1 nm and the other dimensions are larger than 100 nm. In the determination of the particle number-based size distribution, particles with at least two orthogonal external dimensions larger than 100 μm need not be considered."



http://www.davidfunesbiomed.eu/2015/06/nanotechnology-introduction.html

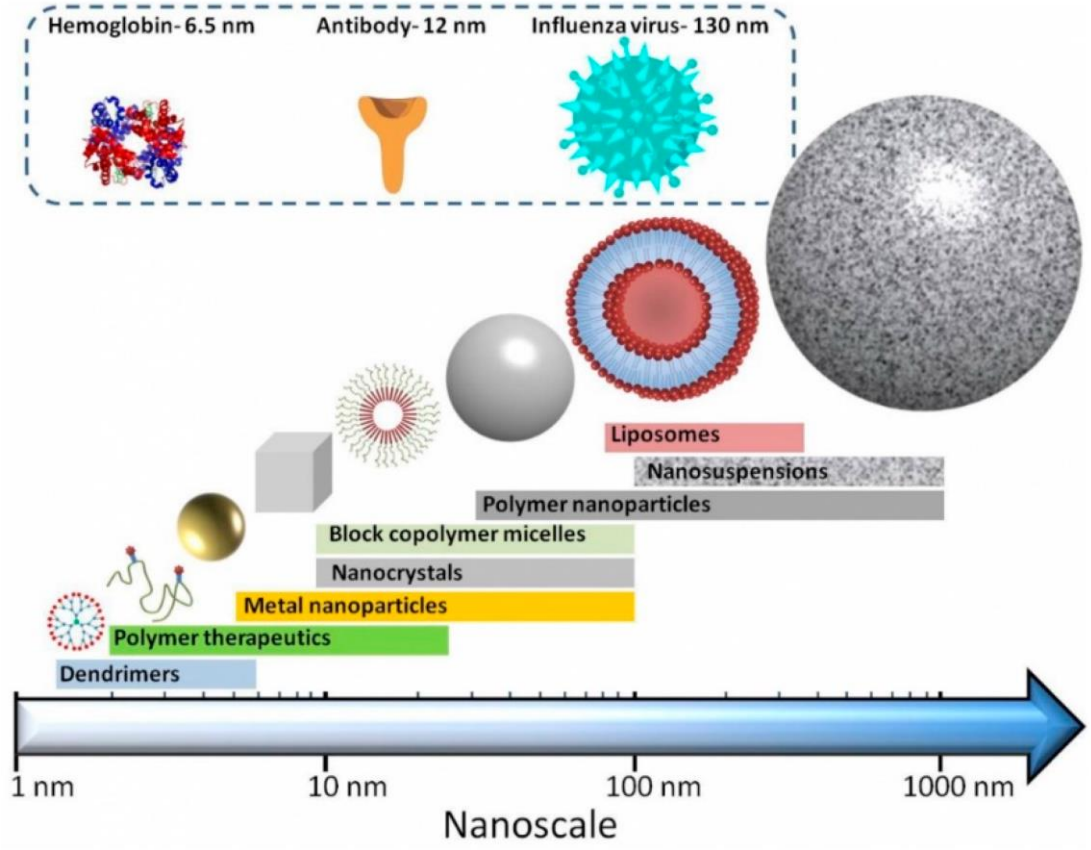
https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX%3A32011H0696

NANOMEDICINES

Nanomedicines: Convergence of nanotechnology and medicine.

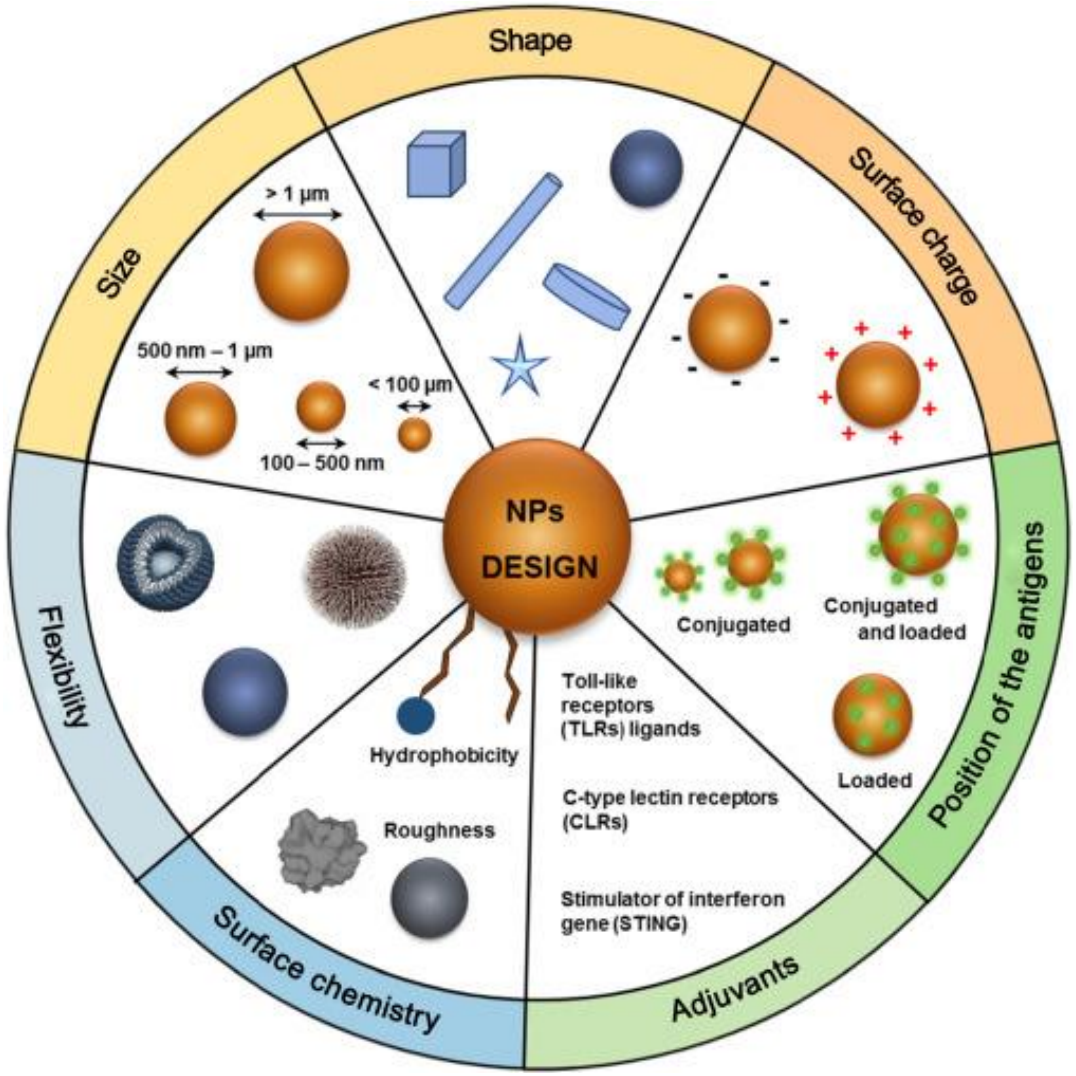
Nanomedicine is the application of nanotechnology to make a medical diagnosis or treat or prevent diseases. It exploits the improved and often novel physical, chemical, and biological properties of materials at nanometric scale.

Nanotherapeutics: improve bioavailability, reduce toxicity, improve dose-response, compared to conventional medicines



*Pharmaceutics **2019**, 11(5), 210; https://doi.org/10.3390/pharmaceutics11050210*

NANOMEDICINES: PHYSICAL FEATURES



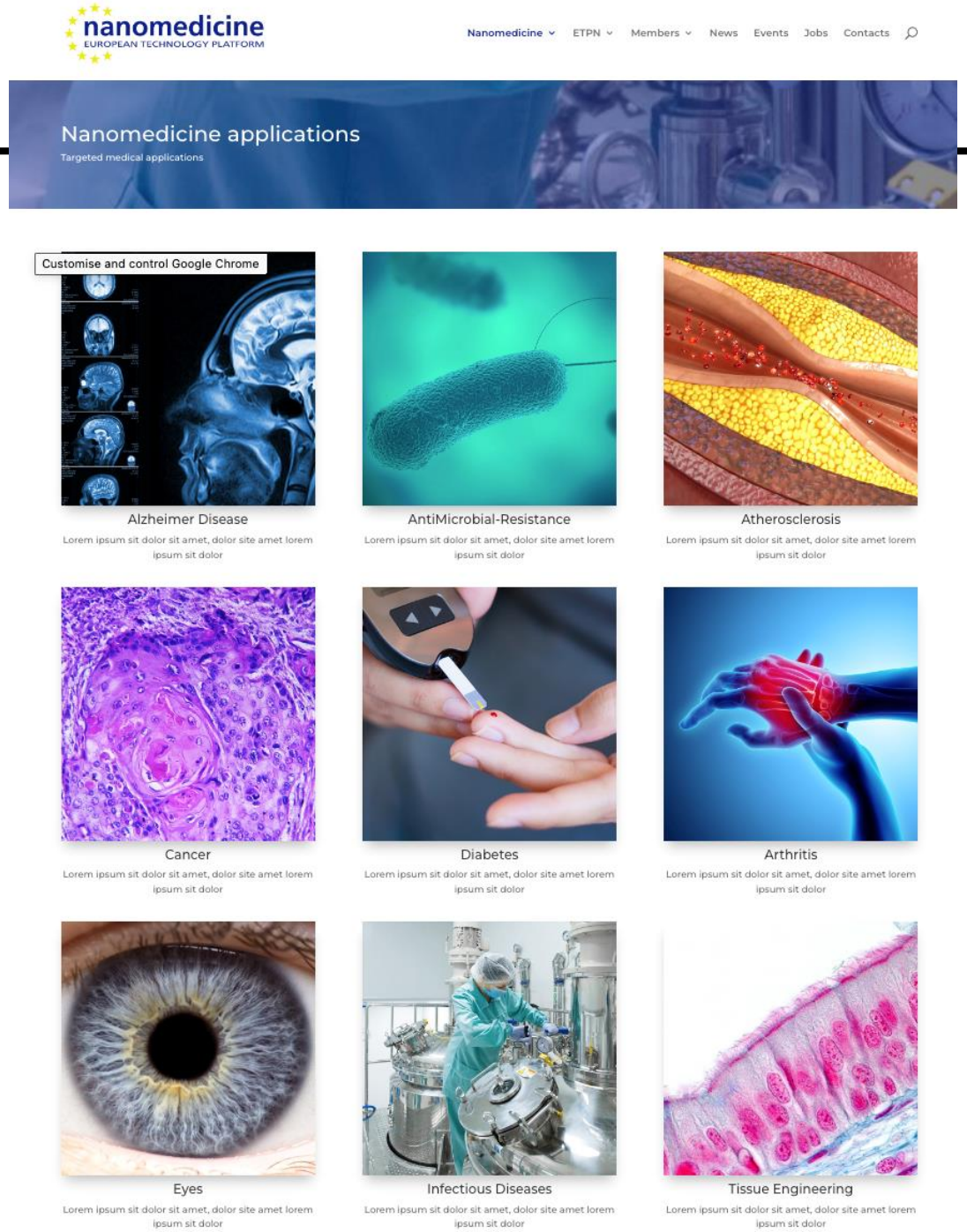
- Size & size distribution:
  - Improved solubility
  - Convert insoluble or poorly soluble drugs into soluble aqueous suspensions
- Shape:
  - Spheres, discs, hemispheres, cones, tubes, wires
  - Hollow, porous, or solid
  - Selection based on target, loading capacity, and transport capabilities
- Surface area and charge :
  - Large surface area relative to size
  - Enhanced solubility
  - Increased circulation time and bioavailability
- Permeability:
  - Size enables to cross physiological barriers to deliver drugs to sites usually not accessible
  - Different uptake mechanisms – structure-activity
  - Passive/Active drug targeting
  - Intracellular targeting to specific organelles- increased therapeutic efficacy, reduced adverse side-effects.

•DOI: [10.3390/app11094305](10.3390/app11094305)

NANOMEDICINES: MARKET & IN CLINICAL DEVELOPMENT

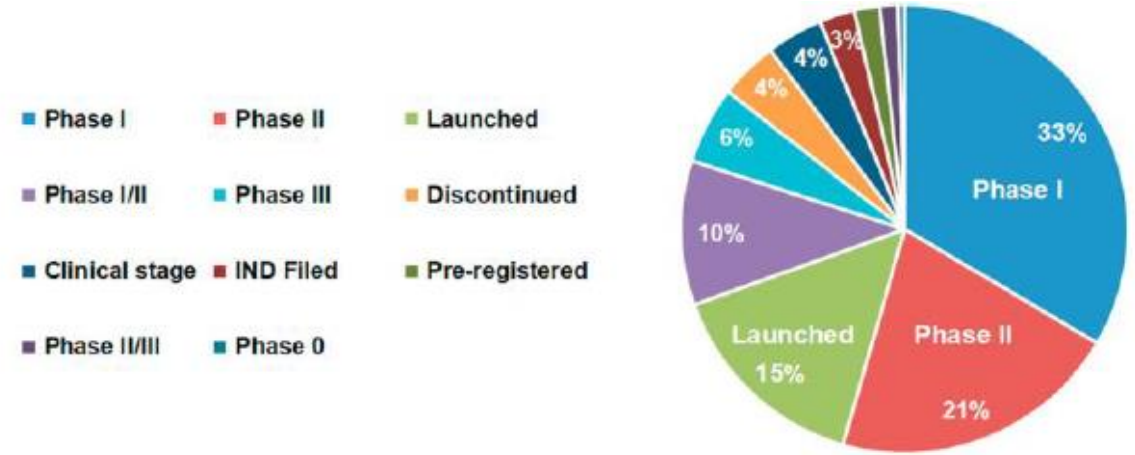
First nanomedicine was authorized by the European Commission in 1996.

- Therapeutics:
  - Drugs, oligonucleotides, macromolecules…
  - Targeted delivery
  - Stimuli-responsive
  - RNA delivery to regulate cells
  - Inorganic NP-mediated cell death
- Vaccines: COVID-19, Melanoma (clinical trials)
- Diagnostics/Theranostic
- Medical Imaging:
  - Computed Tomography
  - MRI
  - Fluorescence Imaging
- Regenerative Medicine (biomaterials' functionalization):
  - Cell-based implants
  - Artificial organs
  - Active tissues

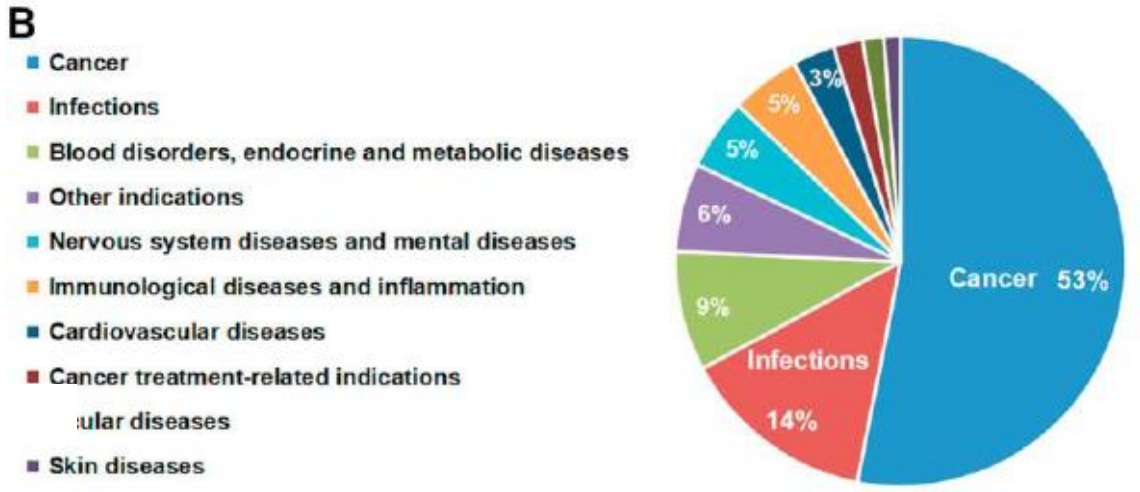


NANOMEDICINES: MARKET & IN CLINICAL DEVELOPMENT

Development

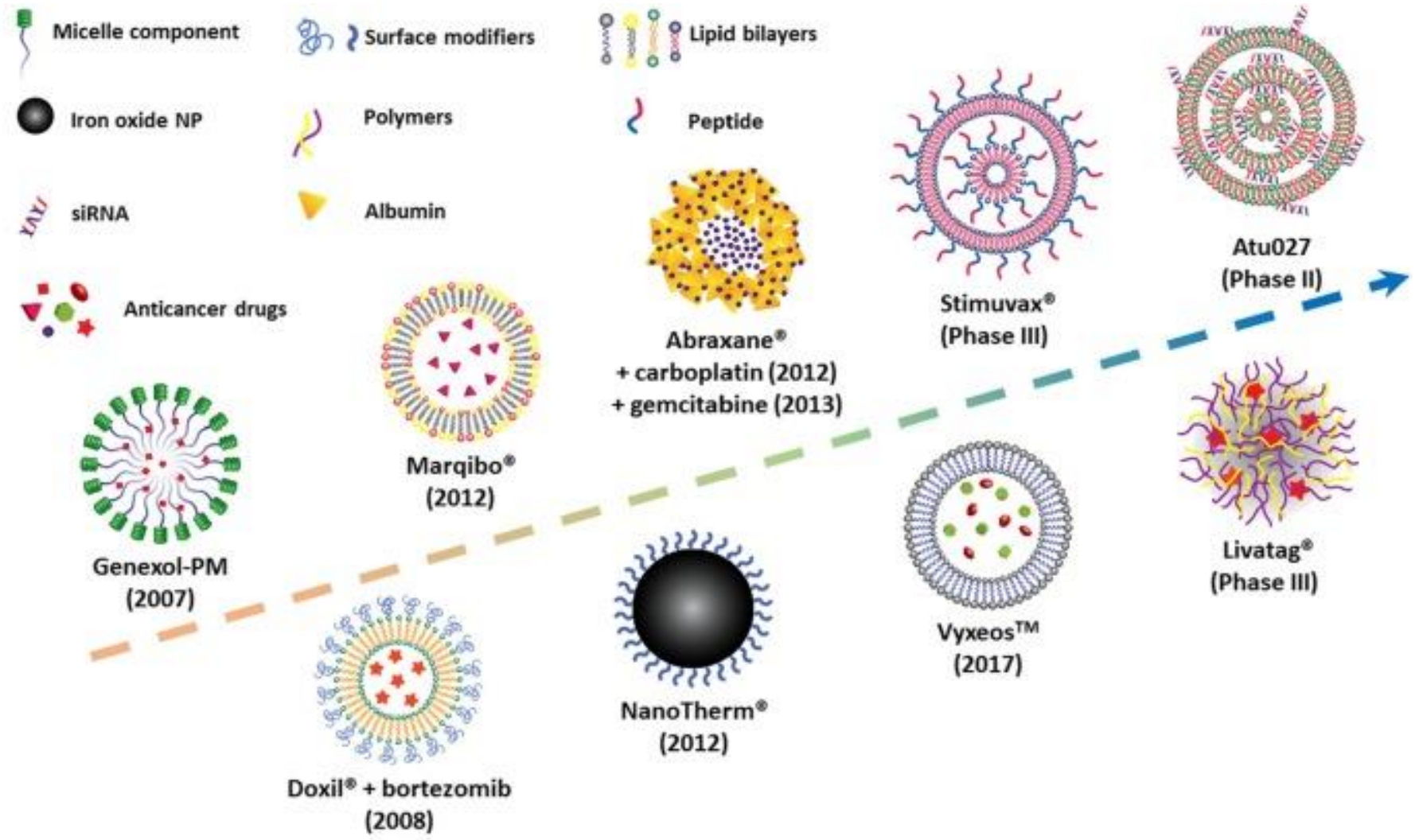


Indications



Acta Pharm Sin B. 2022 Jul;12(7):3028-3048. doi: 10.1016/j.apsb.2022.02.025.

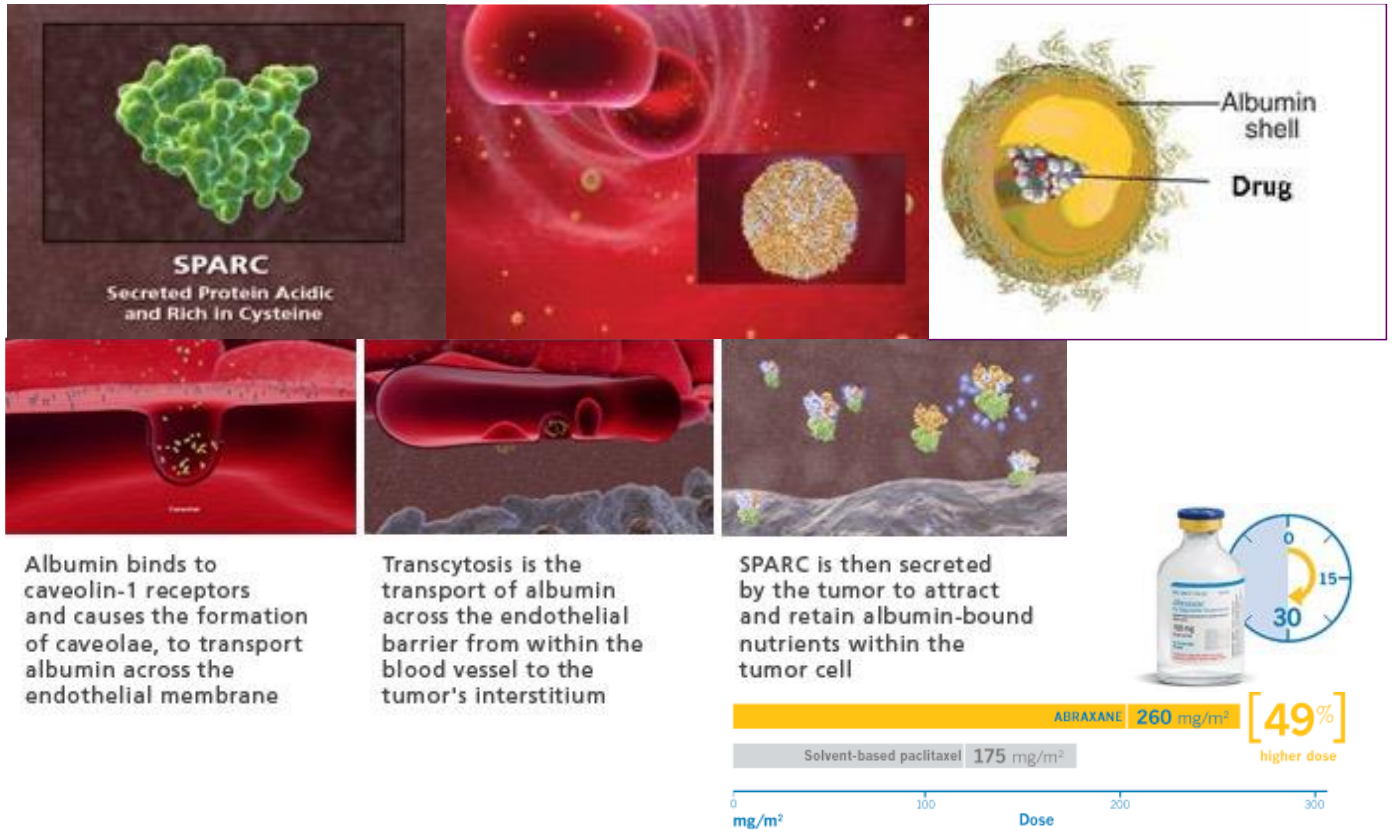
NANOMEDICINES IN THE MARKET



Trends in Pharmacological Sciences

NANOMEDICINES

Abraxane®



- **Paclitaxel-loaded albumin nanoparticles**
  - High-pressure homogenization
  - Albumin concentration similar to the one present in the blood
  - 130 nm – Intravenous administration
  - Lower volume and infusion time
  - Cremophor-EL® free formulation

Abraxane®

Medicine	Active substance and platform/technology	Use	Advantages of nanoformulation
Abraxane	Solvent-free colloidal suspension of albumin-bound paclitaxel (active substance) in spherical nanoparticle form	Treatment of breast cancer, adenocarcinoma of the pancreas, non-small cell lung cancer	Solubility problems of active principle solved (paclitaxel itself insoluble in water). Less frequent and severe toxicity (nausea, vomiting, fatigue, arthralgia, myalgia, alopecia) in comparison to previous formulations

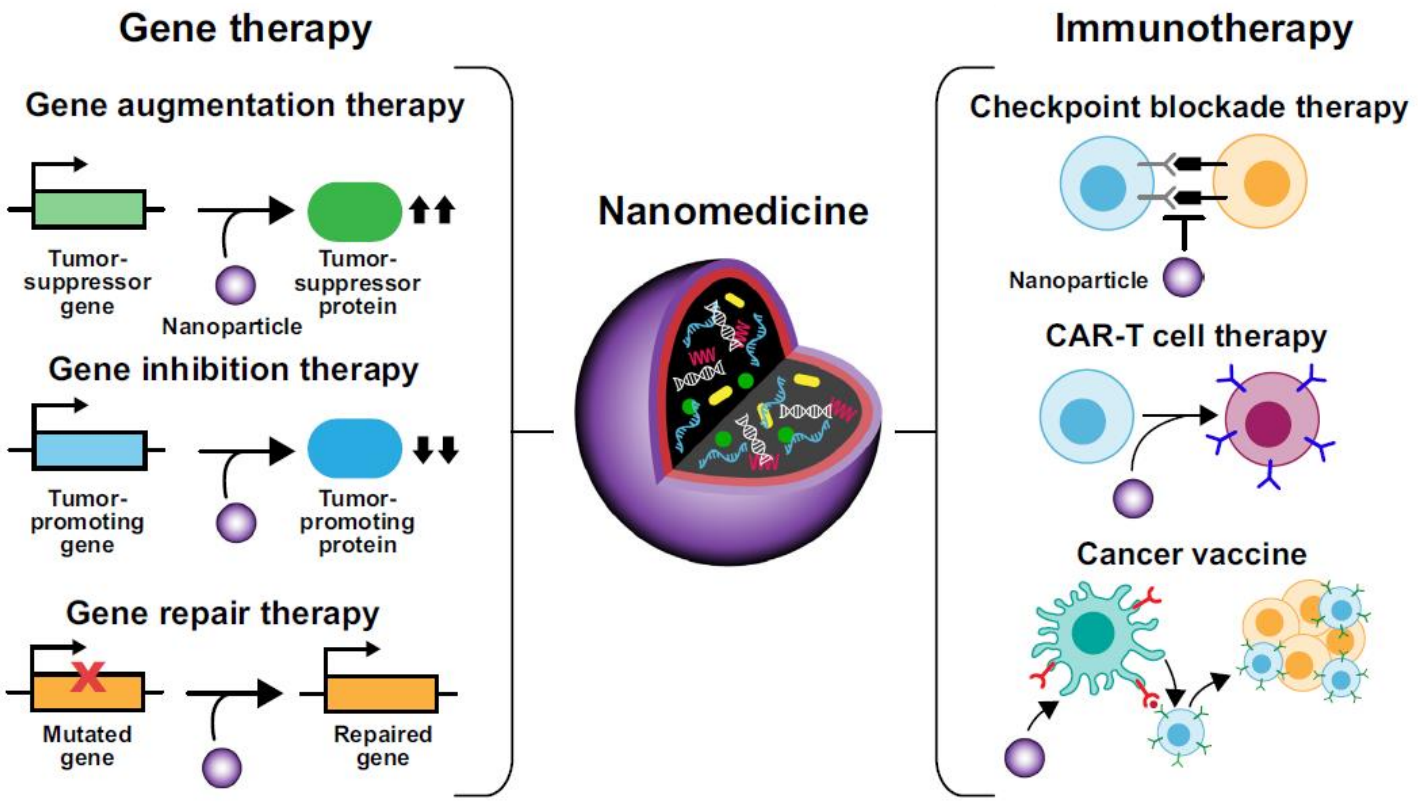
https://euon.echa.europa.eu/nanomedicines-on-the-eu-market

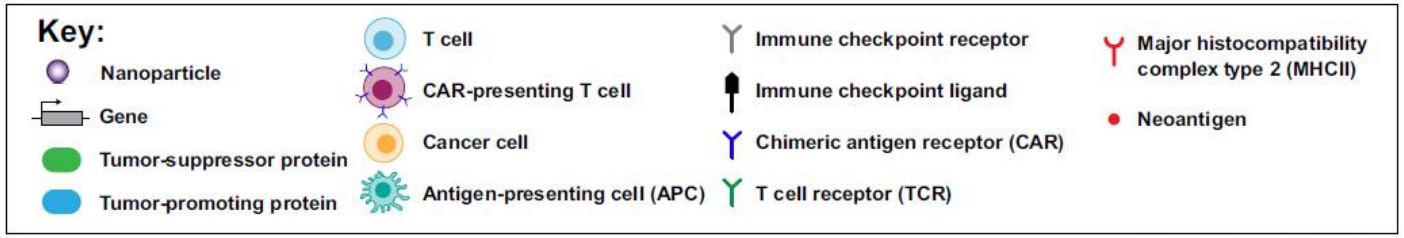
NANOMEDICINES

- **NanoCrystal particles**
  - Size lower than 1 μm
  - 100% drug (no polymeric/lipidic matrix material)
  - Need to be stabilized –surface adsorption
  - Colloidal dispersion
  - Structure crystalline or amorphous
  - Higher dissolution rate, solubility
  - Mainly for poorly water-soluble compounds (higher absorption, lower dose, lower variability, smaller dosage forms)
  - Production: Precipitation, Milling, High pressure homogenization

Marketed
Rapamune (Sirolimus) – oral tablet
Emend (Aprepitant) – oral capsule
TriCor (Fenofibrate)
Herbesser (diltiazem) sustained-release
Megace ES (megestrol acetate) – oral suspension

EVOLVING NANOPHARMACEUTICALS





https://doi.org/10.1016/j.tips.2020.08.001

Trends in Pharmacological Sciences

NANOMEDICINES: CHALLENGES

- **Materials Science:** characterization and stability of nanotechnology-based systems

  - New materials (inorganic nanoparticles, non-biodegradable/ non-biocompatible materials, quantum dots, cationic particles, dendrimeric structures, carbon nanotubes)

  - Physicochemical characterization: size and size distribution, aggregation and agglomeration, surface area, porosity, structure, chemical composition, surface chemistry and charge, water solubility, dissolution kinetics/release.

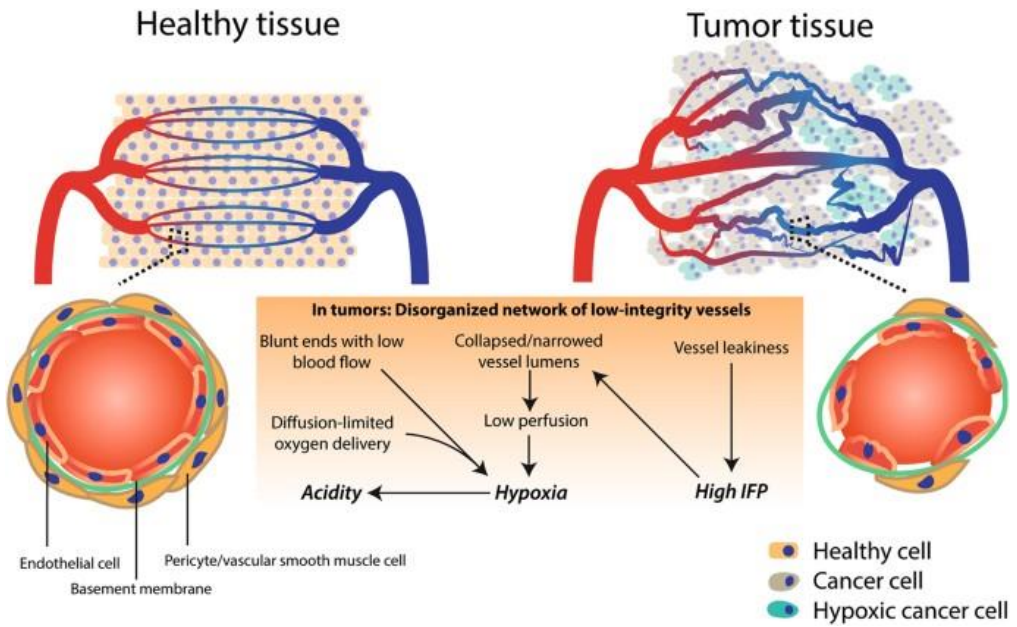
NANOMEDICINES: PROPERTIES

Particle size and size distribution – important for drug loading, drug release, formulation stability, *in vivo* biodistribution, biological fate, toxicity, targeting effect, cellular uptake and intracellular fate.

- Ex: nanoparticles have higher cellular uptake than microparticles and can be available to a wider range of biological targets.
- Determined by photon-correlation spectroscopy or dynamic light scattering
- Confirmed by SEM or TEM

Tumor/Tumor Microenvironment Targeting

- Delivery of a concentrated dose of the drug in the vicinity of the tumor targets
- Enhanced permeability and retention effect
- Active target – targeting ligands
- Specific target of tumor tissue
- Reduce drug exposure to healthy tissues

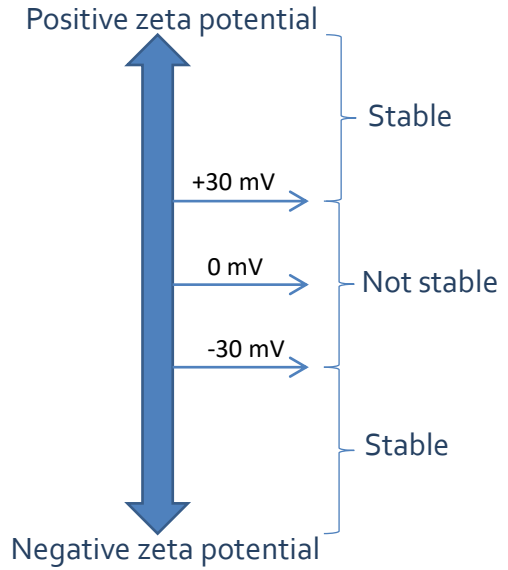


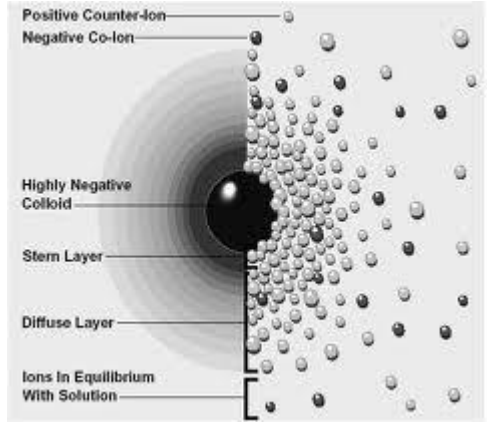
https://www.nature.com/articles/s41419-017-0061-0

NANOMEDICINES: PROPERTIES

Surface properties

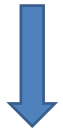
- Zeta potential – the electrical potential of particles (particle aggregation and to assess whether API is entrapped or adsorbed onto particle surface). It depends on particle composition and the medium in which it is dispersed.
- Non-modified nanoparticles are phagocytosed by RES– absorption of opsonin due to surface hydrophobicity.
- Modification of the biodistribution profile- drugs are mainly delivered to the mononuclear phagocyte system (liver, spleen, lung, bone marrow).
- Prolong circulation of nanoparticles *in vivo*





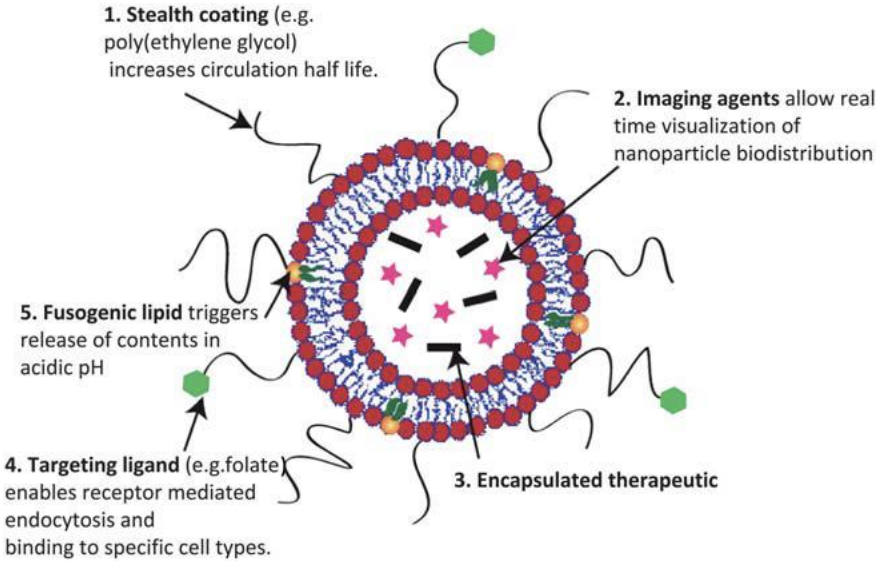
Long circulating nanoparticles

- To avoid mononuclear phagocyte system-rich organs
  - Surface modified NP by hydrophilic polymers – polyethylene glycol, poloxamines, poloxamers and polysaccharides



  - Stealth (PEGylated NPs) – avoid opsonization, not being recognized by macrophages (≤100 nm and hydrophilic surface)
  - non-stealth

- Active targeting
  - Ligand-receptor (folate, peptides or carbohydrates)
  - Antigen-antibody

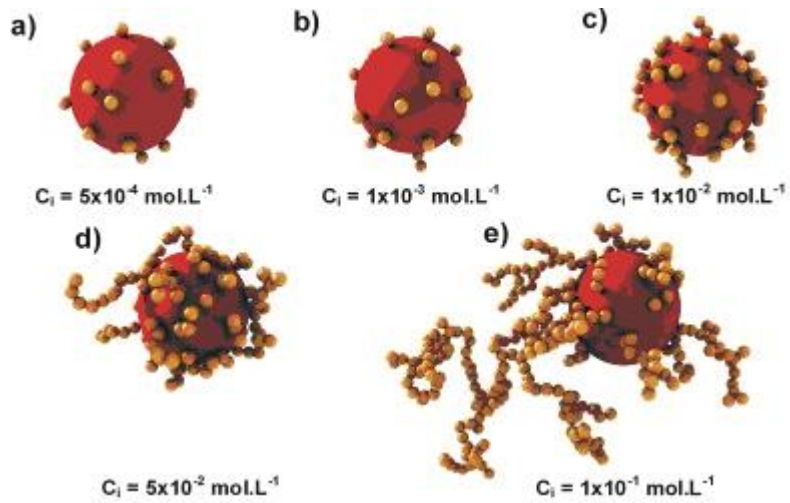


https://doi.org/10.1039/C0SM00534G

NANOMEDICINES: PROPERTIES

Drug loading

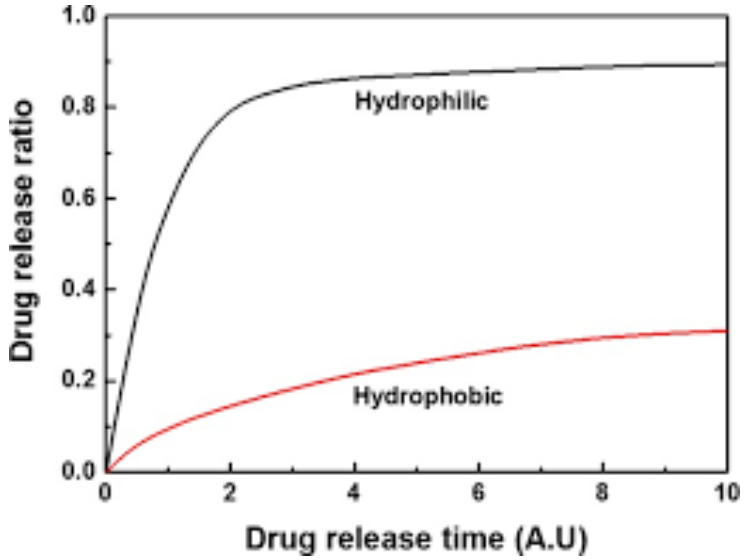
- High-drug loading capacity (reduced amount of matrix materials to be administered)
- Adsorption method *vs* Incorporation method
- Impact on dose administered, colloidal stability, excipient amount, scale-up and quality control
- Depends:
  - Drug solubility in the polymer (dissolved or dispersed) – polymer composition, molecular weight, drug-polymer interaction, presence of end-functional groups
  - Macromolecules – higher loading near their isoelectric point (↑ adsorption)
  - Small molecules – ionic interaction drug-polymer.

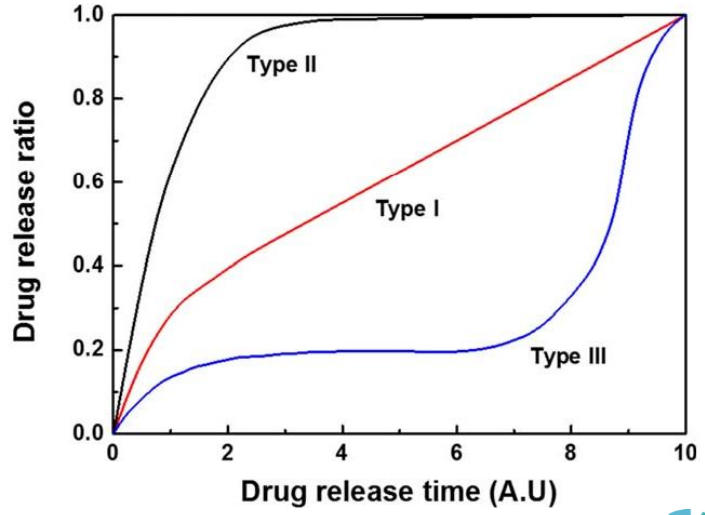


NANOMEDICINES: PROPERTIES

Drug release

- Drug solubility
- Desorption of adsorbed drug
- Drug diffusion through nanoparticle matrix
- Matrix degradation/erosion
- Erosion/diffusion
  - “burst” – weakly bound or adsorbed
  - Better sustained release for the incorporation methods
- Impacts PK and biodistribution, toxicity, time-dependent therapeutic efficacy





https://doi.org/10.1002/jbm.b.33648

NANOMEDICINES: CHALLENGES

- **Manufacturing Process Control & Scale-up**
  - Continuous manufacturing: batch *vs* continuous
    - *Scalable*
    - *Higher quality products*
    - *Adapting existing technologies to new opportunities: Quality by Design (QbD) & Process Analytical Technology (PAT) (fine-control of Quality Attributes, Reduce Human Error)*
  - Automation/robotization of manufacturing processes
  - Customization/adequacy of manufacturing to specific clinical needs
  - Physicochemical characteristics may change during the manufacturing process, impacting the quality and safety of the final product
  - Stability: under biological conditions (e.g. systemic circulation by aggregation/agglomeration in plasma and cell media) and storage

NANOMEDICINES: CHALLENGES

- **Quality Attributes (QA)** – chemical, physical and biological properties or any other relevant characteristic of the nanomaterial

Material Attributes:

- ✓ Polymer/lipid concentration;
- ✓ Lipid/Polymer ratios;
- ✓ Polymer/lipid purity;
- ✓ pKa;
- ✓ Lipid ionizable species;
- ✓ Aqueous phase (salts, organic phases)

Process parameters:

- ✓ Aqueous and organic flow rates;
- ✓ Mixing speed;
- ✓ Aqueous and organic phases temperature;
- ✓ pH of the aqueous phase

Quality attributes:
Particle Size; Polydispersity index; Zeta Potential; Encapsulation Efficiency; Drug loading; Drug Crystal Structure; Residual Solvent

NCI by Rachael Crist and Scott McNeil

NANOMEDICINES: CHALLENGES

- **Process Analytical Technologies (PAT): particle size setup**

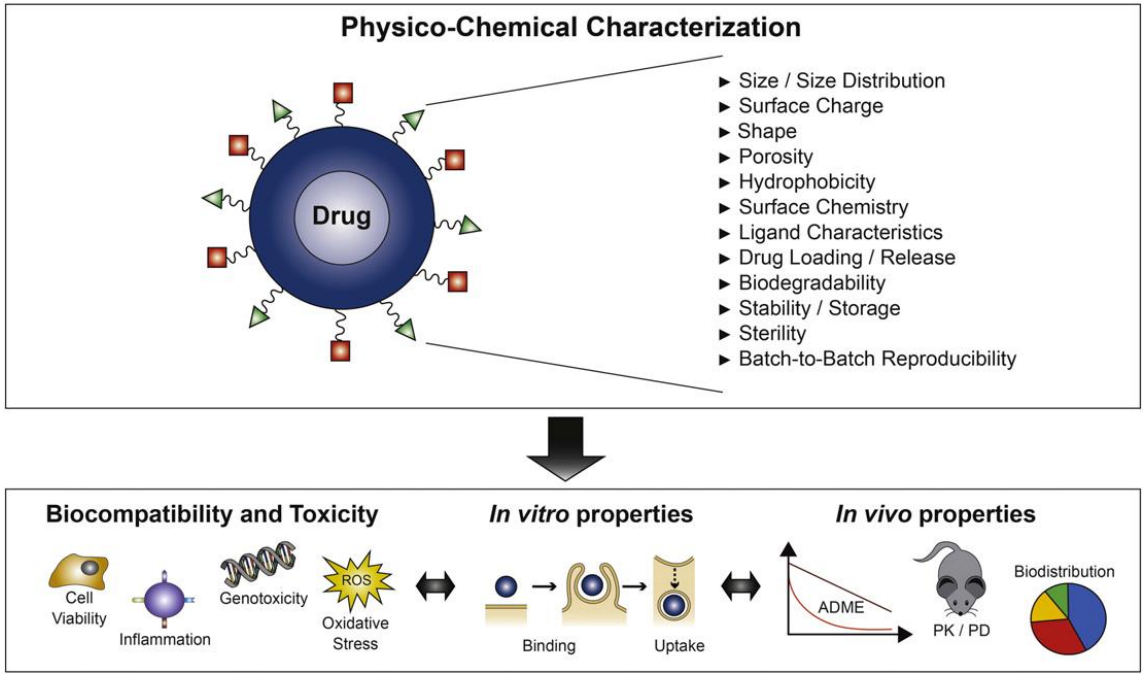
  - ✓ Rapid and Online analysis
  - ✓ Measurement interval: < 6s
  - ✓ Applicable to low and high concentrations
  - ✓ No dilution

https://www.inprocess-lsp.com/

NANOMEDICINES: CHALLENGES

- **Translational Research**
  - *In-vitro/ in-vivo* correlation: predict and evaluate the interaction of drugs with the biological environment (ADME, efficacy, safety)
  - Biocompatibility and safety: *in vivo* studies complemented by *in vitro* assays

- *Physicochemical properties correlation with biocompatibility, biodistribution, clearance, and toxicity*
- *Recommended reference standard for comparative studies*
- *Demonstration of product-controlled release nature*
- *Biopharmaceutics/dissolution, proper choice of apparatus, sink conditions, complete release (>75-80%)*
- *In vivo PK/PD data: the presence of biomaterials, their accumulation, and persistence of degradation products – long-term safety*
- *Characterization of nanosystems reactivity and bio-interface, including coating and "excipients", and related changes in biological interactions*



A.Wicki et al. Journal of Controlled Release 200 (2015) 138–157.

NANOMEDICINES: CHALLENGES

- **Translational Research**
  - Adequacy of non-clinical evaluation before first-in-man use (relevance of, appropriate toxicity efficacy biomarkers and barriers related to disease phase and different routes of administration)

- *Poor translation of preclinical results to clinical trials*
- *Characterization of toxicity and efficacy: appropriate biomarkers*
- *Dose Selection/Schedule*
- *Novel models of the disease (In vitro/Ex vivo/In Vivo): 2D, 3D in vitro/ex vivo cell models; organ-on-a-chip*
- *Translational models adapted to specific questions with “nano” (PK/PD versus specific organ toxicity and differential)*

NANOMEDICINES: CHALLENGES

- **Clinical development**
  - Demonstration of safety and efficacy: non-inferiority *versus* superiority (risk-benefit management)
  - Pharmacokinetics & Pharmacodynamics: absorption, distribution, elimination and metabolism
  - Not standard pharmacokinetics, environmental and accumulation issues, genotoxicity, representativeness of *in vitro* nanotoxicology tests, increased permeation, stability and manufacturing scale-up, nanomorphology and characterization, non-standardized terminology, and regulations

REGULATORY FRAMEWORK ON NANOMEDICINES

EMA scientific guidelines aim to help developers prepare marketing authorization applications for human medicines:

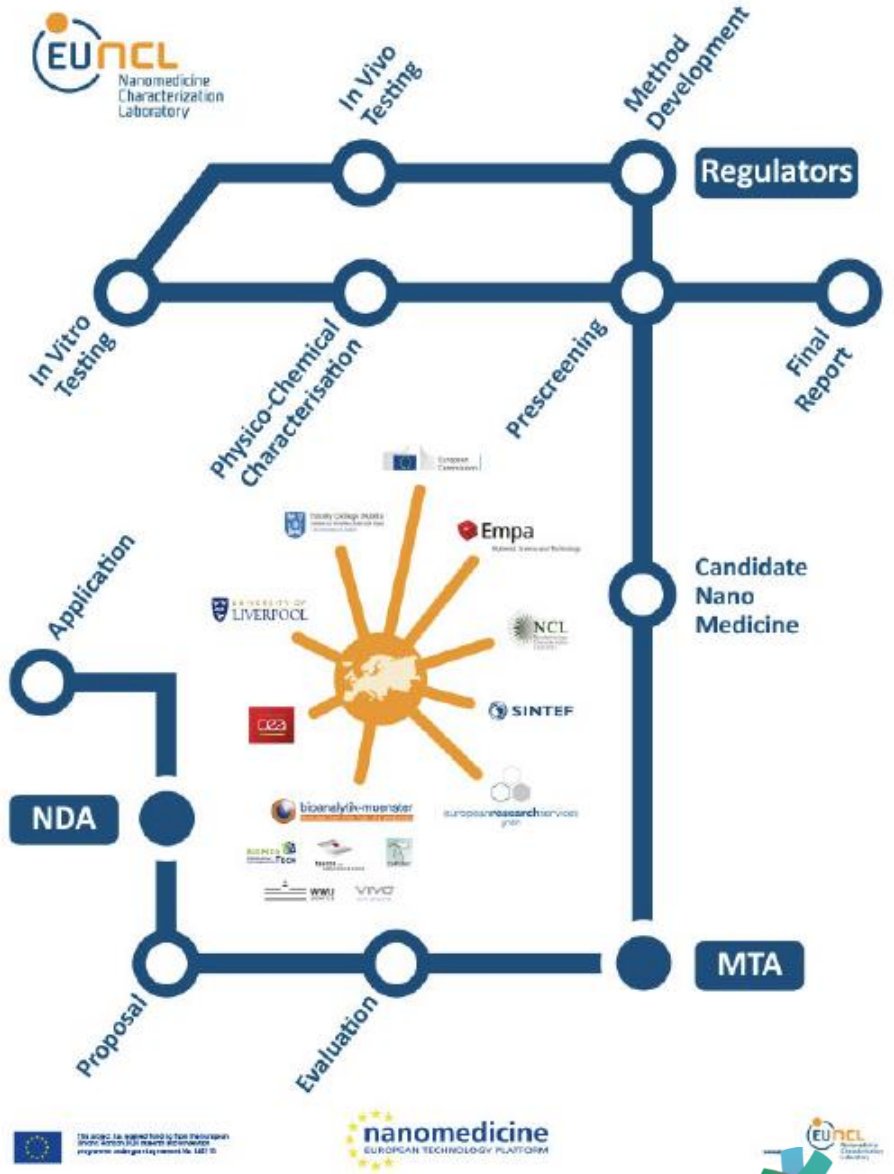
- Data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product
- Data requirements for intravenous liposomal products developed with reference to an innovator liposomal product
- Development of block-copolymer-micelle medicinal products
- Surface coatings: general issues for consideration regarding parenteral administration of coated nanomedicine products

EUROPEAN NANOMEDICINE CHARACTERIZATION LABORATORY

Support the European nanomedicine community towards the clinical translation of their product.

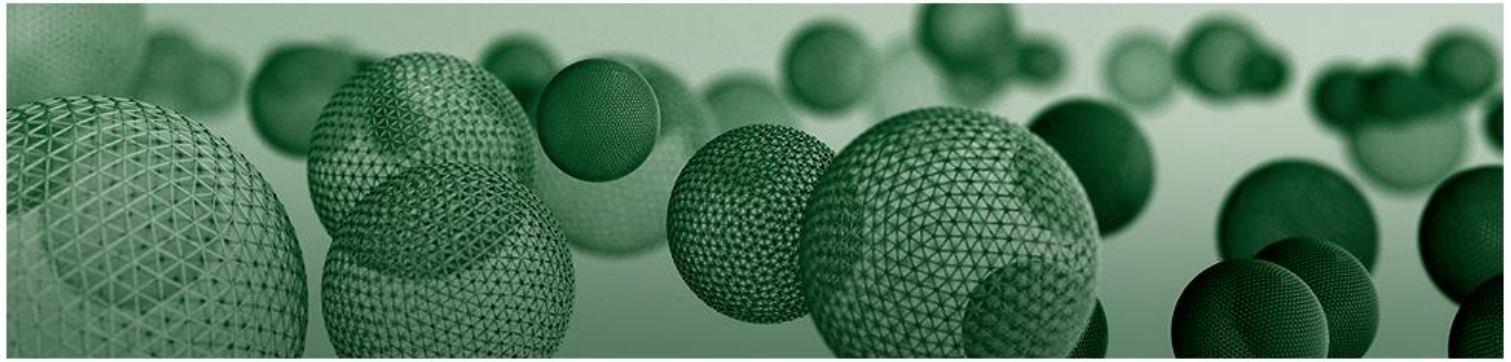
"provide a trans-disciplinary testing infrastructure covering a comprehensive set of preclinical characterisation assays (physical, chemical, in-vitro and in-vivo biological testing) allowing researchers to fully comprehend the biodistribution, metabolism, pharmacokinetics, safety profiles and immunological effects of their Med-NPs"

"The emphasis of the EUNCL is to serve as a nexus for trans-disciplinary research, development, and clinical applications of nanotechnology. Therefore, lessons learned, best practices, knowledge, tools, and methods will be available to the scientific community, such as academic researchers, industry, regulatory bodies, and metrology institutes."



https://www.euncl.org/

NANOTECHNOLOGY CHARACTERIZATION LABORATORY (NCL) BY NCI



Nanotechnology Characterization Laboratory (NCL)

NCI established the Nanotechnology Characterization Laboratory (NCL) to accelerate the progress of nanomedicine by providing preclinical characterization and safety testing of nanoparticles. It is a collaborative effort between NCI, the US Food and Drug Administration (FDA), and the National Institute of Standards and Technology (NIST).

The NCL serves as a resource and knowledge base for all cancer researchers in academia, industry, and government to facilitate the development and clinical translation of nanotechnologies intended as cancer therapeutics and diagnostics. NCL performs:

- characterization of strategies with proven efficacy for cancer therapy, diagnosis, or vaccines
- evaluation of novel, early-stage nanomedicine platforms with potential for cancer therapy and diagnosis
- evaluation of approaches intended to alleviate side effects, toxicities and other adverse effects associated with cancer therapy
- structure activity relationship (SAR) studies, technology advancement, and method development contributing to improvement of the cancer nanotechnology knowledge base

Considering the relevance of nanoparticles to combat COVID-19 pandemic, the NCL also supports the characterization of nanotechnology-based COVID vaccines and therapeutics.

https://www.cancer.gov/nano/research/ncl

Assay cascade:

- ***Sterility and endotoxin*** (e.g. LAL)
- ***Physicochemical characterization*** (*size, size distribution, pH, Zeta potential, PEG quantification, chemical composition by Mass Spectrometry, NP concentration; asymmetric-flow field-flow fractionation*)
- ***Immunology*** (*hematology, Immune cell proliferation, NP impact on APC, cytokine secretion, cytotoxicity, mechanistic immunotoxicology*)
- ***Pharmacology and Toxicology*** (*in vitro* and *In vivo*)

NANOMEDICINES: CONCLUSIONS & PERSPECTIVES

- Nanotechnology is an emerging science with extensive opportunities in drug delivery for diagnostics, theragnostic, and regenerative medicine
- Advances in nanoscience are leading to a new generation of hybrid and complex structures
- Expected new nanopharmaceuticals, imaging agents and combination products
- Improved knowledge of nanomedicines' biological interactions and fate helps their development and supports translation
- Artificial Intelligence-assisted development of nanomedicines

European Union Observatory for Nanomaterials: https://euon.echa.europa.eu/

European Chemicals Agency https://echa.europa.eu/

REACH Regulation *https://environment.ec.europa.eu/topics/chemicals/reach-regulation_en*

NanoREG II: safe-by-design (SbD) concept and application in industrial case studies
Deliverables NANOREG toolbox and the SIA framework

THANK YOU

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e Produtos de Saúde, I.P.

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