

Nanomedicine

Nanomedicine is a multidisciplinary field that applies the knowledge and tools of nanotechnology to the prevention, diagnosis, and treatment of diseases. It involves the use of nanoparticles, nanostructures, and nanoscale materials to develop innovative medical therapies and diagnostic techniques.

Nanomedicine seeks to improve the effectiveness of treatments by **enabling targeted drug delivery**, **reducing side effects**, and enhancing the **bioavailability of therapeutic agents**. It also has the potential to revolutionize diagnostics by developing highly sensitive and accurate devices for detecting diseases at an early stage, which can significantly improve patient outcomes.

Targeted drug delivery: Nanoparticles can be engineered to carry drugs specifically to the site of a disease, improving the efficiency of the treatment and minimizing side effects.

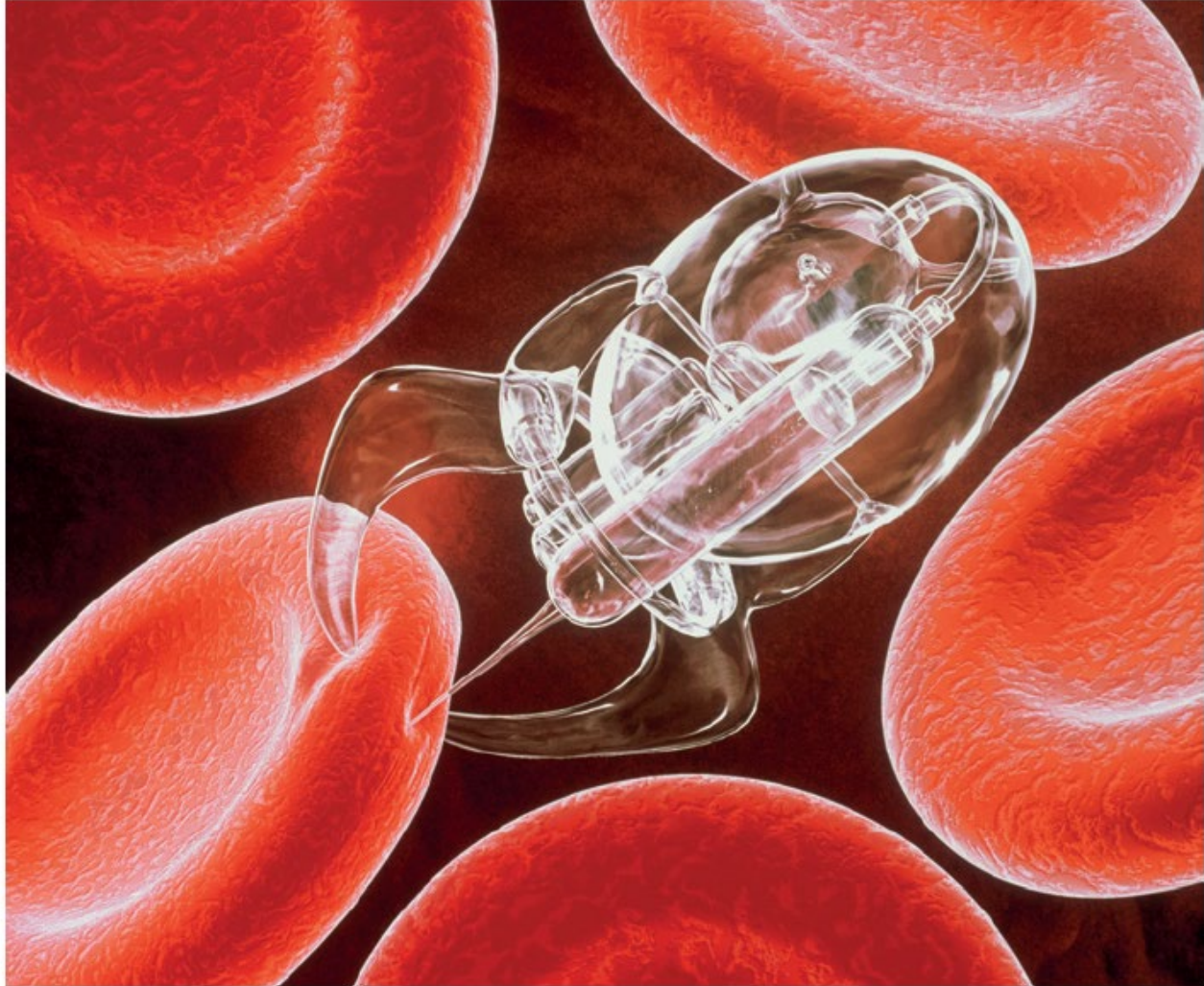
Imaging and diagnostics: Nanoscale materials can be used as contrast agents or imaging probes, enhancing the detection of diseases and providing real-time monitoring of therapeutic responses.

Regenerative medicine: Nanotechnology can be employed to develop scaffolds or nanomaterials that promote tissue regeneration and repair.

Gene therapy: Nanocarriers can be designed to transport genetic material into cells, allowing for the correction of genetic defects or the manipulation of gene expression.

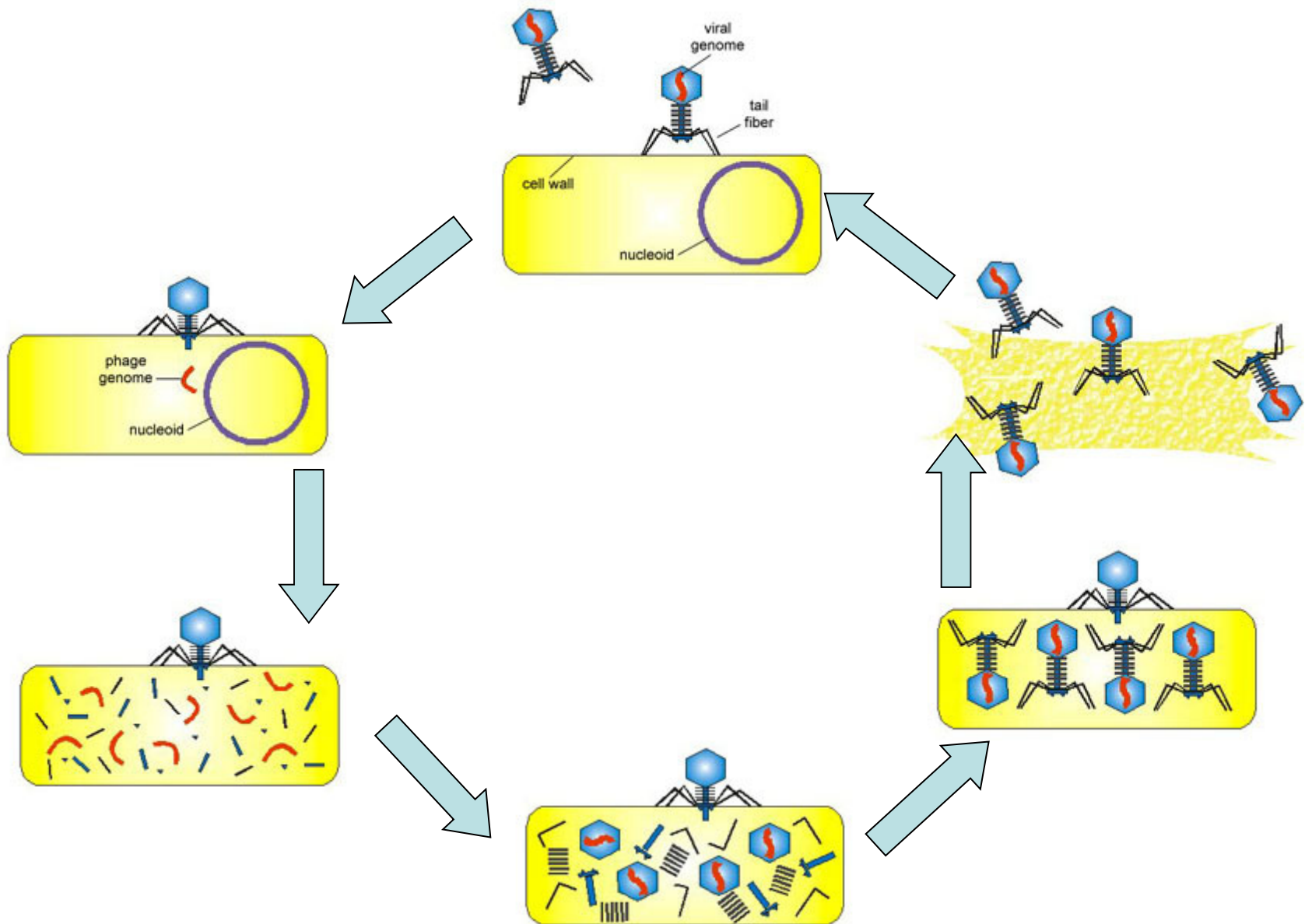
Immunotherapy: Nanoparticles can be used to modulate the immune system, either by enhancing immune responses or suppressing overactive immune reactions.

Nanobots

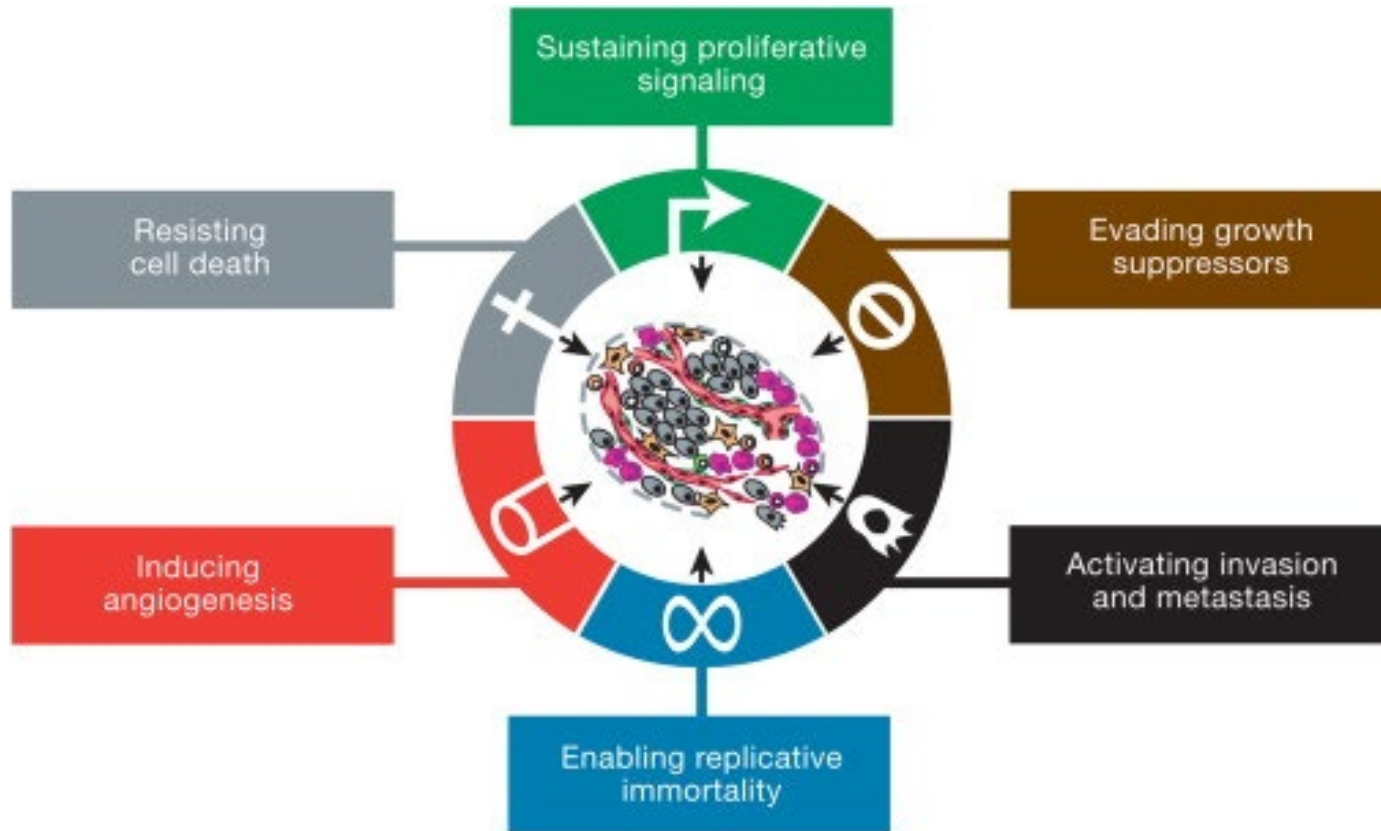


© CONEYLJAY / GETTY IMAGES

Virus Infection



Cancer Hallmark



Sustained proliferative signaling: Cancer cells can stimulate their own growth by producing growth factors or by overactivating growth factor receptors.

Evasion of growth suppressors: Cancer cells can bypass cellular mechanisms that normally inhibit cell growth and division, such as tumor suppressor genes like p53 and Rb.

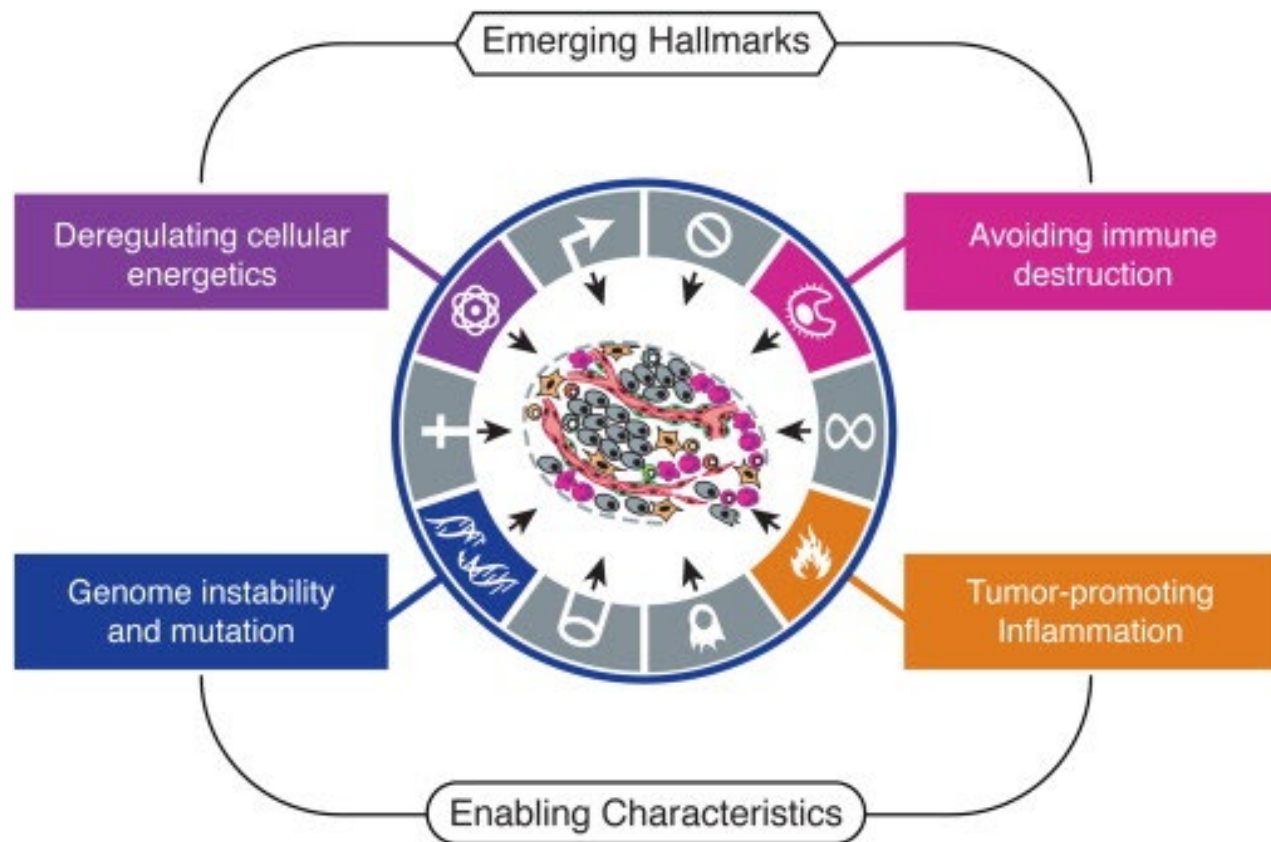
Resistance to cell death: Cancer cells can avoid apoptosis, the programmed cell death that occurs when cells are damaged or dysfunctional, allowing them to survive and multiply.

Replicative immortality: Cancer cells can maintain the length of their telomeres, the protective caps on the ends of chromosomes, which enables them to divide indefinitely.

Induction of angiogenesis: Cancer cells can stimulate the formation of new blood vessels (angiogenesis) to supply the growing tumor with oxygen and nutrients.

Activation of invasion and metastasis: Cancer cells can break away from the primary tumor, invade surrounding tissues, and form secondary tumors in distant sites.

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, 100(1), 57-70.



An increasing body of research suggests that two additional hallmarks of cancer are involved in the pathogenesis of some and perhaps all cancers. One involves the capability to modify, or reprogram, cellular metabolism in order to most effectively support neoplastic proliferation. The second allows cancer cells to evade immunological destruction, in particular by T and B lymphocytes, macrophages, and natural killer cells. Because neither capability is yet generalized and fully validated, they are labeled as emerging hallmarks. Additionally, two consequential characteristics of neoplasia facilitate acquisition of both core and emerging hallmarks. Genomic instability and thus mutability endow cancer cells with genetic alterations that drive tumor progression. Inflammation by innate immune cells designed to fight infections and heal wounds can instead result in their inadvertent support of multiple hallmark capabilities, thereby manifesting the now widely appreciated tumor-promoting consequences of inflammatory responses.

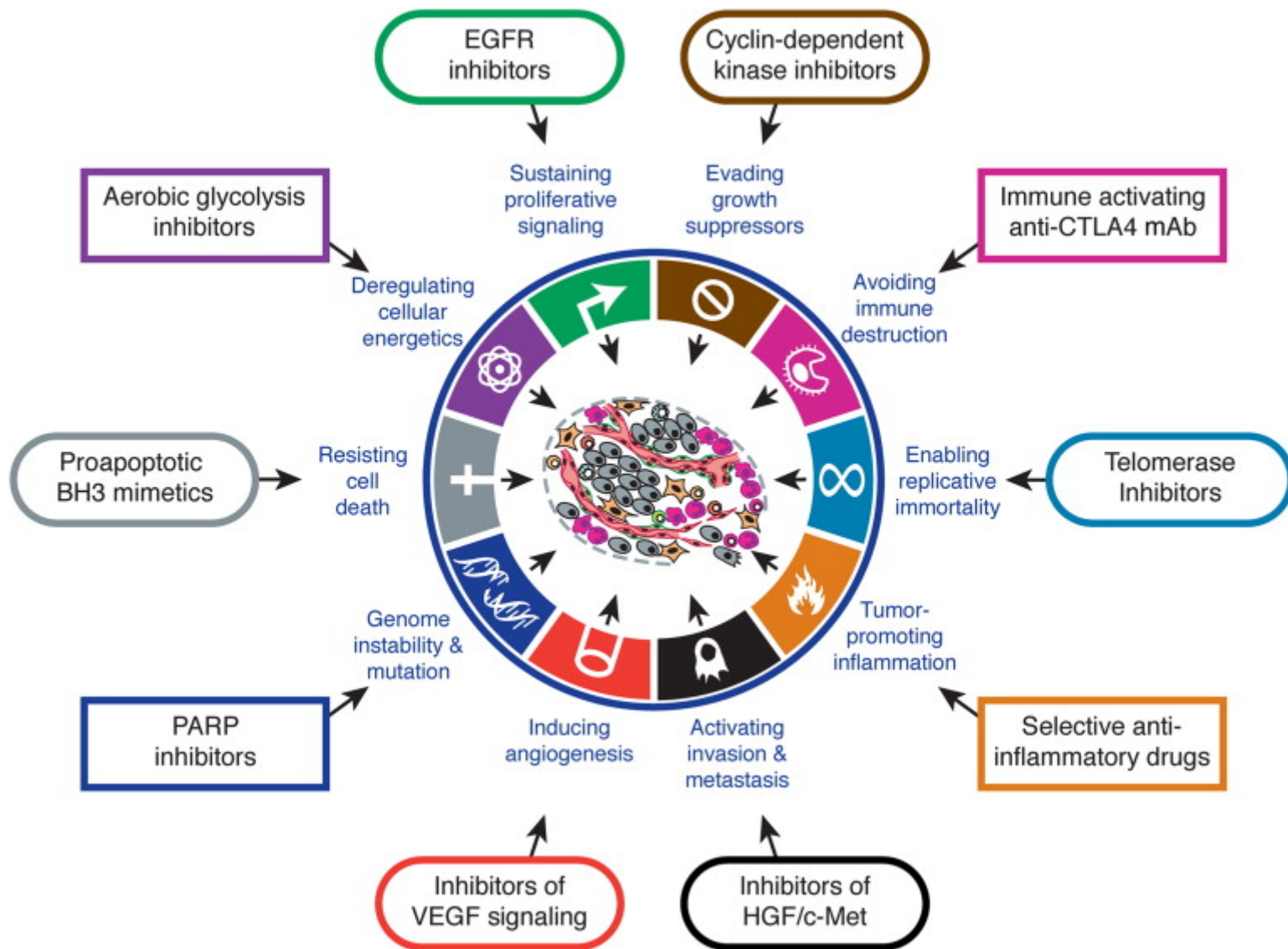
Deregulation of cellular energetics: Cancer cells often exhibit altered metabolism, such as increased glucose uptake and glycolysis, even in the presence of oxygen (a phenomenon known as the Warburg effect).

Avoiding immune destruction: Cancer cells can evade detection and destruction by the immune system through various mechanisms, such as the expression of immune checkpoint proteins.

(Enabling characteristic) **Genome instability and mutation:** Cancer cells often have increased genomic instability, leading to a higher rate of mutations that can drive the acquisition of the other cancer hallmarks.

(Enabling characteristic) **Tumor-promoting inflammation:** Inflammation can create a tumor-promoting environment that fosters the growth, survival, and spread of cancer cells.

Hanahan, D., & Weinberg, R. A. (2011).
Hallmarks of cancer: the next generation.
Cell, 144(5), 646-674.



Cancer nanomedicine: progress, challenges and opportunities

Jinjun Shi¹, Philip W. Kantoff², Richard Wooster³ and Omid C. Farokhzad^{1,4}

Box 1 | Distinctive features of nanotechnology in oncological applications

- Improvement of the drug therapeutic index by increasing efficacy and/or reducing toxicities
- Targeted delivery of drugs in a tissue-, cell- or organelle-specific manner
- Enhancement of the pharmaceutical properties (for example, stability, solubility, circulating half-life and tumour accumulation) of therapeutic molecules
- Enabling of sustained or stimulus-triggered drug release
- Facilitation of the delivery of biomacromolecular drugs (for example, DNA, small interfering RNA (siRNA), mRNA and protein) to intracellular sites of action
- Co-delivery of multiple drugs to improve therapeutic efficacy and overcome drug resistance, by providing more precise control of the spatiotemporal exposure of each drug and the delivery of appropriate drug ratio to the target of interest
- Transcytosis of drugs across tight epithelial and endothelial barriers (for example, gastrointestinal tract and the blood–brain barrier)
- More sensitive cancer diagnosis and imaging
- Visualization of sites of drug delivery by combining therapeutic agents with imaging modalities, and/or real-time feedback on the *in vivo* efficacy of a therapeutic agent
- Provision of new approaches for the development of synthetic vaccines
- Miniaturized medical devices for cancer diagnosis, drug screening and delivery
- Inherent therapeutic properties of some nanomaterials (for example, gold nanoshells and nanorods, and iron oxide nanoparticles) upon stimulation

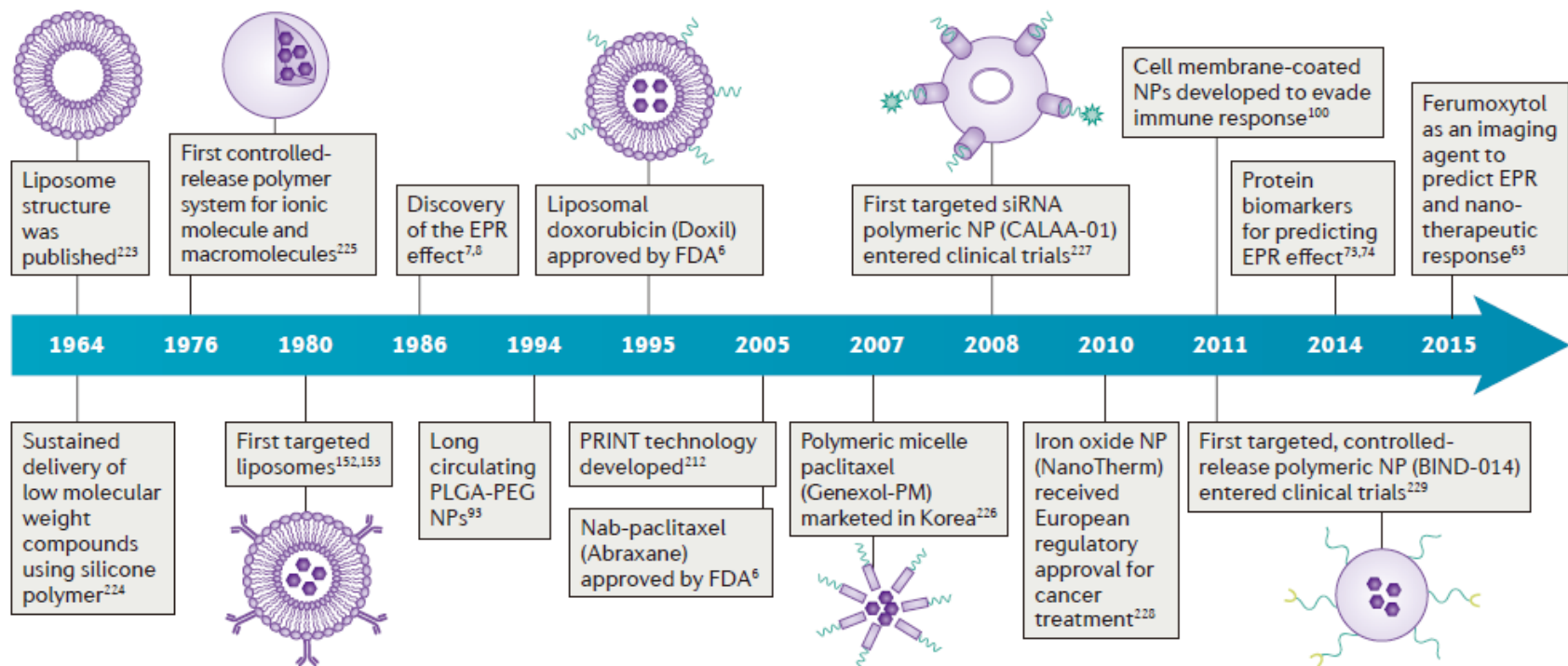
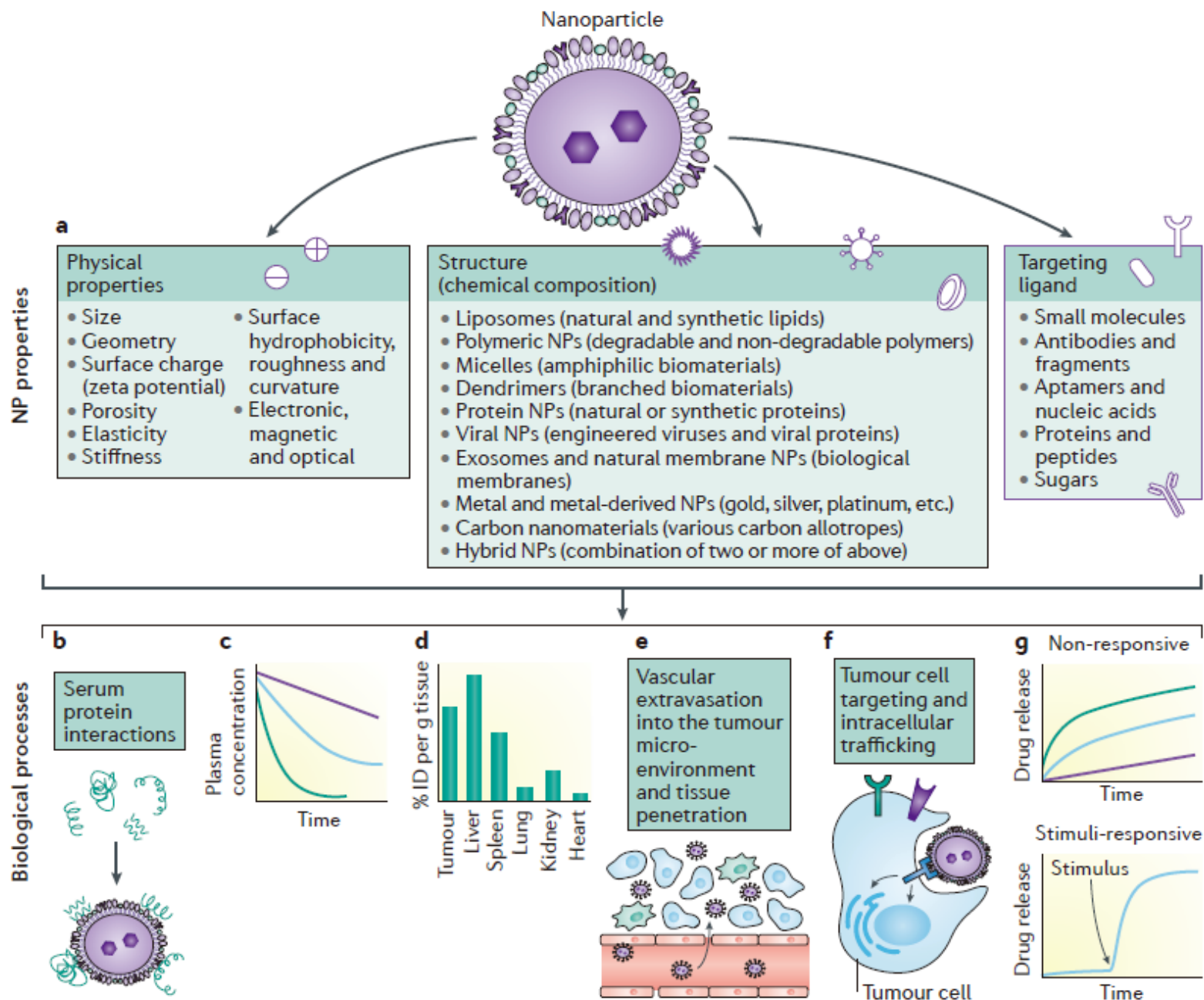
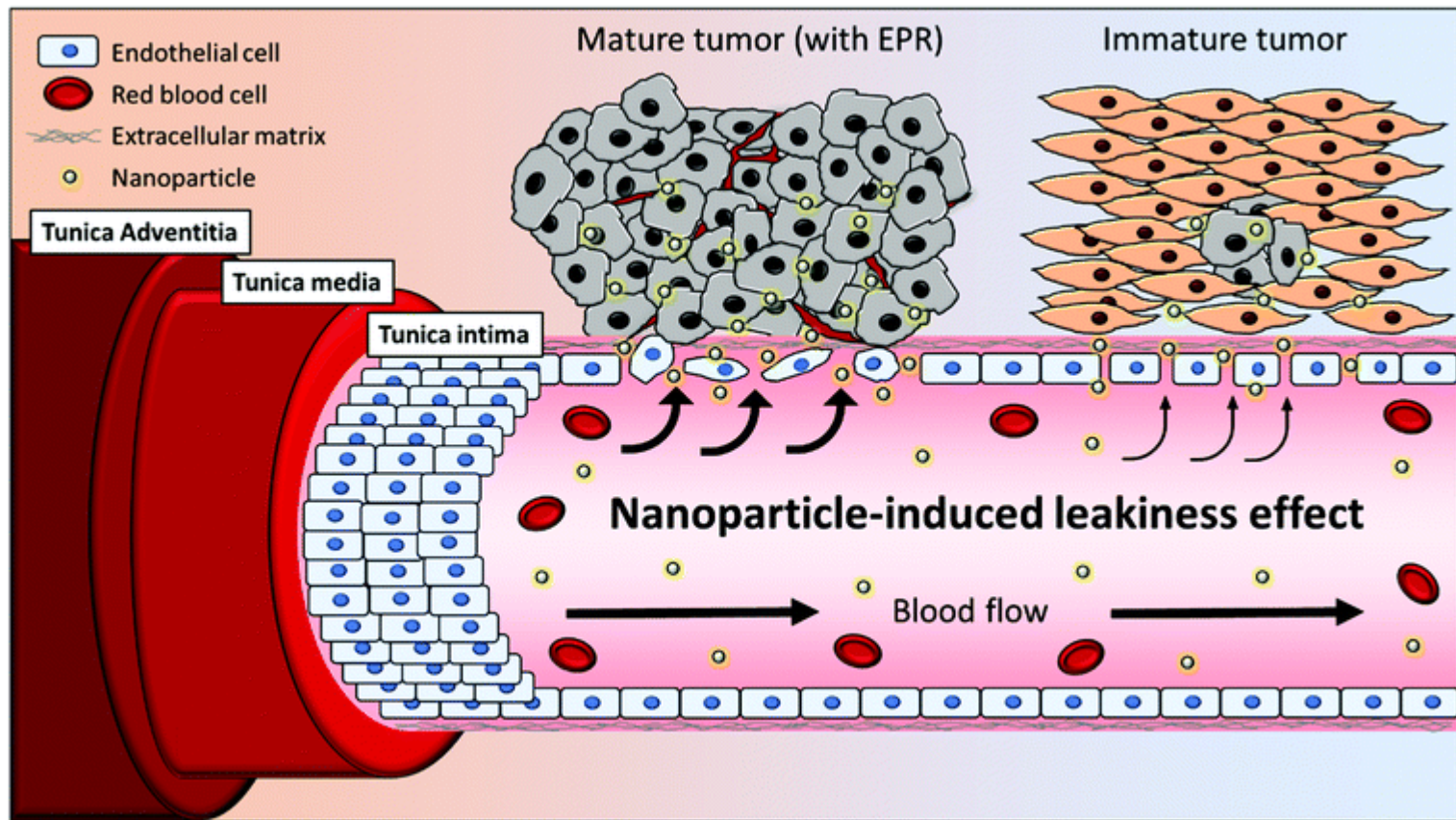


Figure 1 | **Historical timeline of major developments in the field of cancer nanomedicine.** EPR, enhanced permeability and retention; FDA, US Food and Drug Administration; nab, nanoparticle albumin-bound; NP, nanoparticle; PLGA-PEG, poly(D,L-lactic-co-glycolic acid)-b-poly(ethylene glycol); PRINT, particle replication in non-wetting template; siRNA, small interfering RNA.



EPR Effect



The Enhanced Permeability and Retention (EPR) effect is a phenomenon that is commonly exploited in nanomedicine for the passive targeting of nanoparticles to solid tumors. The EPR effect is based on the unique characteristics of the tumor microenvironment, which include leaky blood vessels and impaired lymphatic drainage.

In solid tumors, the rapid growth of cancer cells leads to the formation of **new blood vessels through a process called angiogenesis**. However, these **newly formed blood vessels are often disorganized and have large gaps between endothelial cells**, resulting in **increased permeability**. This enhanced permeability allows nanoparticles, which are typically in the size range of **10-200 nm**, to passively accumulate in the tumor tissue more efficiently than in healthy tissues.

The second key factor contributing to the EPR effect is the **impaired lymphatic drainage in the tumor microenvironment**. The lymphatic system is responsible for removing excess fluids, proteins, and waste products from tissues. However, **in tumors, the lymphatic vessels are often compressed or dysfunctional**, which hinders their ability to efficiently drain the accumulated substances. This **results in the retention of nanoparticles within the tumor tissue for extended periods**, allowing them to exert their therapeutic effects.

The EPR effect provides a passive targeting mechanism for nanomedicines, enhancing their accumulation and retention within solid tumors, while reducing their distribution to healthy tissues. t.

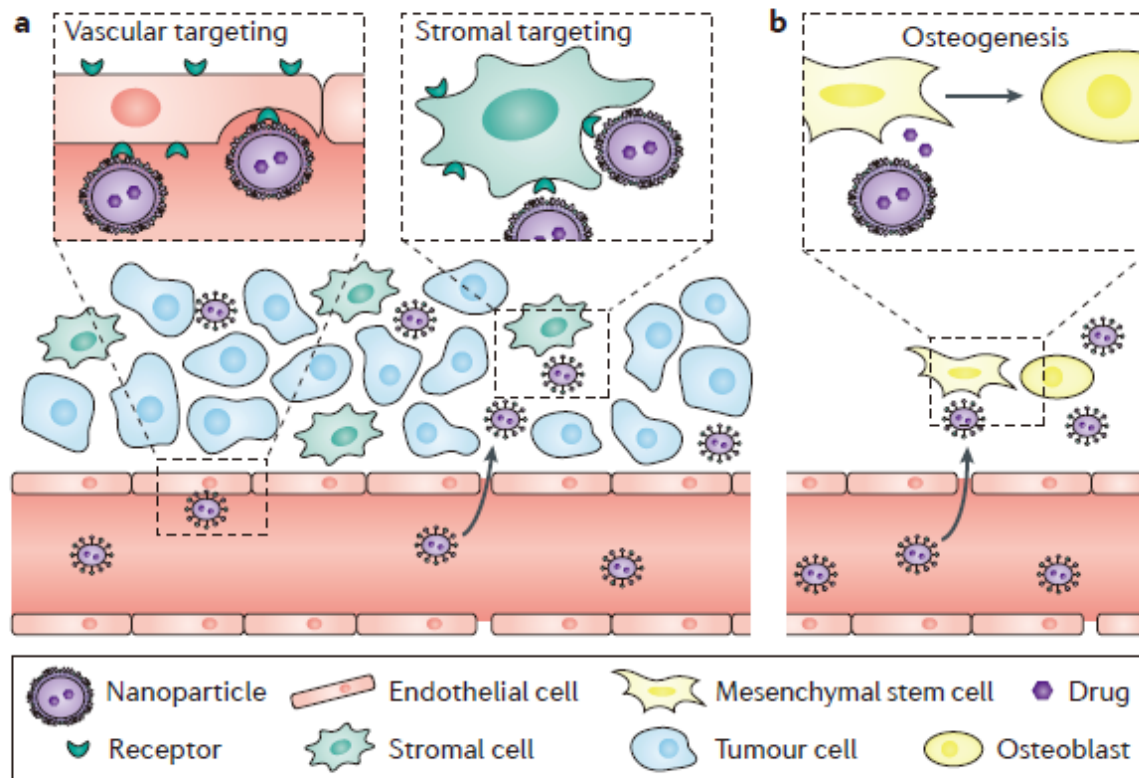
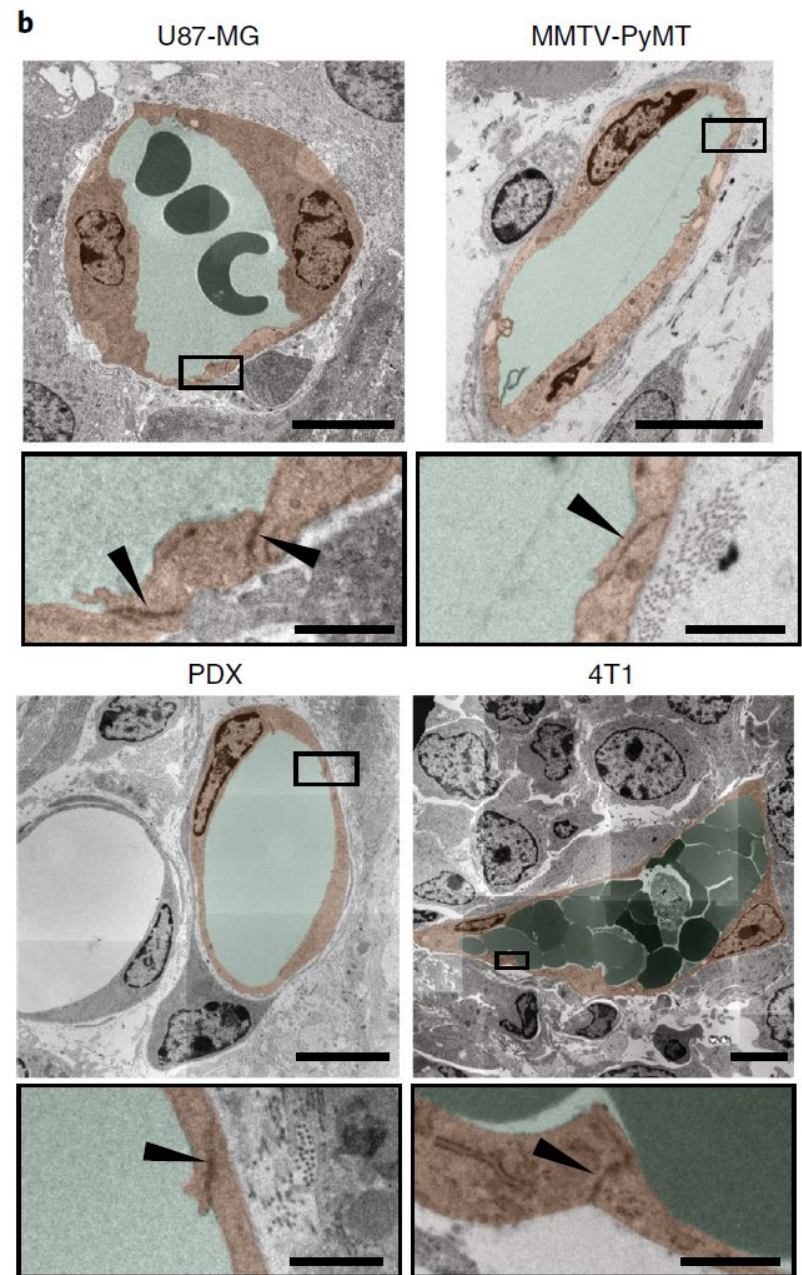
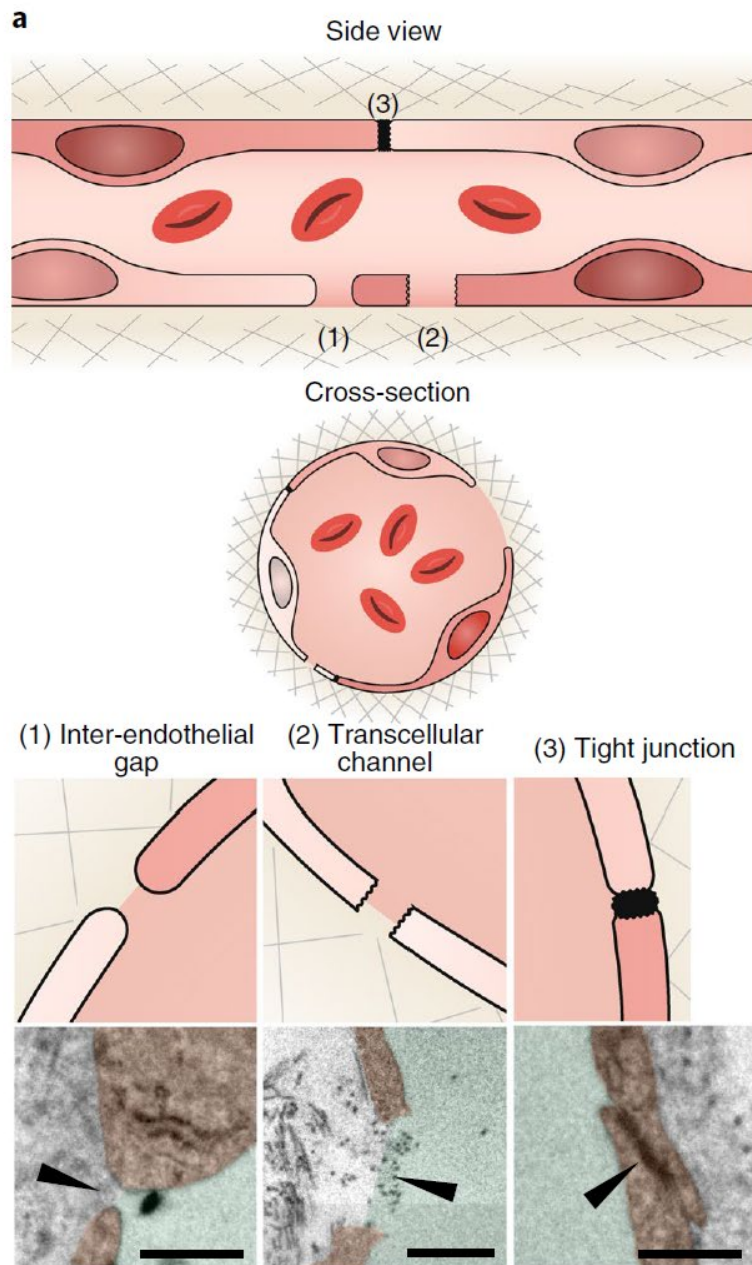
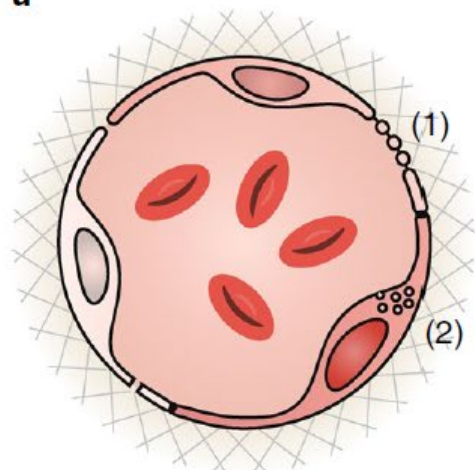


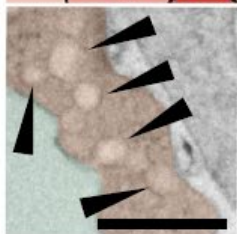
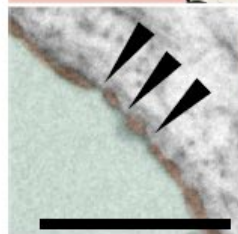
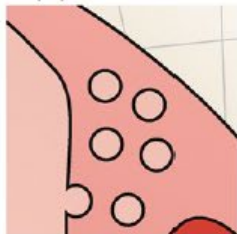
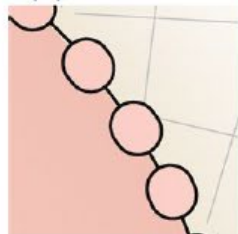
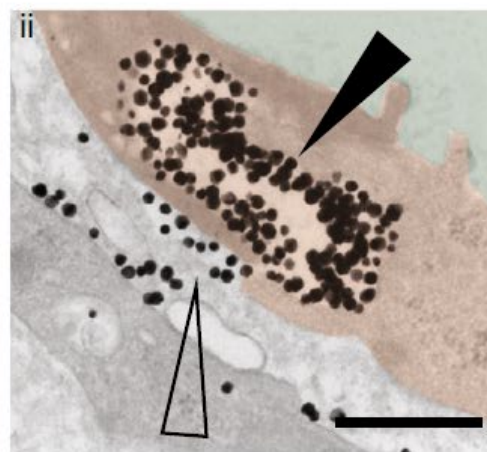
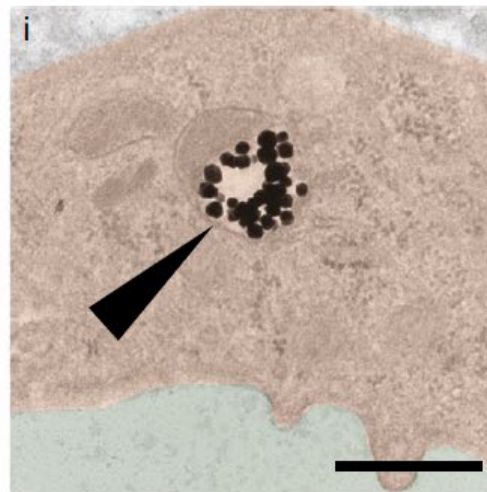
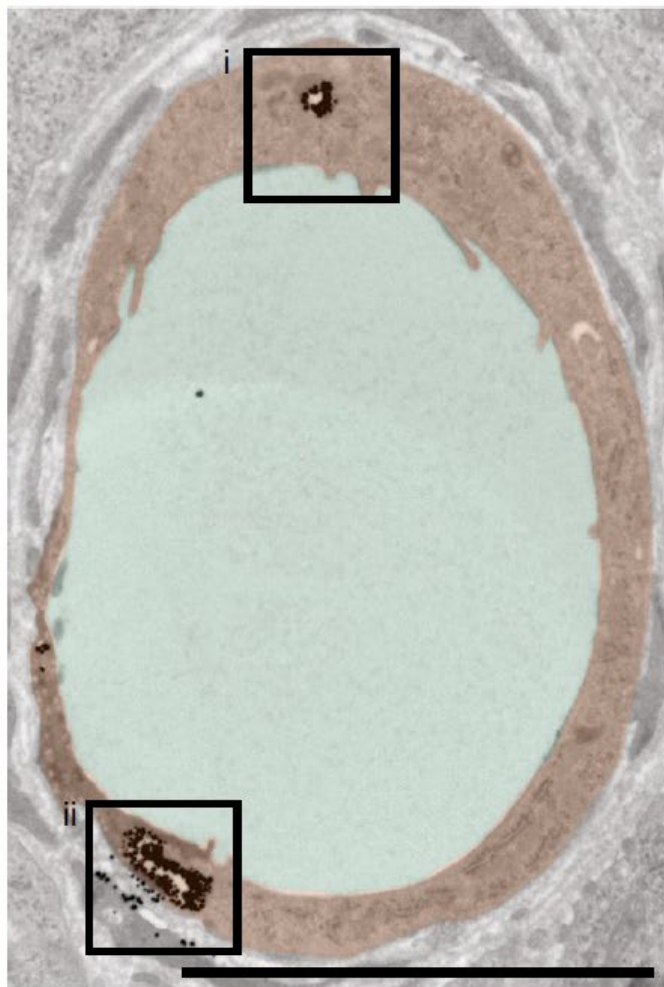
Figure 4 | Nanoparticle targeting of the tumour microenvironment and the premetastatic niche. Targeting of the tumour vasculature or stromal cells in the tumour microenvironment (part **a**) and the premetastatic microenvironments such as the bone marrow niche, where induction of the osteogenic differentiation of mesenchymal stem cells enhances bone strength and volume (part **b**). Cell-specific targeting can be achieved via the modification of nanoparticles (NPs) with ligands that bind to specific receptors (for example, $\alpha\beta_3$ integrin and mannose receptor) on the surface of tumour endothelial cells, stromal cells or other target cells. It should be noted that even without targeting ligands, NPs can be engineered for preferential cellular uptake. The payloads released from NPs localized in tumours or premetastatic tissues can also be nonspecifically taken up by these cells.



a

(1) Fenestrae

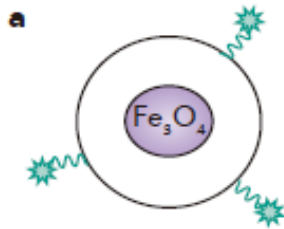
(2) Vacuoles

**b**

Patients with heterogeneous tumour EPR effect



Potential EPR markers



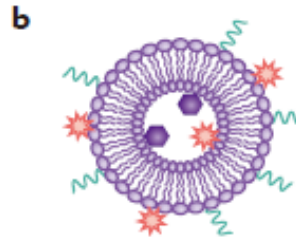
Companion imaging NPs

Pros

- No modification of therapeutic NPs
- Proof-of-concept available in animal models and in patients
- Non-invasive imaging

Cons

- Regulatory, marketing and use complexity



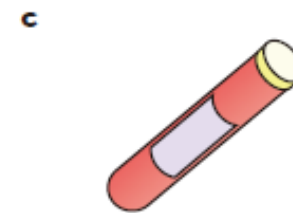
Theranostic NPs

Pros

- More precise tracking
- Proof-of-concept available in animal models and in patients
- Non-invasive imaging

Cons

- Complexity of chemistry and manufacturing



Serum or tissue biomarkers

Pros

- Detection using patient samples
- Proof-of-concept available in animal models

Cons

- Require serum sample or tumour biopsy
- Need more biological understanding



Patients with high EPR effect to receive nanotherapeutics



Potential EPR Markers

Vascular endothelial growth factor (VEGF): VEGF is a key factor that stimulates angiogenesis in tumors, promoting the formation of disorganized and leaky blood vessels. High levels of VEGF in a tumor may suggest an increased likelihood of the EPR effect.

Angiopoietin-2 (Ang-2): Ang-2 is another angiogenic factor that contributes to the formation of leaky blood vessels in tumors. Like VEGF, elevated levels of Ang-2 may indicate a tumor microenvironment conducive to the EPR effect.

Hypoxia-inducible factors (HIFs): HIFs are transcription factors that are activated under low oxygen conditions (hypoxia), which are common in solid tumors. HIFs regulate the expression of various genes involved in angiogenesis, including VEGF, and can, therefore, serve as indirect indicators of the EPR effect.

Endoglin (CD105): Endoglin is a protein that is highly expressed on the surface of endothelial cells involved in angiogenesis. It can be used as a marker to identify newly formed blood vessels in tumors and may provide some indication of the EPR effect.

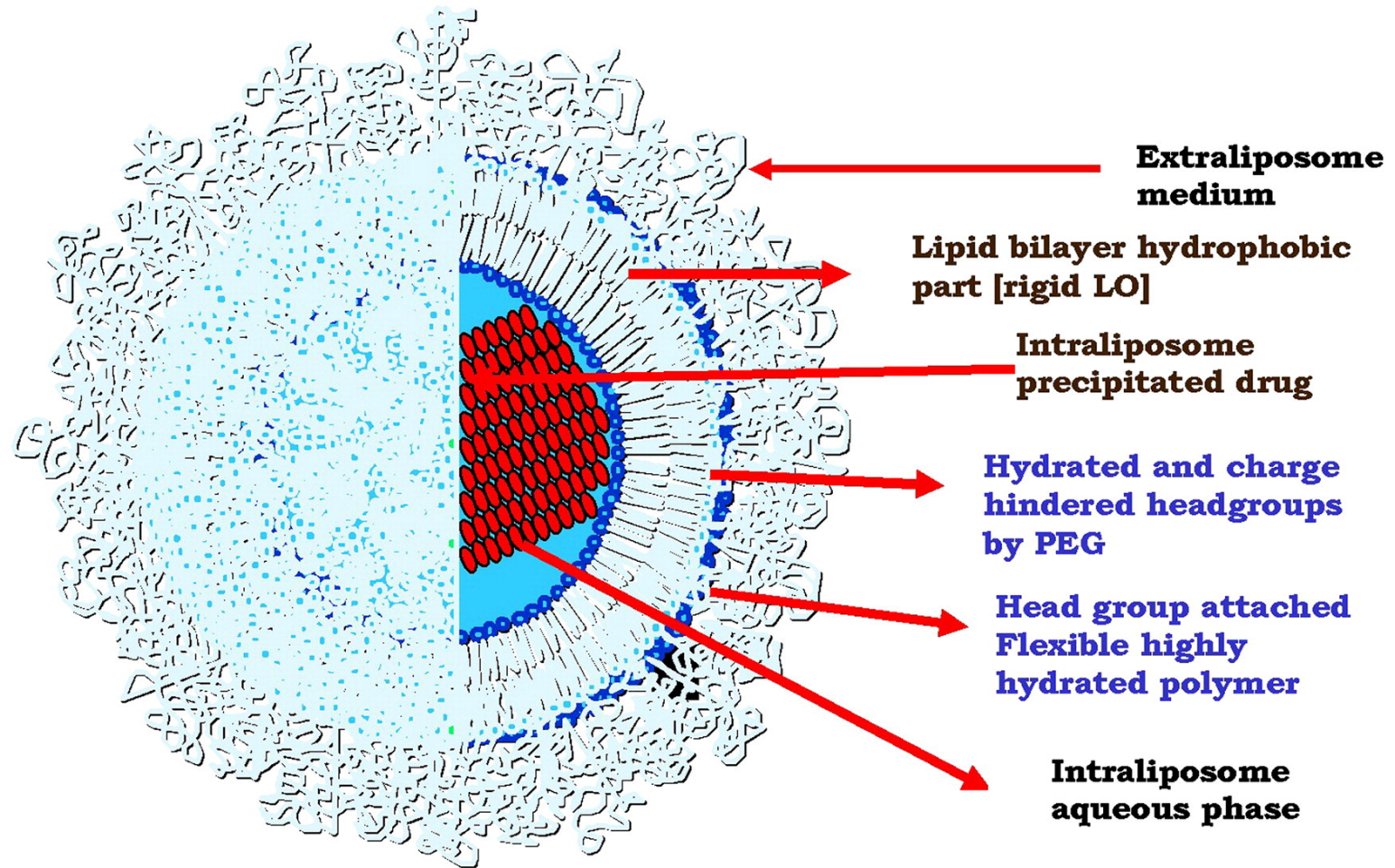
Platelet-derived growth factor (PDGF): PDGF is another factor involved in angiogenesis and blood vessel maturation. Elevated levels of PDGF may be associated with the formation of leaky blood vessels in the tumor microenvironment, which can contribute to the EPR effect.

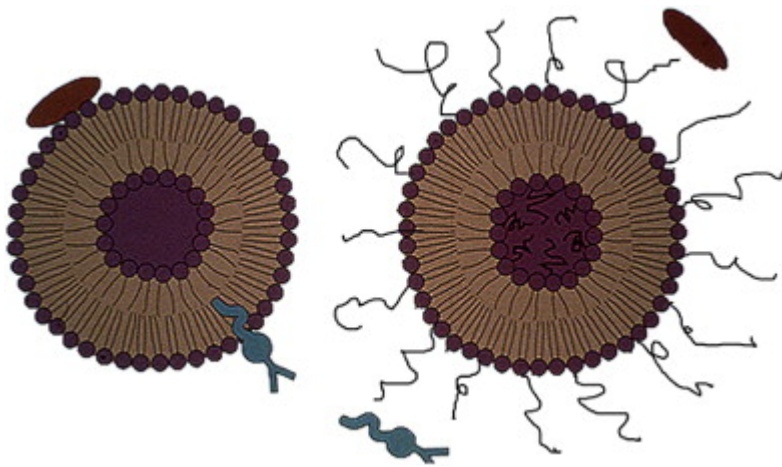
Table 1. Examples of Nanomaterials in Clinical Use.*

Nanomaterial	Trade Name	Application	Target	Adverse Effects	Manufacturer	Current Status
Metallic						
Iron oxide	Feridex	MRI contrast	Liver	Back pain, vaso-dilatation	Bayer Schering	FDA approved
	Resovist	MRI contrast	Liver	None	Bayer Schering	FDA approved
	Combidex	MRI contrast	Lymph nodes	None	Advanced Magnetix	In phase 3 clinical trials
	NanoTherm	Cancer therapy	Various forms	Acute urinary retention	MagForce	In phase 3 clinical trials
Gold	Verigene	In vitro diagnostics	Genetic	Not applicable	Nanosphere	FDA approved
	Aurimmune	Cancer therapy	Various forms	Fever	CytImmune Sciences	In phase 2 clinical trials
Nanoshells	Auroshell	Cancer therapy	Head and neck	Under investigation	Nanospectra Biosciences	In phase 1 clinical trials
Semiconductor						
Quantum dot	Qdots, EviTags, semiconductor nanocrystals	Fluorescent contrast, in vitro diagnostics	Tumors, cells, tissues, and molecular sensing structures	Not applicable	Life Technologies, eBioscience, Nanoco, CrystalPlex, Cytodiagnosics	Research use only
Organic						
Protein	Abraxane	Cancer therapy	Breast	Cytopenia	Abraxis Bioscience	FDA approved
Liposome	Doxil/Caelyx	Cancer therapy	Various forms	Hand-foot syndrome, stomatitis	Ortho Biotech	FDA approved
Polymer	Oncaspar	Cancer therapy	Acute lymphoblastic leukemia	Urticaria, rash	Rhône-Poulenc Rorer	FDA approved
	CALAA-01	Cancer therapy	Various forms	Mild renal toxicity	Calando	In phase 2 clinical trials
Dendrimer	VivaGel	Microbicide	Cervicovaginal	Abdominal pain, dysuria	Starpharma	In phase 2 clinical trials
Micelle	Genexol-PM	Cancer therapy	Various forms	Peripheral sensory neuropathy, neutropenia	Samyang	For phase 4 clinical trials

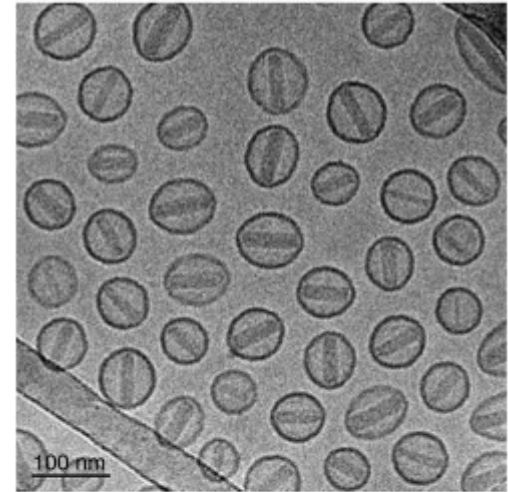
* MRI denotes magnetic resonance imaging.

Doxil/Caelyx (liposomal doxorubicin): Doxil (known as Caelyx outside of the US) is a liposomal formulation of the chemotherapy drug doxorubicin. The liposomal encapsulation improves the pharmacokinetics of the drug, reduces side effects such as cardiotoxicity, and enhances its accumulation in tumors.

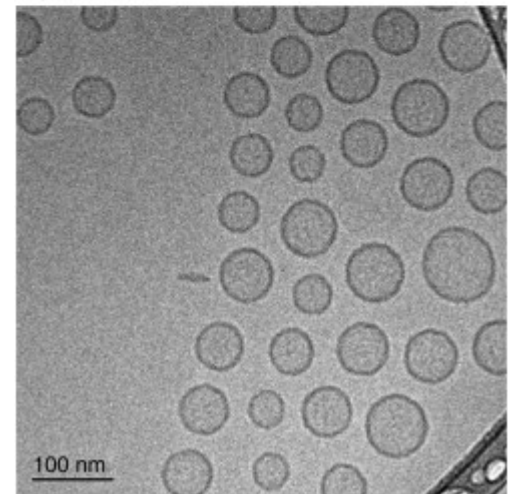




A) Doxil®



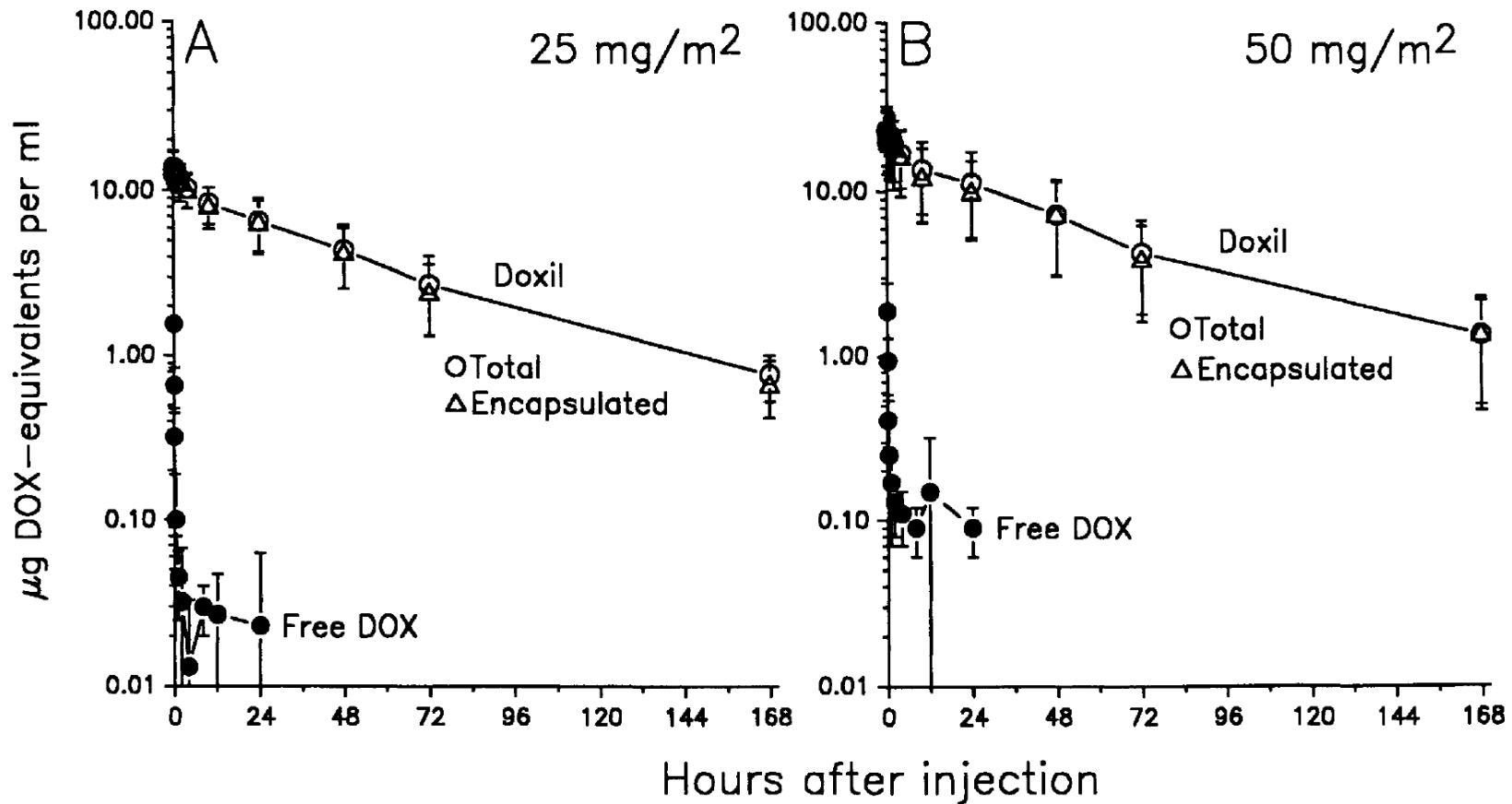
B) DOXG

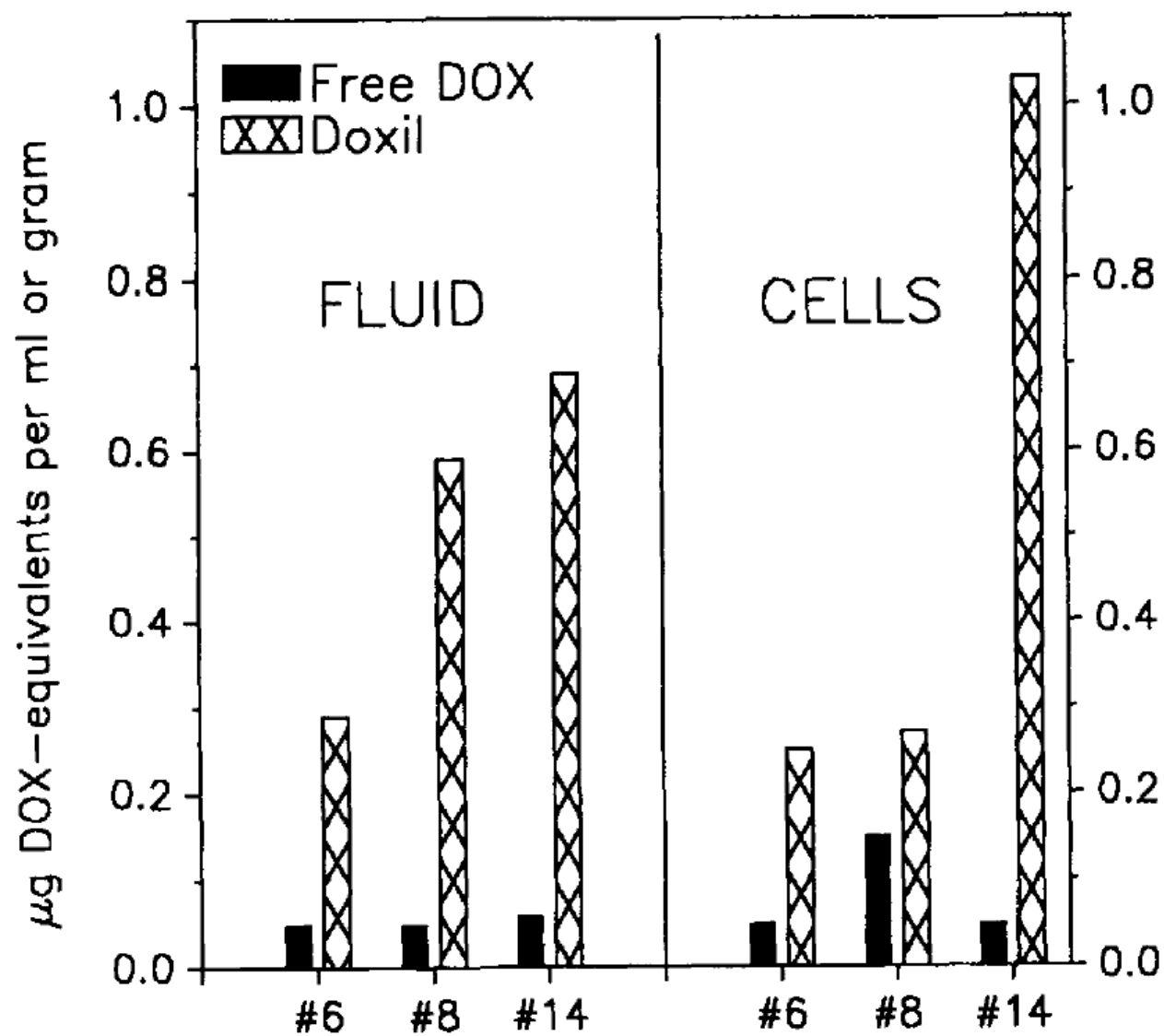


Prolonged Circulation Time and Enhanced Accumulation in Malignant Exudates of Doxorubicin Encapsulated in Polyethylene-glycol Coated Liposomes¹

Alberto Gabizon,² Raphael Catane, Beatrice Uziely, Bela Kaufman, Tamar Safra, Rivka Cohen, Francis Martin, Anthony Huang, and Yechezkel Barenholz

Sharett Institute of Oncology [A. G., R. C., B. U., B. K., T. S.], Hadassah University Hospital, and Department of Membrane Biochemistry [R. C., Y. B.], Hebrew University-Hadassah Medical School, Jerusalem, Israel; and Liposome Technology, Inc., Menlo Park, California 94025 [F. M., A. H.]





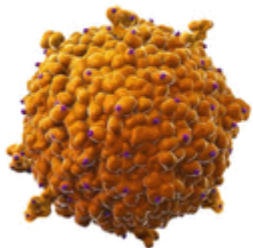
Abraxane (albumin-bound paclitaxel)

Abraxane is a nanoparticle-based formulation of the chemotherapy drug paclitaxel, bound to albumin protein. It is used for the treatment of breast cancer, non-small cell lung cancer, and pancreatic cancer. The albumin-bound formulation enhances the solubility of paclitaxel and allows for better drug delivery to tumors.

Journal of Clinical Oncology, 23(31), 7794-7803.

Phase III Trial of Nanoparticle Albumin-Bound Paclitaxel Compared With Polyethylated Castor Oil-Based Paclitaxel in Women With Breast Cancer

nab-Paclitaxel nanoparticle



Cross section

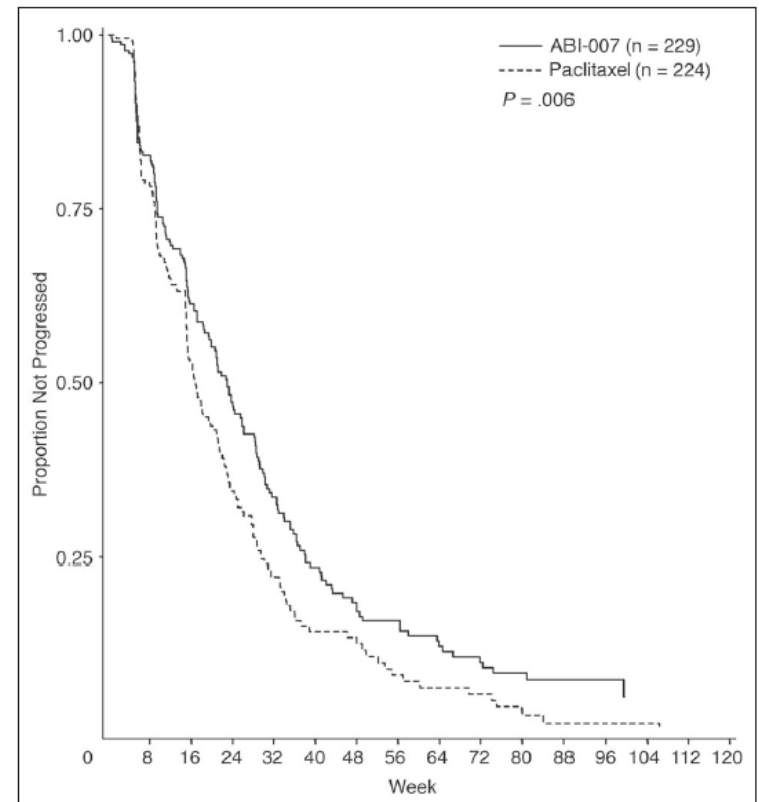
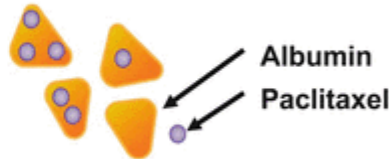


Fig 1. Median time to disease progression.

Results

ABI-007 demonstrated significantly higher response rates compared with standard paclitaxel (33% v 19%, respectively; $P = .001$) and significantly longer time to tumor progression (23.0 v 16.9 weeks, respectively; hazard ratio = 0.75; $P = .006$). The incidence of grade 4 neutropenia

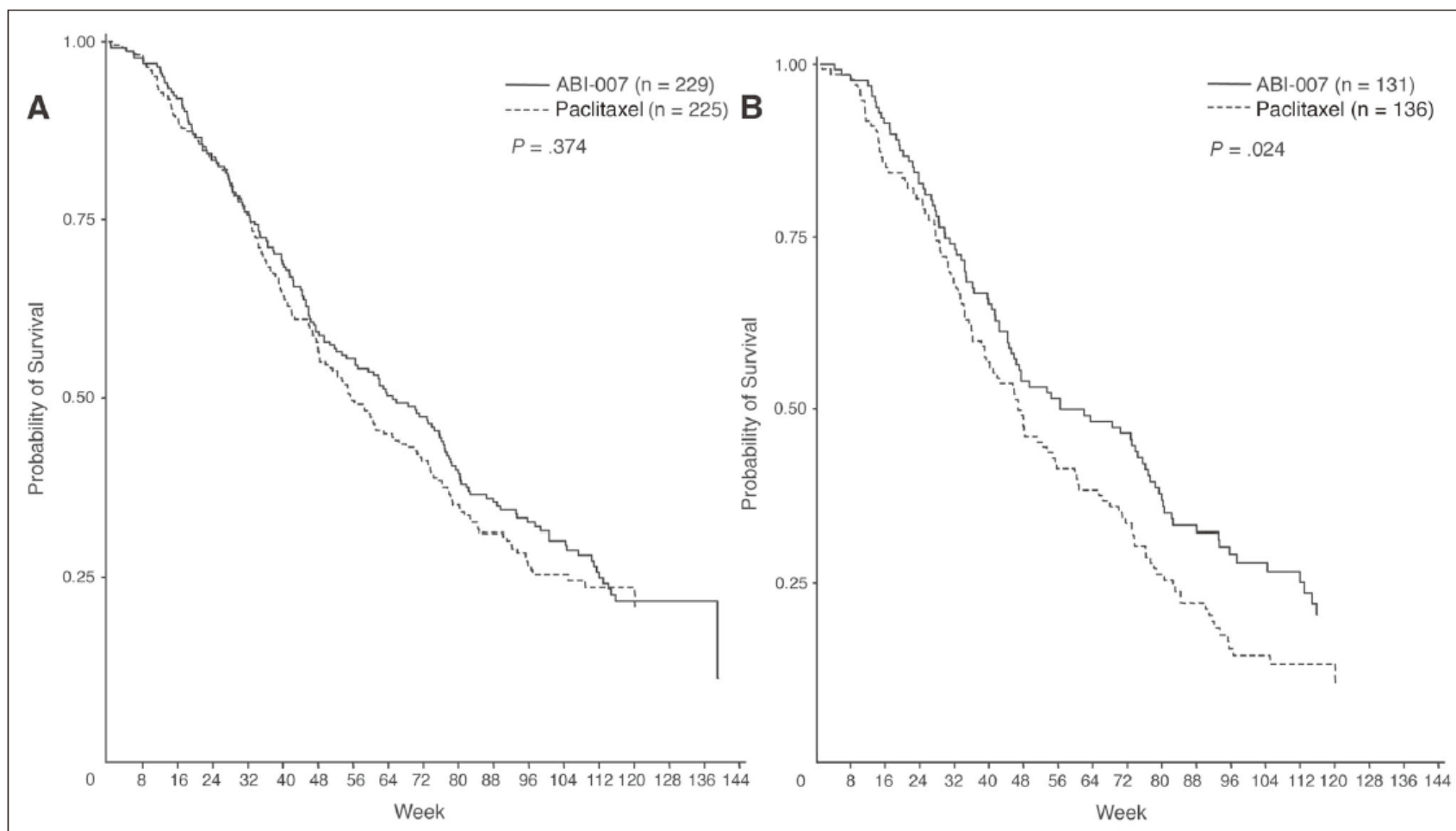
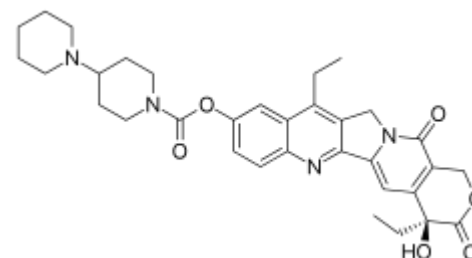
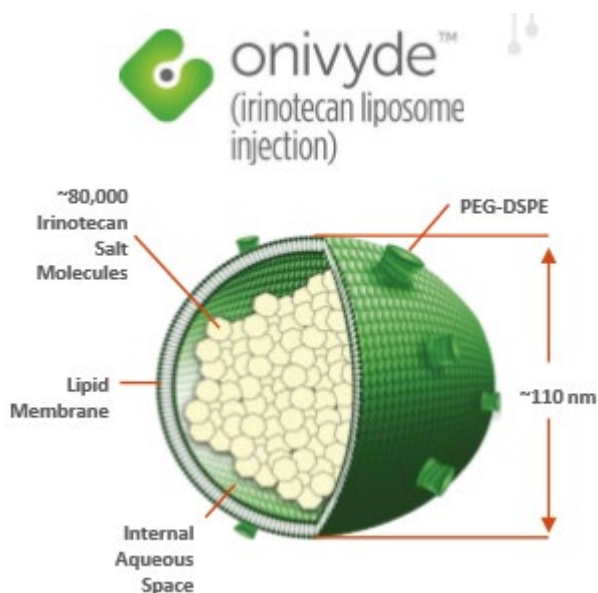


Fig 2. (A) Patient survival over time. (B) Patient survival over time in patients who received second-line or greater therapy. P values from log-rank test. Survival indicates time from first dose of study drug to date of death.

Onivyde (liposomal irinotecan): Onivyde is a liposomal formulation of the chemotherapy drug irinotecan, approved for use in combination with other drugs for the treatment of metastatic pancreatic cancer. The liposomal encapsulation improves the pharmacokinetics and biodistribution of irinotecan, increasing its therapeutic effect.



Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial

The Lancet, 387(10018), 545-557.

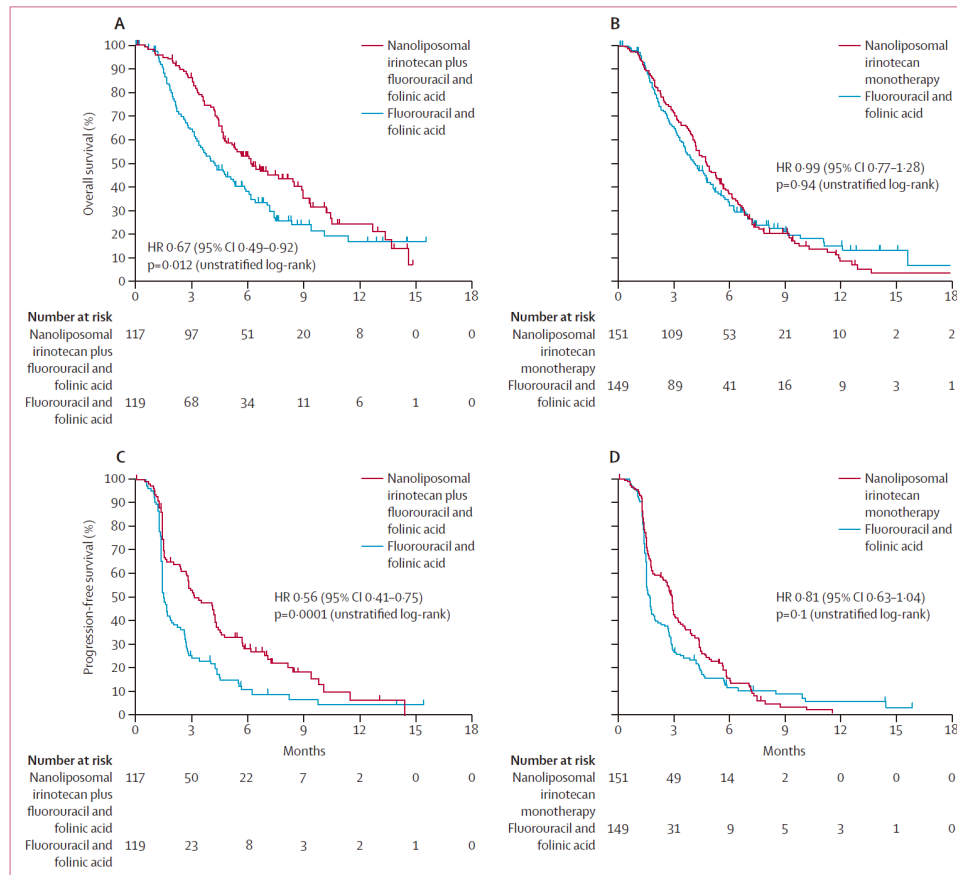


Figure 2: Kaplan-Meier survival analyses

HR=hazard ratio. (A) Overall survival with nanoliposomal irinotecan plus fluorouracil and folinic acid versus fluorouracil and folinic acid. (B) Overall survival with nanoliposomal irinotecan monotherapy versus fluorouracil and folinic acid. (C) Progression-free survival with nanoliposomal irinotecan plus fluorouracil and folinic acid versus fluorouracil and folinic acid. (D) Progression-free survival with nanoliposomal irinotecan monotherapy versus fluorouracil and folinic acid.

Targeted Delivery

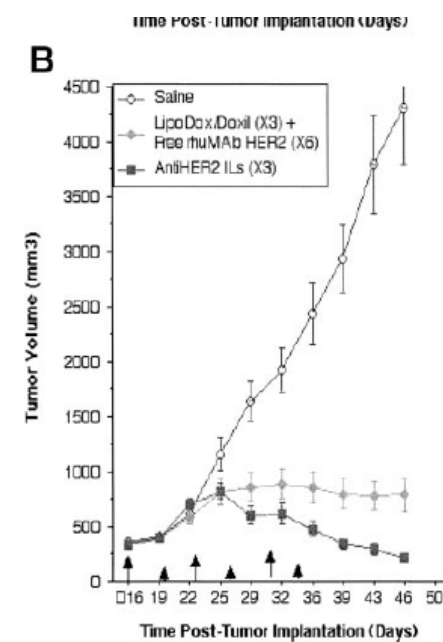
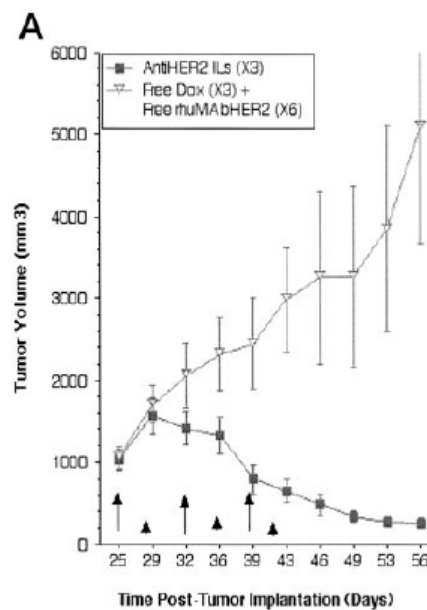
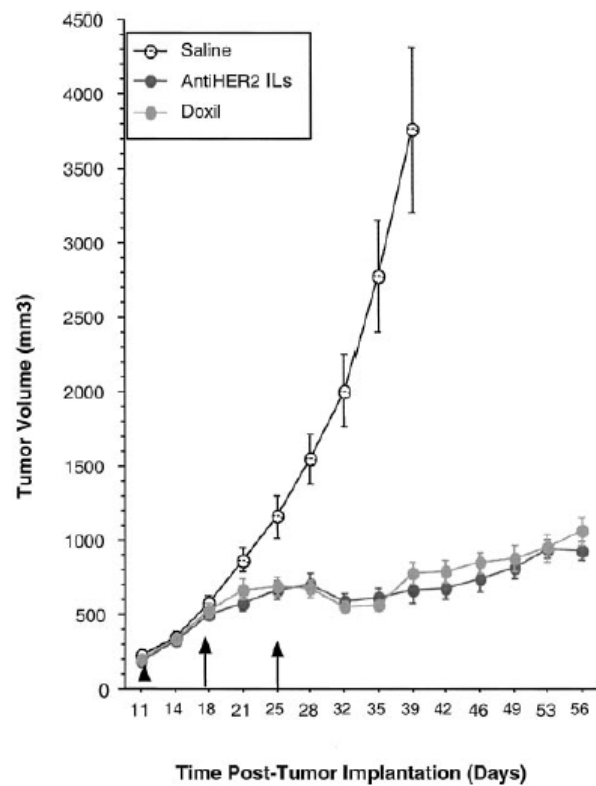
Active targeting: Active targeting involves the functionalization of nanocarriers with ligands that specifically recognize and bind to receptors or other molecular targets overexpressed on cancer cells or tumor-associated blood vessels. This approach can enhance the selectivity and efficacy of nanomedicines.

Liposomes functionalized with antibodies: Doxil® is a PEGylated liposomal formulation of doxorubicin, an anticancer drug, that passively targets tumors through the EPR effect. Researchers have developed immunoliposomes, which are liposomes conjugated with antibodies specific to cancer cell surface antigens, such as HER2, to actively target cancer cells.

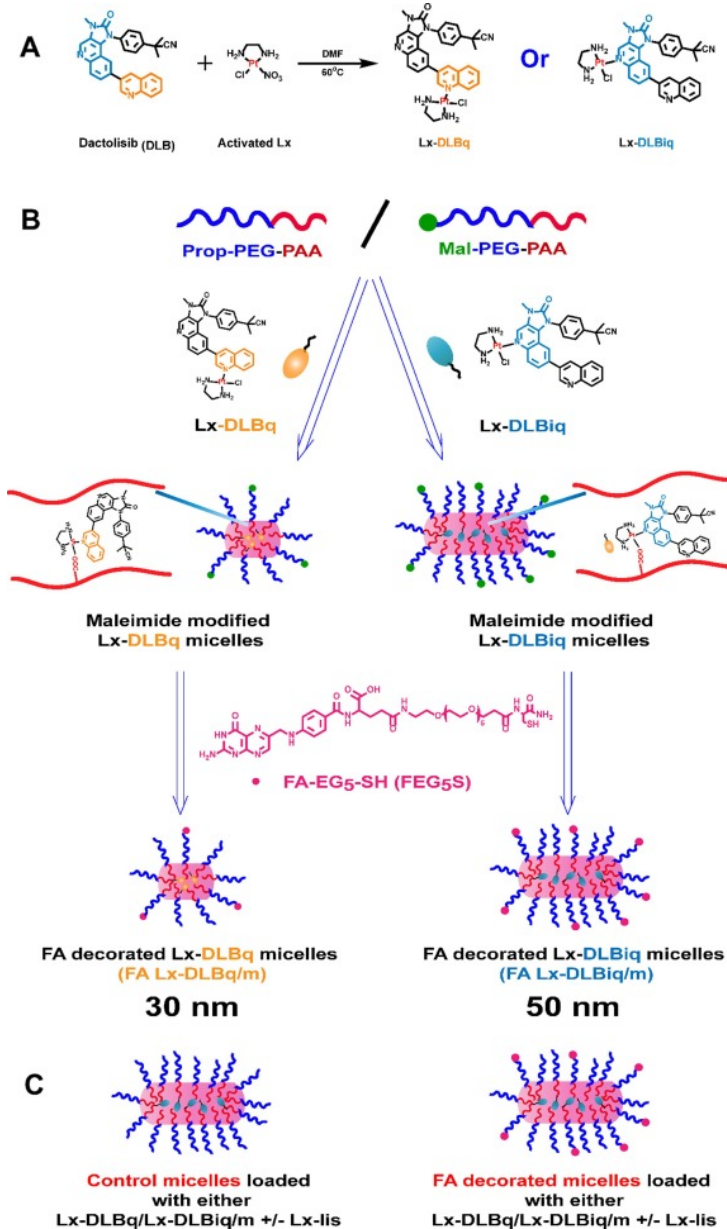
Polymeric nanoparticles: Polymeric nanoparticles have been developed to deliver chemotherapeutic agents, such as paclitaxel, to tumors. For example, Genexol-PM is a formulation of paclitaxel-loaded polymeric micelles that passively target tumors through the EPR effect. Active targeting can be achieved by conjugating ligands, such as folic acid, to polymeric nanoparticles to target cancer cells that overexpress folate receptors.

Gold nanoparticles: Gold nanoparticles have been used for the targeted delivery of anticancer drugs and photothermal therapy. For example, researchers have functionalized gold nanoparticles with aptamers, which are short nucleic acid sequences that specifically bind to target molecules, to actively target cancer cells that overexpress the EpCAM antigen.

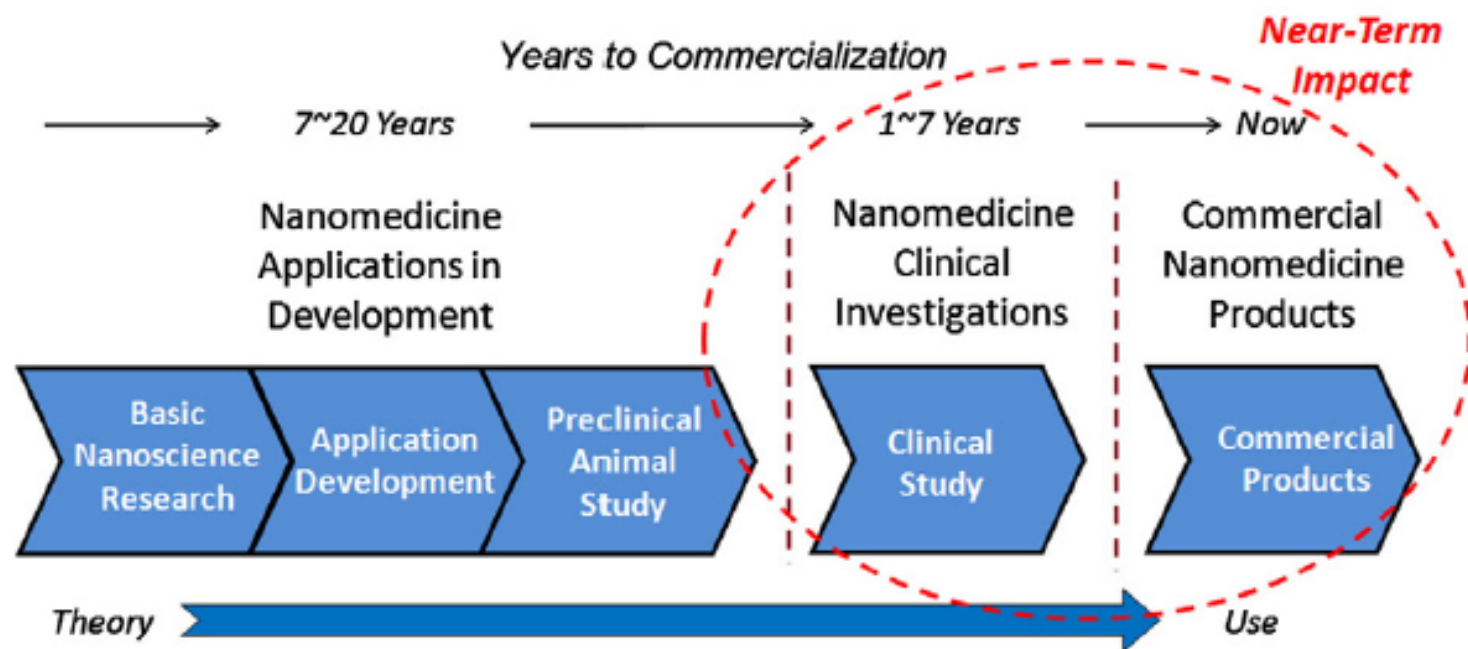
Anti-HER2 Immunoliposomes: Enhanced Efficacy Attributable to Targeted Delivery¹



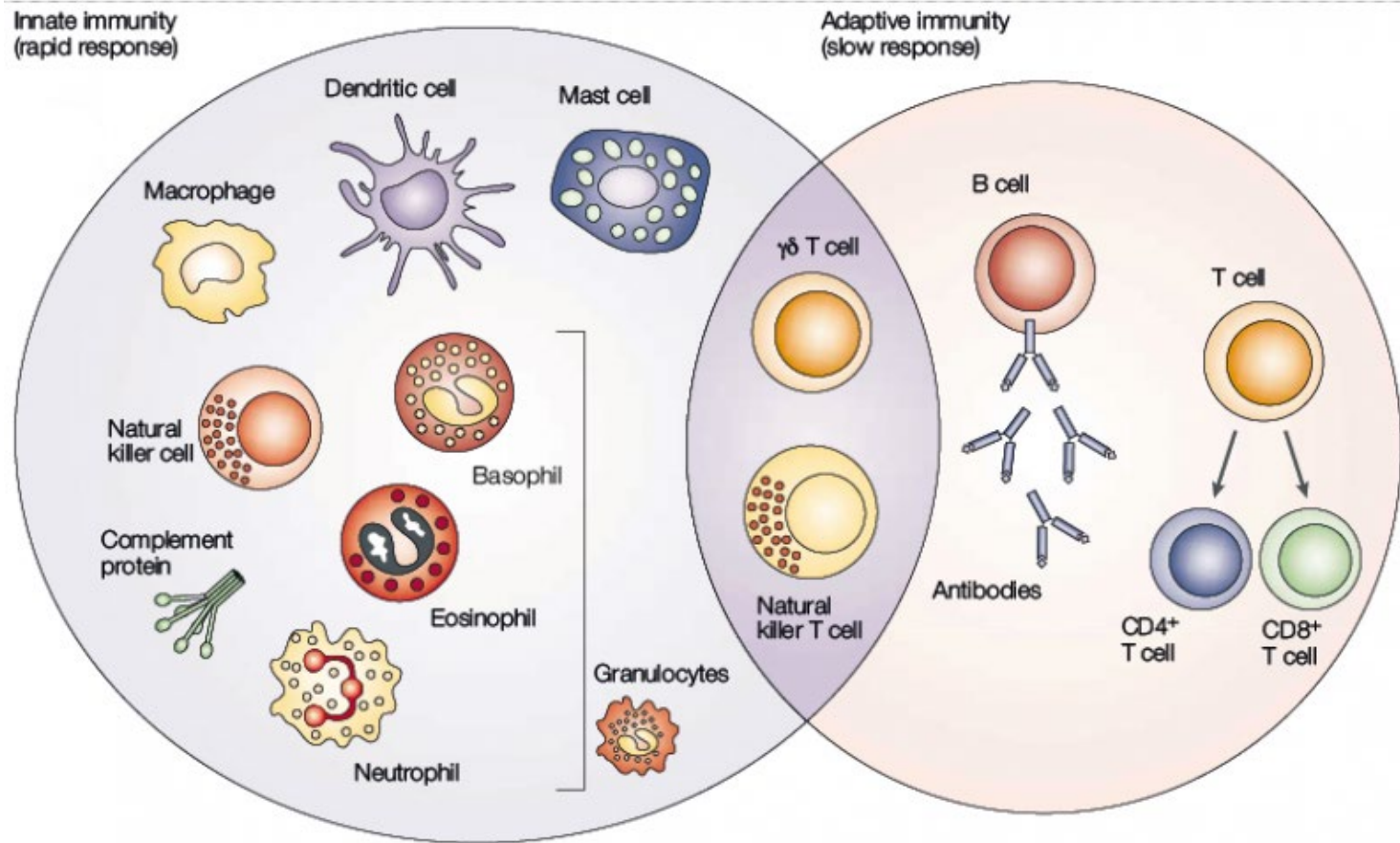
Folate decorated polymeric micelles for targeted delivery



Nanomedicine Technology Development Pipeline



Immune Cells



Immune System

Innate immunity: This is the first line of defense and provides a rapid, nonspecific response to harmful substances or pathogens. Innate immunity consists of various physical and chemical barriers (e.g., skin, mucous membranes, stomach acid), immune cells (e.g., **neutrophils, macrophages, dendritic cells, natural killer cells**), and proteins (e.g., complement system, cytokines). The innate immune system can recognize common molecular patterns associated with pathogens, such as lipopolysaccharides on the surface of bacteria, through pattern recognition receptors (PRRs).

Adaptive immunity: This component of the immune system provides a highly specific, targeted response to specific pathogens or antigens. It is slower to develop than innate immunity but has the advantage of immunological memory, which allows for a faster and more robust response upon subsequent encounters with the same pathogen. Adaptive immunity is mainly mediated by two types of white blood cells: **B lymphocytes (B cells)** and **T lymphocytes (T cells)**.

Innate immune cells

Neutrophils: These are the most abundant type of white blood cells and are the first to arrive at the site of infection. They are phagocytic cells, meaning they engulf and destroy pathogens. They also release enzymes and antimicrobial peptides to kill microbes and recruit other immune cells to the site of infection.

Macrophages: These large phagocytic cells play a critical role in both innate and adaptive immunity. They engulf and destroy pathogens, remove dead cells, and secrete cytokines that regulate inflammation and recruit other immune cells. Macrophages also function as antigen-presenting cells, displaying fragments of foreign substances to T cells and initiating an adaptive immune response.

Dendritic cells: These cells act as sentinels in tissues exposed to the external environment, such as the skin and mucosal surfaces. They capture and process foreign substances and present them to T cells, thus bridging the gap between innate and adaptive immunity.

Natural killer (NK) cells: NK cells are lymphocytes that can recognize and kill virus-infected cells and cancer cells without prior sensitization. They play a crucial role in the early defense against infections and tumors.

Adaptive immune cells

B lymphocytes (B cells): B cells are responsible for producing antibodies, which are proteins that can recognize and neutralize specific pathogens or infected cells. When a B cell encounters its specific antigen, it differentiates into plasma cells that produce and secrete large amounts of antibodies, and memory B cells that provide long-lasting immunity against the same antigen.

T lymphocytes (T cells): T cells play a central role in the adaptive immune response. They can be divided into several types, including:

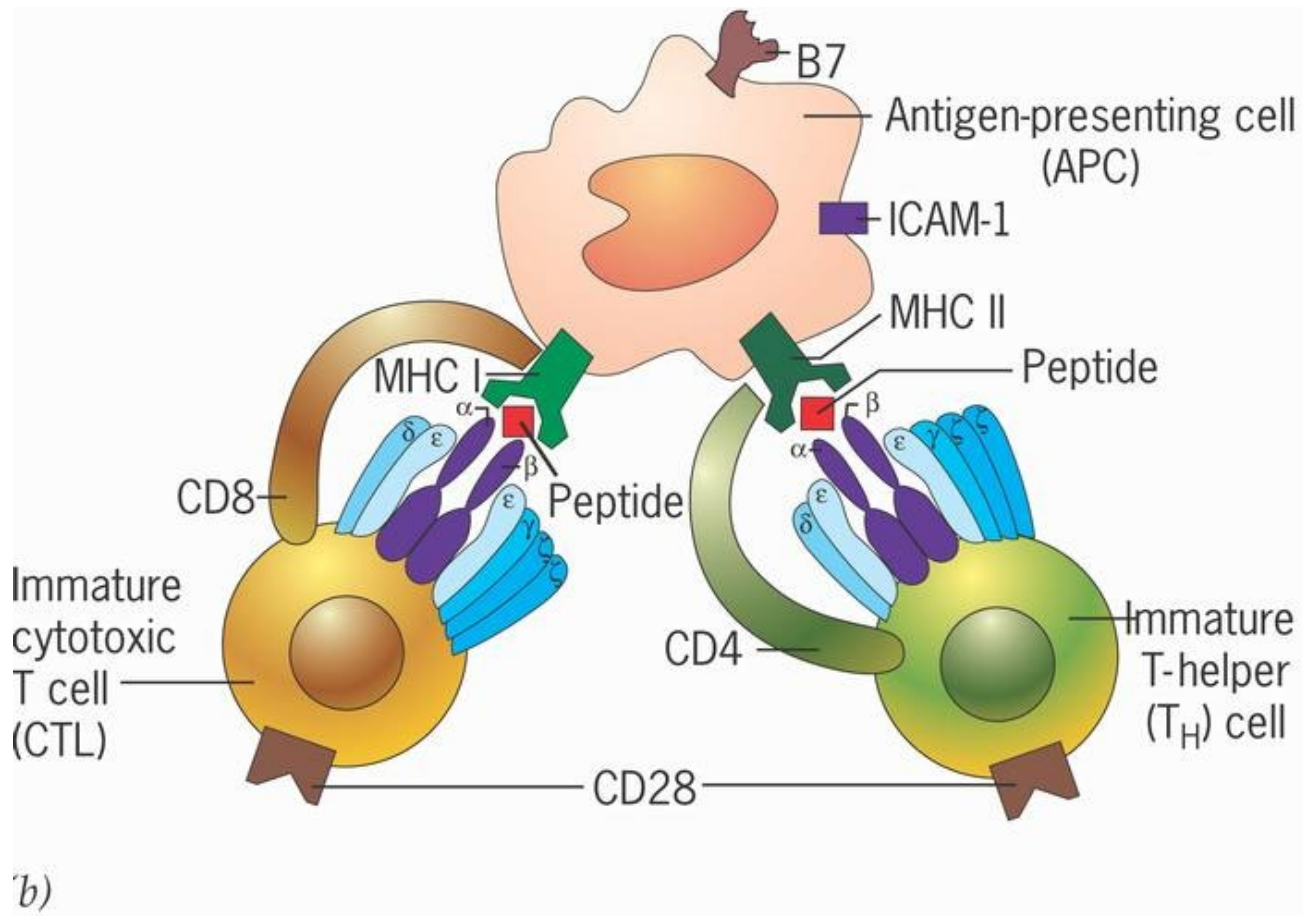
a. **Helper T cells (CD4+ T cells):** These cells coordinate the immune response by secreting cytokines that activate and regulate other immune cells, such as B cells, cytotoxic T cells, and macrophages.

b. **Cytotoxic T cells (CD8+ T cells):** These cells can directly kill infected or abnormal cells by releasing cytotoxic molecules, such as perforin and granzymes, that induce apoptosis (cell death).

c. **Regulatory T cells (Tregs):** These cells help maintain immune tolerance by suppressing overactive immune responses and preventing autoimmune reactions.

Memory T cells: These long-lived T cells are generated following an immune response and provide long-lasting immunity against the same antigen. They can rapidly differentiate into effector T cells upon re-exposure to the antigen, resulting in a faster and more robust immune response.

APC



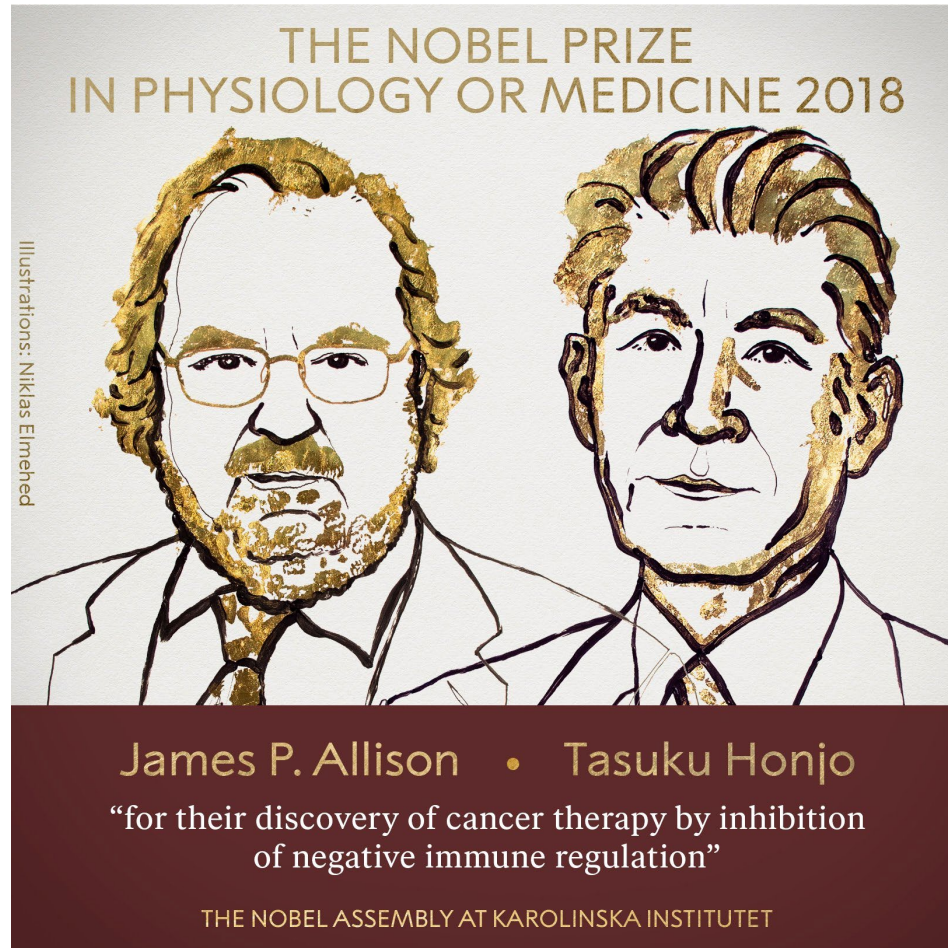
An **antigen-presenting cell (APC)** is a type of immune cell that plays a critical role in initiating and shaping the adaptive immune response. APCs capture, process, and present antigens—foreign substances or protein fragments derived from pathogens—on their surface in association with major **histocompatibility complex (MHC) molecules**. T cells can recognize these antigen-MHC complexes, allowing them to mount a specific immune response against the foreign substance.

Dendritic cells: These cells are often considered the most potent APCs due to their ability to initiate naive T cell responses. Dendritic cells are located in various tissues, especially those exposed to the external environment, such as the skin and mucosal surfaces. They capture and process antigens and then migrate to lymphoid organs, where they present the antigens to T cells.

Macrophages: Macrophages are phagocytic cells involved in both innate and adaptive immunity. They can engulf and destroy pathogens, as well as present antigens to T cells. Macrophages are widely distributed throughout the body and play a crucial role in the immune response against intracellular pathogens, such as bacteria and fungi.

B lymphocytes (B cells): B cells can also act as APCs, primarily in the context of the humoral immune response. When B cells encounter their specific antigen, they internalize, process, and present it to helper T cells, which in turn provide signals for B cell activation, differentiation, and antibody production.

Immunotherapy



Cancer Immunotherapies

Cancer immunotherapy refers to a group of therapeutic approaches that aim to harness the power of the immune system to recognize and eliminate cancer cells. By stimulating or restoring the immune response against cancer, immunotherapies can offer a targeted and potentially long-lasting treatment for various types of cancer.

There are several types of cancer immunotherapy, including:

- Immune checkpoint inhibitors:

- Cancer vaccines

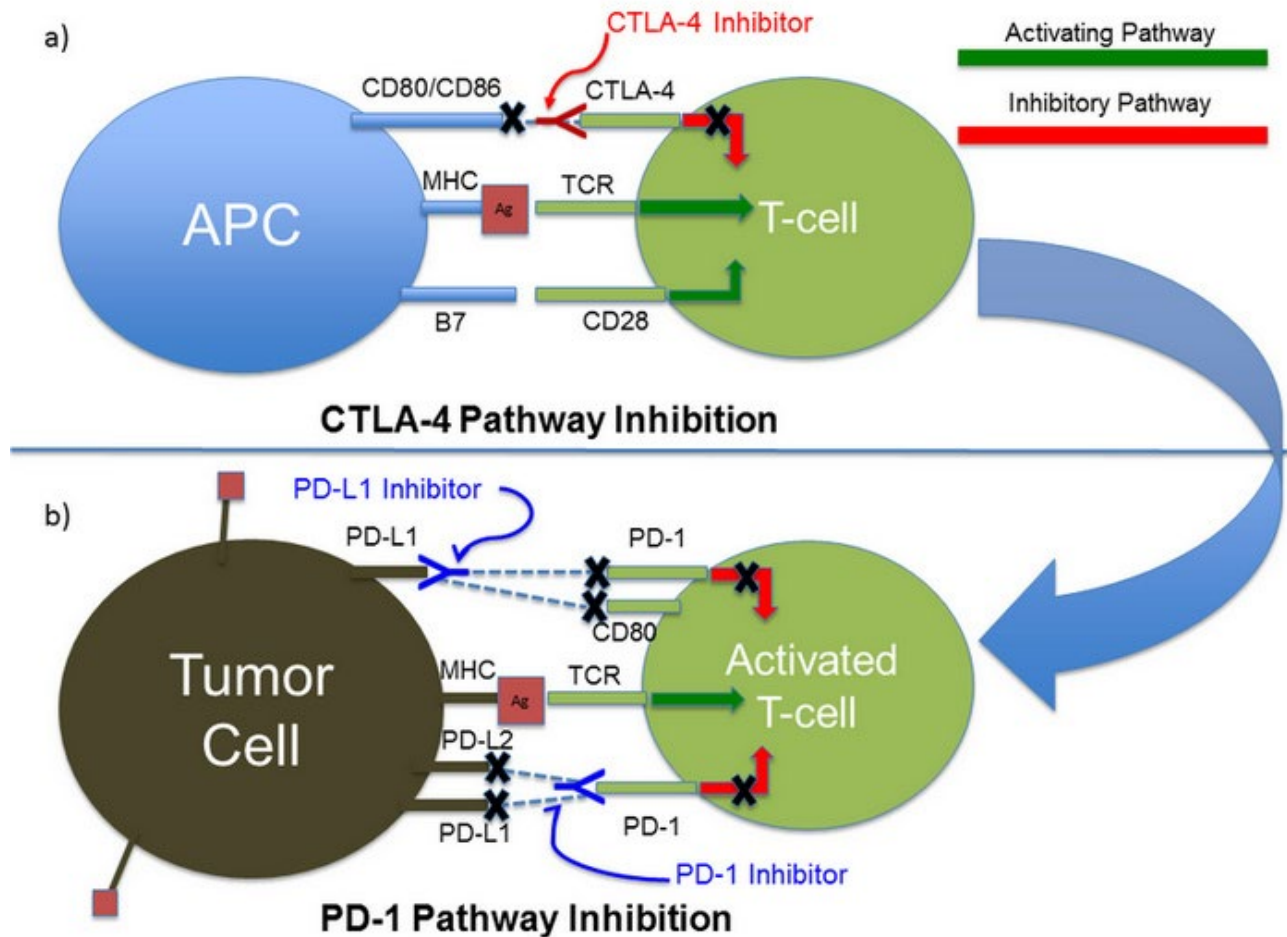
- Adoptive cell transfer (ACT):

- Oncolytic viruses:.

- Cytokines:

- Bispecific antibodies

Immune Checkpoint Blockade



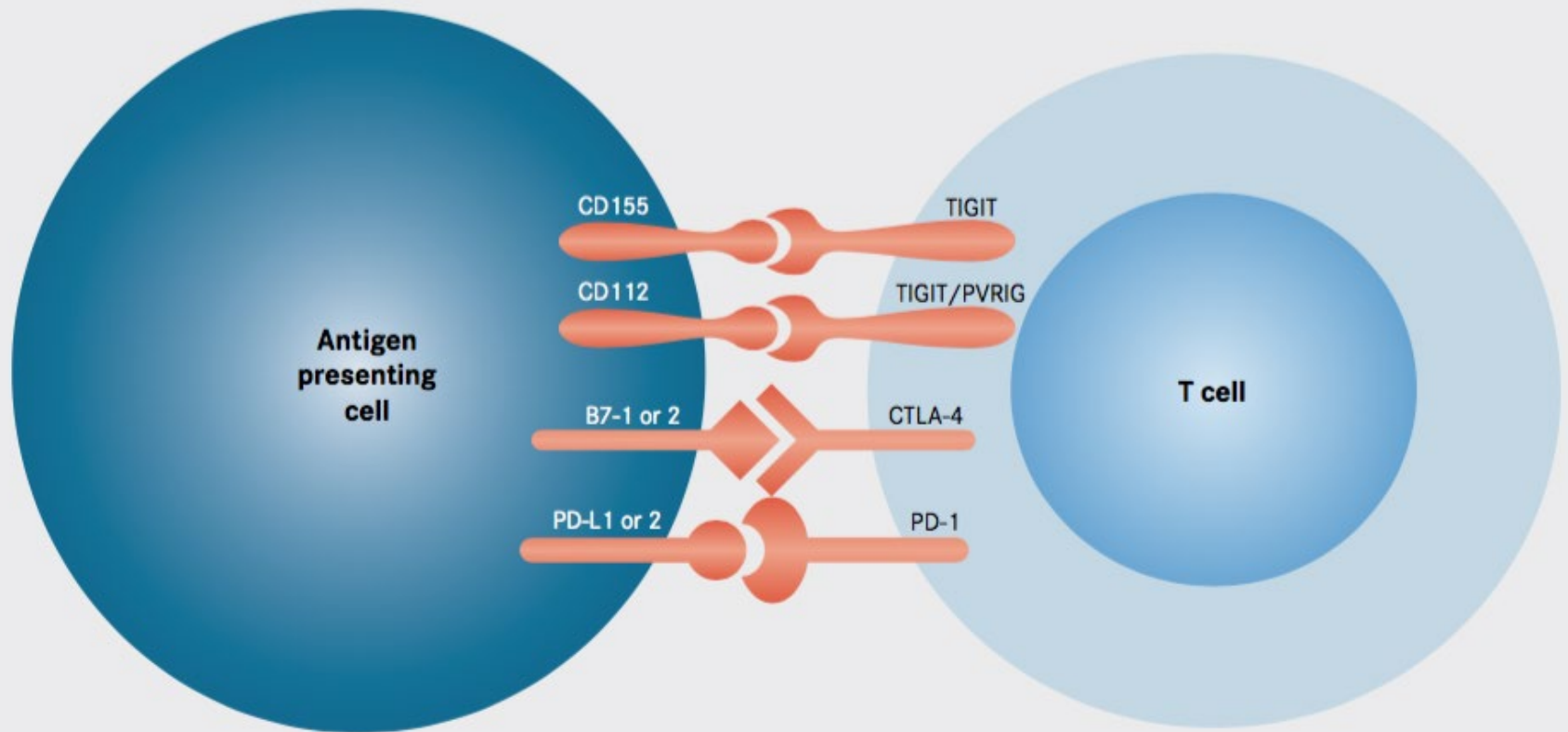
Immune checkpoint blockade involves using monoclonal antibodies that bind to and block the interaction between checkpoint proteins and their ligands, thereby "releasing the brakes" on the immune system and allowing T cells to target and kill cancer cells more effectively.

CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4): CTLA-4 is an inhibitory molecule expressed on the surface of T cells. It competes with the stimulatory molecule CD28 for binding to the B7 molecules (CD80 and CD86) on antigen-presenting cells. When CTLA-4 binds to B7 molecules, it inhibits T cell activation, preventing an overactive immune response.

PD-1 (Programmed cell death protein 1): PD-1 is another inhibitory receptor found on the surface of T cells, as well as some other immune cells. It binds to its ligands, PD-L1 and PD-L2, which can be expressed on cancer cells and some immune cells in the tumor microenvironment. The interaction between PD-1 and its ligands leads to T cell exhaustion, a state of dysfunction that impairs the T cells' ability to effectively eliminate cancer cells.

PD-L1 (Programmed cell death ligand 1): PD-L1 is the primary ligand for PD-1 and is often expressed on cancer cells and some immune cells within the tumor microenvironment. Blocking PD-L1 can disrupt the inhibitory signaling pathway mediated by PD-1, leading to enhanced T cell function and anti-tumor immune response.

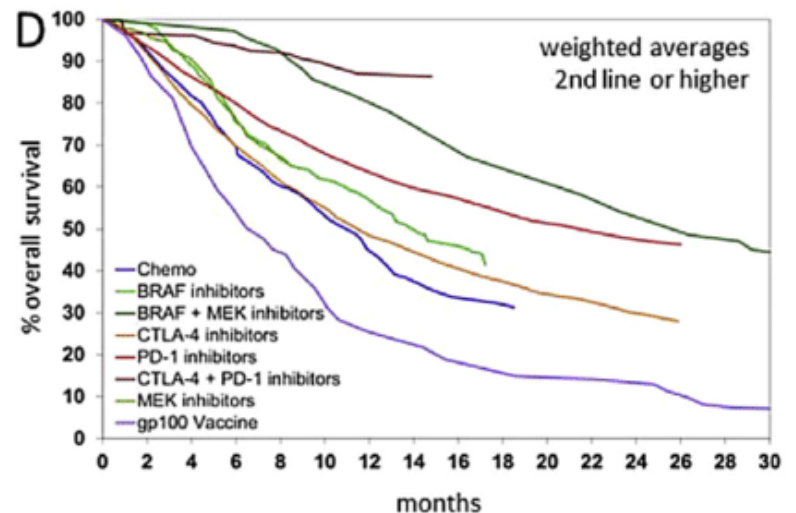
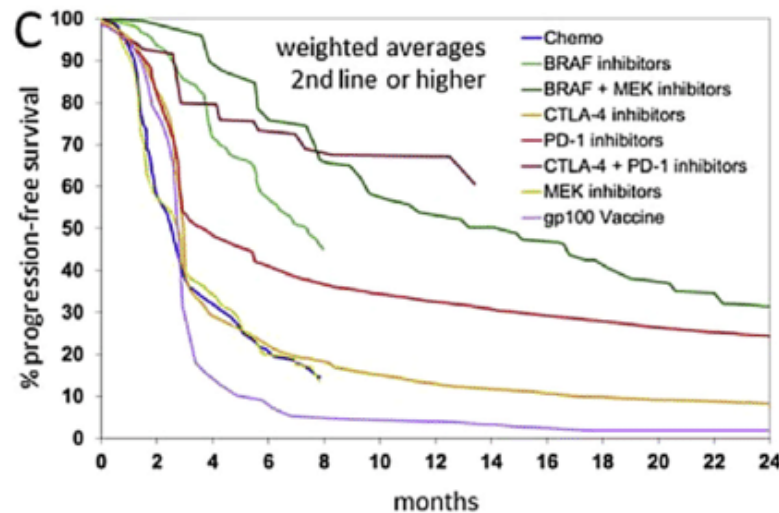
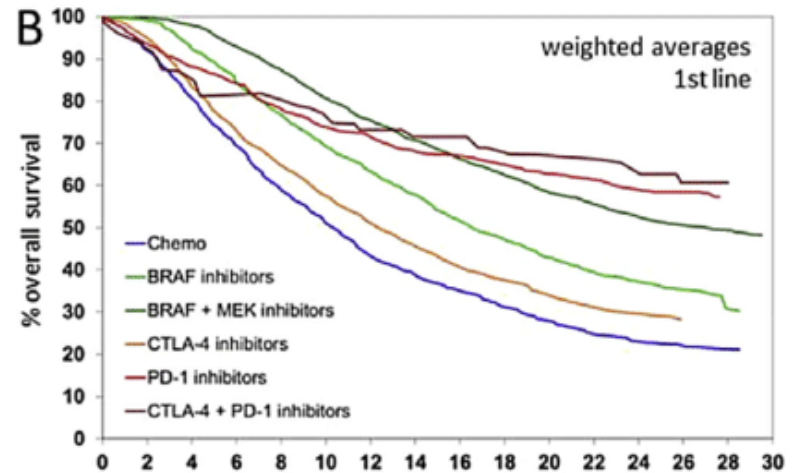
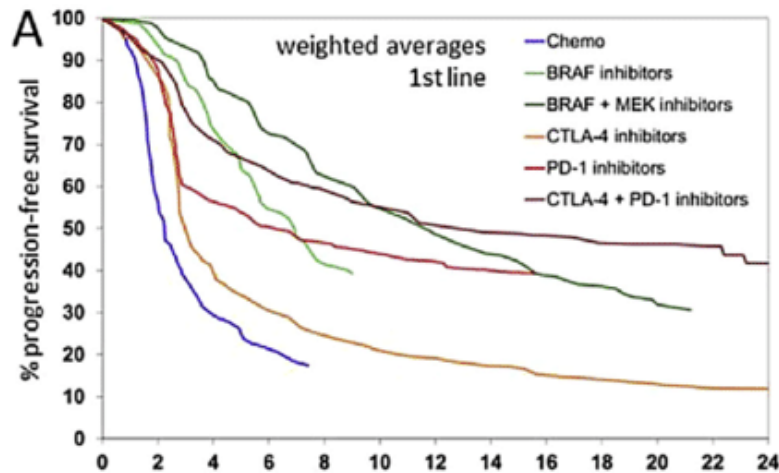
Partners in Immune System Signaling



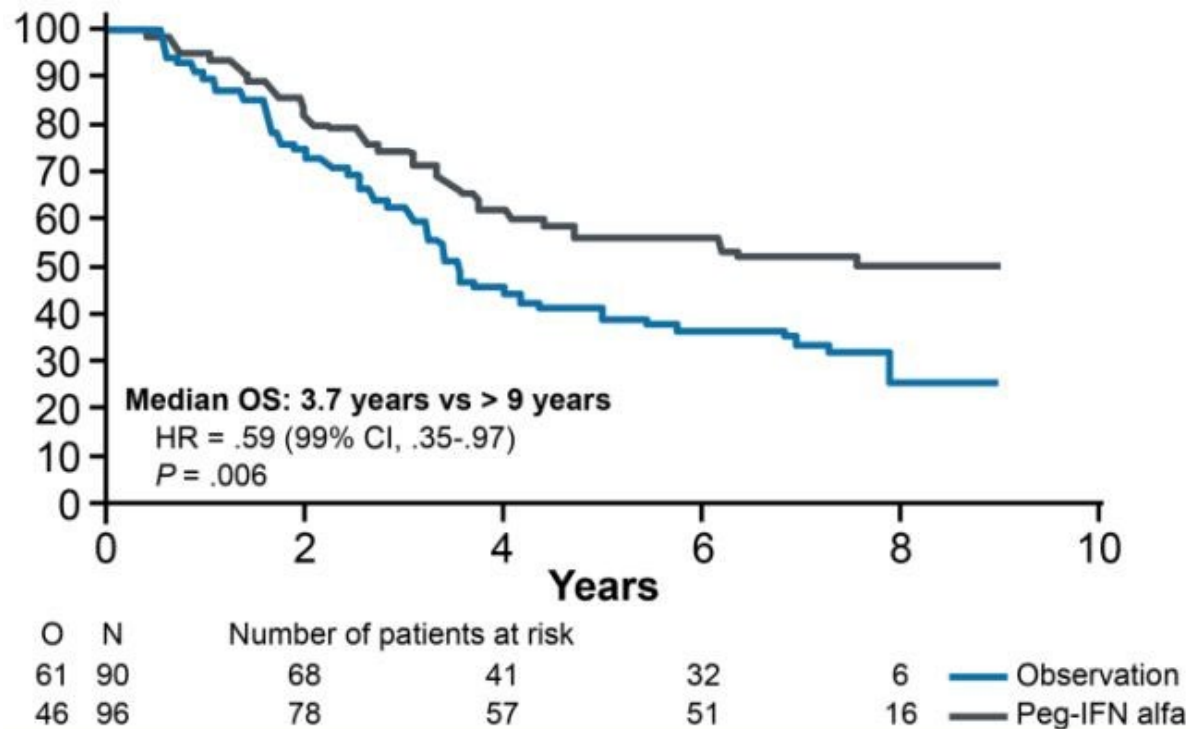
TIGIT is among the T-cell receptors that interact with proteins expressed by antigen presenting cells to send inhibitory signals to the immune system. Dysregulated interaction between TIGIT and its ligands serves to suppress immunity when under attack from cancer cells, similar to the activity of the PD-1 and CTLA-4 pathways.

Immune checkpoint inhibitors: These are drugs that target immune checkpoint proteins, such as CTLA-4, PD-1, and PD-L1, which normally act as "brakes" on the immune system to prevent overactivity and damage to healthy tissues. Cancer cells can exploit these checkpoints to evade immune recognition and destruction. By blocking these checkpoint proteins, immune checkpoint inhibitors can unleash the immune system's full potential to attack cancer cells. **Examples include ipilimumab (anti-CTLA-4), pembrolizumab (anti-PD-1), and atezolizumab (anti-PD-L1).**

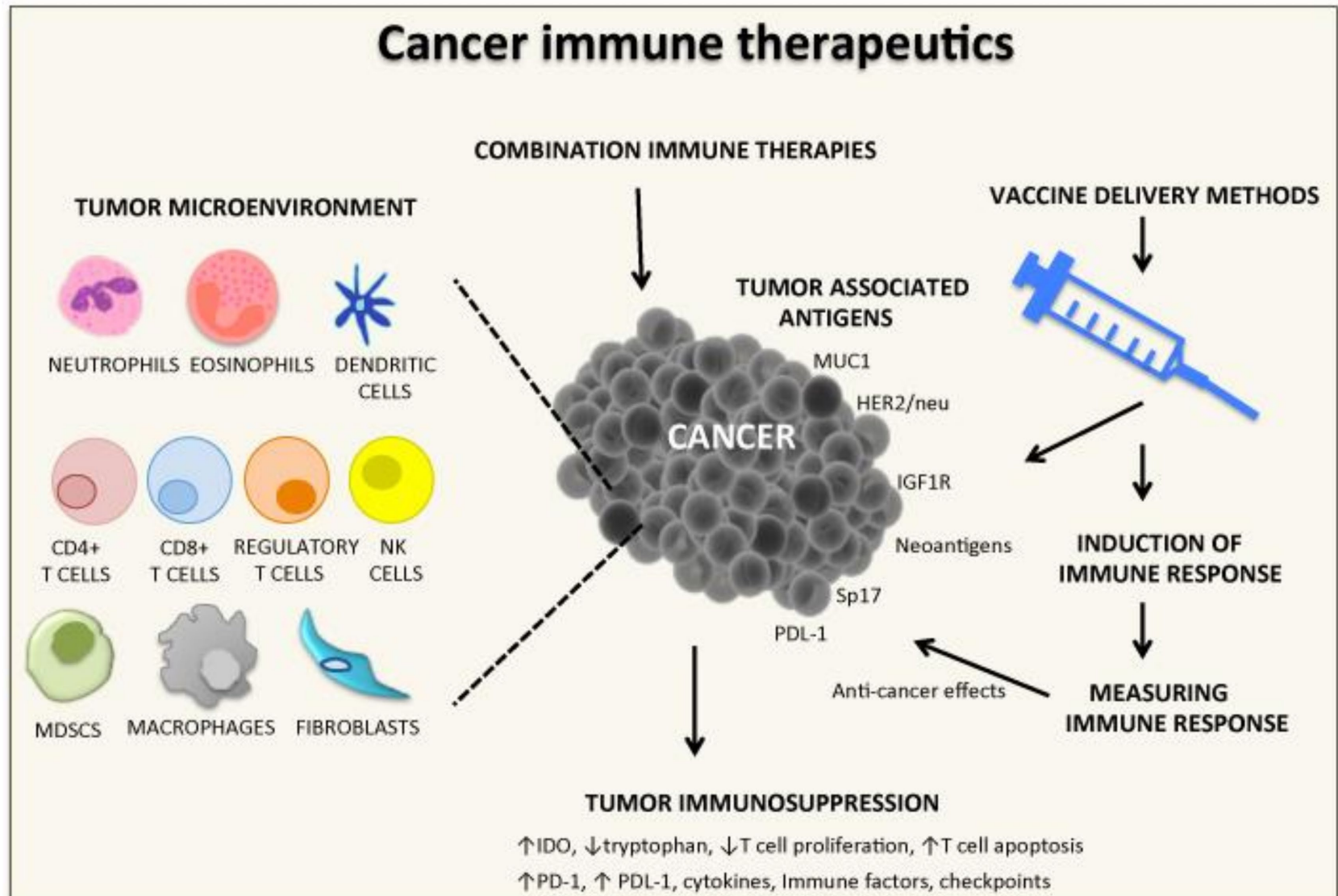
Overall Survival



Long Term Survival



Cancer Vaccine



Cancer Vaccine

A cancer vaccine is a type of immunotherapy that aims to stimulate the immune system to recognize and destroy cancer cells. Cancer vaccines can be divided into two main categories: preventive vaccines and therapeutic vaccines.

Preventive vaccines: These vaccines are designed to protect against cancer-causing viruses, such as human papillomavirus (HPV) and hepatitis B virus (HBV). By preventing viral infections, these vaccines indirectly reduce the risk of developing cancers associated with these viruses, such as cervical cancer (HPV) and liver cancer (HBV). Examples of preventive cancer vaccines include Gardasil and Cervarix for HPV, and the hepatitis B vaccine.

Therapeutic vaccines: These vaccines are designed to treat existing cancers by stimulating the immune system to recognize and attack cancer cells. Therapeutic cancer vaccines usually **target specific tumor-associated antigens (TAAs)**, which are proteins expressed by cancer cells but not (or rarely) by normal cells. By targeting these antigens, the vaccine aims to train the immune system to recognize and eliminate cancer cells without harming healthy cells.

Cancer Vaccine

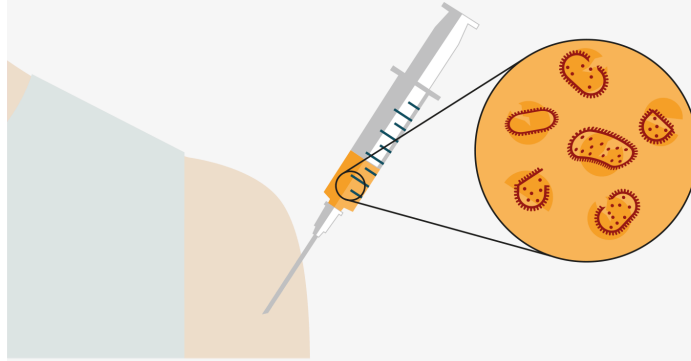
There are several types of therapeutic cancer vaccines, including:

- a. **Peptide-based vaccines:** These vaccines contain short fragments of proteins (peptides) derived from TAAs to stimulate an immune response against cancer cells.
- b. **Tumor cell-based vaccines:** These vaccines use whole cancer cells or parts of cancer cells to provoke an immune response. The cells can be taken from the patient's own tumor (autologous) or from another individual (allogeneic).
- c. **Dendritic cell-based vaccines:** These vaccines use dendritic cells, which are potent antigen-presenting cells that can activate the immune system. Dendritic cells are loaded with TAAs and then reintroduced into the patient to stimulate a targeted immune response against cancer cells.
- d. **DNA/RNA-based vaccines:** These vaccines use genetic material (DNA or RNA) encoding TAAs to stimulate the production of the target antigens in the patient's own cells, leading to an immune response against the cancer cells.

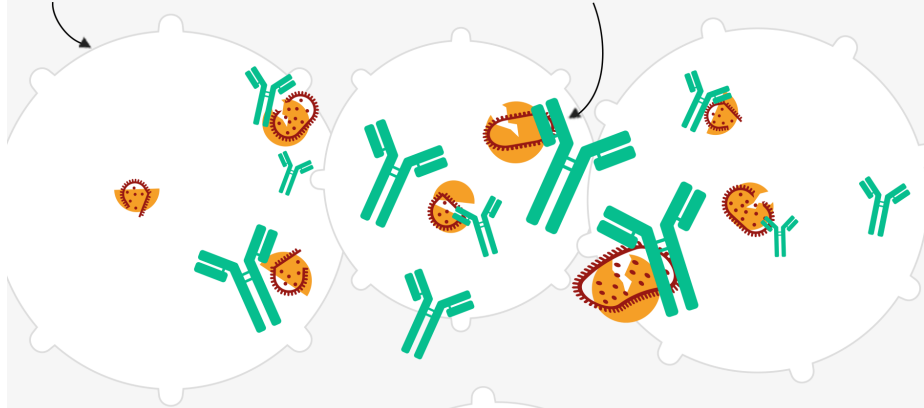
One example of an FDA-approved therapeutic cancer vaccine is sipuleucel-T (Provenge), a dendritic cell-based vaccine used to treat metastatic castration-resistant prostate cancer.

How vaccines work

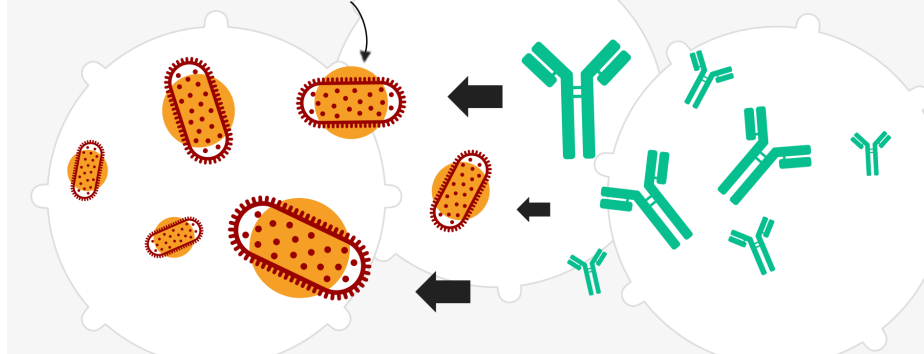
Weakened or dead disease bacteria introduced into the patient, often by injection



White blood cells triggered to produce antibodies to fight the disease



If patient encounters disease later, antibodies neutralise the invading cells

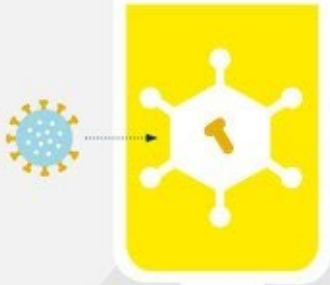


How do different Covid-19 vaccines work?



Viral vector

Uses a harmless virus which is altered to contain part of Covid-19's genetic code



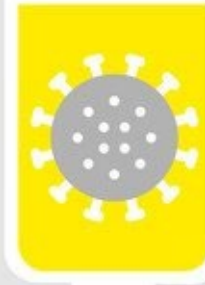
RNA (nucleic acid)

Contains a synthetic version of part of Covid-19's genetic code (messenger RNA)



'Whole' virus

Contains a weakened or inactivated version of the Covid-19 virus



Protein subunit

Uses pieces of the Covid-19 virus - sometimes fragments of the 'spike' protein

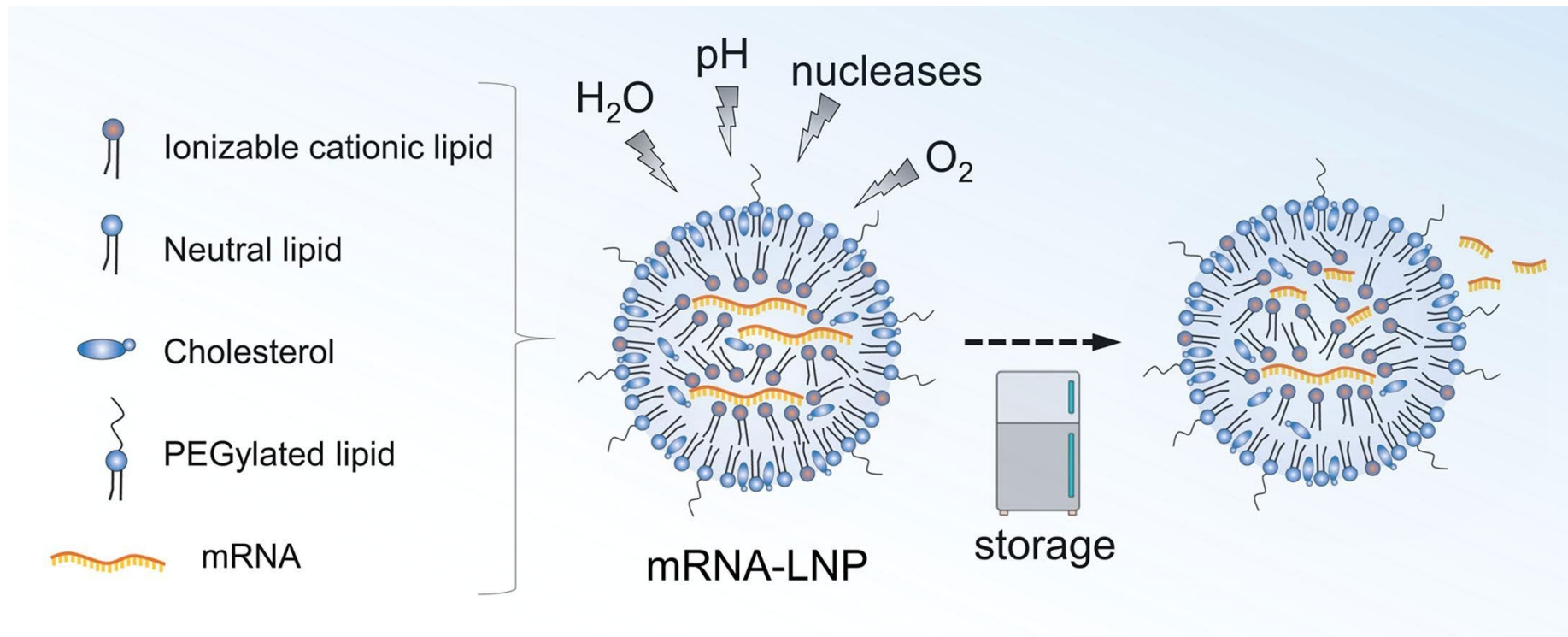


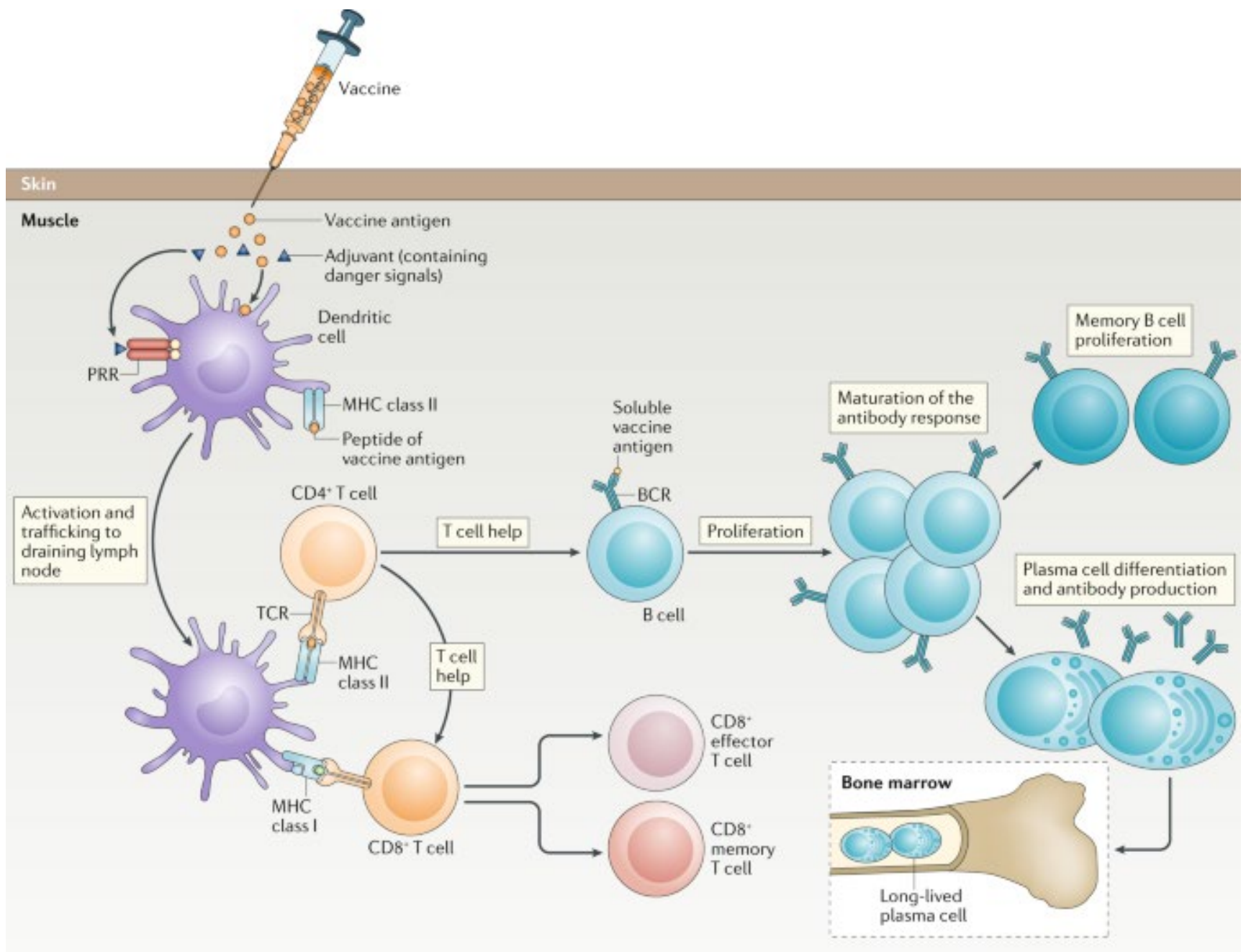
The code tells our cells to make the Covid-19 'spike' protein, which triggers an immune response

This triggers an immune response



Lipid Nanoparticle for Vaccine





Adjuvants

Adjuvants play a crucial role in the development and effectiveness of cancer vaccines. They are substances added to vaccines to enhance the immune response against tumor-associated antigens (TAAs). Adjuvants work by activating and modulating the immune system in various ways, which can ultimately lead to a more robust and long-lasting anti-tumor response. Some of the roles of adjuvants in cancer vaccines include:

- Activation of antigen-presenting cells (APCs).

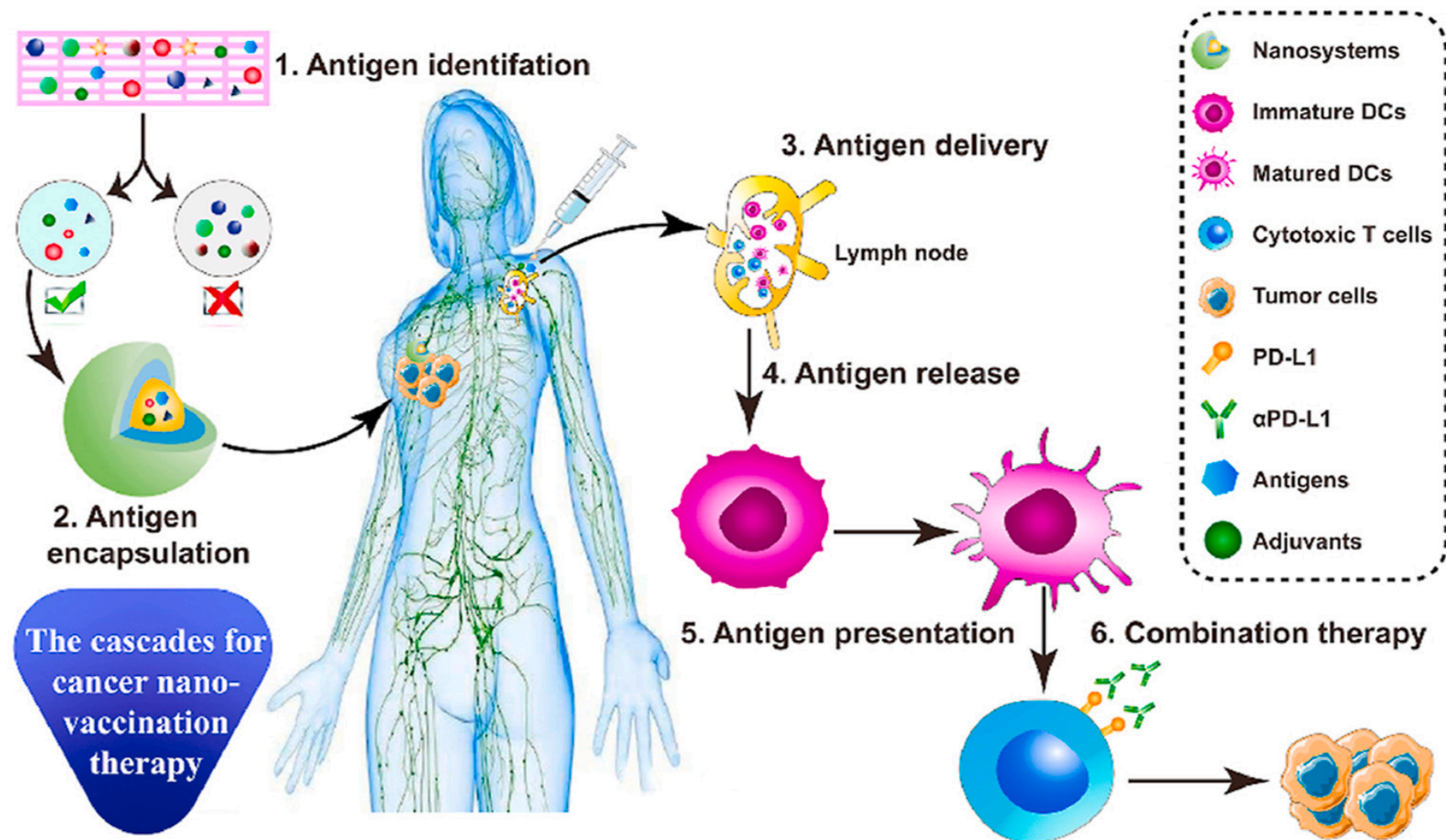
- Recruitment and activation of immune cells:

- Enhanced cytokine and chemokine production:

- Induction of long-lasting immunity

- Modulation of the immune response

Various adjuvants are used in cancer vaccines, including aluminum salts, oil-in-water emulsions, liposomes, and TLR agonists. The choice of adjuvant depends on the specific vaccine formulation and the desired immune response. Ongoing research is focused on developing novel adjuvants and optimizing their use in cancer vaccines to improve their safety and effectiveness in stimulating anti-tumor immune responses.



Neoantigen

Neoantigens are novel protein fragments or peptides that arise from somatic mutations within tumor cells. These mutations can result in the production of abnormal proteins that are not present in normal cells. Since neoantigens are specific to cancer cells and not found in healthy cells, they represent attractive targets for cancer immunotherapy.

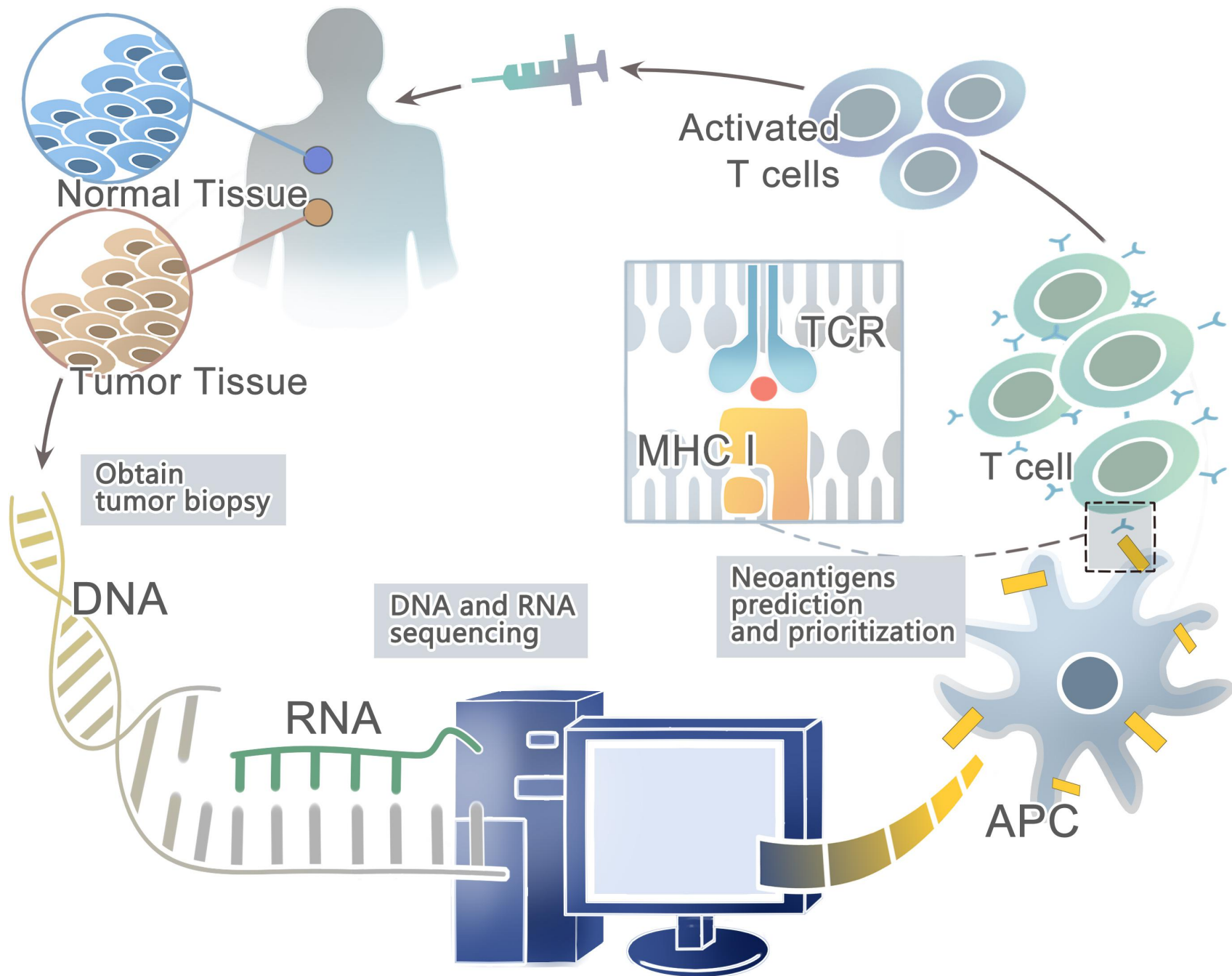
The immune system can recognize neoantigens as foreign substances, which can trigger an immune response against the cancer cells expressing these neoantigens. T cells, a type of immune cell, can identify and target these neoantigens presented on the surface of cancer cells by major histocompatibility complex (MHC) molecules. This immune recognition can lead to the activation and expansion of T cells that are capable of selectively killing cancer cells while sparing healthy cells.

Neoantigens play a critical role in several cancer immunotherapy strategies, including:

- Personalized cancer vaccines:

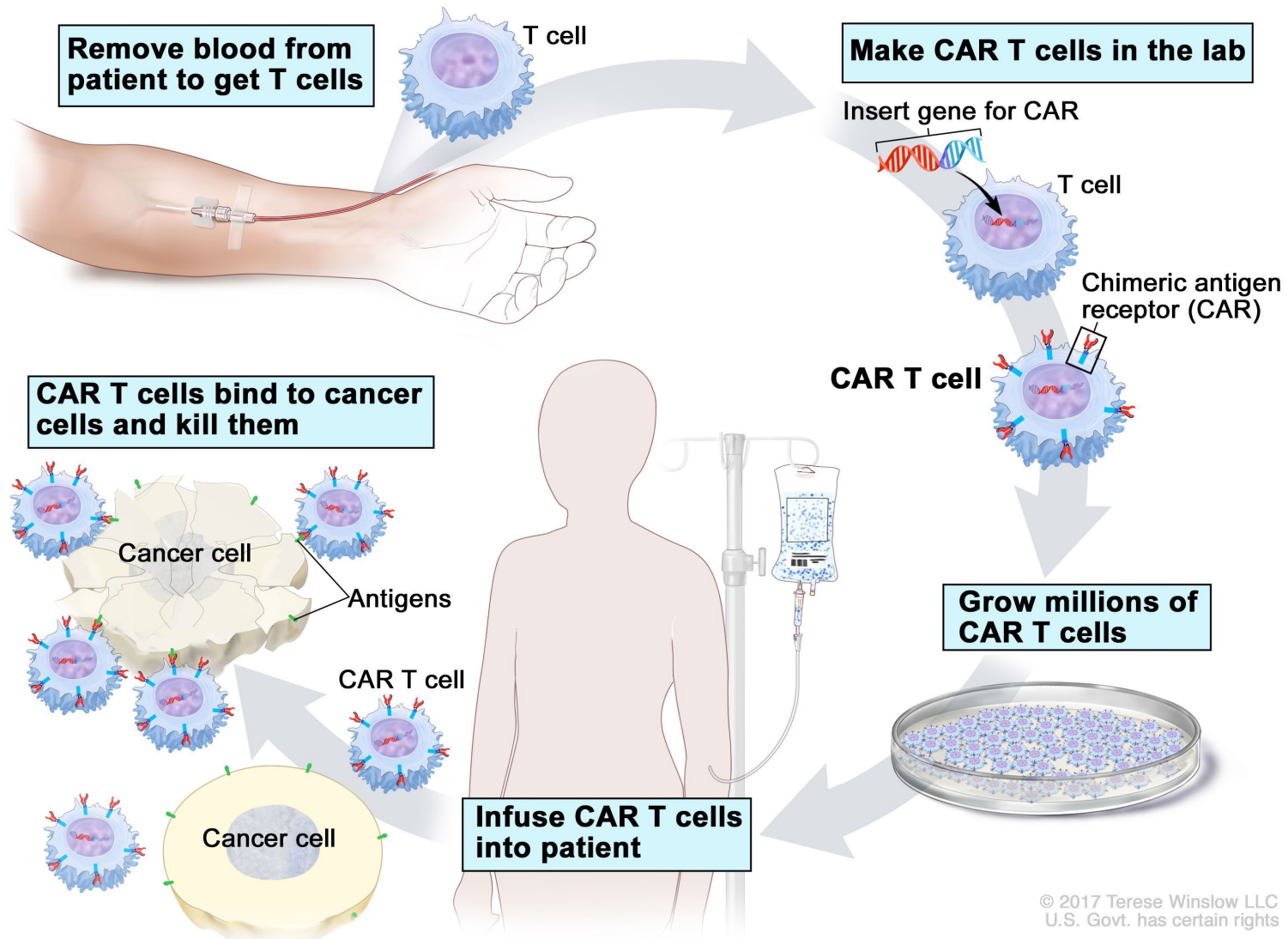
- Adoptive T cell therapy:

- T cell receptor (TCR)-engineered T cells.



(CAR) T-cell therapy

CAR T-cell Therapy



Chimeric Antigen Receptor (CAR) T-cell therapy is a type of adoptive cell therapy that harnesses the power of a patient's immune system to fight cancer. It involves the genetic engineering of a patient's T cells to express a chimeric antigen receptor (CAR) on their surface, which is designed to recognize and bind to a specific antigen expressed by cancer cells.

The principle behind CAR T-cell therapy can be broken down into several key steps:

T-cell collection:

Genetic engineering: The isolated T cells are then genetically modified, typically using viral vectors, to express a chimeric antigen receptor (CAR) on their surface. The CAR is a synthetic protein that combines an extracellular antigen-binding domain, usually derived from an antibody, with intracellular signaling domains responsible for activating the T cell upon antigen recognition.

T-cell expansion:

Patient preparation

CAR T-cell infusion

The first FDA-approved CAR T-cell therapies, Kymriah (tisagenlecleucel) and Yescarta (axicabtagene ciloleucel), target the CD19 antigen, which is commonly expressed on the surface of B-cell malignancies.

Hot Tumors and Cold Tumors

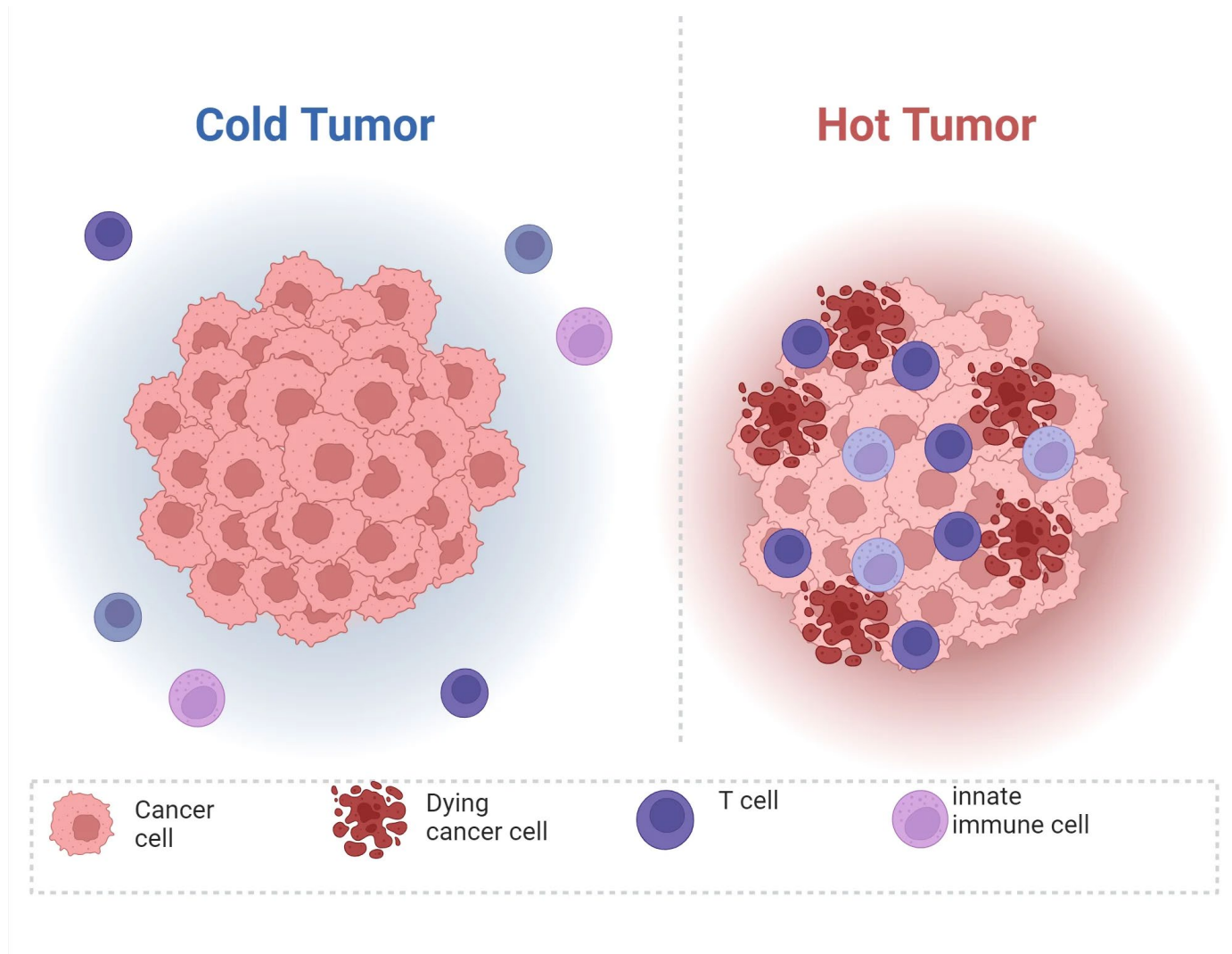
Hot Tumors:

Hot tumors are characterized by their inflamed nature, which means they contain a high number of infiltrating immune cells, such as T-cells. These tumors often express higher levels of immune checkpoint proteins and are generally more visible to the immune system due to the presence of these immune cells and the expression of neoantigens (new antigens produced by tumor mutations). Because of this immune activity, hot tumors are typically more responsive to immunotherapies such as checkpoint inhibitors. These therapies help to further activate the immune system against the tumor.

Cold Tumors:

Cold tumors, in contrast, are non-inflamed and contain few or no infiltrating immune cells. They have a lower expression of neoantigens and immune checkpoint molecules, making them less visible or even invisible to the immune system. This lack of immune activity makes cold tumors less responsive to immunotherapy, as there is minimal existing immune response to enhance. Cold tumors often require different strategies to become treatable with immunotherapy, such as combining immunotherapy with other treatments that can help to turn a cold tumor into a hot one, making it more susceptible to immune attacks.

Hot Tumors and Cold Tumors



Converting Cold Tumors into Hot Tumors

Delivery of Immunomodulatory Agents:

Nanoparticles can be engineered to deliver immunostimulatory agents directly to the tumor microenvironment. These agents can include cytokines (like interleukin-2 or interleukin-12), toll-like receptor (TLR) agonists, or STING (Stimulator of Interferon Genes) agonists, which can activate immune cells and promote inflammation within the tumor.

Combination Therapy:

Combining nanomedicine with other treatments, such as chemotherapy or radiotherapy, can induce immunogenic cell death, releasing tumor antigens that attract immune cells to the tumor site. Nanoparticles can be designed to deliver chemotherapy drugs and immunotherapy agents simultaneously, allowing localized combination therapy that minimizes systemic side effects.

Checkpoint Inhibitors:

Nanoparticles can be used to deliver checkpoint inhibitors, such as PD-1 or CTLA-4 inhibitors, directly to the tumor site. This localized delivery can enhance the immune response against the tumor while reducing the immune-related adverse effects often associated with systemic administration of these drugs.

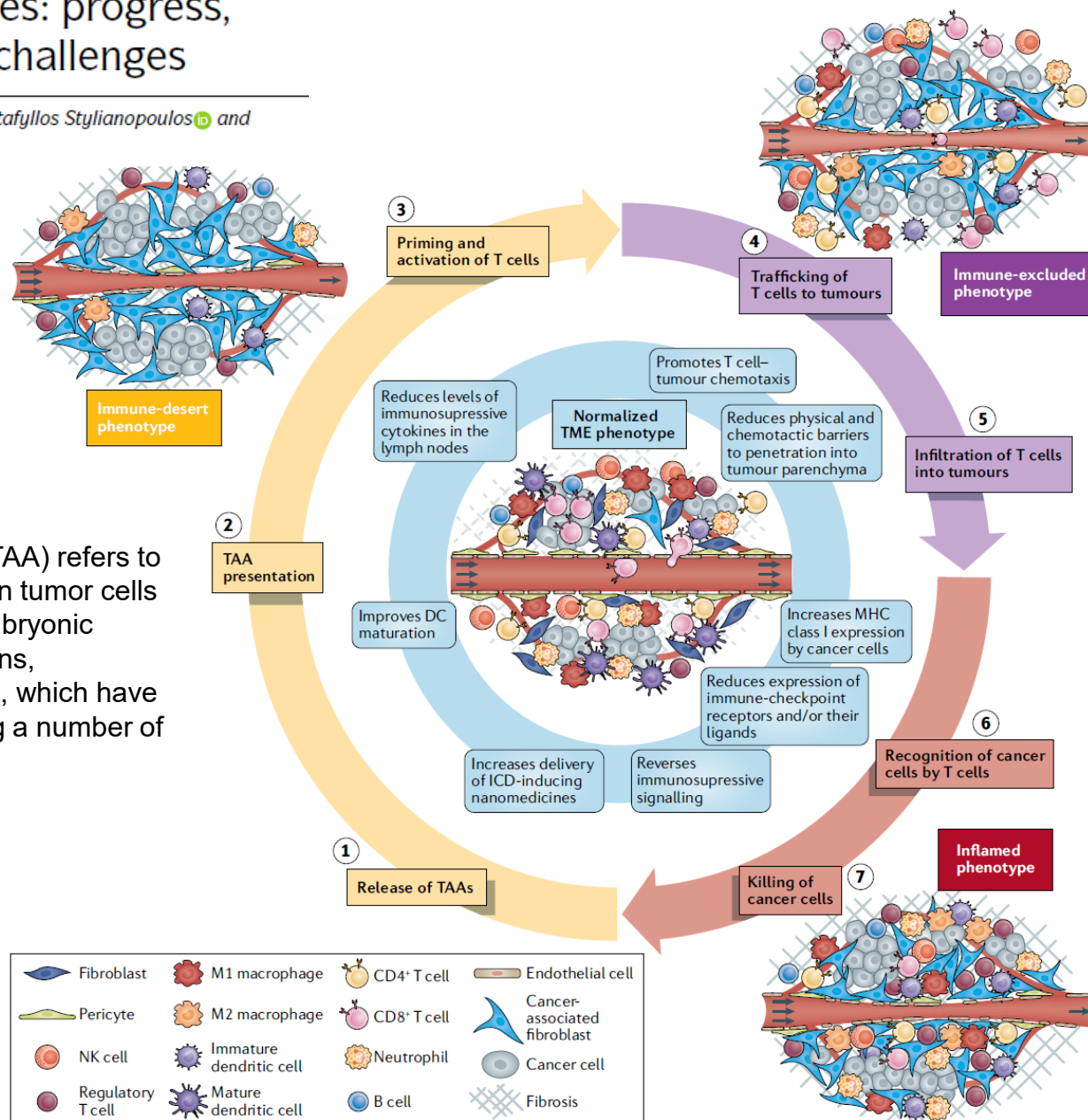
Targeting the Tumor Microenvironment:

Nanoparticles can be designed to modulate the suppressive tumor microenvironment. This can involve the delivery of agents that deplete immunosuppressive cells (like regulatory T cells or myeloid-derived suppressor cells) or reprogram the fibroblasts and other stromal cells that often support tumor growth and immune evasion.

Improving cancer immunotherapy using nanomedicines: progress, opportunities and challenges

John D. Martin¹, Horacio Cabral², Triantafyllos Stylianopoulos¹ and Rakesh K. Jain¹

Tumor-associated antigen (TAA) refers to antigen molecules present on tumor cells or normal cells, including embryonic proteins, glycoprotein antigens, squamous cell antigens, etc., which have been widely used for treating a number of tumors.



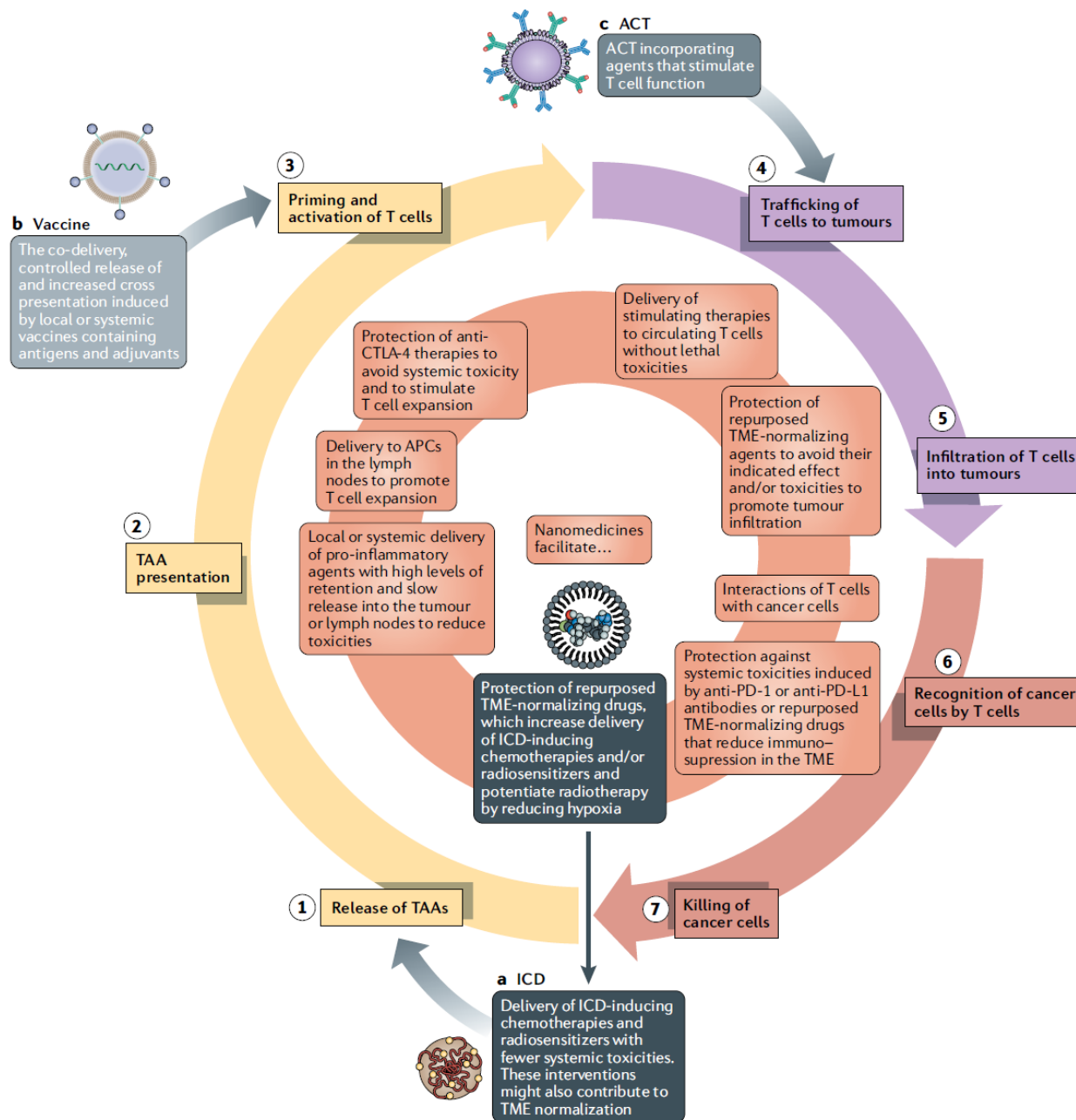
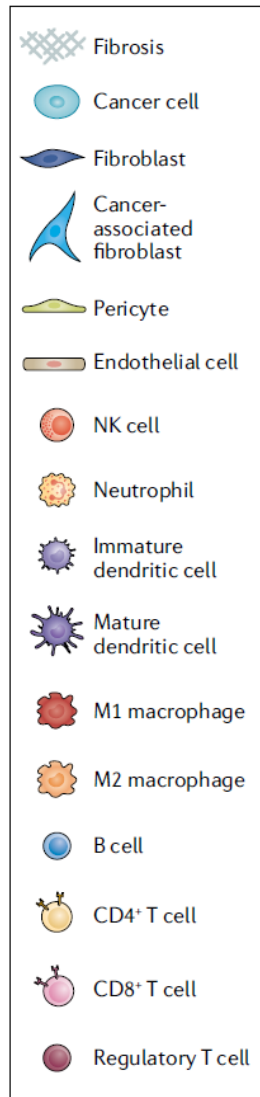
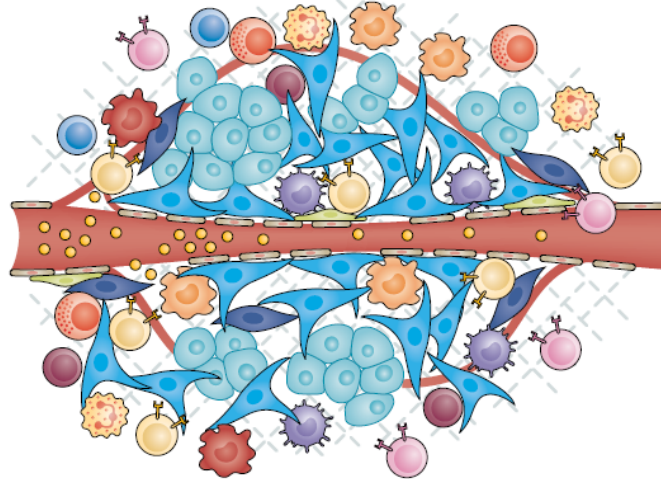


Fig. 2 | **How nanomedicines can be used to perpetuate the cancer-immunity cycle.** The goal of nanomedicine-based immunotherapy is to ensure that the cancer-immunity cycle (the seven numbered steps) perpetuates. Initially, in order to ensure that T cells are capable of attacking cancer cells, we highlight three nanomedicine-based starting points: (a) immunogenic cell death (ICD)-inducing therapy, (b) vaccines and (c) nanoparticle-loaded T cells.

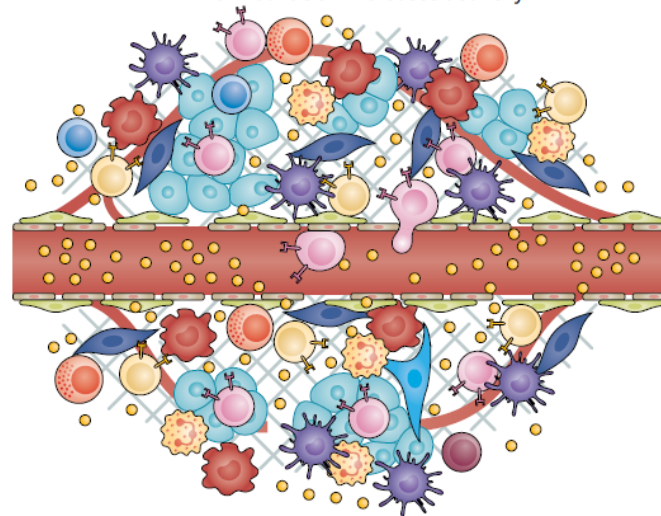


The TME compromises delivery



Targeted, functionalized and/or combination nanomedicines (such as those responding to pH, temperature, heat, or sound waves) to enhance intratumoural drug distribution are compromised by the TME

TME normalization increases delivery



TME normalization increases nanomedicine distribution

Targeted, functionalized and/or combination nanomedicines widely distribute throughout tumour lesion

Vaccine or ACT

TME normalization increases immune cell distribution

Enhancing cancer immunotherapy with nanomedicine

Darrell J. Irvine^{1,2,3,4,5*} and Eric L. Dane¹

NATURE REVIEWS | IMMUNOLOGY

VOLUME 20 | MAY 2020 | 321

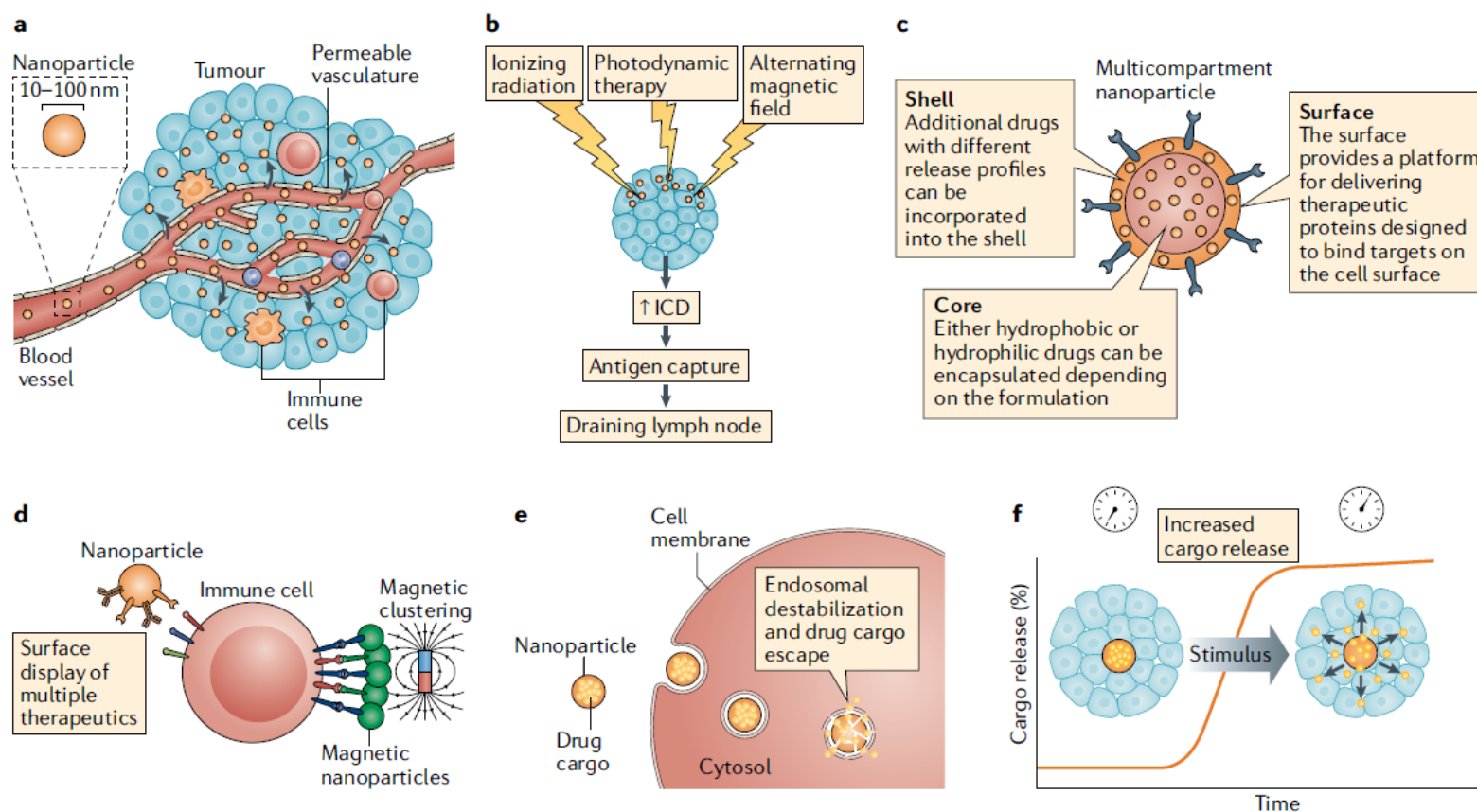


Fig. 1 | Nanomedicines allow unique modes of action in immunotherapy. **a** | Nanomedicines, such as nanoparticles, accumulate within tumours via the enhanced permeation and retention effect, concentrating the drug in tumour sites. **b** | Nanoparticles can be designed to interact with external energy sources, such as ionizing or non-ionizing radiation or magnetic fields to enhance immunogenic cell death (ICD). **c** | Nanomedicines allow combinations of therapeutics, including drugs with very different properties, to be co-delivered to tumour sites. **d** | Multiple ligands can be arrayed on the surfaces of polymers and nanoparticles to enhance engagement of immunostimulatory receptors. **e** | Nanoparticles can be formulated to destabilize endosomal membranes and promote drug delivery into the cytosol. **f** | Nanoparticles allow control of the kinetics of drug release, either preprogrammed through the particle chemistry or through responsiveness to an external stimulus such as light or heat.

Table 1 | **Clinical translation of cancer immunotherapy nanomedicines**

Developer	Concept	Indications	Clinical stage	ClinicalTrials.gov identifiers	Refs
NanoBiotix	Metal nanoparticle radioenhancers in combination with checkpoint blockade	Various solid tumours	Phase I–III	NCT03589339	25–27
Oslo University Hospital/Bristol-Myers Squibb	Pegylated liposomal doxorubicin in combination with checkpoint blockade	Metastatic breast cancer	Phase IIb	NCT03409198	16
Nektar Therapeutics	Reversibly pegylated IL-2 in combination with checkpoint blockade	Various solid tumours	Phase I, phase II	NCT02983045, NCT03138889, NCT03282344, NCT03635983, NCT03785925, NCT03729245, NCT03435640	81–83
Exicure	Intratumoural administration of TLR9 agonist-functionalized nanoparticles in combination with checkpoint blockade	Various solid tumours	Phase Ib/II	NCT03684785	49,50
Torque Therapeutics	Nanoparticle-functionalized antigen-primed T cell therapy	Various solid tumours and lymphomas	Phase I	NCT03815682	113,115,120
Rimo Therapeutics	Metal–organic framework nanoparticles as radioenhancers combined with IDO inhibitors and/or checkpoint blockade	Various solid tumours	Phase I	NCT03444714	29
Coordination Pharma	Nanoscale coordination polymer-based particles	Various solid tumours	Phase I	NCT03781362, NCT03953742	35–37
Moderna Therapeutics	Lipid nanoparticle-delivered mRNA encoding OX40L, IL-23 and IL-36γ with or without checkpoint blockade	Relapsed or refractory solid tumour malignancies or lymphoma	Phase I	NCT03739931	53
Moderna Therapeutics	Lipid nanoparticle-delivered mRNA encoding OX40L	Relapsed or refractory solid tumour malignancies or lymphoma	Phase I	NCT03323398	53
OncoNano	STING-activating polymer micelles	TBD	Phase I projected 2020–2021	NA	48,142
Tidal Therapeutics	Nanoparticles for gene transfer to macrophages and lymphocytes	TBD	Phase I projected 2020–2021	NA	127,128

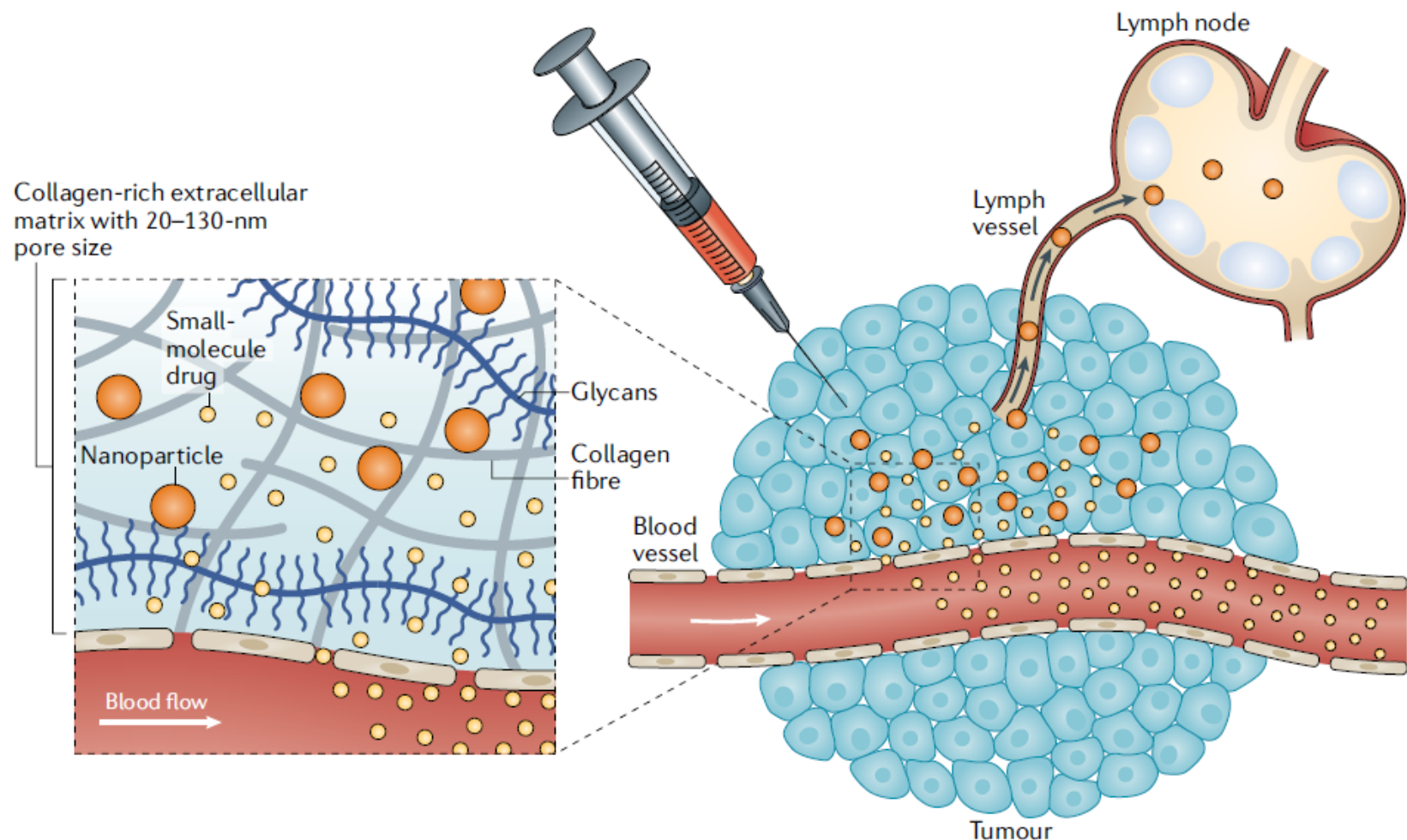


Fig. 2 | Nanomedicines improve tumour retention and lymph node trafficking. When nanomedicines are administered locally, the tumour's dense extracellular matrix, composed of a collagen-rich hydrogel with a pore size of 20–130 nm, preferentially retains nanomedicines and promotes their trafficking to the lymph node, whereas small-molecule drugs are rapidly cleared into the systemic circulation due to their small size and the high interstitial fluid pressure in tumours.

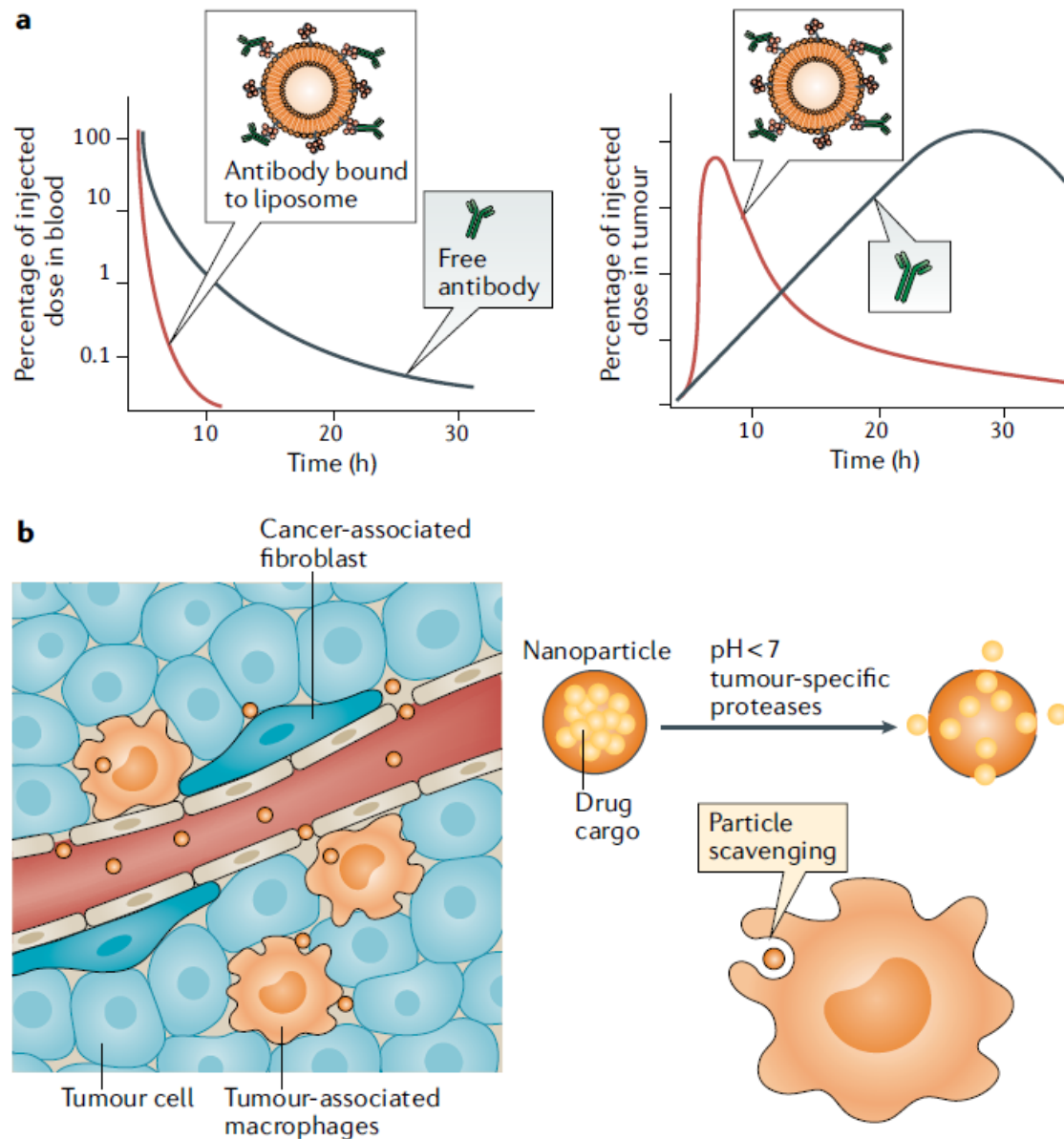


Fig. 3 | Systemic targeting of tumours by intravenously administered nanomedicines. a | Nanoparticles alter the pharmacokinetics of immunomodulatory drugs in a manner that can increase safety and efficacy. Illustrated is the case of an

Regulating trained immunity with nanomedicine

VOLUME 7 | JUNE 2022 | 465

NATURE REVIEWS | MATERIALS

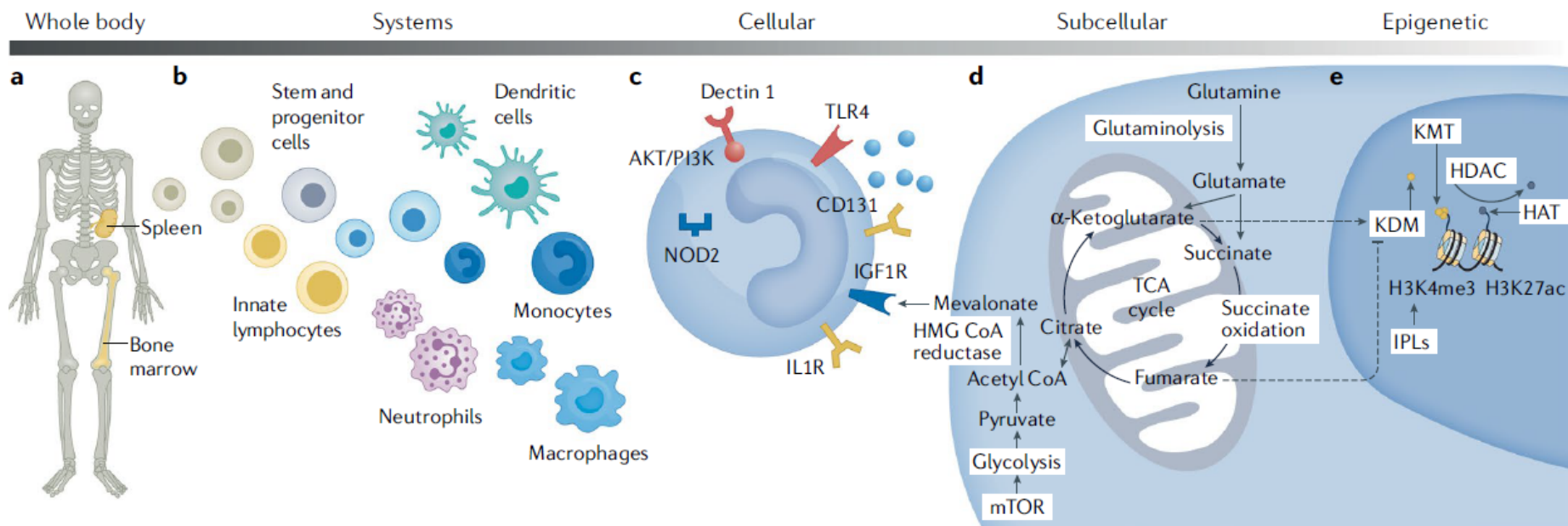
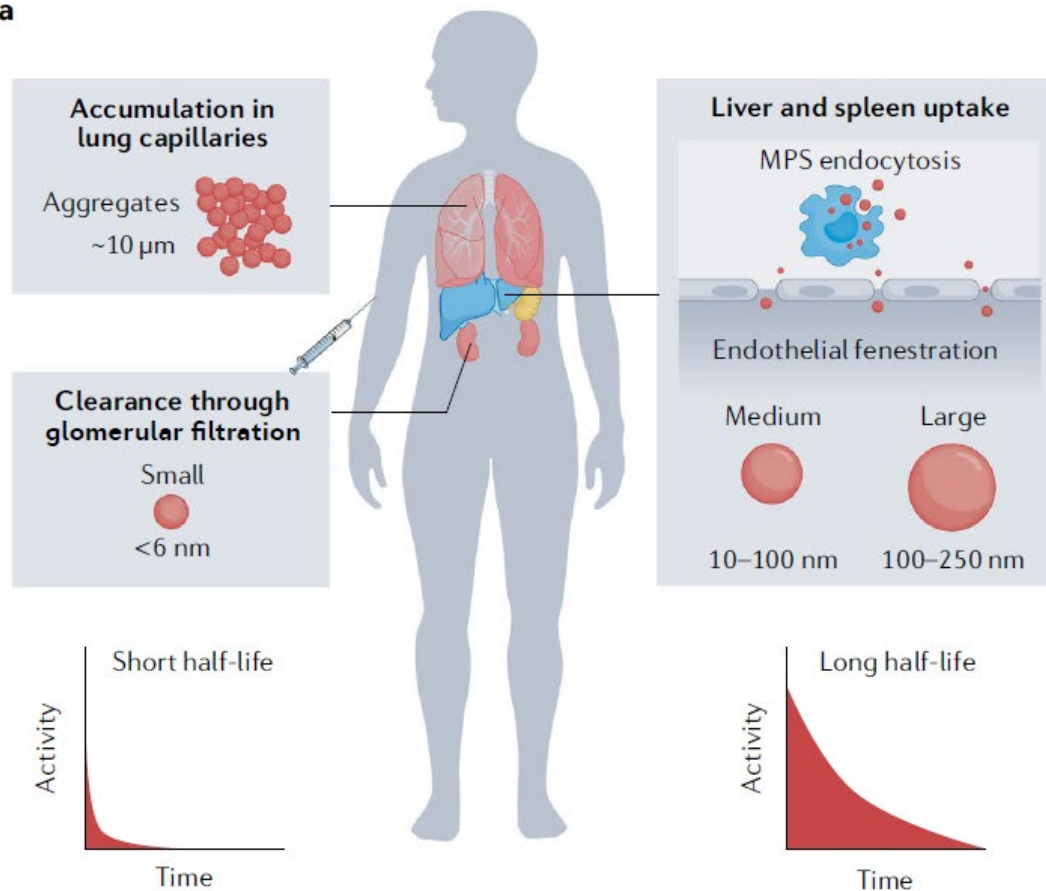
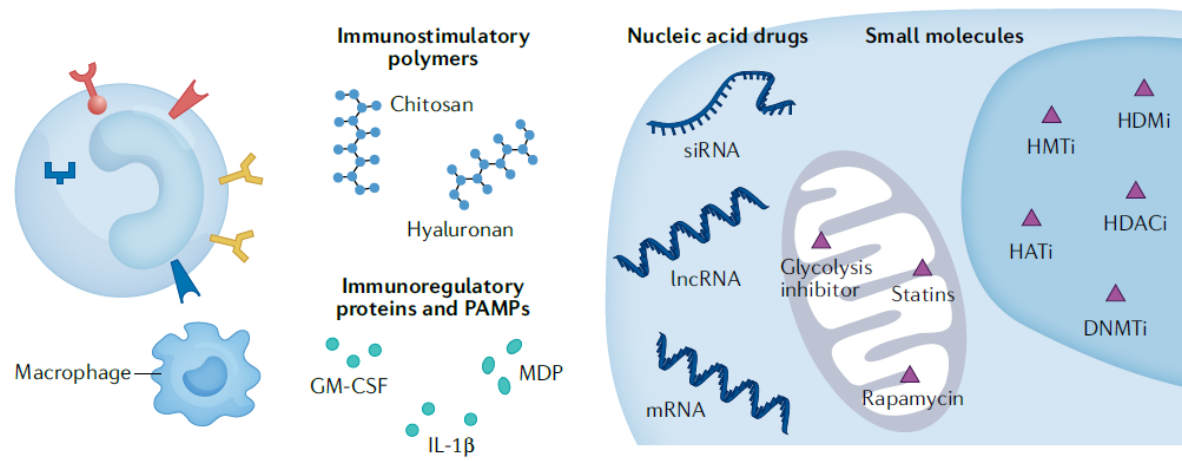
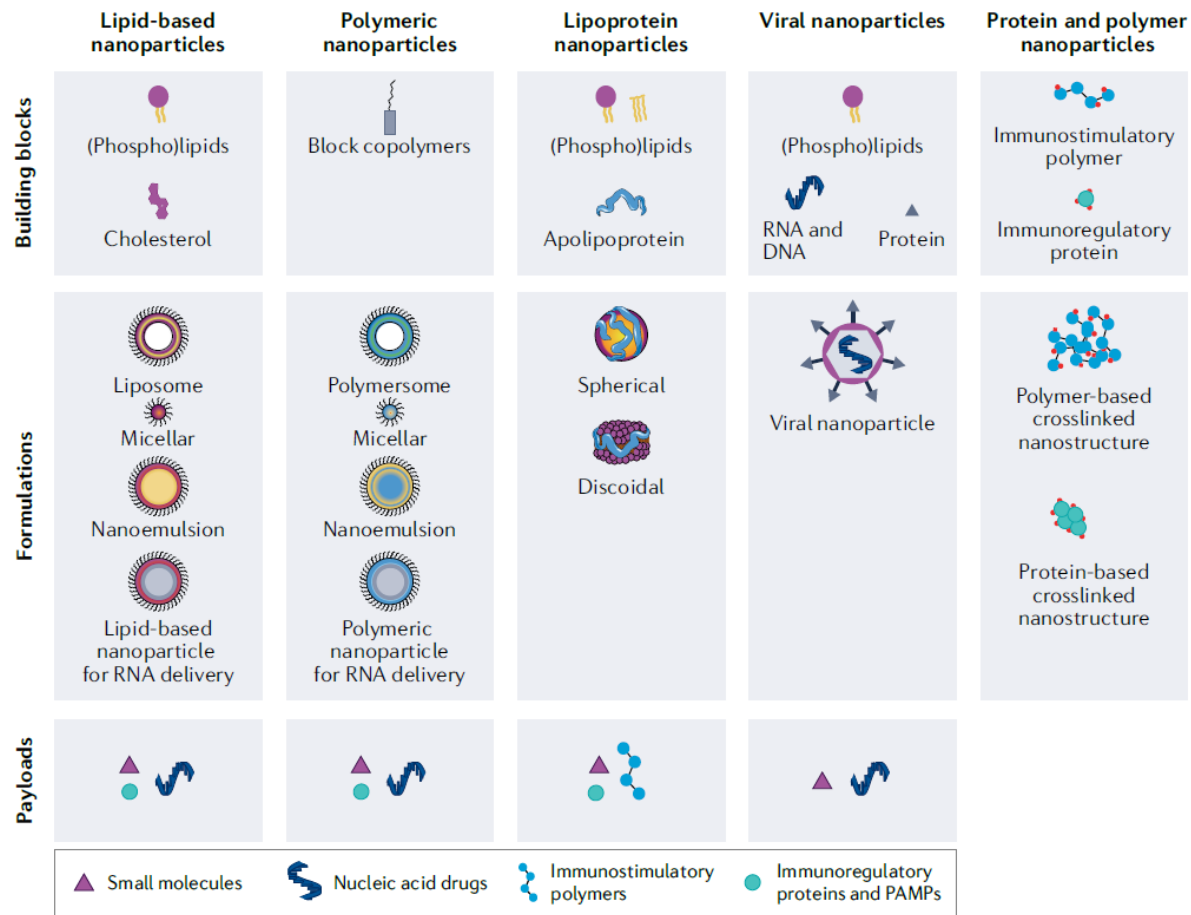
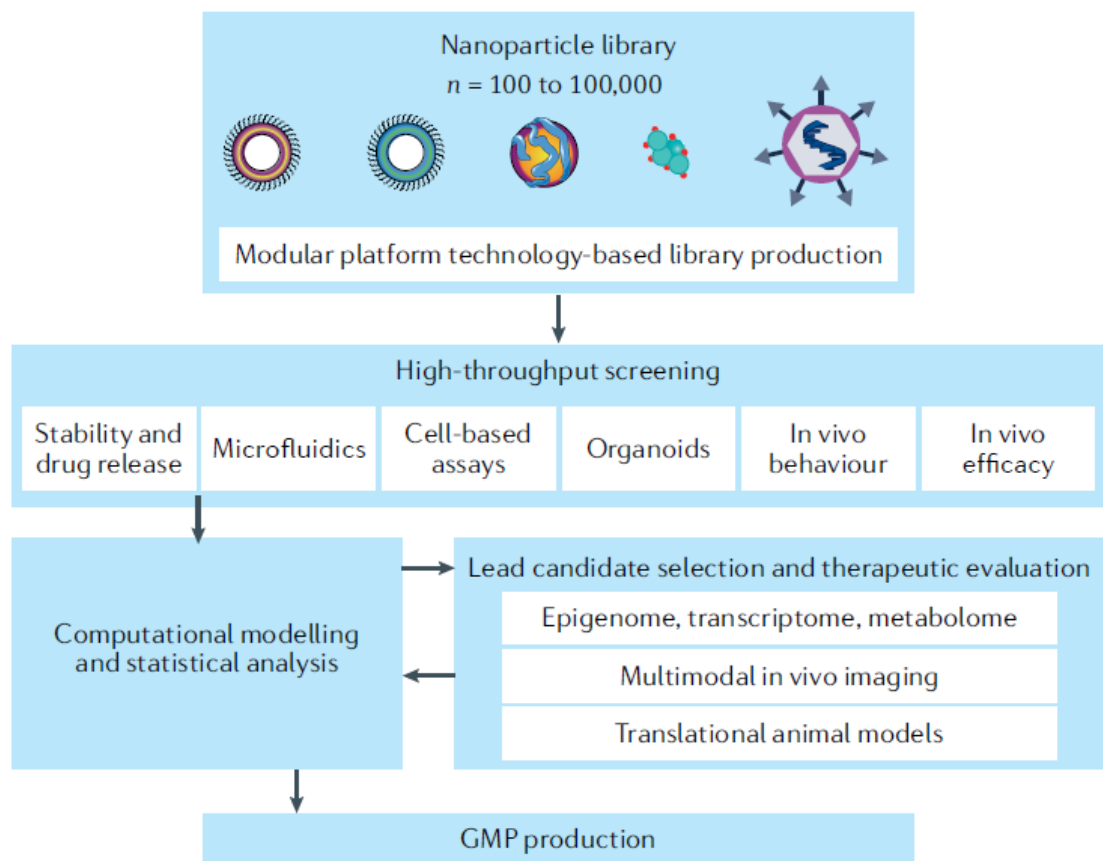


Fig. 1 | Trained immunity targeting levels. **a** | The spleen and bone marrow are important target organs, because they produce and contain large numbers of innate immune cells. **b** | Mature innate immune cells (innate lymphocytes, dendritic cells, monocytes, neutrophils and macrophages) and haematopoietic stem and progenitor cells can be targeted to prevent or enhance trained immunity. **c** | Pattern recognition receptors play an important part in trained immunity. Examples include dectin 1, Toll-like receptor 4 (TLR4) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2). These receptors recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). CD131 is the common β -subunit of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) receptors. The IL-1 receptor (IL-1R)

binds to IL-1 β . Insulin-like growth factor 1 receptor (IGF1R) recognizes extracellular mevalonate. **d** | Intracellular metabolic pathways that can be targeted include glycolysis⁴ (through interference with glycolytic enzymes or indirect through mechanistic target of rapamycin (mTOR) inhibition), cholesterol metabolism⁵⁴ (by targeting HMG CoA reductase), glutaminolysis⁶⁰ (through glutaminase inhibitors) and the tricarboxylic acid cycle (TCA) cycle (for example, by restricting succinate oxidation). **e** | H3K4me3 and H3K27ac are hallmark epigenetic signatures of trained immunity, which can be modified by targeting lysine demethylase (KDM), lysine methyltransferase (KMT), histone deacetylase (HDAC) and histone acetyltransferase (HAT) activity. Immune gene-priming long non-coding RNAs (IPLs) facilitate trimethylation of cytokine promoters⁶⁶. PI3K, phosphatidylinositol 3-kinase.

a**b**





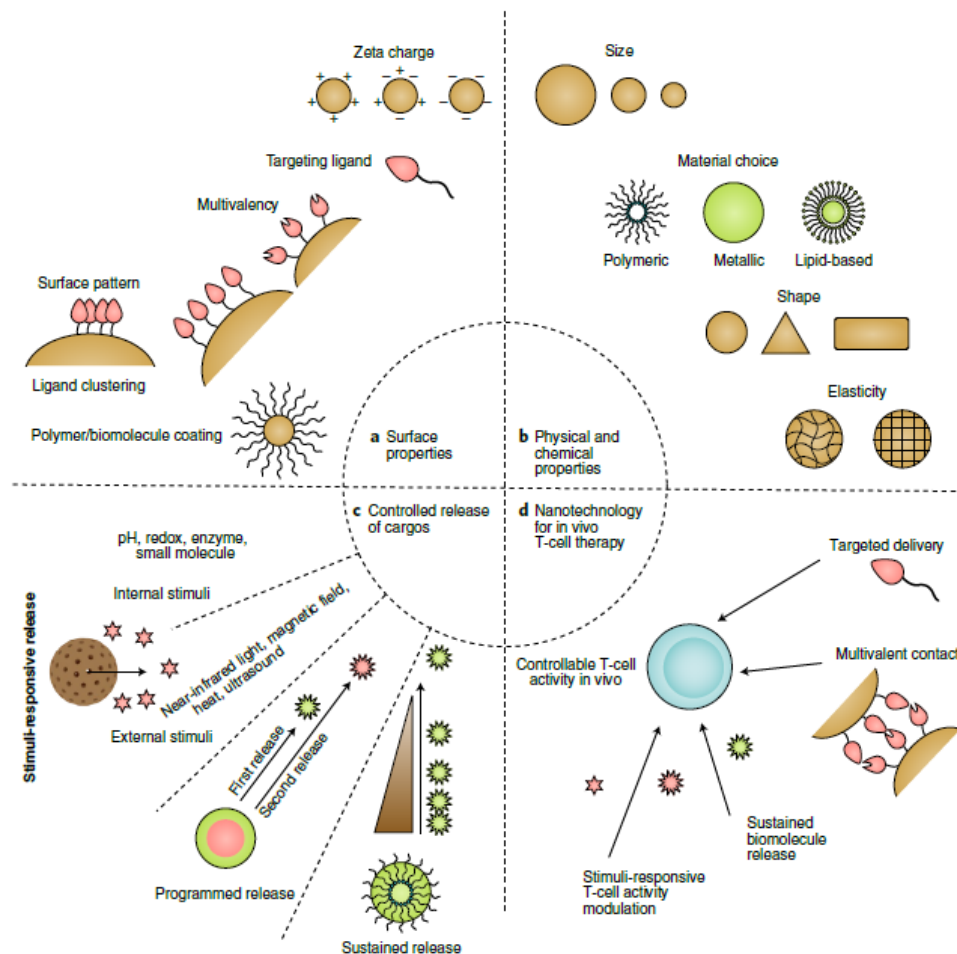


Fig. 2 | The current nanomaterial toolbox can be applied to in vivo T-cell therapies. a-c, Current strategies for expanding the functionalities of nanotechnologies include surface characteristics (a), physicochemical properties (b) and encapsulation and release features (c) of nanomaterials. **d,** Nanomaterials with optimized features could greatly benefit future T-cell cancer immunotherapies in vivo.

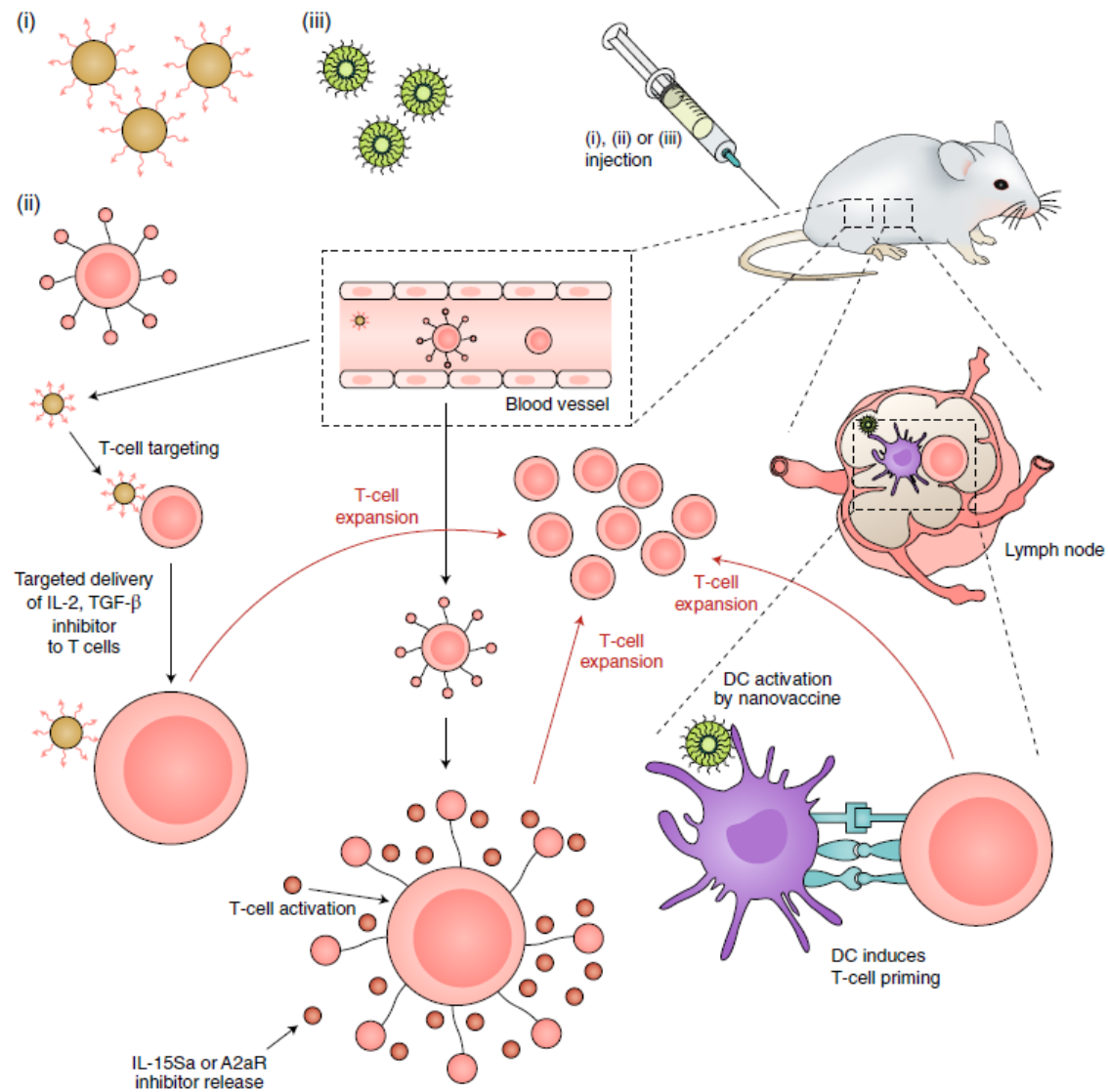


Fig. 3 | Nanomaterials for in vivo T-cell expansion. Nanomaterials can be designed for targeted delivery to T cells and induce T-cell activation and expansion in vivo (i). Backpacking nanoparticles are attached to the T-cell surface and release their cargo of stimulatory cues in response to environmental or applied stimuli, leading to precise control over the expansion of T cells in vivo (ii). Vaccine nanoparticles that target antigen-presenting cells, such as DCs, can activate these cells and induce T-cell expansion in vivo (iii).

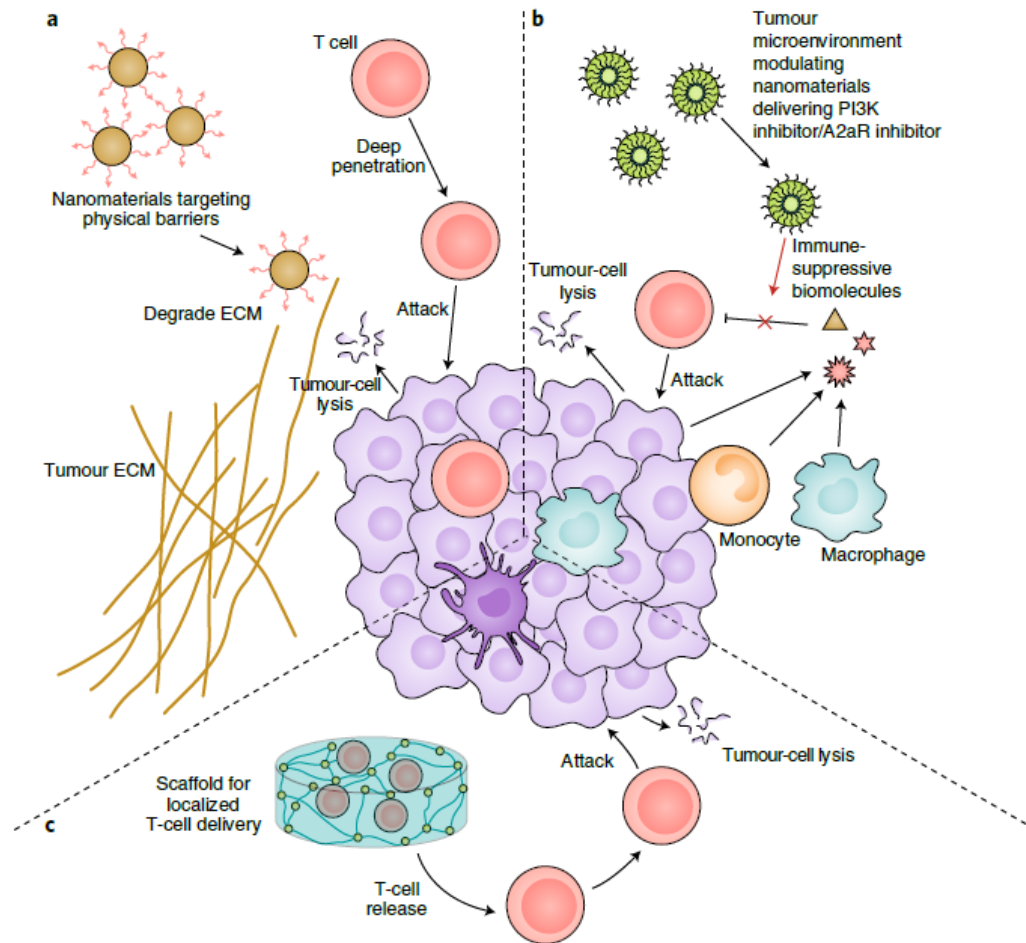


Fig. 4 | Nanomaterials overcome physical barriers and immune-suppressive environments for T-cell therapy. **a**, Nanomaterials can be designed to target the ECM and degrade the physical barriers inhibiting T-cell penetration and tumour cell targeting. **b**, Nanomaterials targeting the tumour microenvironment can deliver stimulatory cues to the tumour tissue and reverse the suppressive tumour microenvironment (immunological barrier), thus activating T-cell activity. **c**, Nanomaterials can locally deliver T cells directly to the tumour tissue with sustained release, which enhances tumour cell killing.

Table 2 | Preclinical and clinical studies of nanomaterials-based T-cell cancer immunotherapies

Nanomaterials	Cargo molecules	Model/indication	Stage
Nanomaterials for T-cell expansion in vivo	T-cell-targeted delivery		
	Poly(beta-amino ester)-based nanomaterial	Plasmids encoding a 194-1BBz CAR and a piggyBac transposase	TBD Phase 1 projected 2020–2021
	Liposome	IL-2-Fc fusion protein	Mouse melanoma Preclinical
	Liposome	TGF- β inhibitor (SB525334)	Mouse melanoma Preclinical
	PLGA-PEG nanomaterial	TGF- β receptor inhibitor (SD-208)	Mouse colon cancer Preclinical
	T-cell (Treg)-targeted hybrid nanomaterial	STAT3/STAT5 pathway inhibitor (imatinib)	Mouse melanoma Preclinical
	Iron nanomaterial	Anti-CD137 and anti-PD-L1	Mouse melanoma Preclinical
	Liposome-coated polymeric gel	Mouse IL-2 and a TGF- β inhibitor (SB505124)	Mouse melanoma Preclinical
	Backpacking nanomaterials		
	IL-15 superagonist complex nanogel	IL-15 superagonist complex	Various solid tumours and lymphomas Phase 1
	Multilamellar liposomal vesicles	A2a adenosine receptor inhibitor (SCH-58261)	Mouse model of human ovarian cancer Preclinical
	Nanomaterials-based vaccines		
	Amphiphile ligands (EGFRvIII peptide-conjugated DSPE-PEG)	NA	Mouse glioma expressing EGFRvIII ⁺ Preclinical
	Lipid nanomaterial	mRNA encoding the tight junction protein claudin 6 (CLDN6)	Mouse melanoma expressing CLDN6 Preclinical

		junction protein claudin 6 (CLDN6)	expressing CLDN6		
Nanomaterials overcome physical barriers and hostile tumour microenvironments	Nanomaterials that target physical barriers				
	PLGA nanomaterial	Photothermal agent indocyanine green	Mouse melanoma	Preclinical	104
	Calcium phosphate nanomaterials with lipid bilayer coating	An antifibrotic compound α -mangostin and a plasmid encoding the stimulatory cytokine LIGHT	Mouse pancreatic cancer	Preclinical	59
	Nanomaterials that reverse the immune-suppressive environment				
	Lipid nanomaterial	A PI3K inhibitor (PI-3065) and a T-cell stimulator (7DW8-5)	Mouse breast cancer	Preclinical	61
	Multilamellar liposomal vesicles	A2a adenosine receptor inhibitor (SCH-58261)	Mouse model of human ovarian cancer	Preclinical	93
	Nanomaterials for local T-cell delivery				
	Macroporous alginate scaffolds	IL-15 superagonists, antibodies for CD3, CD28 and CD137	Mouse breast cancer, mouse ovarian cancer	Preclinical	60
Nanomaterials as NBiTEs	Nickel-titanium alloys	Antibodies for CD3, CD28, CD137	Mouse model of human pancreatic cancer expressing receptor tyrosine kinase-like orphan receptor (ROR1)	Preclinical	111
	Liposome	Human epidermal growth factor receptor 2 (HER2) and CD20 antibodies	Mouse breast cancer	Preclinical	131
	Polystyrene nanomaterial	Antibodies for HER2 and calreticulin protein	Mouse breast cancer	Preclinical	132
	Exosome	Exosome expressing antibodies for CD3 and epidermal growth factor receptor (EGFR)	Mouse breast cancer	Preclinical	58
TBD, to be determined; NA, not applicable; LIGHT, tumour necrosis factor superfamily 14.					

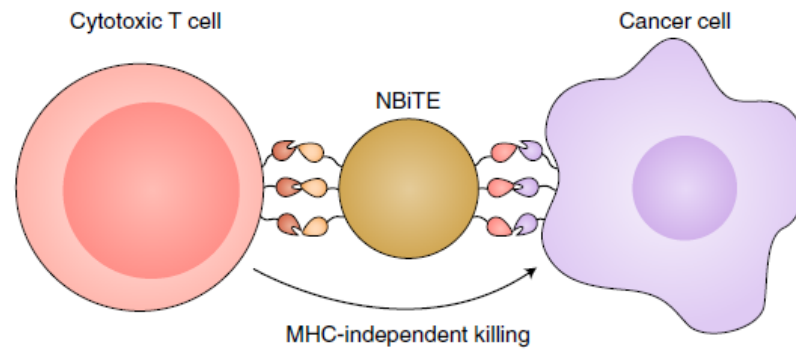


Fig. 5 | NBIETs for cancer immunotherapy. A typical NBIET is developed by adding two scFvs on the nanoparticle surface, with one scFv targeting a T-cell-specific antigen while the other targets a tumour-specific antigen. The multivalent contact at the nanomaterial/cell interfaces makes NBIETs bridge T cells and tumour cells more effectively than traditional BiETs and induces potent tumour cell killing.

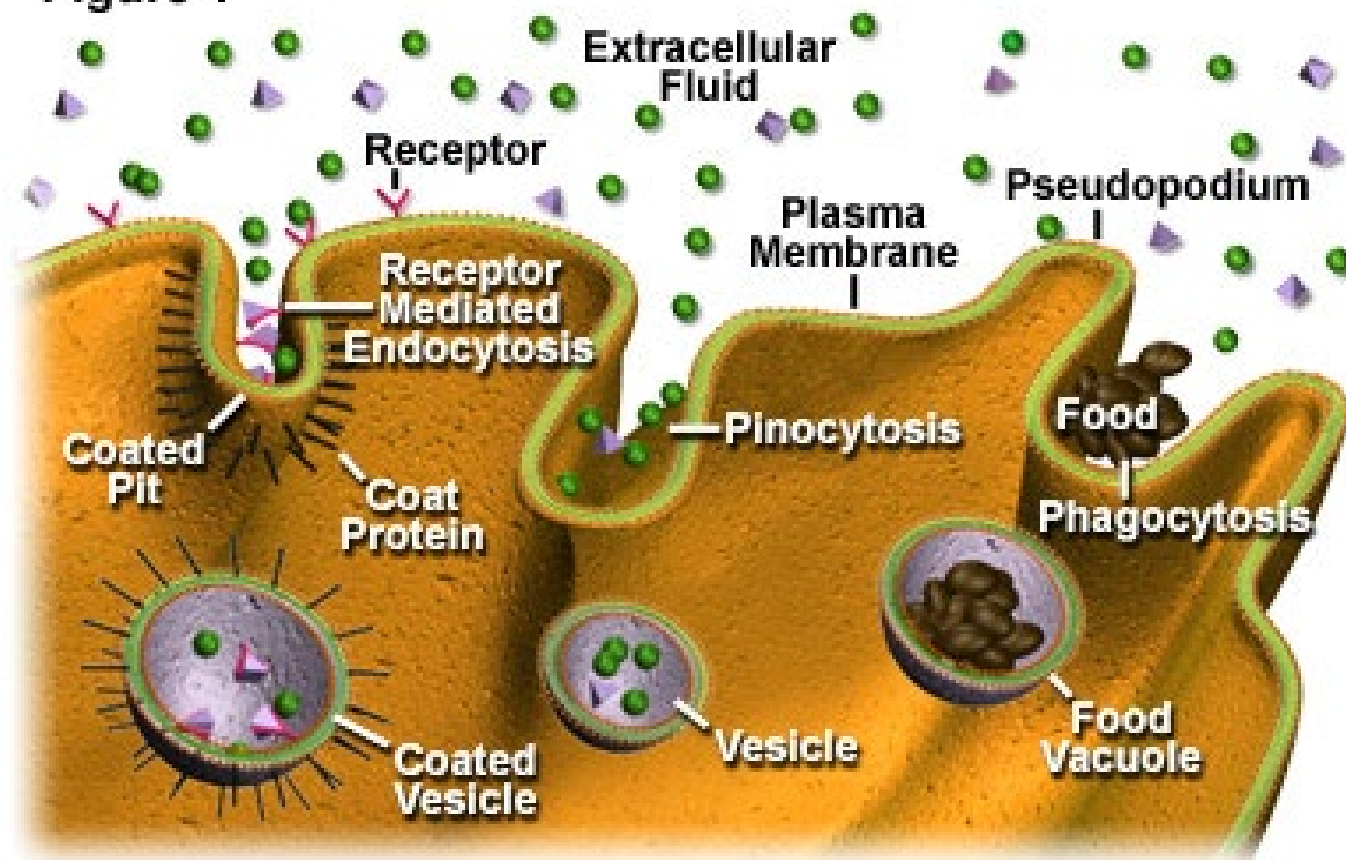
Gene Delivery

- Transfection- the delivery of foreign molecules such as DNA and RNA into eukaryotic cells
- Naked DNA is not suitable for in-vivo transport of genetic materials-> degradation by serum nucleases
- Ideal gene delivery system
 - Biocompatible
 - Non-immunogenic
 - Stable in blood stream
 - Protect DNA during transport
 - Small enough to extravagate
 - Cell and tissue specific

Endocytosis

Figure 1

Endocytosis in Animal Cells



Endocytic pathway in mammalian cells

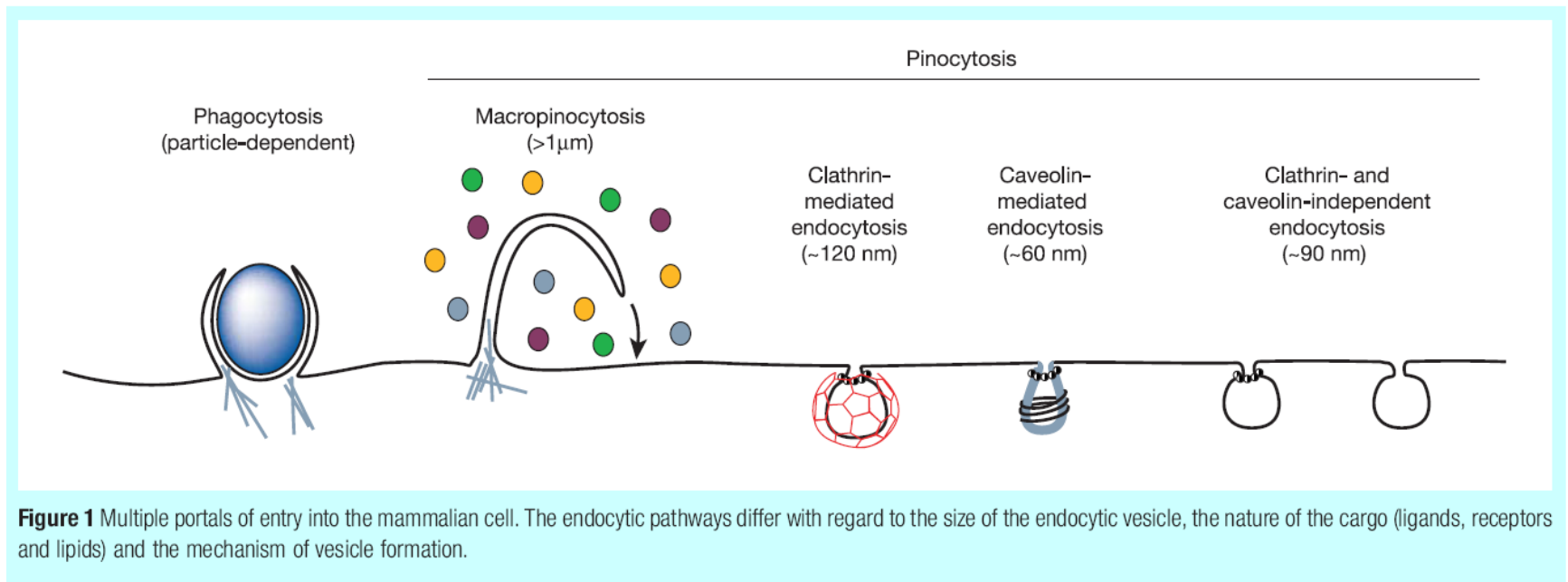


Figure 1 Multiple portals of entry into the mammalian cell. The endocytic pathways differ with regard to the size of the endocytic vesicle, the nature of the cargo (ligands, receptors and lipids) and the mechanism of vesicle formation.

Barrier to non-viral gene delivery

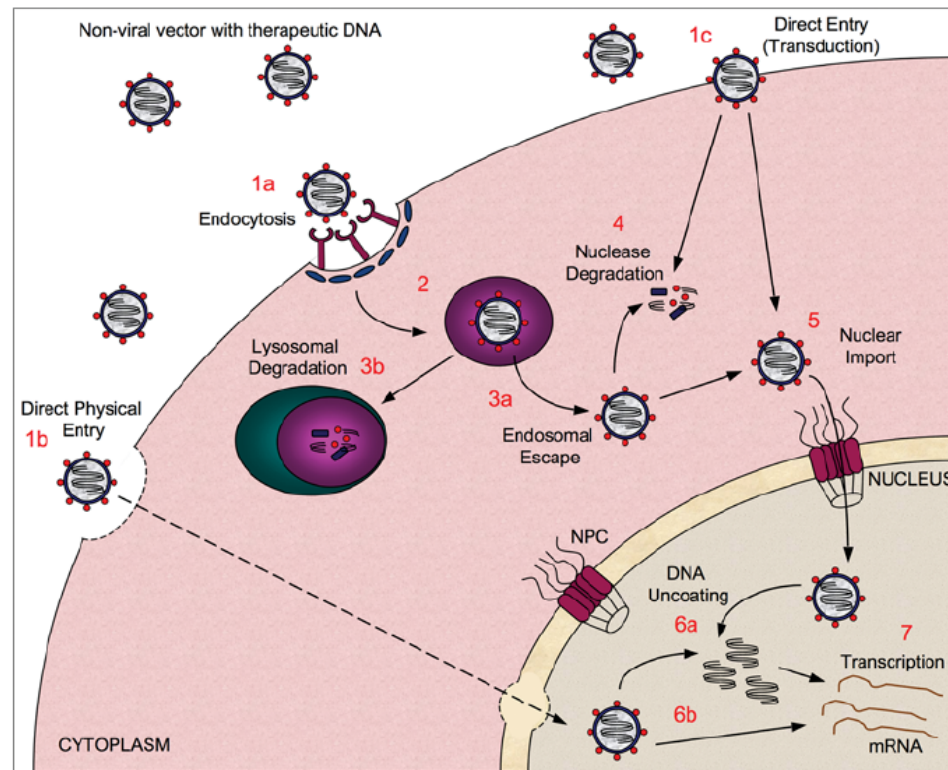


Figure 1 Barriers to non-viral gene delivery

Representation of the route travelled by a non-viral gene-delivery vector carrying therapeutic DNA to the nucleus. A non-viral vector, formed by interaction of the DNA with a carrier compound, must cross the plasma membrane to enter the cell. This can be via several routes, including endocytosis-based entry (1a), direct physical entry routes, such as electroporation or ballistic delivery (1b), or direct entry via protein transduction (1c). Depending on the mode of cellular entry, the vector may become encapsulated in an endosome (2), from which it must escape (3a) or it will become degraded when the endosome fuses with a lysosome (3b). The DNA will at some point be subjected to degradation by cytosolic nucleases (4), as it traverses through the cytoplasm to reach the nucleus. Finally, the vector must undergo nuclear transport (5) through NPCs embedded in the NE in order to gain access to the nucleoplasm. Once in the nucleus, the DNA may (6a) or may not (6b) need to be uncoated, depending upon the vector used, before it can ultimately be transcribed (7).

NLS-mediated nuclear import

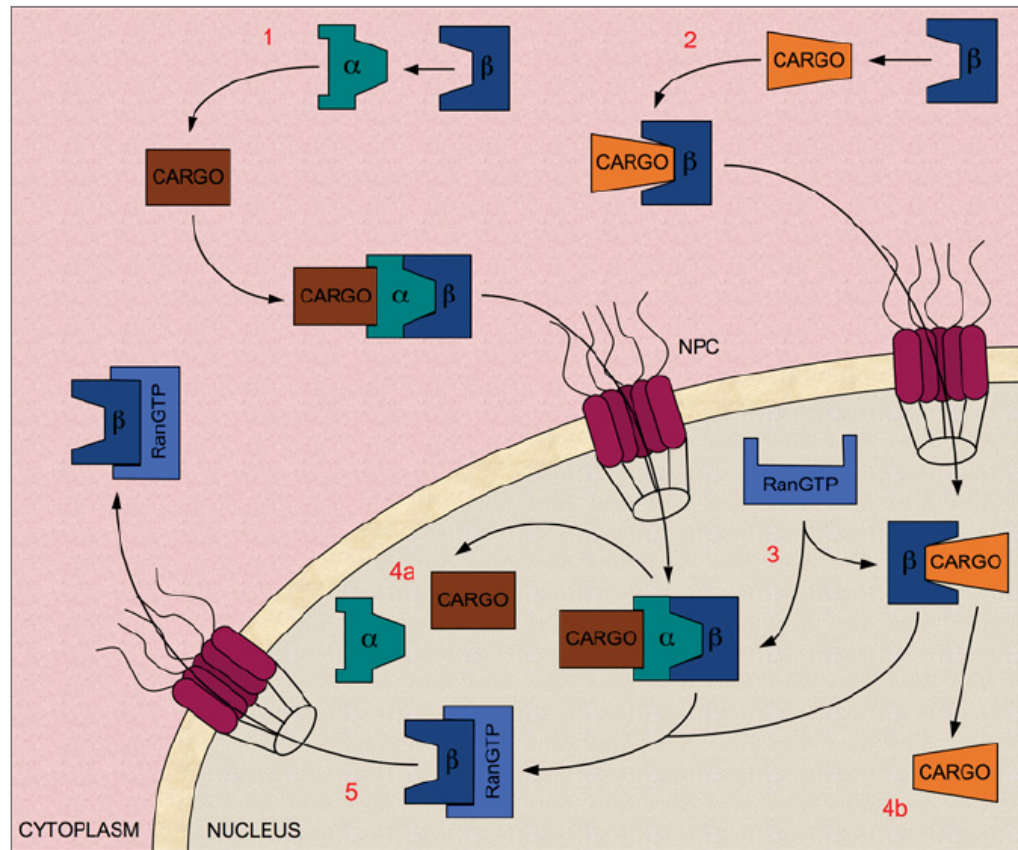


Figure 2 NLS-mediated nuclear import pathways

In classical nuclear import, the NLS found in cargo bound for the nucleus is recognized by the Imp α subunit of the Imp α/β heterodimer (1). However, there are also many examples where Imp β or one of its many homologues can mediate nuclear import or cargo proteins independently of Imp α (2). In both cases, transient interactions between the Imp β and the nucleoporin proteins that line the NE-embedded NPCs mediate translocation into the nucleus. Once inside, RanGTP binds to Imp β (3), releasing Imp α and the cargo into the nucleoplasm (4a and 4b). RanGTP itself is then recycled back to the cytoplasm (5), where it is converted into its RanGDP state (not shown). An animated version of this Figure can be found at <http://www.BiochemJ.org/bj/406/0185/bj4060185add.htm>

Barriers to DNA Delivery

BOX 1

A number of challenges and barriers face the successful delivery of therapeutic DNA to target cells in the body. Physicochemical, economic and sterilization challenges complicate formulation; the complex environment of the human body hinders its successful transport to the target cell population; and endocytic pathway barriers hinder its successful transport to the nucleus of the cell (the site of action). Each known and major barrier is listed in Fig. B1, using nanoscale DNA-delivery systems as representative examples. Each barrier exists independent of length scale. L = lysosome. A number of clever systems have been devised to overcome these barriers, the general design criteria of which are given in Tables B1 and B2.

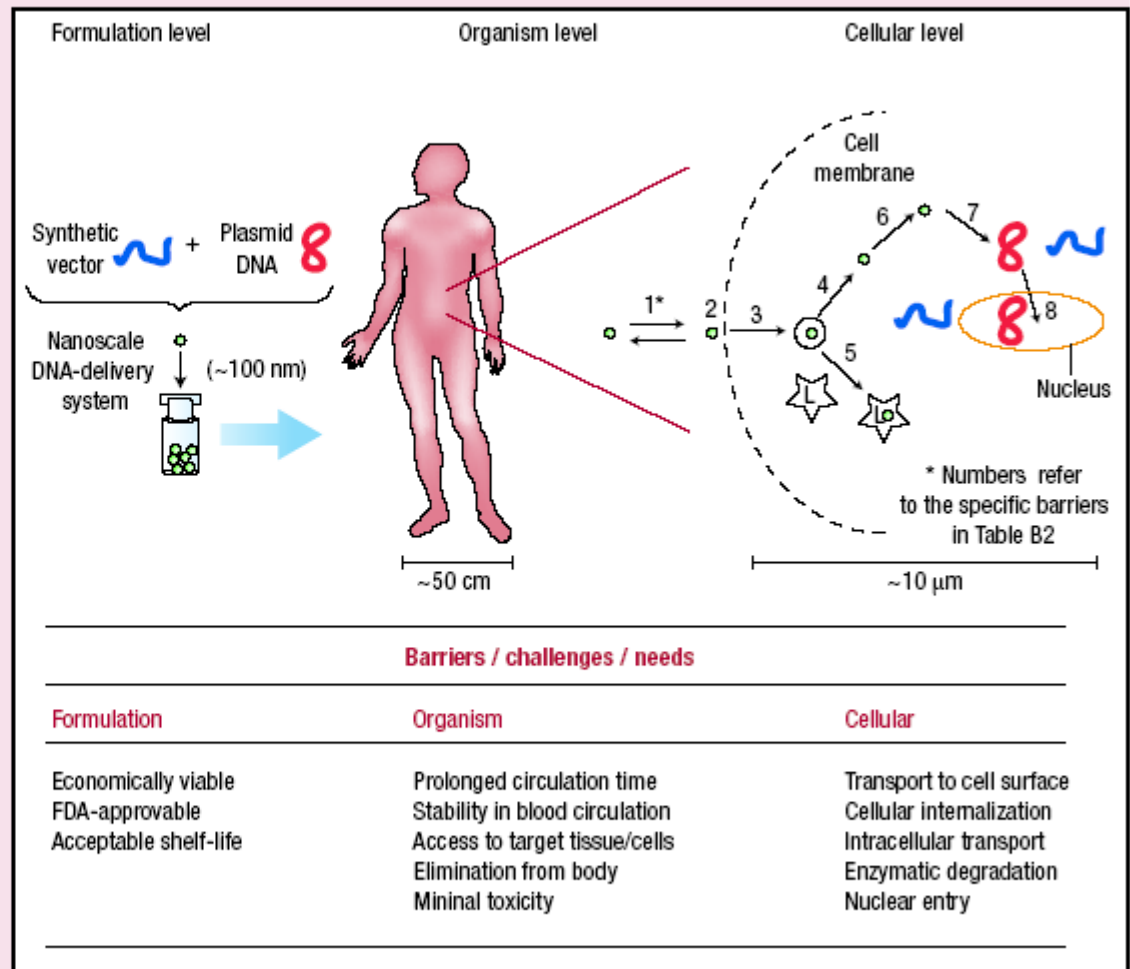


Figure B1 Barriers to DNA delivery.

Organism Level			
Barrier/challenge/need	Rationale	Example approaches	Materials design criteria
Prolonged circulation time	Maximize total flux past target cell type	PEG conjugates to minimize interaction with serum proteins	Hydrophilic Uncharged
Stability within blood circulation	Maintenance of designed functionality	Crosslinking to maximize overall stability	Stable crosslinks within bloodstream, but reversible upon entry into target cell
Access to target tissue/cells	Transport from capillary lumen to extracellular space to reach target cell surface	Vaso-active protein conjugates (for example, vascular endothelial growth factor)	Retention of protein activity post conjugation
		Targeting restricted to 'leaky' vessel tissues (for example, tumour, liver, spleen).	Small diameter delivery system (for example, <100 nm)
Elimination from body	Minimal build-up of delivery vector over time	Control over molecular weight	Filterable through kidneys
		Engineered biodegradation sites	Biodegradable
Minimal toxicity and immunogenicity	Safety over treatment duration and beyond that required for FDA-approval	Minimize cation density	Non-cytotoxic
		Avoid protein-based materials/conjugates	Non-immunogenic

Cellular Level

Barrier number (from Fig. B1)	Barrier/challenge/need	Example approaches	Materials design criteria
1, 2 and 3	Transport to cell surface, association with cell membrane, internalization	<p>Receptor/ligand interaction (for example, antibody/polymer conjugates, recombinant protein–polymer fusions, carbohydrate conjugates)</p> <p>Non-specific interaction with cell surface (for example, positive zeta potential, lipid conjugates)</p>	<p>Cell-type specificity, low cross reactivity, if desired</p> <p>Promiscuous attachment, high cross reactivity, if desired (for example, positive zeta potential, lipid conjugation)</p> <p>Endocytic pathway trigger (for example, clathrin-dependent, clathrin-independent, caveolin-dependent)</p>
4 and 5	Escape endosomal vesicle and avoid transport to lysosome	<p>Buffering capacity between pH ~7.2 and ~5.0</p> <p>Fusogenic peptide conjugate</p>	<p>Ability to disrupt endosomal membrane and/or fusion of endosome with lysosome</p>
6	Transport through cytosol to perinuclear space with minimal degradation	<p>‘Higher’ molecular weight to maintain complex stability within cytosol</p>	<p>Thermodynamic and kinetic stability of complex within cytosol</p> <p>Minimize DNA degradation within cytosol</p>
7	Separation of complex to allow nuclear translocation	<p>Hydrolytically or reductively degradable polymers to reduce molecular weight</p>	<p>‘Triggered’ degradation of polymer to reduce thermodynamic and kinetic stability of complex. Release of intact DNA at or near nuclear envelope</p>
8	Nuclear entry	<p>Nuclear localization sequence conjugates</p> <p>Mitosis</p>	<p>Facilitate nuclear uptake of DNA using virus-derived signals</p> <p>Facilitate nuclear uptake during mitosis when the nuclear envelope is dissolved.</p>

CANCER NANOTECHNOLOGY: OPPORTUNITIES AND CHALLENGES

NATURE REVIEWS | **CANCER** | VOLUME 5 | MARCH 2005 | **161**

Summary

- Nanotechnology concerns the study of devices that are themselves or have essential components in the 1–1,000 nm dimensional range (that is, from a few atoms to subcellular size).
- Two main subfields of nanotechnology are nanovectors — for the administration of targeted therapeutic and imaging moieties — and the precise patterning of surfaces.
- Nanotechnology is no stranger to oncology: liposomes are early examples of cancer nanotherapeutics, and nanoscale-targeted magnetic resonance imaging contrast agents illustrate the application of nanotechnology to diagnostics.
- Photolithography is a light-directed surface-patterning method, which is the technological foundation of microarrays and the surface-enhanced laser desorption/ionization time-of-flight approach to proteomics. Nanoscale resolution is now possible with photolithography, and will give rise to instruments that can pack a much greater density of information than current biochips.
- The ability of nanotechnology to yield advances in early detection, diagnostics, prognostics and the selection of therapeutic strategies is predicated based on its ability to ‘multiplex’ — that is, to detect a broad multiplicity of molecular signals and biomarkers in real time. Prime examples of multiplexing detection nanotechnologies are arrays of nanocantilevers, nanowires and nanotubes.
- Multifunctionality is the fundamental advantage of nanovectors for the cancer-specific delivery of therapeutic and imaging agents. Primary functionalities include the avoidance of biobarriers and biomarker-based targeting, and the reporting of therapeutic efficacy.
- Thousands of nanovectors are currently under study. By systematically combining them with preferred therapeutic and biological targeting moieties it might be possible to obtain a very large number of novel, personalized therapeutic agents.
- Novel mathematical models are needed, in order to secure the full import of nanotechnology into oncology.

Protein Corona

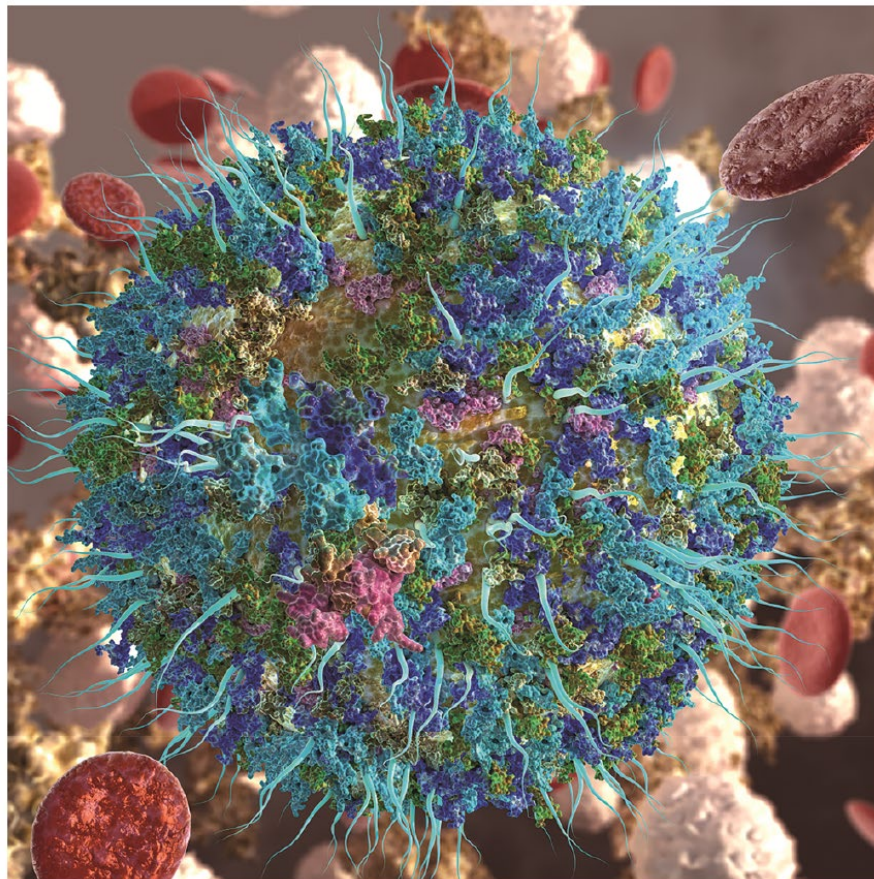


Figure 1. A nanoparticle gains a new biological identity upon its dynamic interactions with biological fluids, giving rise to a protein corona (shown as adsorbed green, blue, and cyan globules), which consequently influences drug delivery and targeting of the functionalized nanoparticle (illustrated as aqua blue fibrils).

The protein corona effect refers to the phenomenon that occurs when nanoparticles are introduced into a biological environment, such as blood or tissue fluids. Upon exposure to these environments, proteins and other biomolecules in the fluid can adsorb onto the surface of the nanoparticles, forming a layer or "corona" around them. This protein corona can significantly impact the physicochemical properties, biological interactions, and the overall fate of the nanoparticles in the body.

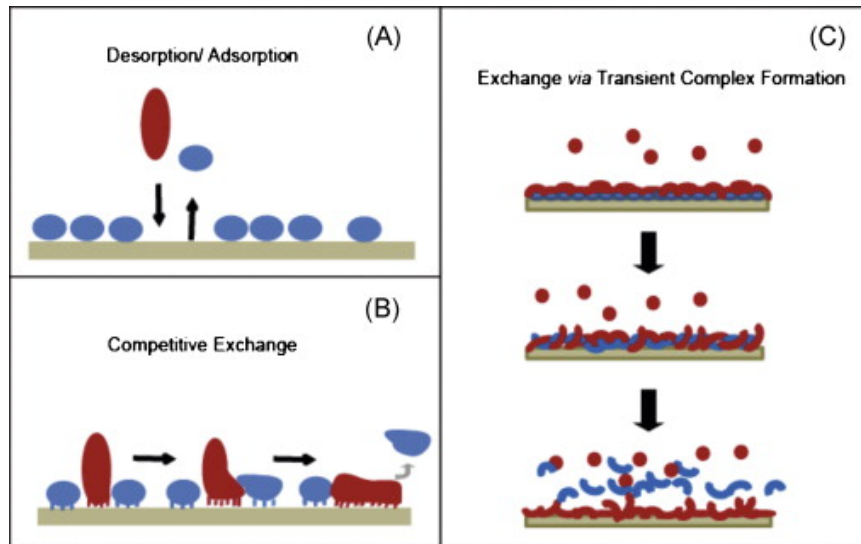
Altered physicochemical properties: The protein corona can change the size, shape, and surface charge of the nanoparticles, which may affect their stability, aggregation, and circulation half-life in the body.

Impact on cellular uptake and targeting: The protein corona can influence how nanoparticles interact with cells, as it may mask the surface ligands or functional groups on the nanoparticles that are intended for targeted delivery. This can lead to a reduced targeting efficiency and unintended uptake by other cells, including immune cells.

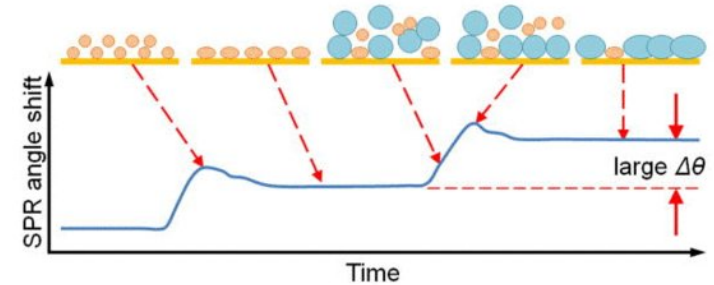
Immunogenicity and toxicity: The presence of a protein corona can potentially trigger an immune response, leading to the clearance of the nanoparticles from the body or causing adverse side effects. The composition of the protein corona may also influence the potential toxicity of the nanoparticles.

Biodistribution and clearance: The protein corona can affect the biodistribution of nanoparticles in the body and their subsequent clearance by altering the recognition and interaction of nanoparticles with various organs and tissues.

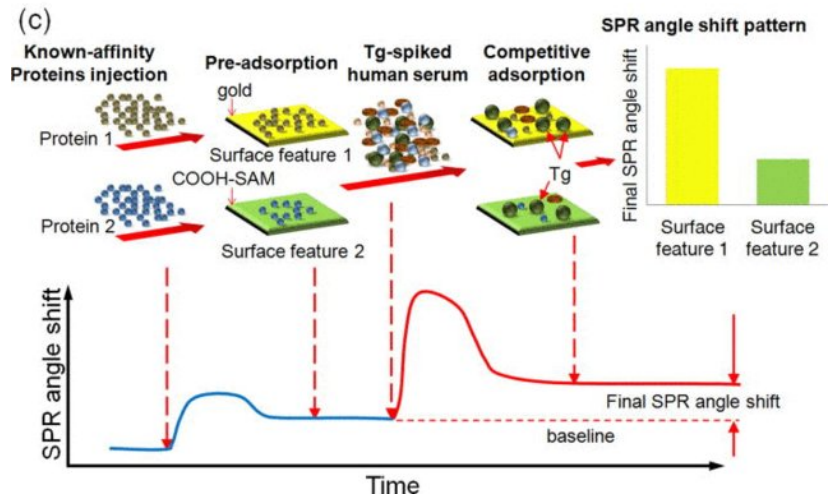
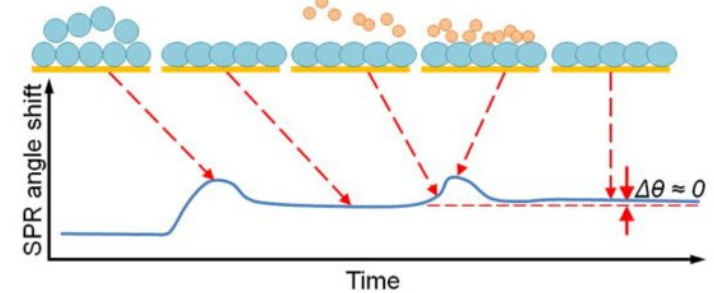
Vroman Effect



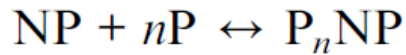
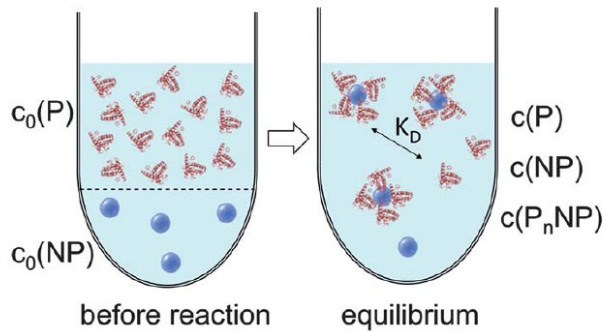
(a) low-affinity proteins \rightarrow high-affinity proteins



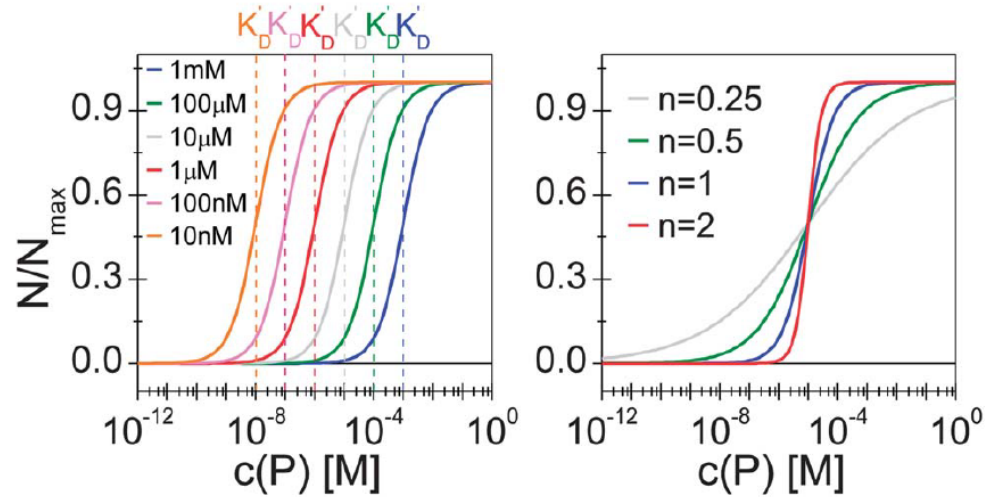
(b) high-affinity proteins \rightarrow low-affinity proteins



Vroman Effect



$$K_D = \frac{c(NP)c^n(P)}{c(P_n NP)} = \frac{k_{\text{off}}}{k_{\text{on}}}$$



The Vroman effect, also known as the Vroman's adsorption-desorption principle, is a phenomenon named after Dr. Leo Vroman, who first described the process in the context of blood-material interactions in the 1960s. The Vroman effect refers to the dynamic exchange of proteins adsorbing onto and desorbing from a surface over time, with higher affinity proteins displacing those with lower affinities. In the context of nanoparticles, the Vroman effect has important implications for understanding the protein corona that forms around nanoparticles in biological fluids.

When nanoparticles are introduced into biological fluids, they rapidly interact with proteins and other biomolecules present in the fluid. Initially, high-abundance proteins with relatively low affinity for the nanoparticle surface may adsorb quickly due to their higher concentration. However, over time, these proteins can be replaced by lower-abundance proteins that have higher affinity for the nanoparticle surface. This dynamic exchange of proteins can lead to changes in the composition of the protein corona over time.

Cellular uptake and targeting: Changes in the protein corona composition can affect the targeting efficiency and cellular uptake of nanoparticles, potentially leading to off-target effects and unintended interactions with immune cells.

Immunogenicity and toxicity: The Vroman effect can influence the immunogenicity and potential toxicity of nanoparticles, as different proteins in the corona can trigger immune responses or cause adverse side effects.

Biodistribution and clearance: The dynamic nature of the protein corona can affect the biodistribution of nanoparticles in the body and their subsequent clearance by altering the recognition and interaction of nanoparticles with various organs and tissues.

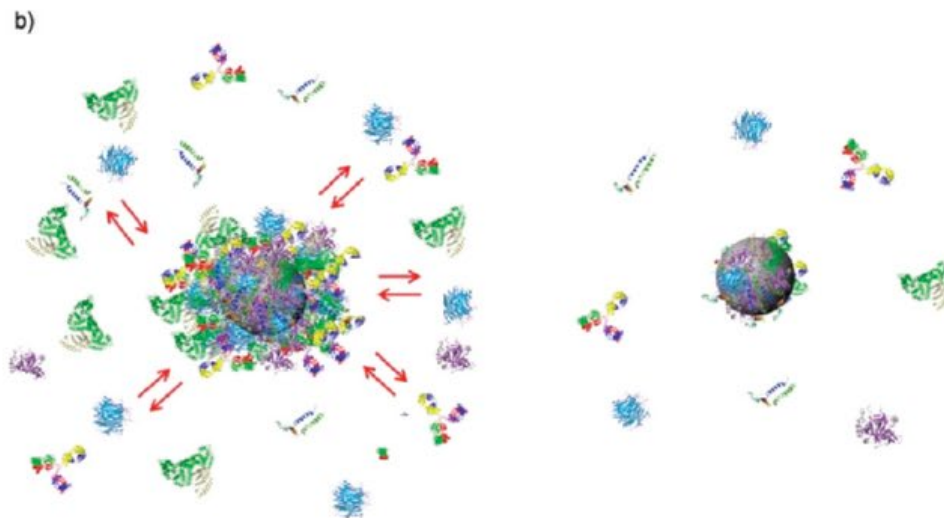
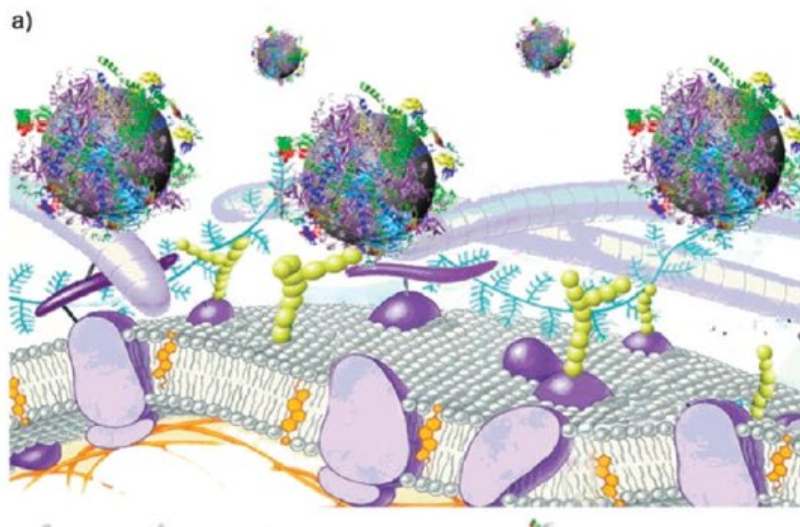


Figure 1. (a) Cartoon representation of the possible exchange/interaction scenarios at the bionanointerface at the cellular level. (b) Schematic drawing of the structure of NP–protein complexes in plasma: the “core” nanoparticle is surrounded by the protein corona composed of an outer weakly interacting layer of protein (left, full red arrows) rapidly exchanging with a collection of free proteins and a “hard” slowly exchanging corona of proteins (right). Diagram is not to scale in representing the proportions of the different objects.

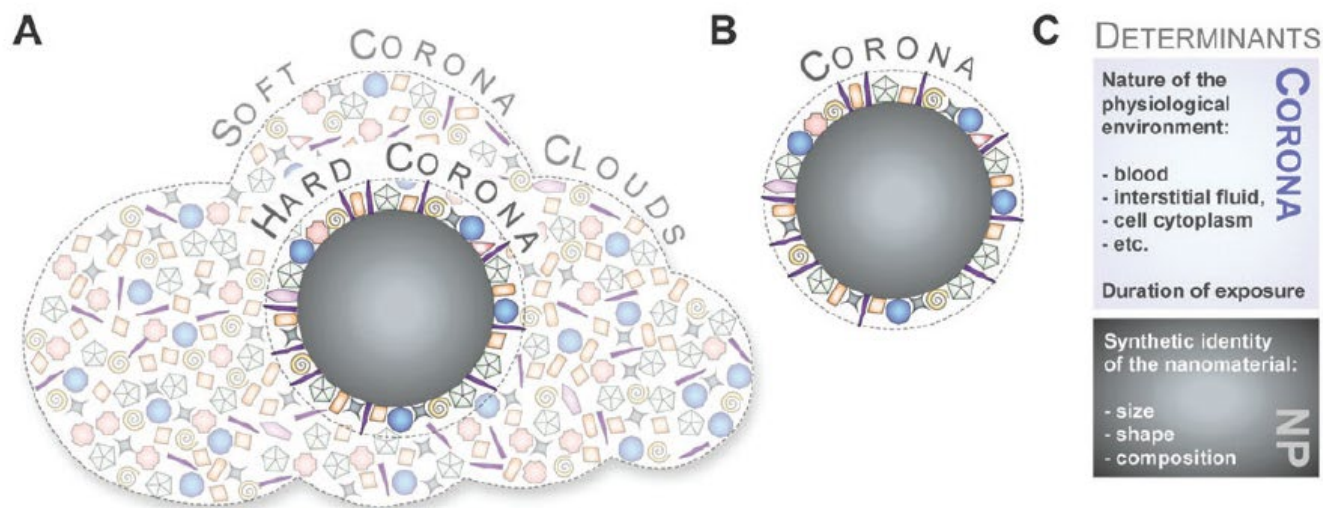


Fig. 3 Illustration of the old and new models referring to the description and determinants of the protein corona. (A) Hard and soft coronas, as well as protein clouds. (B) Coronas as analytically accessible NP–protein complexes. (C) Determinants of corona formation include not only the synthetic identity of the nanomaterial, but also the nature of the physiological environment.

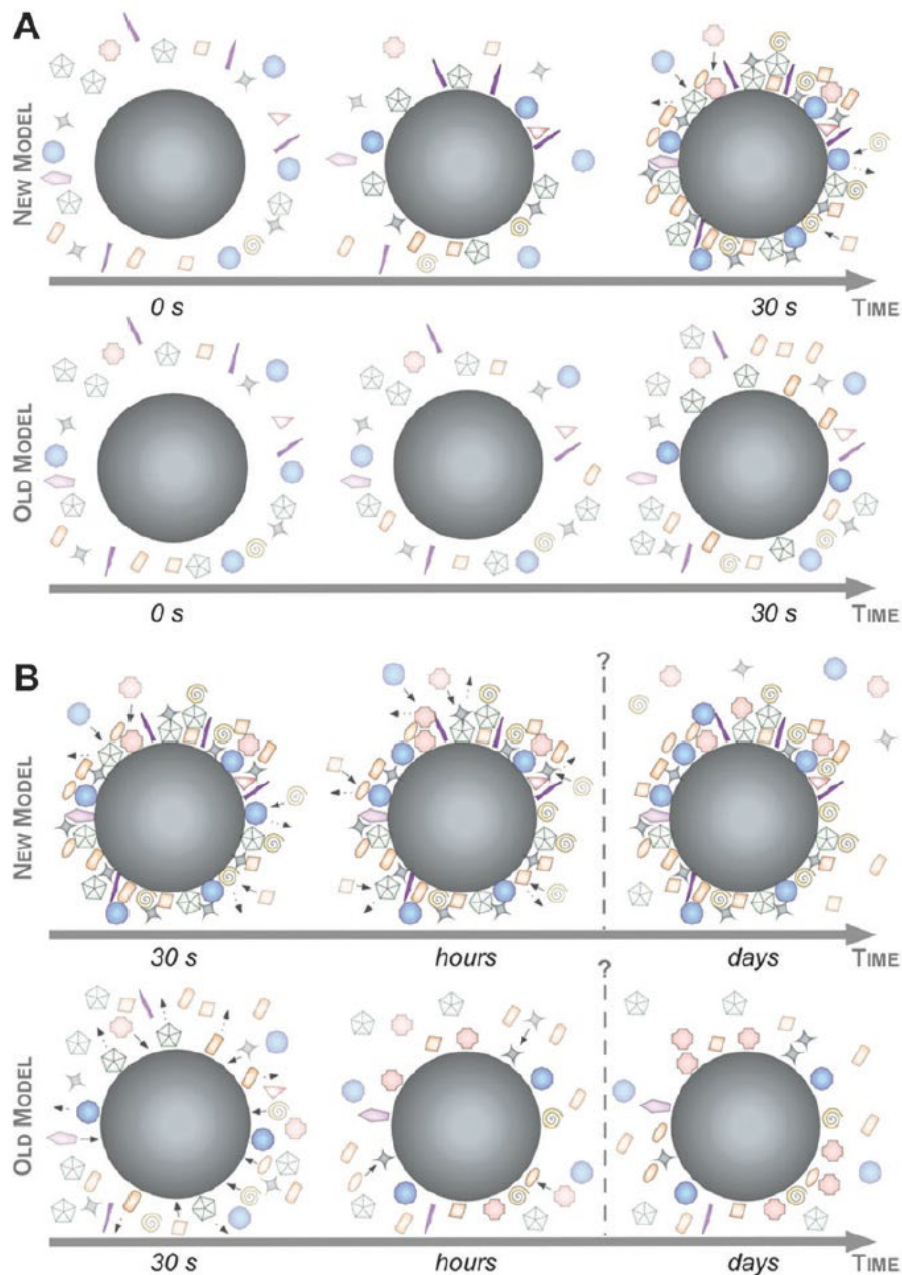


Fig. 6 Complexity and evolution of the biomolecule corona – the old versus the new model. (A) The early phase: a highly complex corona is established already in 30 sec, which may be composed of multiple core-shell structures ('new'). A corona of low complexity evolves slowly ('old'). (B) The late phase: corona composition *ex situ* remains stable and changes predominantly quantitatively rather than qualitatively over time with Vroman-effect dependent and independent binding kinetics ('new'). A highly dynamic protein corona, changes significantly over time, controlled by the 'Vroman-effect' ('old'). Note that the objects are not drawn to scale.

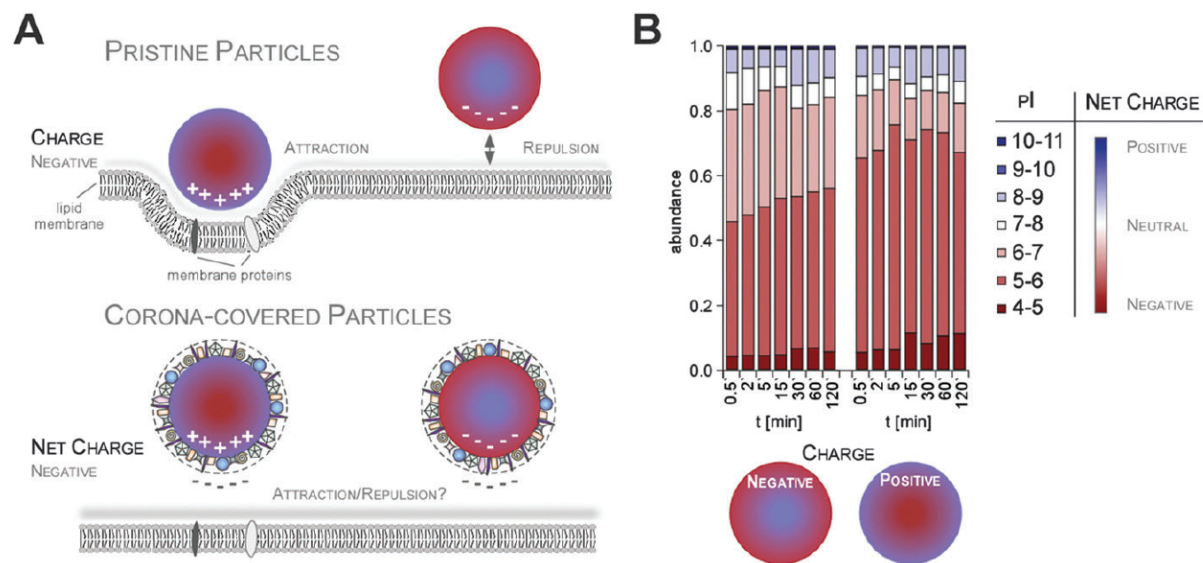


Fig. 7 Impact of NP charge on cellular uptake in the absence or presence of the protein corona. (A) Improved cellular uptake of positively charged NPs may be mediated by enhanced interaction with the negatively charged cell membrane only for pristine NPs (upper panel). In contrast, plasma corona covered NPs are overall negatively charged *in situ*, probably preventing NP-charge driven cell membrane interaction. (B) Plasma corona covered NPs are overall negatively charged, irrespective of the NPs' negative or positive surface functionalization.

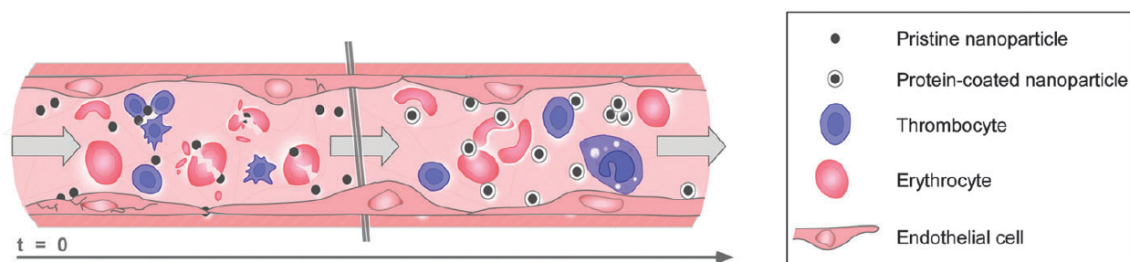
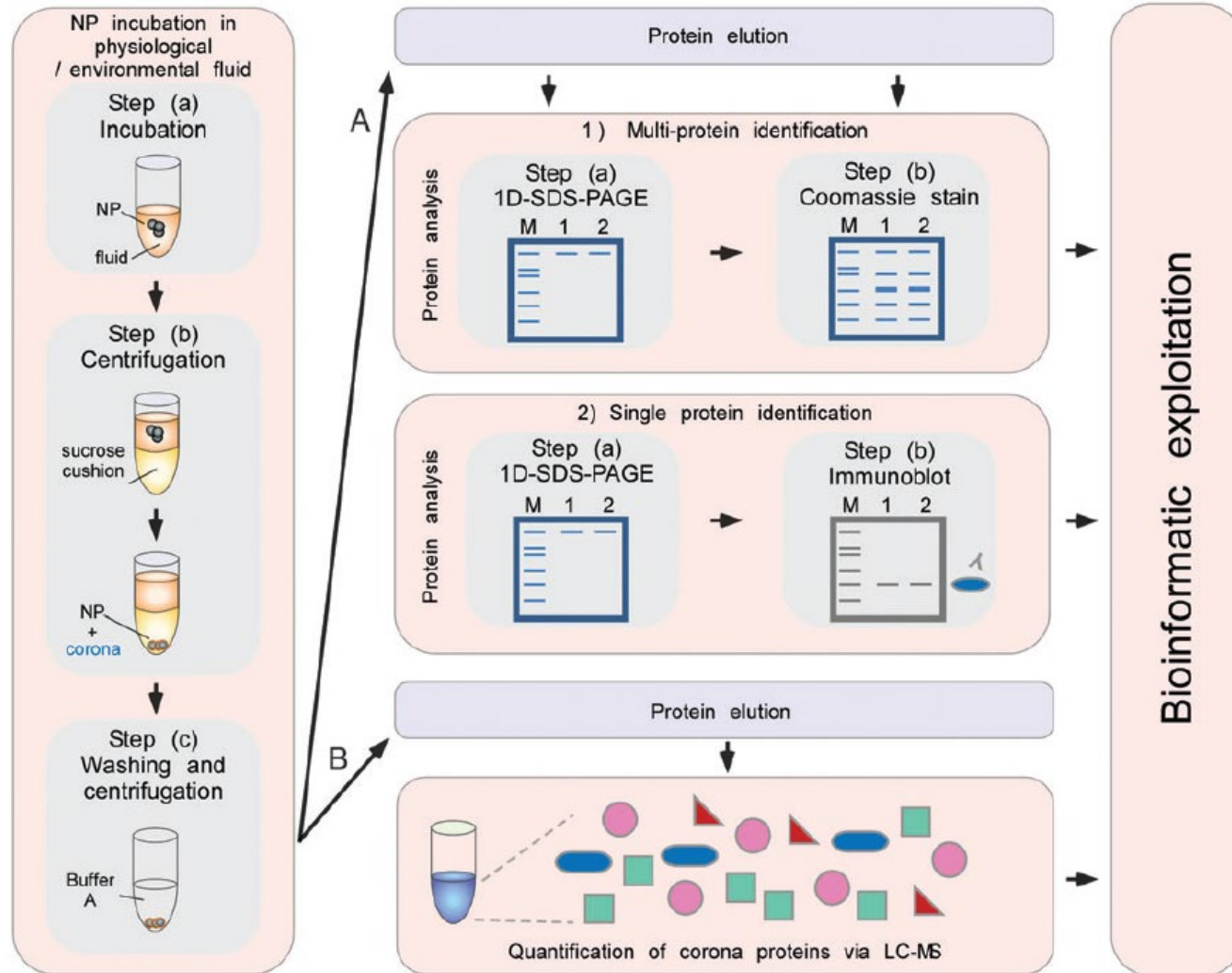
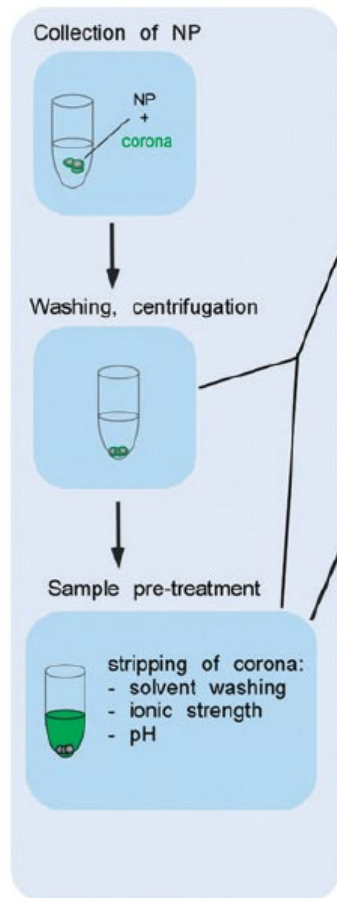


Fig. 8 Illustration of how rapid corona formation kinetically impacts early nanopathology in the human blood system. Upon entry or parenteral application, pristine NPs only exist for a short period of time, but are still capable of immediately affecting the vitality of endothelial cells, triggering thrombocyte activation and aggregation, and may also induce hemolysis. Formation of the biomolecule corona rapidly modulates the NPs' decoration with bioactive proteins protecting the cells of the blood system against nanoparticle-induced (patho)biological processes, and can also promote cellular uptake. Note, the elements are not drawn to scale.

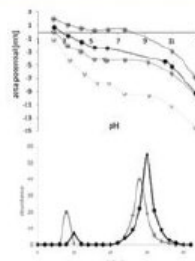


A - environmental corona



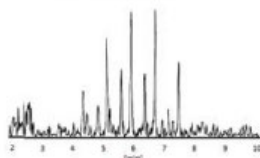
characterization of NP-corona

- size
- charge
- stability



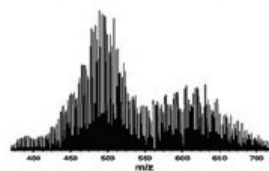
characterization of corona

- GC
- NMR
- LCMS
- ...



identification and quantification of certain compounds from complex matrix

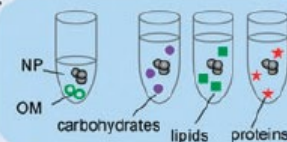
fingerprinting:
- e.g. FTICR-MS



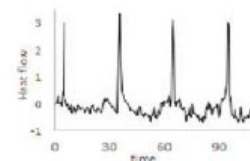
general corona pattern
(corona memory?)

B - artificial corona

NP incubation with NOM or single compounds in changing environment (pH, ionic strength etc.)

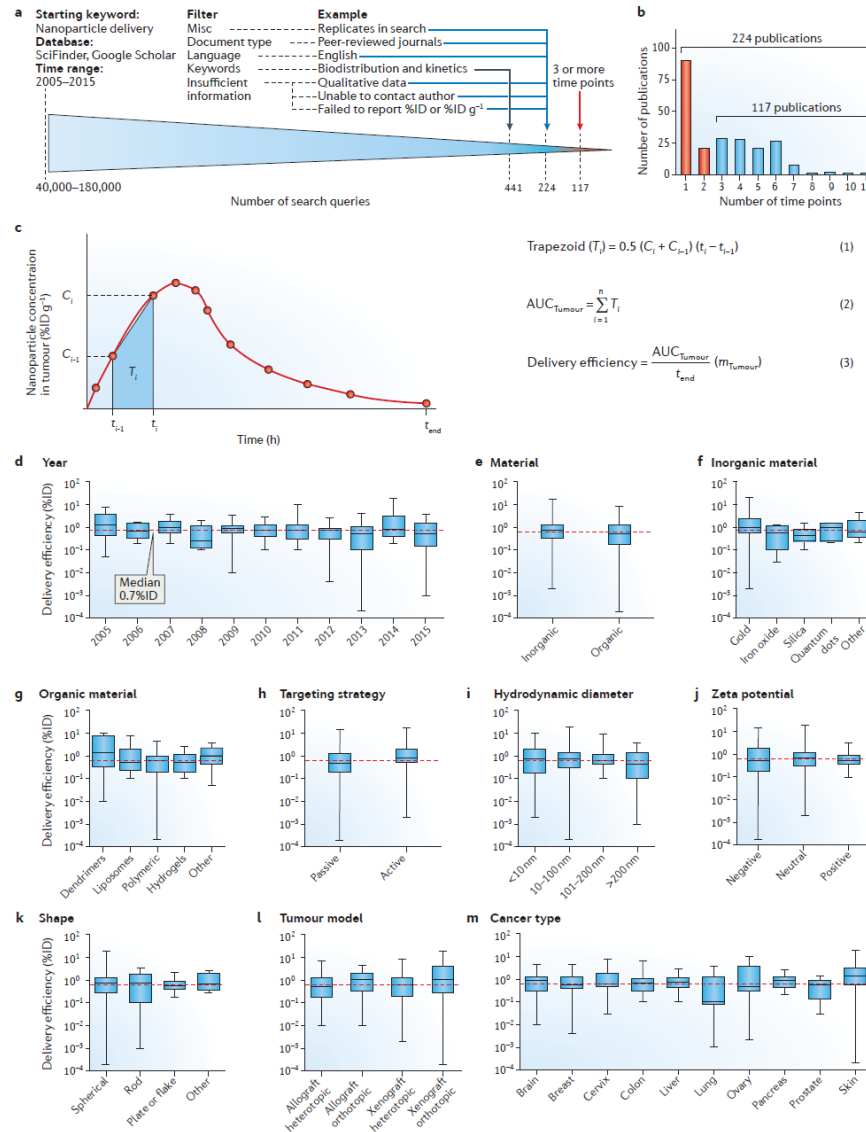


- sorption isotherms
- binding affinity
- thermodynamic parameters

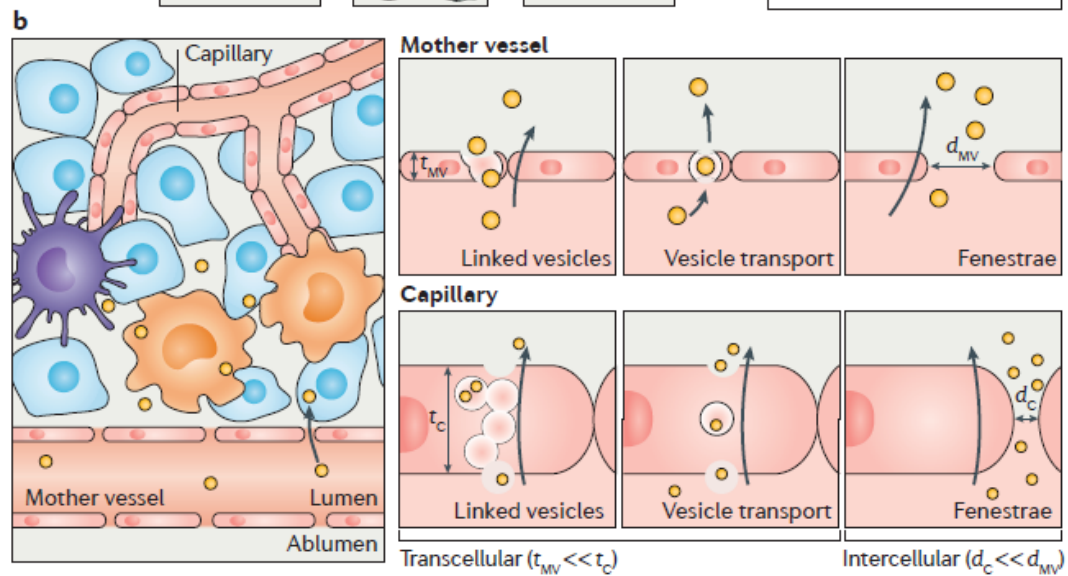
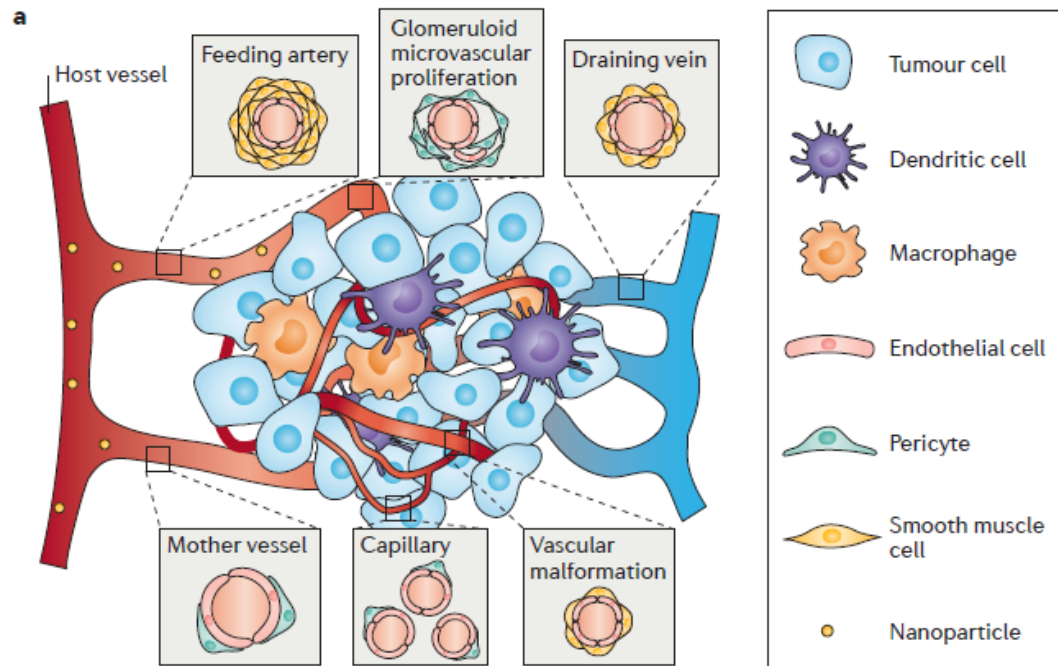


understanding of NP-biomolecule interactions

Delivery Efficiency



< 1%ID



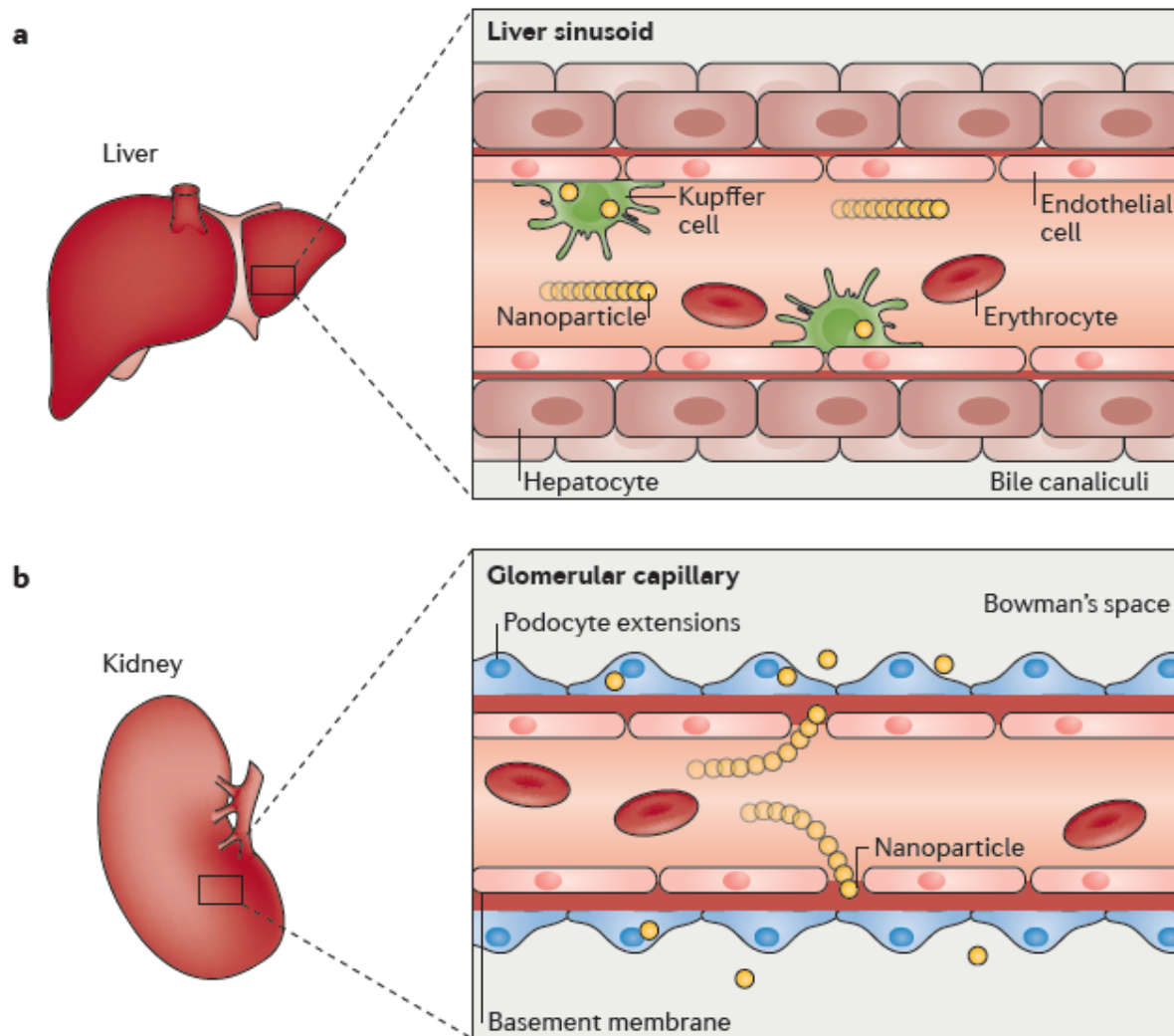
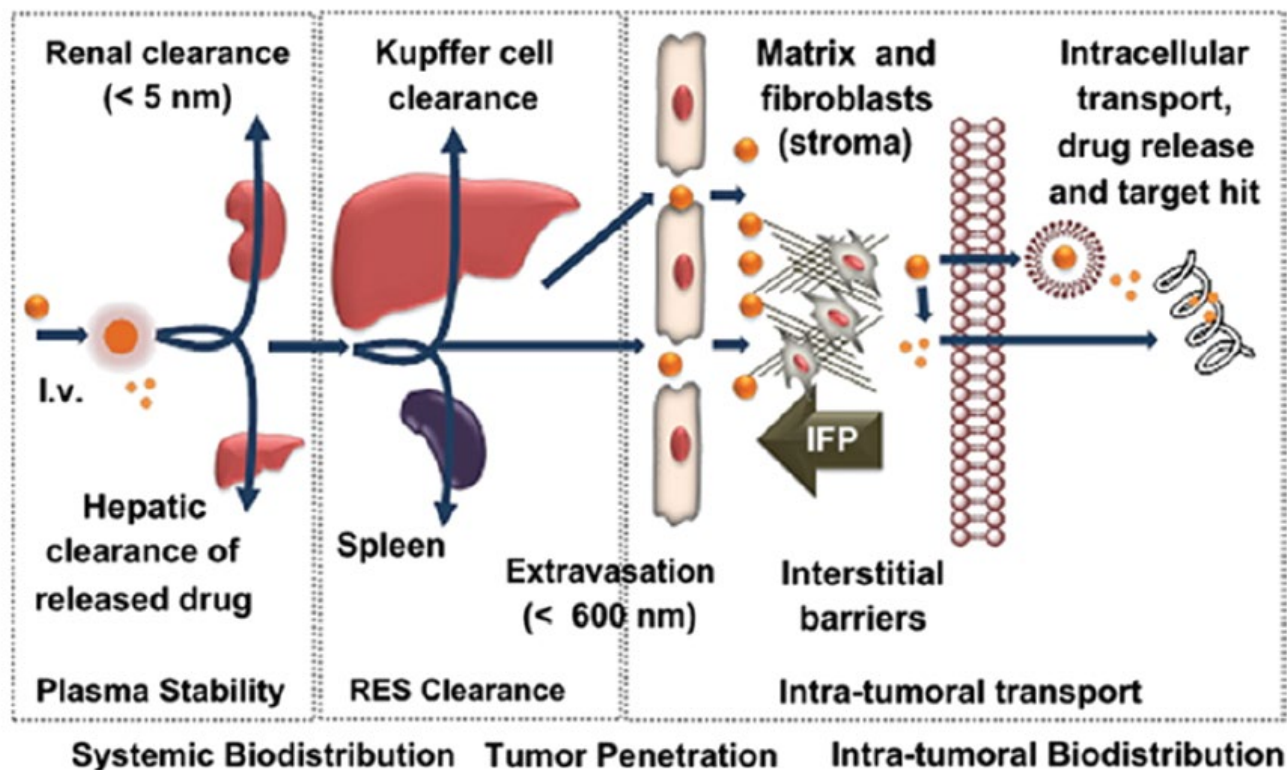


Figure 4 | **Mechanisms for nanoparticle elimination from the bloodstream.** **a** | The liver is the primary organ of the mononuclear phagocytic system that entraps a vast majority of the administered nanoparticle dose. Phagocytic cells, such as Kupffer cells, line the liver sinusoid. **b** | If the hydrodynamic diameter of a nanoparticle is smaller than 5.5 nm, it may be filtered from the blood via the kidneys and excreted in urine. Other major organs that are involved in the removal of nanoparticles from the bloodstream include the spleen, lymph nodes and the skin.

(A)



(B)

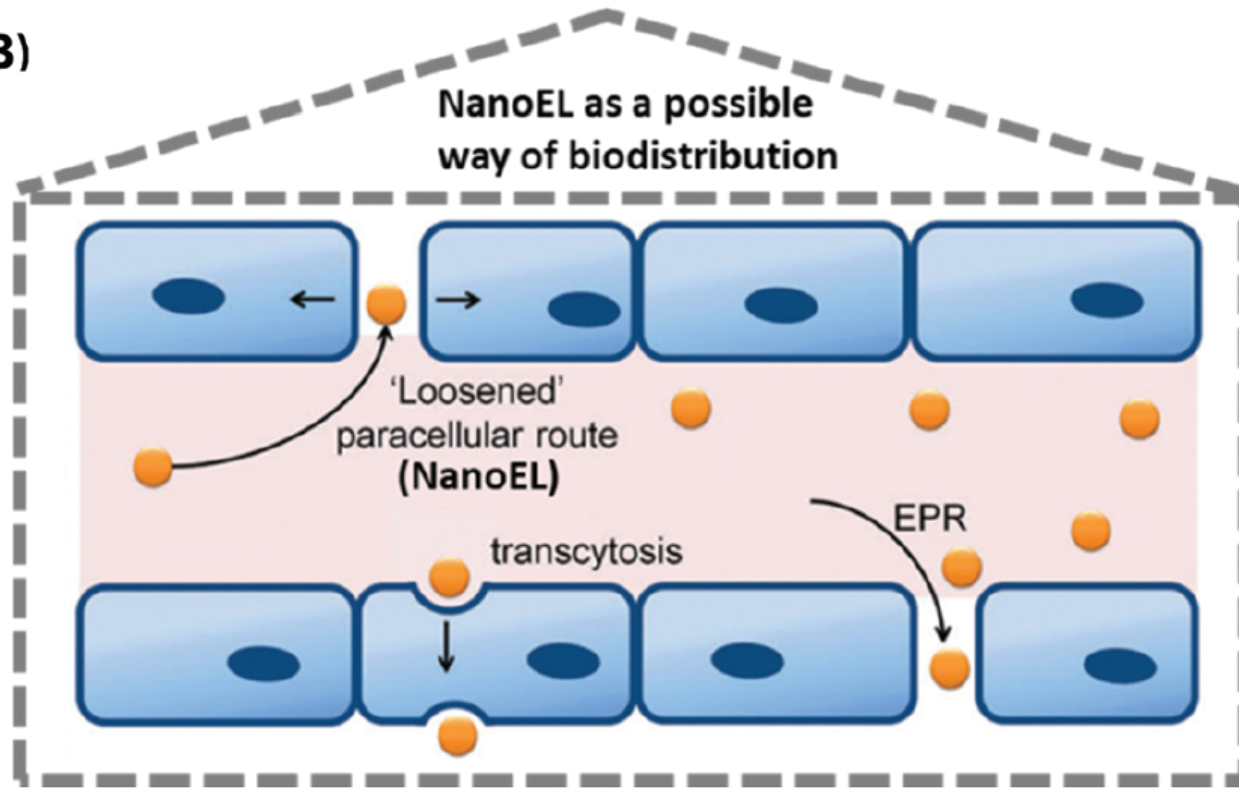
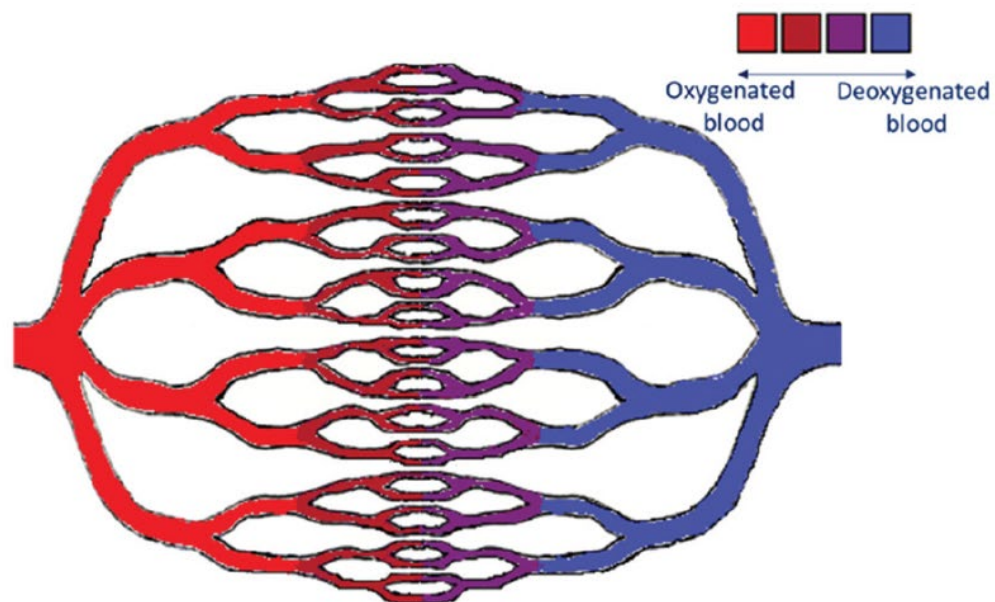
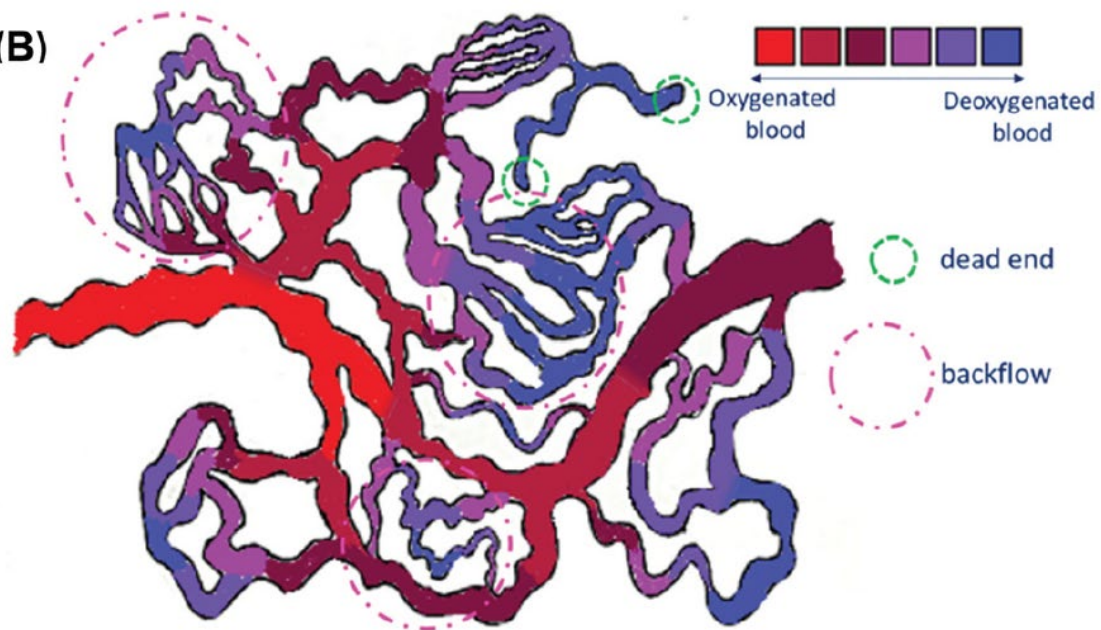


Fig. 1 Drug delivery by NPs are subjected to various factors that limit the overall dose of drugs reaching the target site. (A) These factors can be classified into three separate phases, namely, systemic biodistribution, tumor penetration and intra-tumoral biodistribution. Reproduced with permission from Ernsting *et al.*²⁴ Copyright 2013 Elsevier. (B) Nanomaterial-induced endothelial leakiness (NanoEL) may be viewed as an emerging strategy to improve the biodistribution of nanomedicine to target sites. Adapted from Setyawati *et al.*²⁵ with permission from the Royal Society of Chemistry 2015. IFP, interstitial fluid pressure.

(A)



(B)



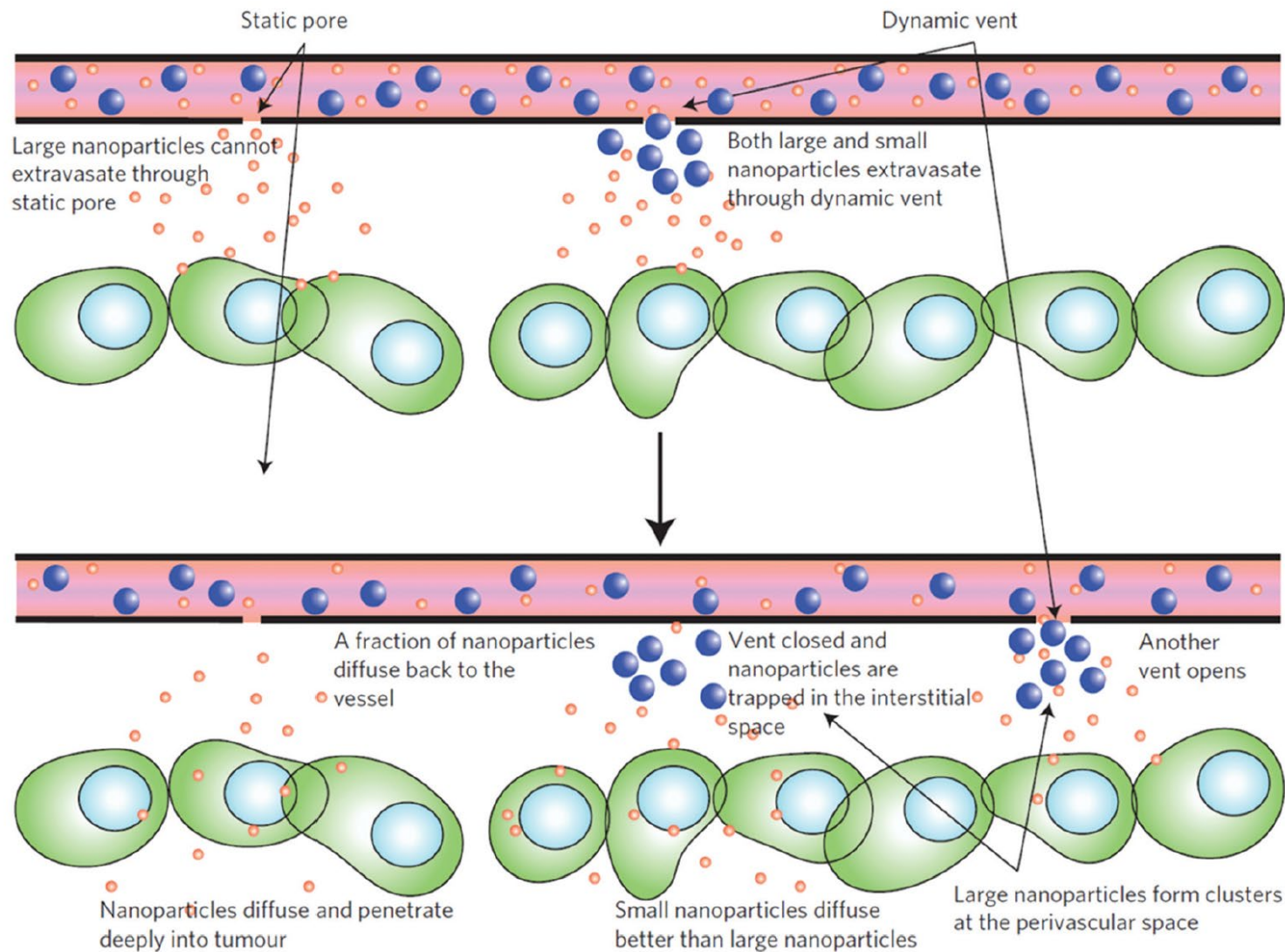
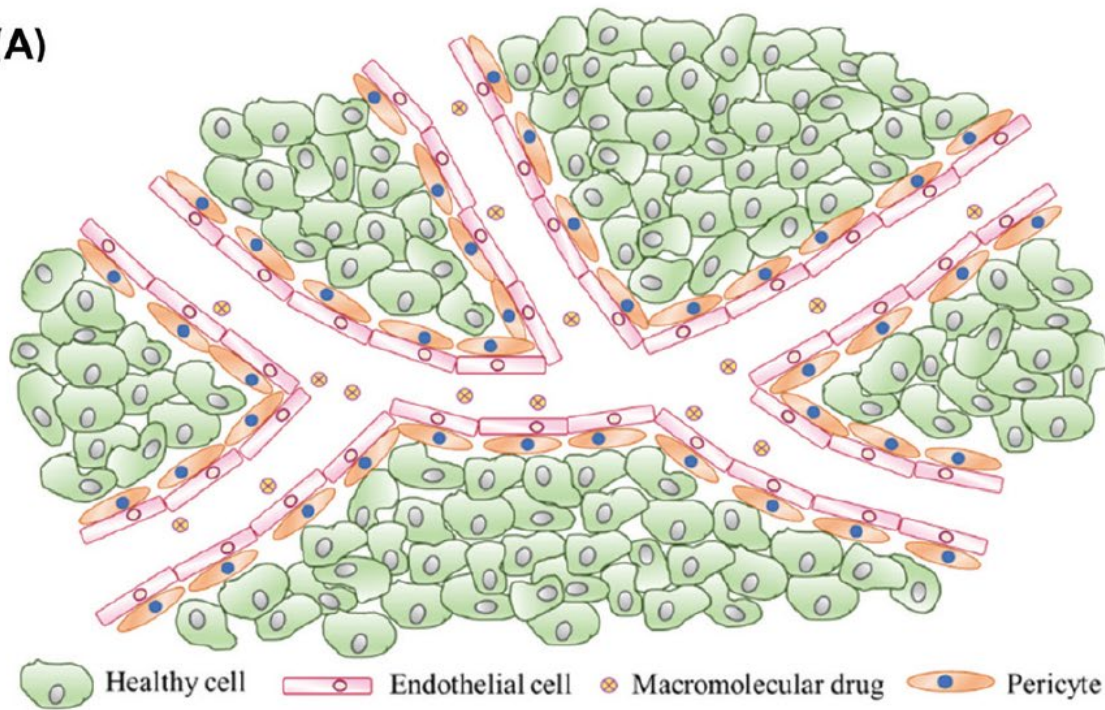
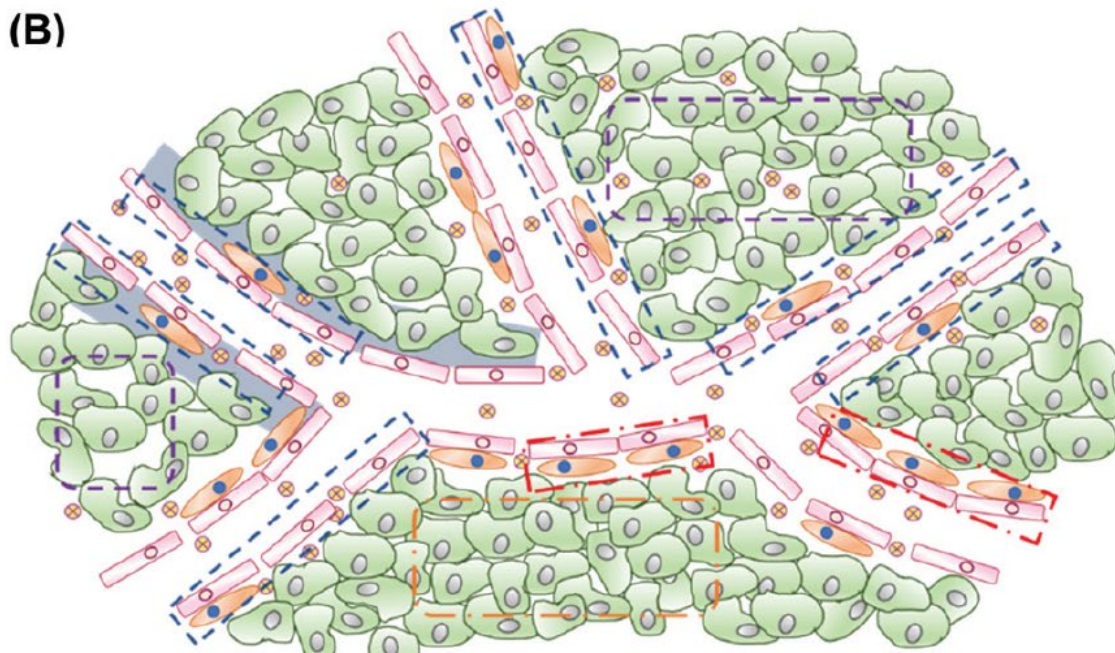


Fig. 3 Presence of static pores and dynamic vents in tumor vessels allows for NPs of differential permeability of NPs to penetrate through the vessel walls to reach the tumor sites. Predominantly for small NPs, static pores promote a deeper penetration of these NPs for a longer period of time. On the other hand, dynamic vents form transient openings which allow both small and large NPs to diffuse across the vessel walls, but over a shorter period of time. Reproduced with permission from Matsumoto *et al.*¹⁰⁷ Copyright 2016 Springer Nature.

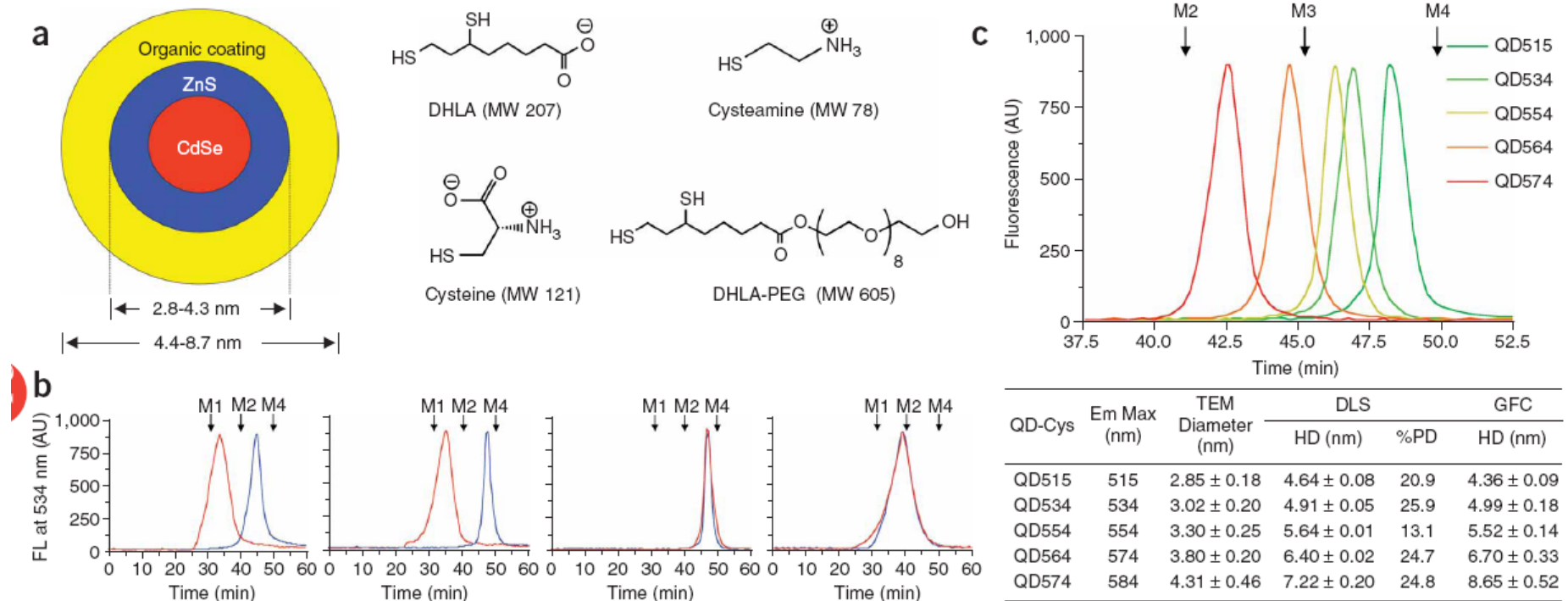
(A)

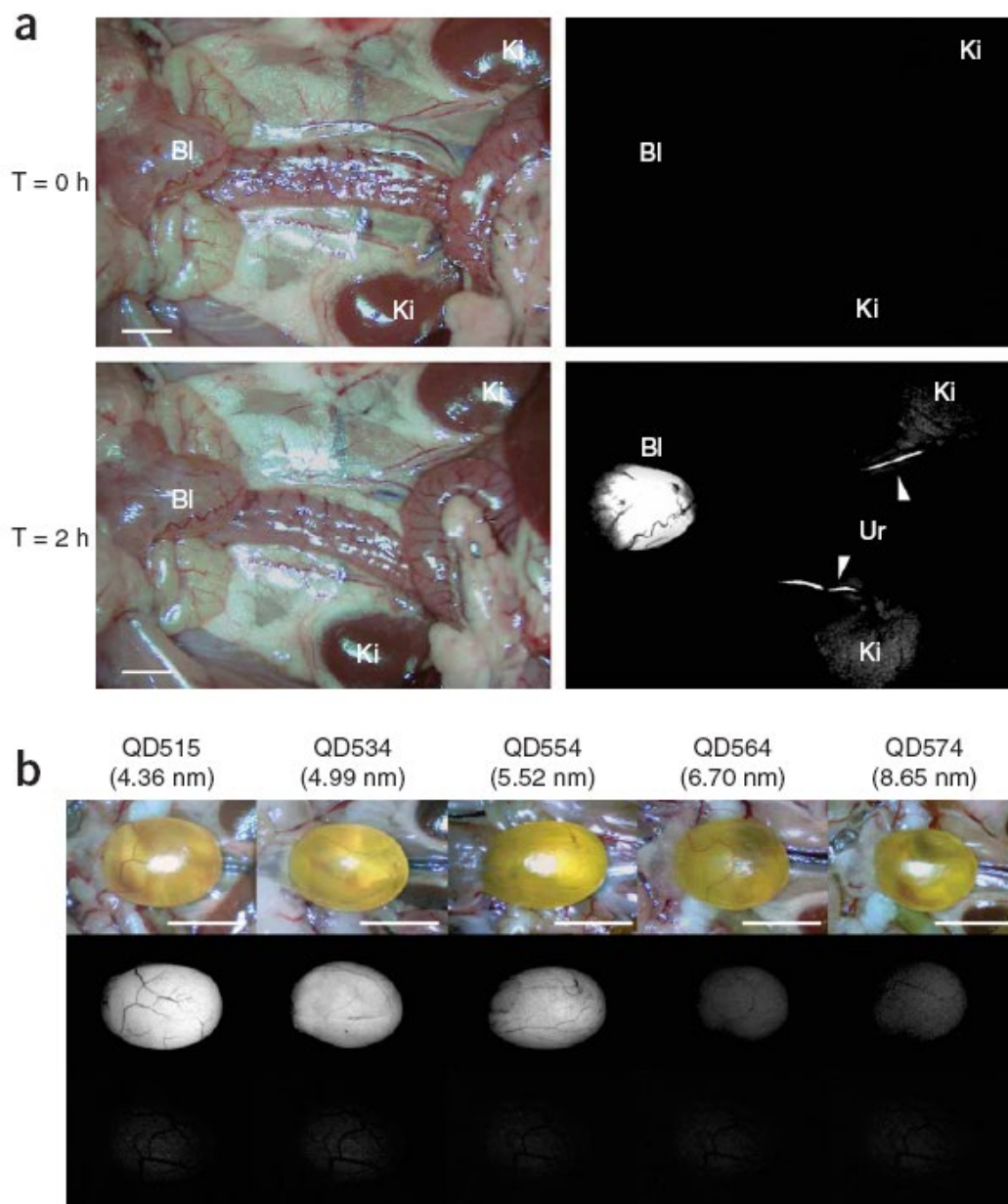


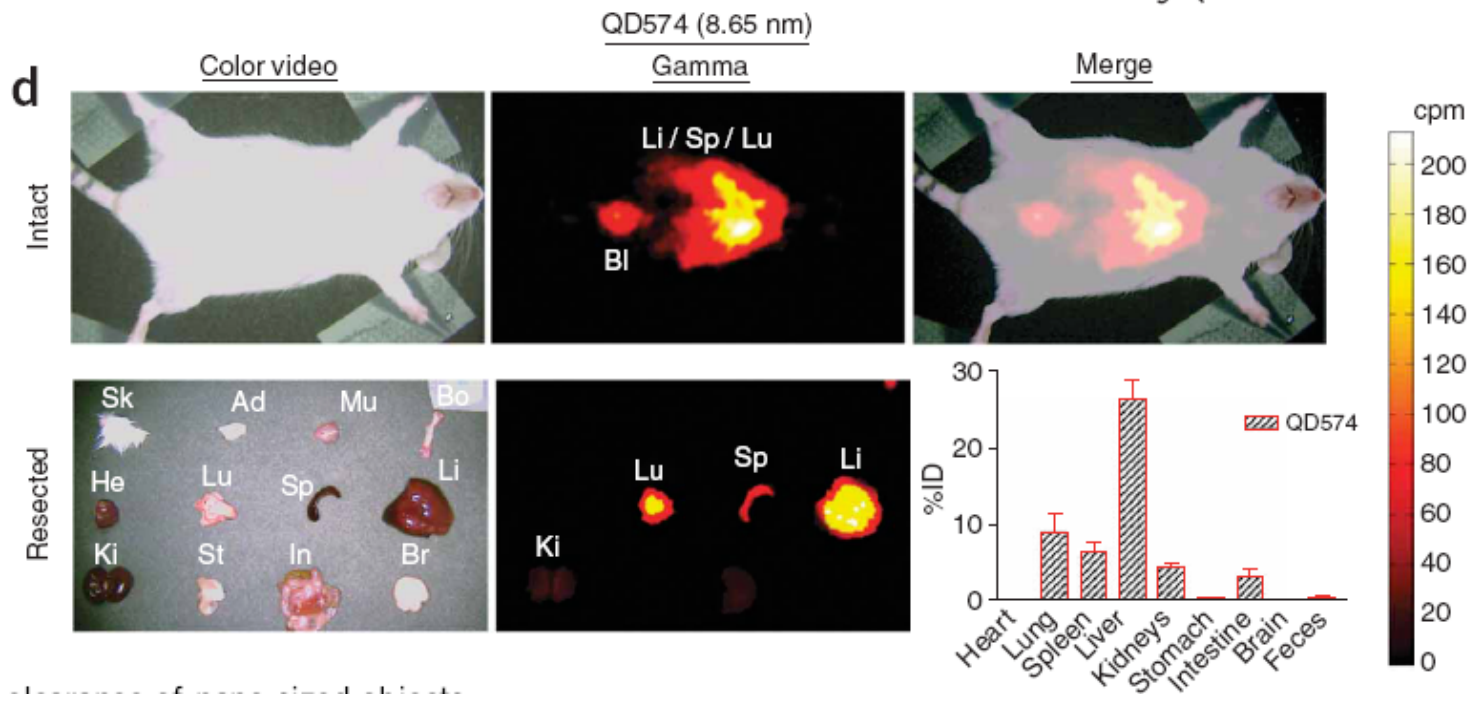
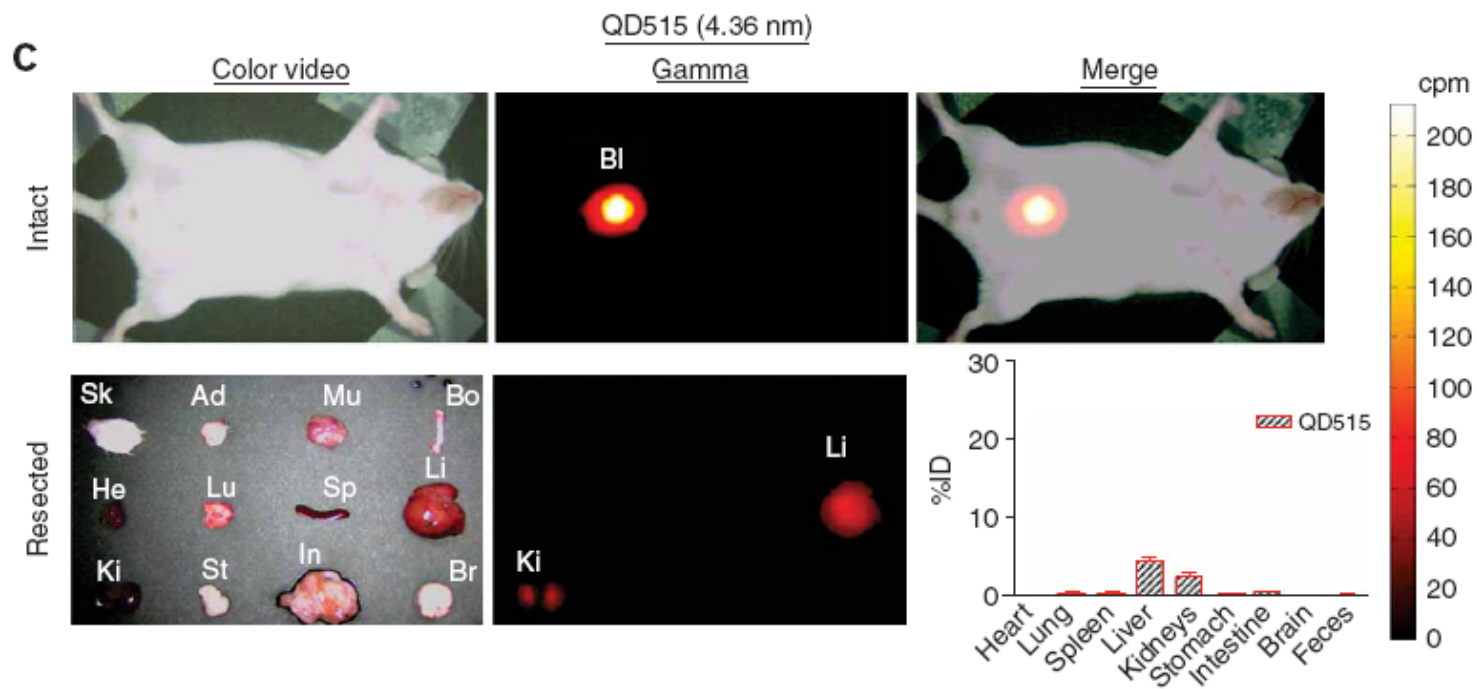
(B)



Renal clearance of quantum dots







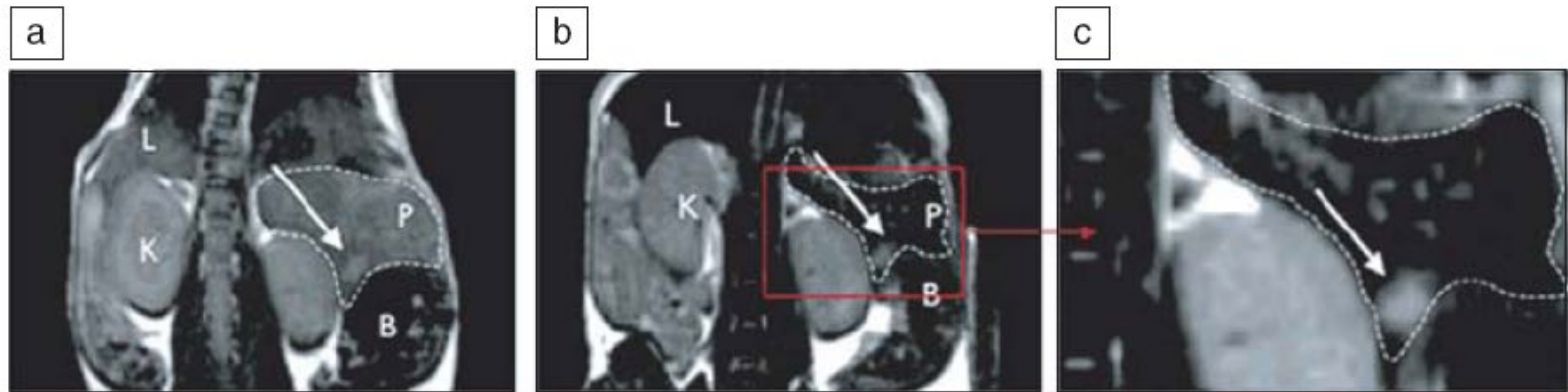


Figure 1. Cross-linked iron oxide (CLIO) nanoparticles for T_2 -weighted images of rodent pancreatic cancer: (a) preinjection of CLIO, (b) postinjection of CLIO, and (c) higher magnification of postinjection image with the arrow indicating tumor. L, liver; P, pancreas; K, kidney; B, bowel.¹⁶

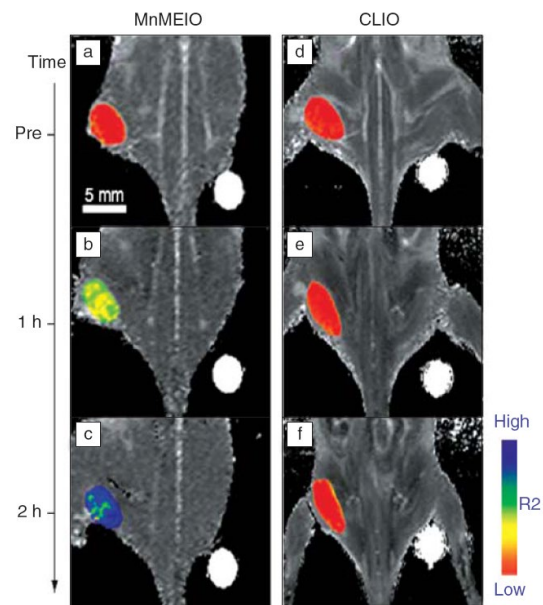
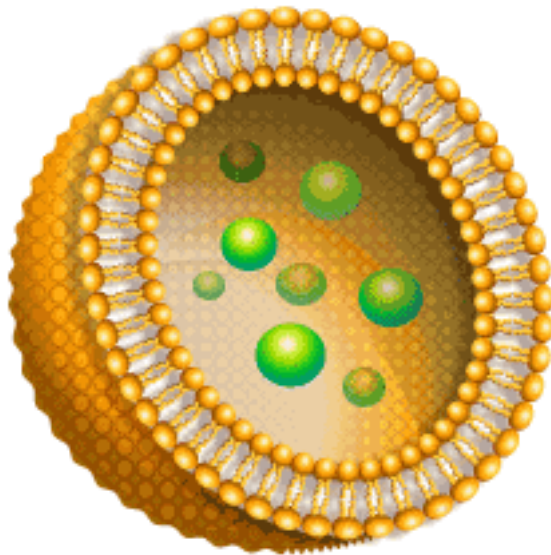
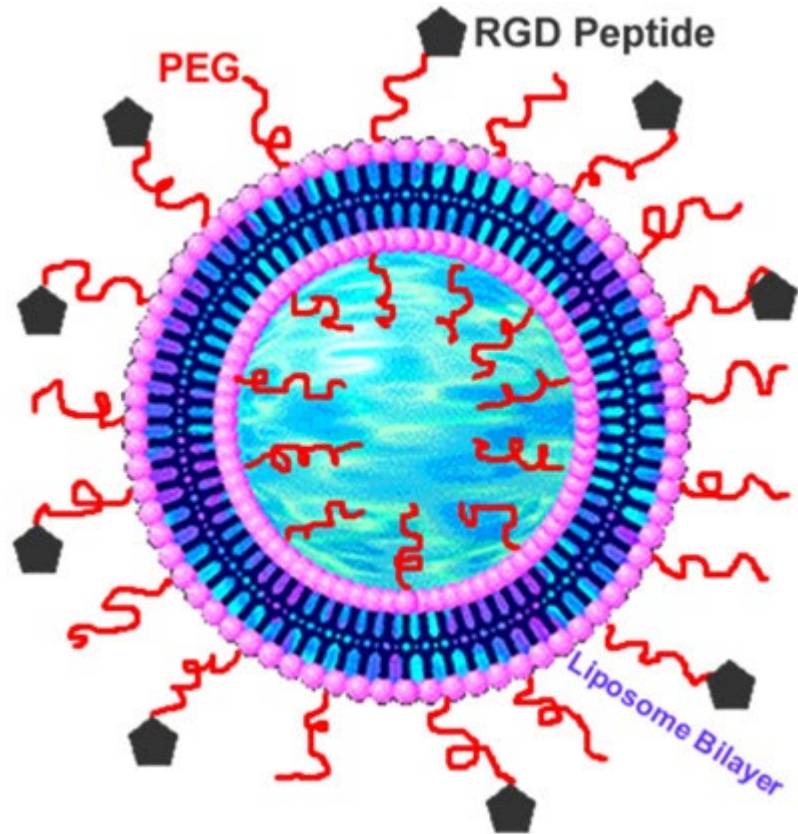


Figure 2. *In vivo* magnetic resonance detection of cancer after administration of magnetic nanoparticles Herceptin conjugates. MnFe_2O_4 nanoparticles (MnMEIO) (a–c) show higher signal enhancement than cross-linked iron oxide (CLIO) (d–f).²⁴ R_2 , inverse of transverse relaxation time.

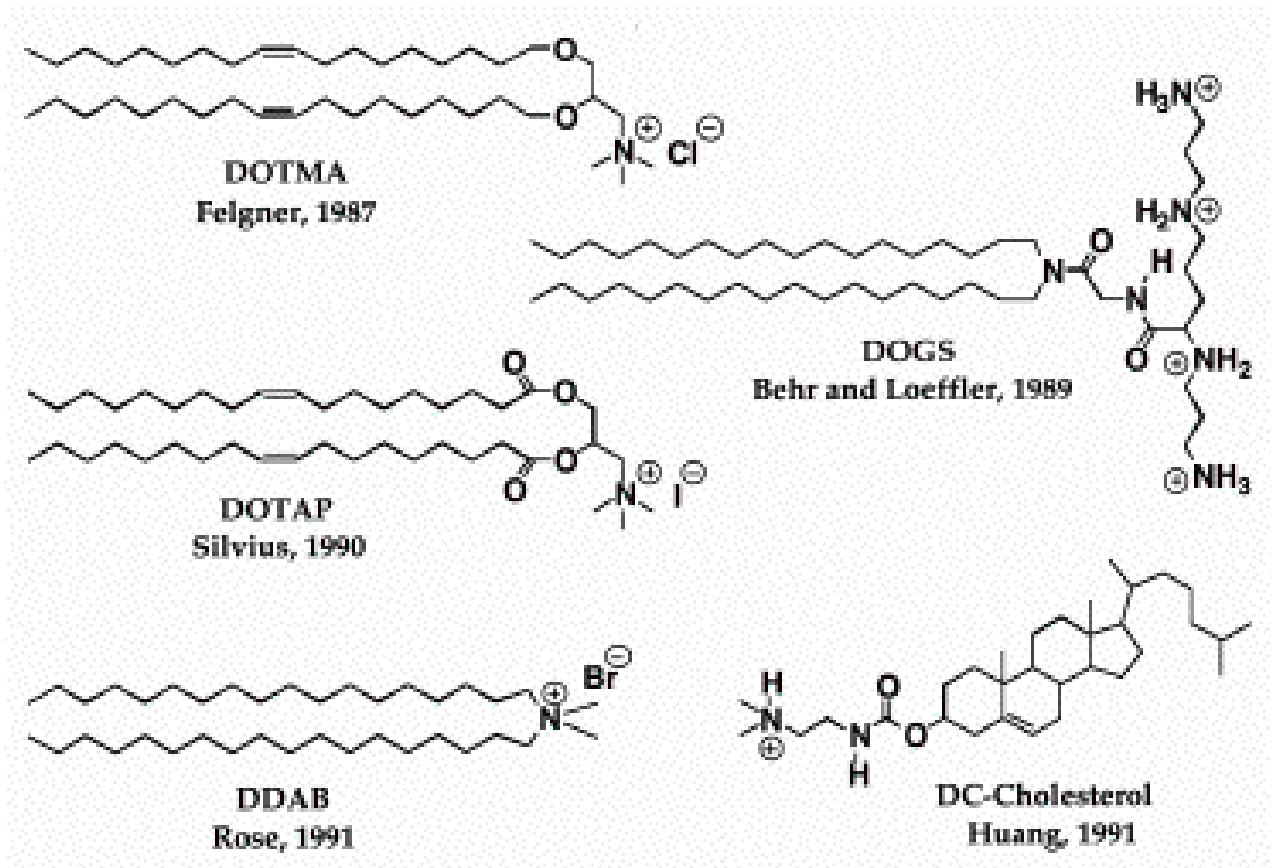
Liposome



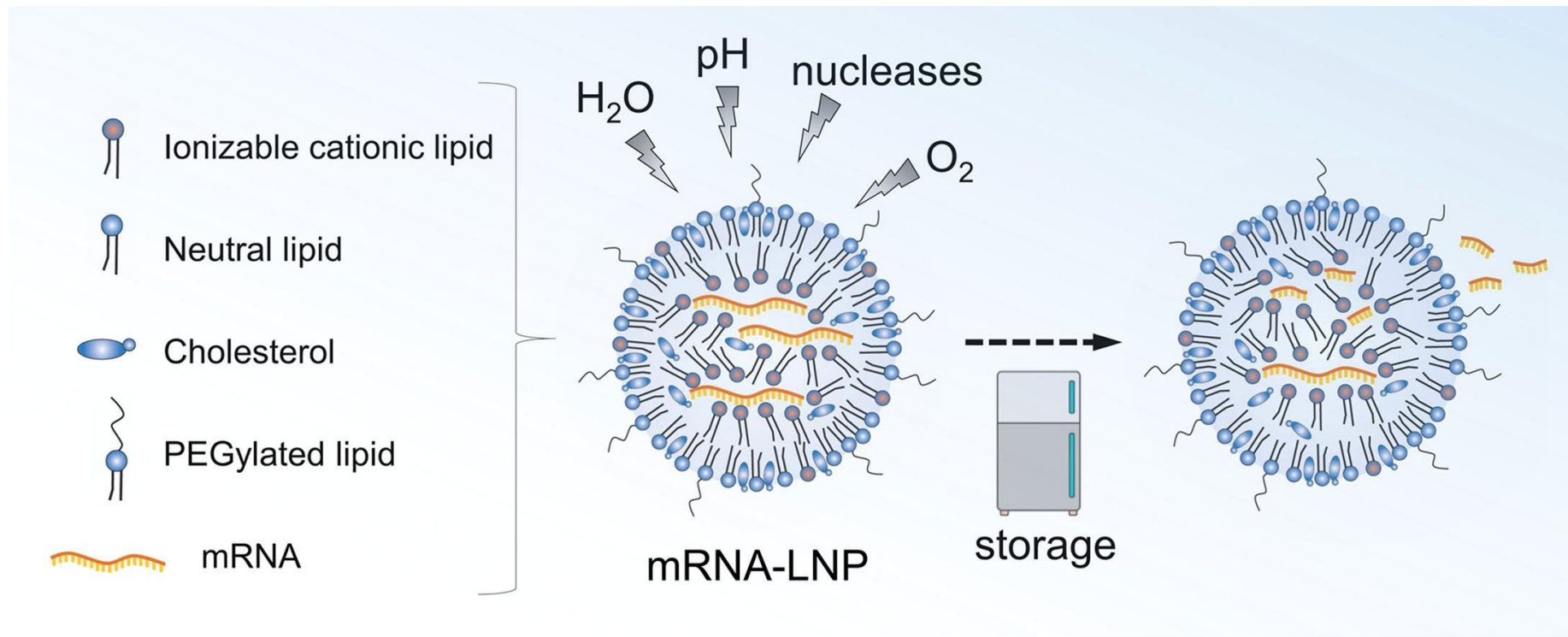
Liposome



Cationic Lipids



Lipid Nanoparticle for Vaccine

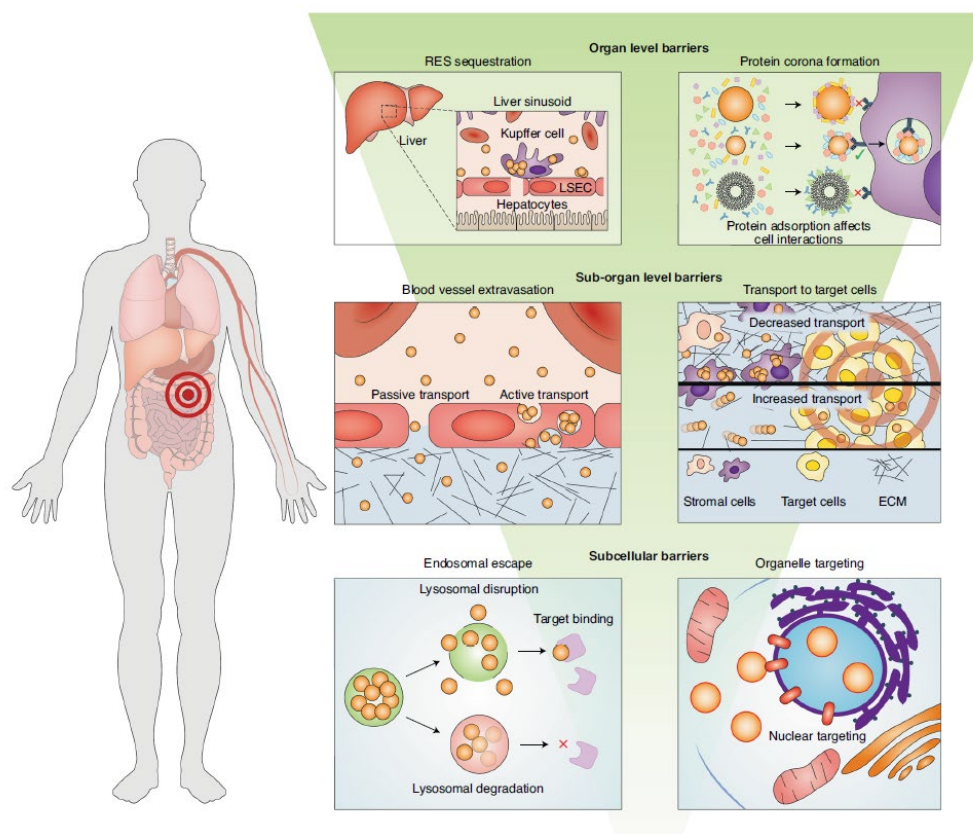




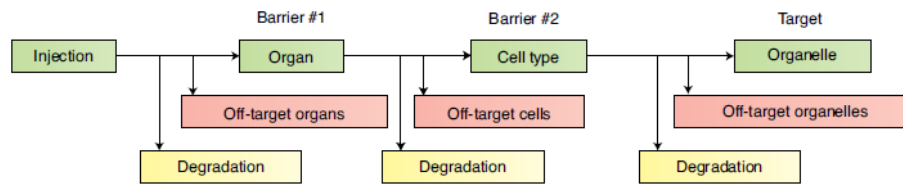
A framework for designing delivery systems

Wilson Poon^{1,2,7}, Benjamin R. Kingston^{1,2,7}, Ben Ouyang^{1,2,3}, Wayne Ngo^{1,2} and Warren C. W. Chan^{1,2,4,5,6} ✉

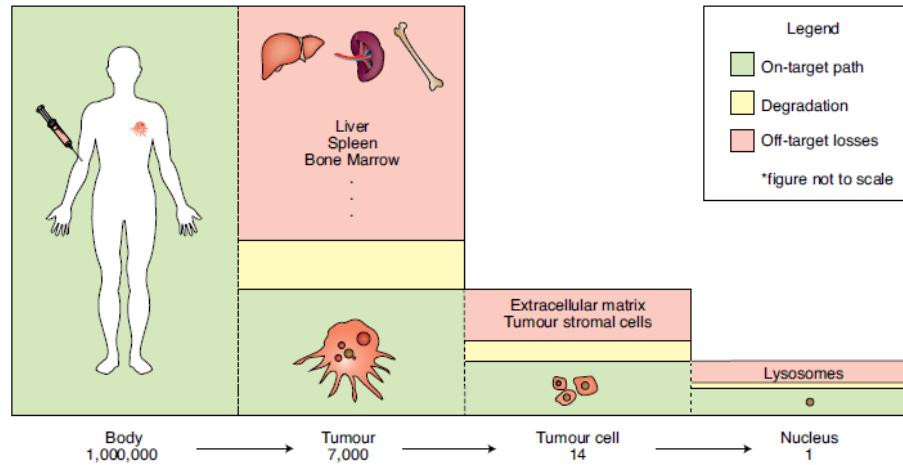
NATURE NANOTECHNOLOGY | VOL 15 | OCTOBER 2020 | 819–829



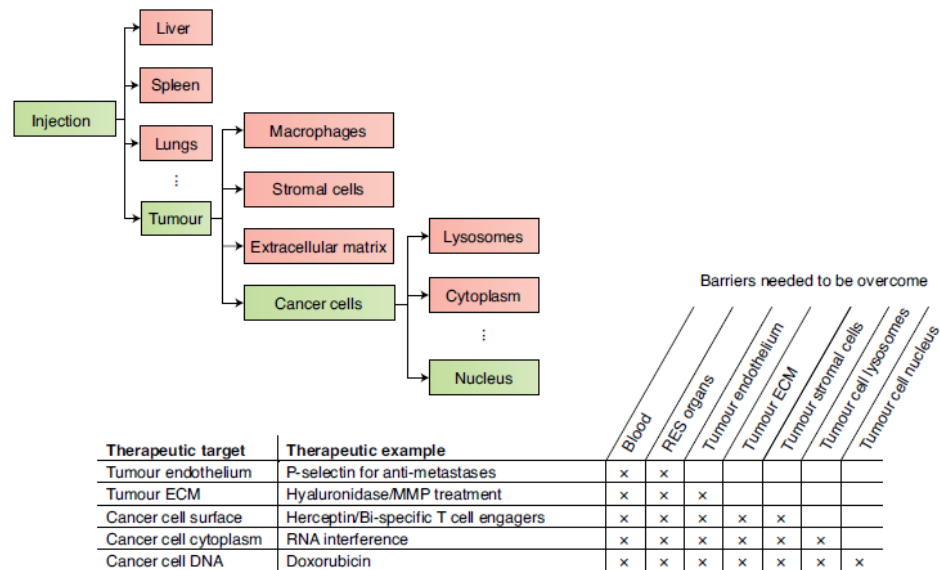
a



b



c



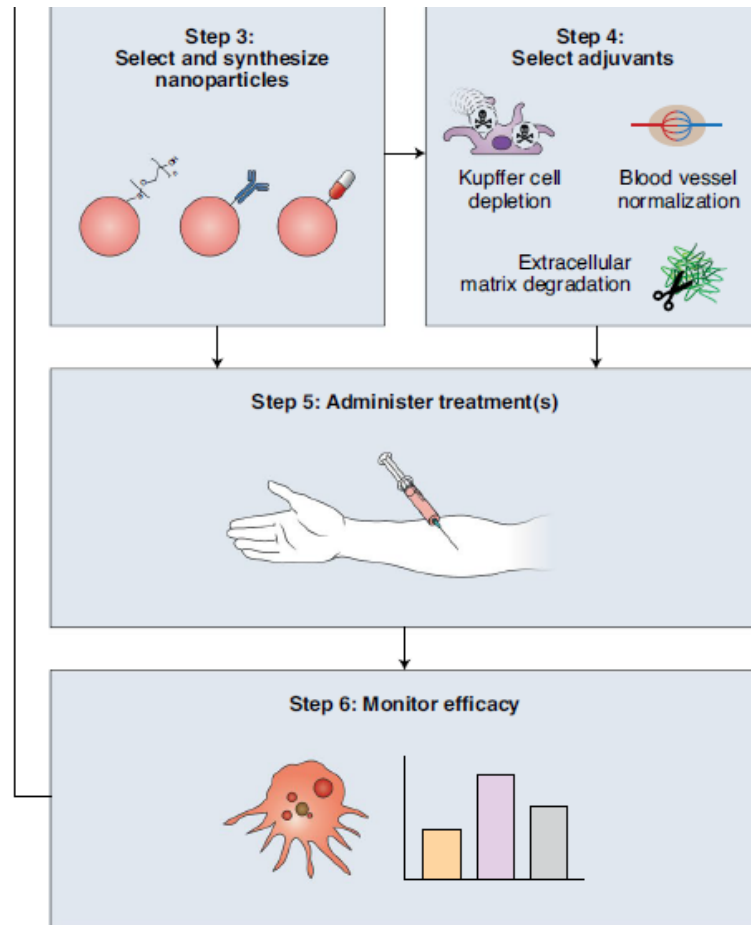
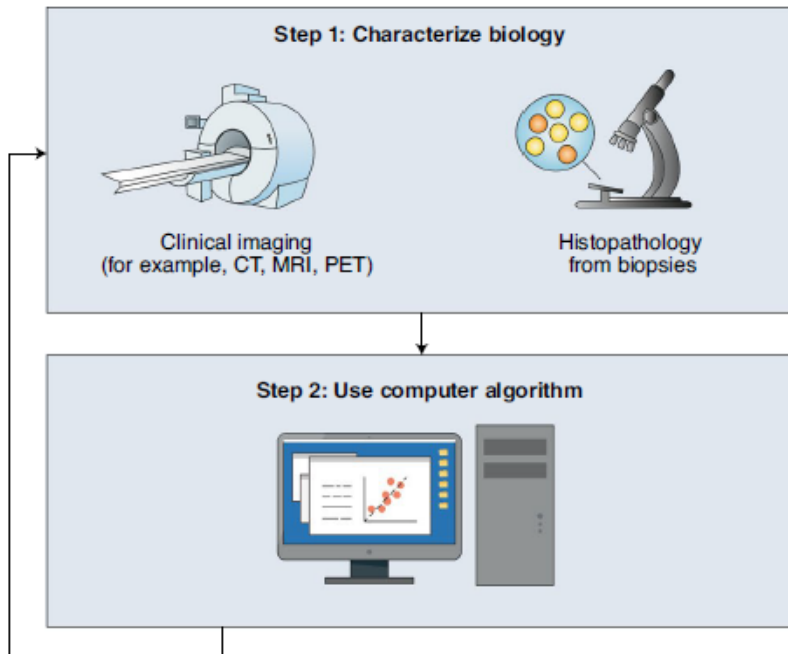
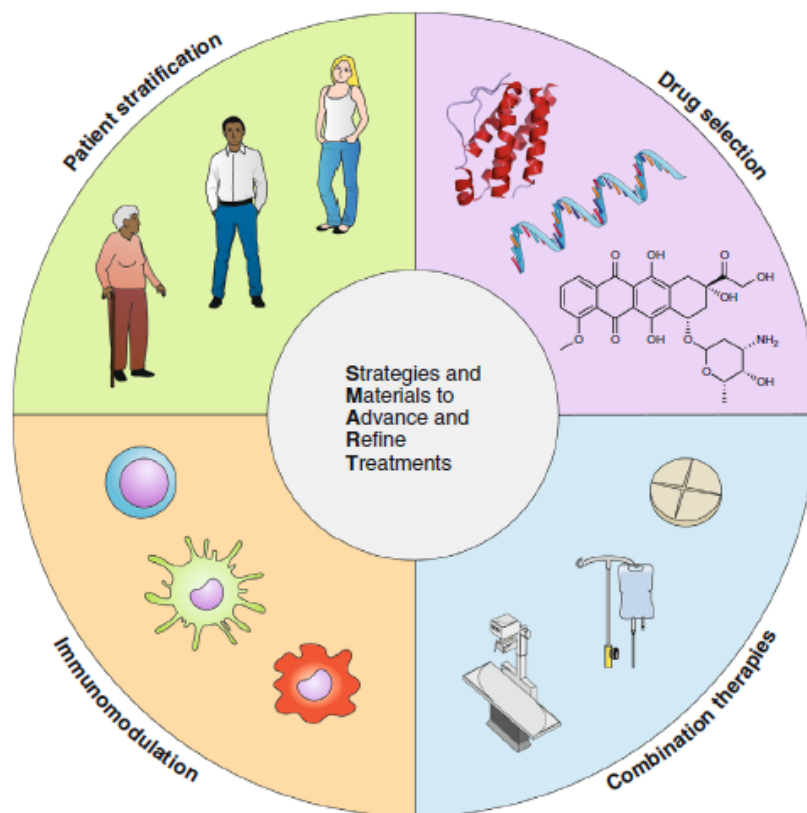


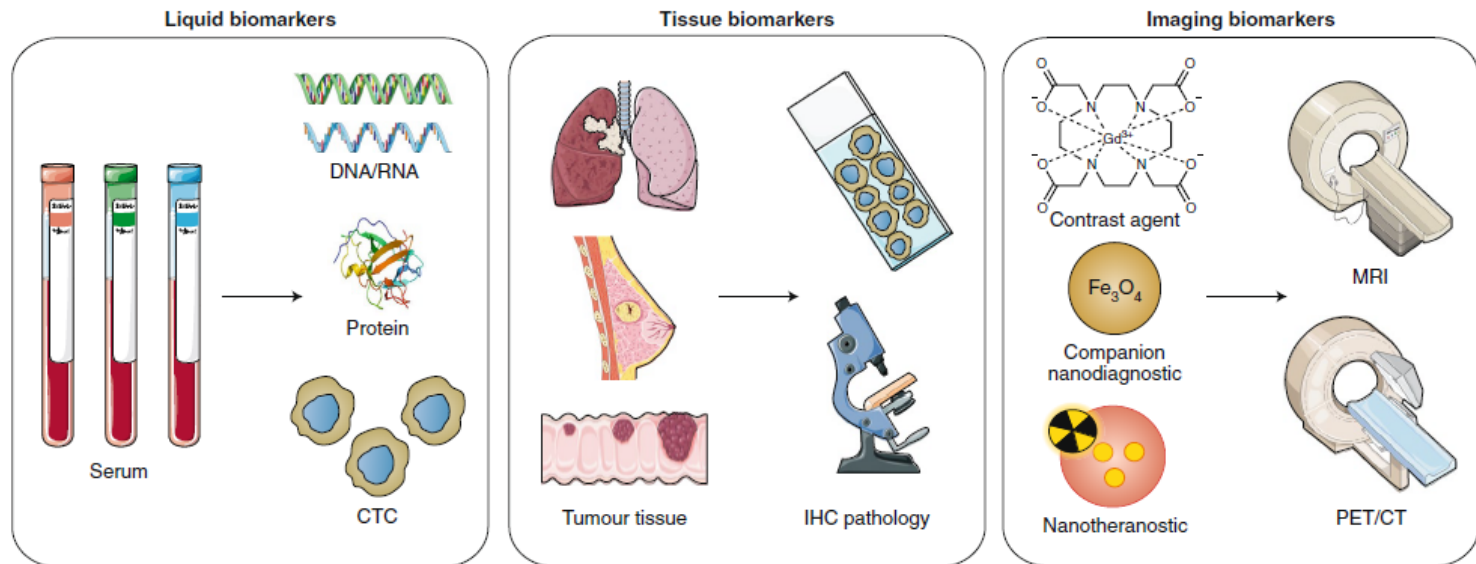
Table 1 | Critical questions for designing nanoparticle delivery systems

Question	Rationale for the question
(1) Where is the delivery target?	The specific biological target (organ or tissue, cell type and subcellular location) defines design of the nanoparticle strategy.
(2) What is the cargo or active agent that needs to be delivered to the target location?	This defines the chemistry for incorporating the agents into the nanoparticle for delivery.
(3) Where is the site of administration?	The location of administration and the delivery target location define the delivery pathway.
(4) What are the specific organs, tissues and cells encountered along the delivery pathway?	This defines the barriers that the nanoparticle will encounter.
(5) What are the interactions between the nanoparticle carrier and the body in each of these biological environments along the delivery pathway?	These interactions will determine if the formulation is degraded or sequestered before it can reach its intended target location.
(6) What strategies are available to overcome the barriers at each step in the delivery pathway?	This allows the development of specific strategies to overcome the barriers.
(7) How will any administered components leave the disease site and be excreted from the body?	This helps to define locations of toxicity and elimination routes.

Smart cancer nanomedicine

NATURE NANOTECHNOLOGY | VOL 14 | NOVEMBER 2019 | 1007-1017 |



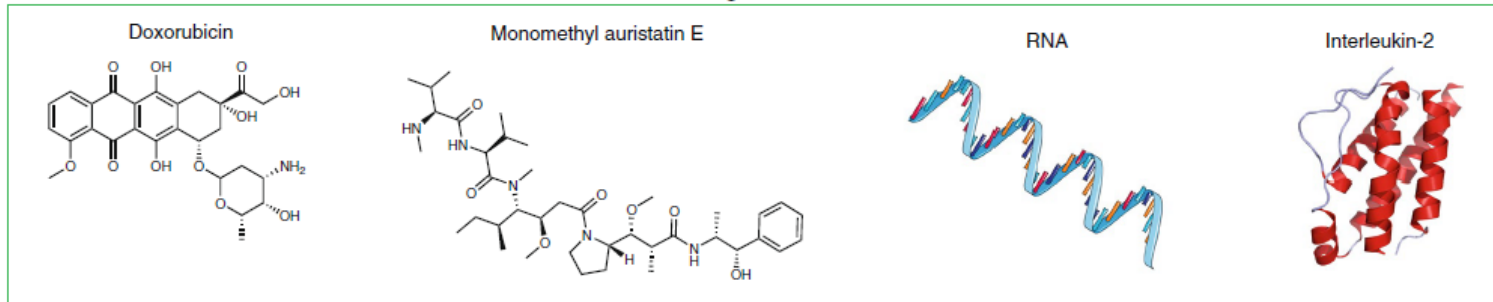


Simplicity

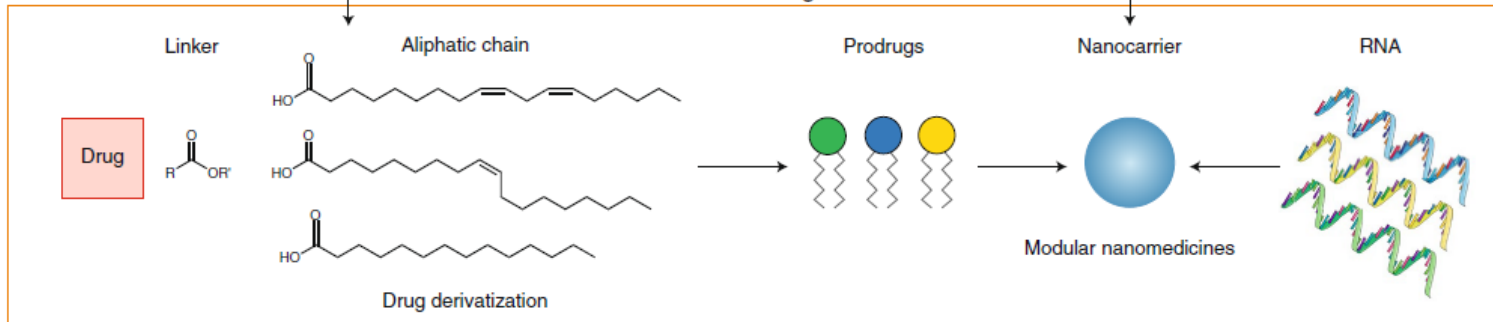
Specificity

Figure 1. Biomarkers and their detection methods. The diagram illustrates the detection methods for three types of biomarkers: Liquid biomarkers (Serum, DNA/RNA, Protein, CTC), Tissue biomarkers (Tumour tissue, IHC pathology), and Imaging biomarkers (Contrast agent, Companion nanodiagnostic, Nanotheranostic, MRI, PET/CT). The diagram also shows a gradient bar indicating the trade-off between Simplicity and Specificity.

Drug classes



Modular design



Library screening

