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SMART BIODEGRADABLE NANOCARRIERS FOR CONTROLLED AND SITE-SPECIFIC CANCER TREATMENT

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ABSTRACT: According to the World Health Organization (WHO), cancer is the second leading cause of death globally, following ischemic heart disease. As of 2020, cancer accounted for approximately 10 million deaths-equating one in six worldwide. Although many cancers can be cured if detected early, but conventional chemotherapy faces serious limitations, including systemic toxicity, poor selectivity, and damage to healthy tissues. These challenges have spurred the development of more targeted and effective therapeutic strategies. Biodegradable nanocarriers have emerged as a promising advancement in cancer therapy. By enabling site-specific drug delivery, they significantly reduce toxicity and resistance while maximizing the pharmacological action at the tumor site. Their biodegradable nature ensures that they break down into non-toxic by products within the body, offering additional biocompatibility advantages. However, the application of nanocarrier-based therapies presents its own set of challenges. Designing nanoparticles with the optimal size, shape, and appropriate inner and outer layers tailored to specific cancer types remains a complex task. Overcoming these obstacles requires advanced approaches, including the development of massively parallel pooled screening methods and other innovative research tools. Such strategies hold the potential to fine-tune nanocarrier systems for maximum therapeutic impact in future oncological treatments.

INTRODUCTION: Most recent data about cancer: Global New Cases (Incidence) – 2020 data (Comparable to “2025” estimates).

1. Lung: 2.21 M (12.4%).
2. Breast (female): 2.26 M (11.6%)
3. Colorectal: 1.93 M (9.6%)
4. Prostate: 1.41 M (7.3%)
5. Skin (non-melanoma): 1.20 M (\approx 6.7%).
6. Stomach: 1.09 M (4.9%)

Global Cancer Deaths: 2020 data with percentages:

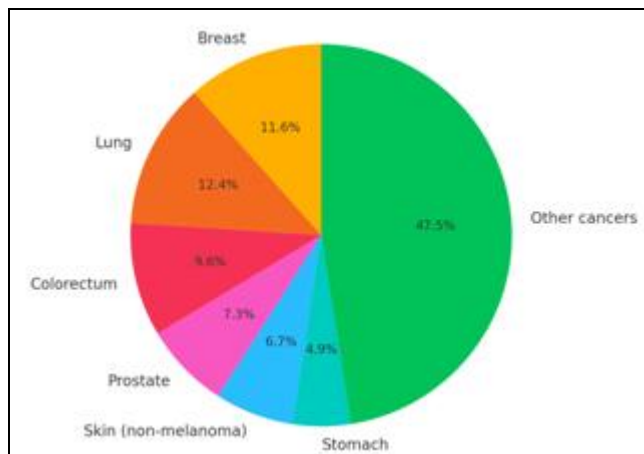
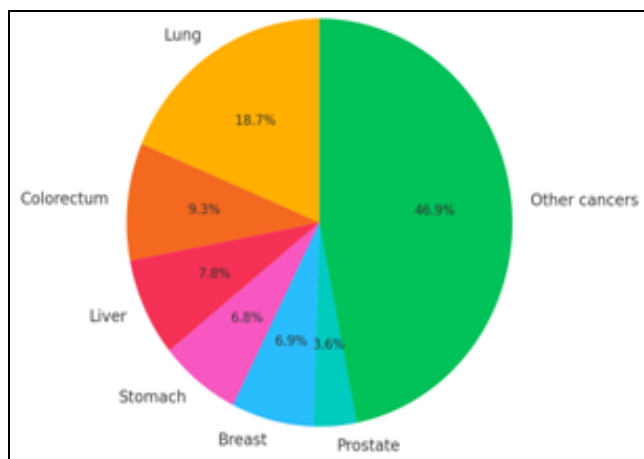
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1. Lung: 1.80 M deaths → 18.7% of all cancer deaths.
2. Colorectal: 916,000 → 9.3%.
3. Liver: 830,000 → 7.8%.
4. Breast: 685,000 → 6.9%.
5. Stomach: 769,000 → 6.8%^{1, 5, 15}.

TABLE 1:

| Cancer type | % of new cases | % of cancer deaths |
|---------------------|----------------|--------------------|
| Lung | ~12-13% | ~19% |
| Breast | ~11.6% | ~6.9% |
| Colorectal | ~9.6% | ~9.3% |
| Prostate | ~7.3% | ~3.-4% |
| Skin (non-melanoma) | ~6-7% | <0.1% |
| Melanoma (skin) | Low (1%) | ~0.5% |
| Stomach | ~4.9% | ~6.8-9% |

Lung cancer ranking #6 individually among all global causes of death (WHO -2021-2023 data).

**FIG. 1: PROPORTION OF GLOBAL CANCER INCIDENCE BY TYPE****FIG. 2: ESTIMATED PROPORTION OF GLOBALE CANCER DEATHS IN 2020 (WORLD, BOTH SEXES, ALL AGES) (2020)**

Now Due to the well-documented limitations of conventional chemotherapy such as systemic toxicity, non-specific distribution, rapid clearance, and poor therapeutic index nanocarrier-based drug delivery systems have emerged as promising alternatives in cancer therapy. For oral administration, nanocarriers must exhibit sufficient stability in the gastrointestinal tract, followed by targeted absorption. Once in systemic circulation, these carriers are engineered to evade renal

clearance (prevent kidney filtration), avoid opsonization by serum proteins, resist phagocytic uptake, and protect the therapeutic payload from enzymatic degradation, thereby prolonging circulation time and enhancing bioavailability. In the case of parenteral delivery, formulations like Doxil® (liposomal doxorubicin), administered via intravenous infusion, have demonstrated reduced cardiotoxicity compared to free doxorubicin, marking a significant advancement in clinical

oncology^{2, 3}. Nanocarriers are constructed from a range of natural and synthetic materials. Natural polymers such as chitosan (derived from chitin in crustacean shells), alginate, hyaluronic acid, collagen, and dextran have shown utility in drug and gene delivery, as well as tissue engineering. On the synthetic side, inorganic nanoparticles such as gold and iron oxide serve critical roles in targeted therapy, imaging, and diagnostics, while organic nanocarriers, including lipid-based systems like those in Doxil®,^{4, 13} are widely applied in the treatment of cancers such as breast and ovarian malignancies. Additionally, polymeric nanocarriers, derived from both natural and synthetic sources, are tailored based on tumor type, target organ, and specific pathophysiological conditions, allowing for a more personalized and efficient approach to cancer treatment^{1, 2}.

Formulation of Biodegradable Multilayered Nano-Carriers:

The design of multilayered

nanocarriers, such as PLGA- and liposome-based systems, involves the strategic incorporation of charged polymeric layers to enhance cellular targeting and therapeutic delivery. These nanoparticles are often formulated using a positively charged (cationic) inner layer composed of materials like poly (ethyleneimine) (PEI) or poly-L-lysine. The outer layer is constructed with negatively charged (anionic) polymers such as poly-L-aspartate, poly-L-glutamate, or hyaluronic acid. This configuration, known as layer-by-layer (LBL) assembly **Fig. 3**, enables precise control over surface chemistry and biological interactions. Hyaluronic acid, when used as the outermost layer, exhibits strong affinity for CD44 receptors, which are overexpressed on the surface of various tumor cells, including ovarian cancer cells. This receptor-mediated interaction facilitates targeted drug delivery, improving therapeutic efficacy while minimizing off-target effects^{2, 5}.

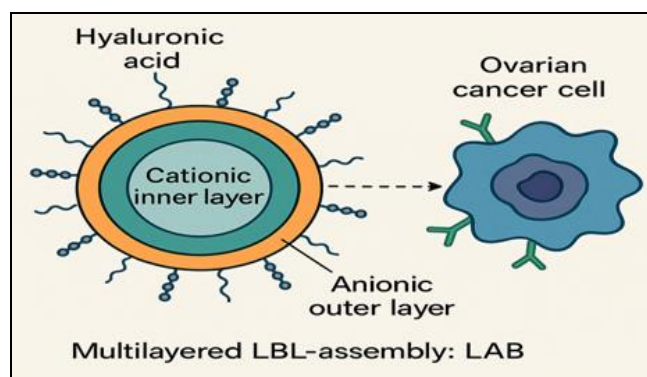


FIG. 3: MULTILAYERED LBL-ASSEMBLY

Types of Biodegradable Polymeric Nanocarriers

Fig. 3: Biodegradable polymeric nanocarriers can be broadly classified into four categories^{7, 8}:

- A. Solid nanoparticles,
- B. Core-shell structures,
- C. Polymeric micelles, and
- D. Polyplexes.

The selection of a particular nanoparticle formulation is largely determined by the physicochemical properties of the polymer and the therapeutic cargo. Polymeric nanoparticles are typically synthesized *via* either self-assembly or emulsion-based methods. Self-assembly involves both intramolecular and intermolecular interactions

between the polymer chains and the encapsulated cargo. A prominent example is formation of polyplexes **Fig. 4**, which result from the electrostatic complexation of cationic polymers with anionic nucleic acids⁸.

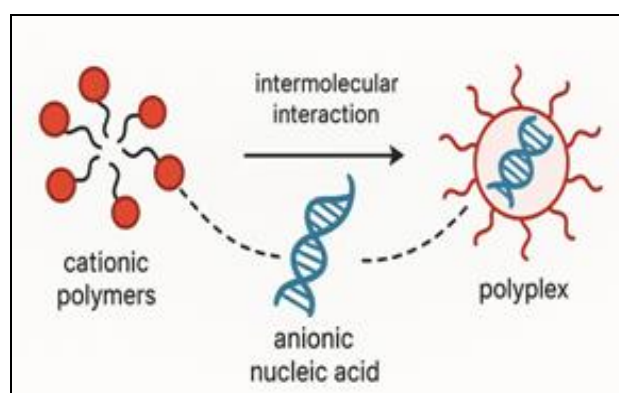


FIG. 4: FORMATION OF POLYPLEX

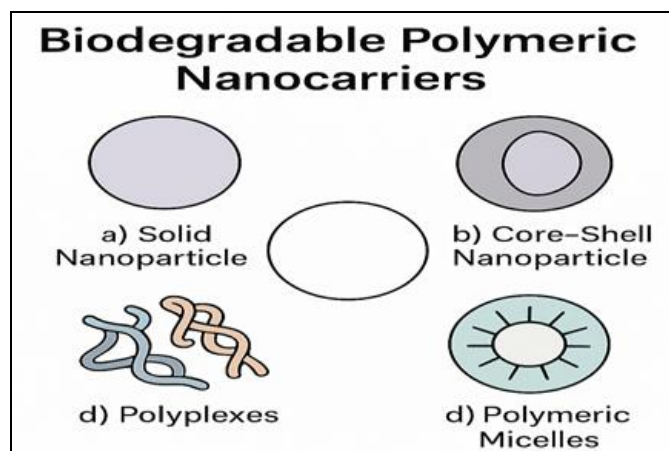


FIG. 5: DIFF. TYPES OF BIODEGRADABLE POLYMERIC NANOCARRIERS

siRNA Based Tumor Therapy and Role of Nanoparticles (NPs): Small interfering RNA (siRNA) is a short double-stranded RNA molecule, typically 20–25 nucleotides in length, generated by the cleavage of longer double-stranded RNA precursors by the Dicer enzyme. siRNA plays a pivotal role in cancer therapy by silencing specific gene expression^{8, 9}. Upon entering the cell, the siRNA forms the RNA-induced silencing complex (RISC) with other proteins, which unwinds the strands. The thermodynamically less stable strand at the 5' end remains bound to the RISC and acts as the guide strand. This guide strand targets complementary messenger RNA (mRNA), leading to its cleavage and degradation, thereby inhibiting the synthesis of proteins crucial for tumor progression, including oncogenes involved in cell proliferation, angiogenesis, and metastasis. In addition to gene silencing, siRNA offers therapeutic benefits by overcoming multidrug resistance (MDR).

It can downregulate genes such as MDR1 and P-glycoprotein (P-gp), thus enhancing the efficacy of chemotherapeutic agents. Furthermore, siRNA can modulate the tumor microenvironment and induce apoptosis by targeting anti-apoptotic genes **Fig. 6**.

Challenges and Resolution: Despite its therapeutic potential, siRNA faces significant delivery challenges: Instability in circulation due to rapid degradation by nucleases. Poor cellular uptake, as the negatively charged, hydrophilic siRNA molecules cannot easily cross cellular membranes. Off-target effects, which can lead to unintended gene silencing.

To address these obstacles, nanoparticle-based delivery systems particularly gold nanoparticles (AuNPs) have shown considerable promise. siRNA can be conjugated to AuNPs *via* covalent gold–thiol (Au–S) bonds **Fig. 6** or through electrostatic layer-by-layer (LBL) assembly¹⁰.

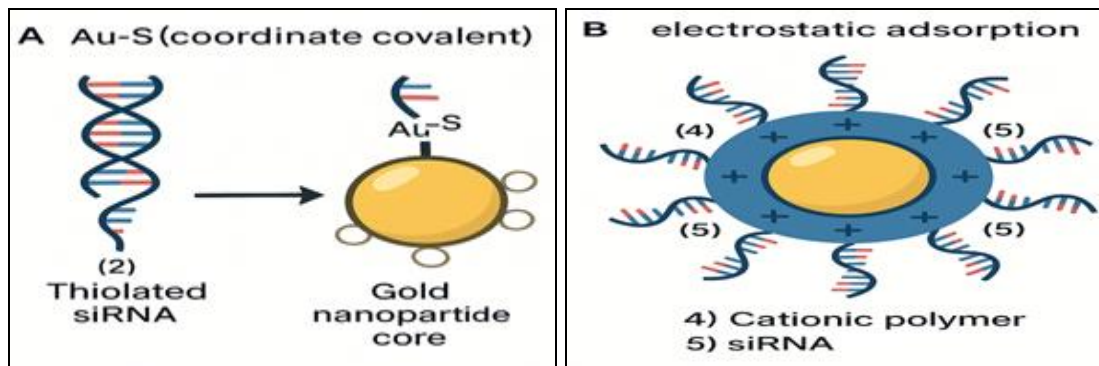


FIG. 6: BOND FORMATION

The incorporation of disulfide linkers between AuNPs and siRNA enables redox-responsive release within the cytosol, where the reducing

environment cleaves the bond and releases the therapeutic cargo. In electrostatic systems, pH- or salt-responsive polymers can be employed to

facilitate endosomal escape; these polymers swell under acidic conditions, freeing the siRNA into the cytoplasm. AuNPs and other NPs enhance siRNA-based therapy by: Protecting siRNA from enzymatic degradation in the bloodstream, which otherwise renders naked siRNA non-functional within minutes. Improving cellular uptake, as AuNPs functionalized with ligands or coated with cationic polymers enter cells *via* endocytosis,

mimicking a "Trojan horse." Enabling tumor-specific targeting, through surface modification with ligands such as folic acid or monoclonal antibodies, minimizing off-target effects and systemic toxicity. These multifunctional capabilities of AuNPs make them potent vehicles for siRNA delivery, overcoming biological barriers and enabling targeted, efficient, and controlled gene silencing in cancer therapy^{5, 10, 11}.

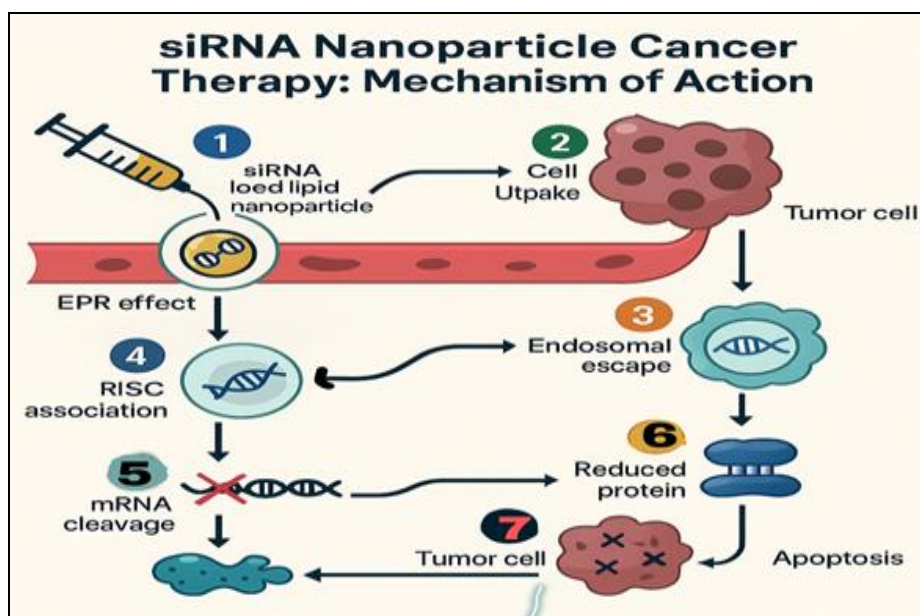


FIG. 7: MOA OF SIRNA NANOPARTICLE FOR CANCER THERAPY

Challenges in Ovarian Cancer Treatment and the Role of Biodegradable Nanocarriers:

Ovarian cancer remains one of the most lethal gynecological malignancies, primarily due to its asymptomatic nature in early stages and consequent late diagnosis. Originating mainly from the surface epithelium of the ovary, the disease is often detected only at advanced stages. Conventional chemotherapy using agents such as paclitaxel and cisplatin faces significant limitations, including:

1. Late diagnosis – due to non-specific clinical symptoms.
2. Drug resistance – tumor cells often develop resistance to first-line chemotherapeutics.
3. Systemic toxicity – non-specific distribution of drugs harms healthy tissues.
4. High recurrence rates – even after remission, the disease frequently returns^{12, 13}.

To address these challenges, nanoparticle-based delivery systems particularly those with a layer-by-

layer (LBL) architecture have emerged as promising alternatives. These nanocarriers typically consist of a positively charged core surrounded by a negatively charged outer layer designed for active targeting. The outer layer, functionalized with anionic polymers such as hyaluronic acid or carboxylated/ sulfated polyelectrolytes, demonstrates strong affinity toward ovarian cancer cells, particularly those overexpressing CD44 receptors¹³.

TABLE 2:

| Carboxylated surface chemistry family | Sulfated surface chemistry family |
|---------------------------------------|-----------------------------------|
| Poly-L- Aspartate | Dextran sulfate |
| Poly-L-glutamate | Sulfated poly (beta-cyclodextrin |
| Polyacrylate | Fucoidan |
| Sodium hyaluronate | |

In clinical strategies, intraperitoneal administration of such nanoparticles allows for direct exposure to the primary tumor and metastatic sites within the abdominal cavity.

This localized approach enhances drug retention at the tumor site, improves cellular uptake, and minimizes off-target toxicity. A focused investigation using a mini-LBL library explored

various anionic surface chemistries including carboxylated and sulfated formulations to optimize tumor binding, penetration, and therapeutic efficacy against solid ovarian tumors¹⁴.

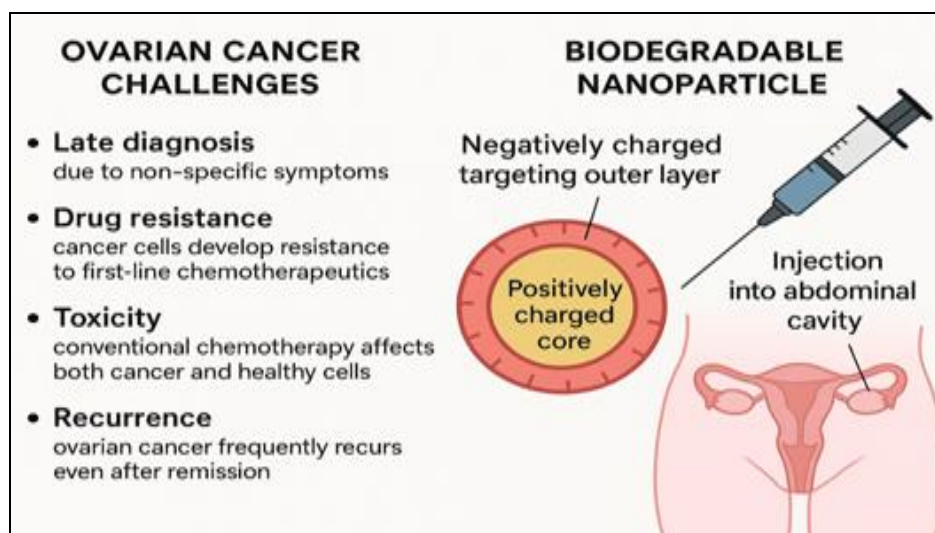


FIG. 8: OVARIAN CANCER

How these anionic polymers (outer layer) matters in ovarian cancer ?

TABLE 3:

| Anionic polymers | Advantages in ovarian cancer |
|-------------------|---|
| Poly-L-glutamate | Degradable, used in polymydrug conjugates (paclitaxel – polyglutamate e-lcojugate), enhances solubility |
| Poly-L- Aspartate | Biodegradable, biocompatible, useful for pH sensitive delivery |
| Hyaluronic acid | Targets CD44 receptor-overexpressed in ovarian cancer cell |
| Heparin | Can inhibit angiogenesis, bind to growth factor like “VEGF” |

Challenges in Brain Cancer Treatment and Role of Biodegradable NPs: Brain cancer therapy remains one of the most formidable challenges in oncology due to the presence of biological barriers chief among them the blood-brain barrier (BBB). The BBB restricts nearly 98% of chemotherapeutic agents from reaching the brain, making effective drug delivery exceedingly difficult. Glioblastoma multiforme (GBM), the most aggressive form of brain tumor, is particularly hard to treat due to its protection by the BBB, its high degree of genetic heterogeneity, and its invasive nature. The tumor cells infiltrate surrounding healthy brain tissue, which makes complete surgical resection nearly impossible^{15, 16}. Furthermore, glioblastoma rapidly develops resistance to standard chemotherapy and radiotherapy, especially to first-line drugs such as temozolomide. Despite aggressive multimodal treatment including surgery, radiation, and chemotherapy median survival remains less than one year for most patients¹⁶. To address these

limitations, researchers are increasingly turning to gene therapy approaches facilitated by biodegradable nanocarriers. Rather than directly administering cytotoxic agents, this strategy involves delivering therapeutic genes into tumor cells. These genes encode enzymes capable of converting systemically administered prodrugs (which can cross the BBB) into active cytotoxic compounds specifically within the tumor microenvironment thereby sparing healthy brain tissue^{17, 18}. A prominent example of this strategy is the use of the Herpes Simplex Virus thymidine kinase (HSVtk) gene. This gene is delivered into brain tumor cells using poly (β -amino ester) (PBAE)-based biodegradable nanoparticles. Once transfected, the patient is systemically administered ganciclovir, a non-toxic prodrug. Within the tumor cells expressing HSVtk, ganciclovir is enzymatically converted into a toxic nucleotide analog, leading to selective tumor cell death. PBAE nanoparticles play a critical role in ensuring safe

and targeted gene delivery, enhancing cellular uptake, and minimizing off-target toxicity^{17, 19}.

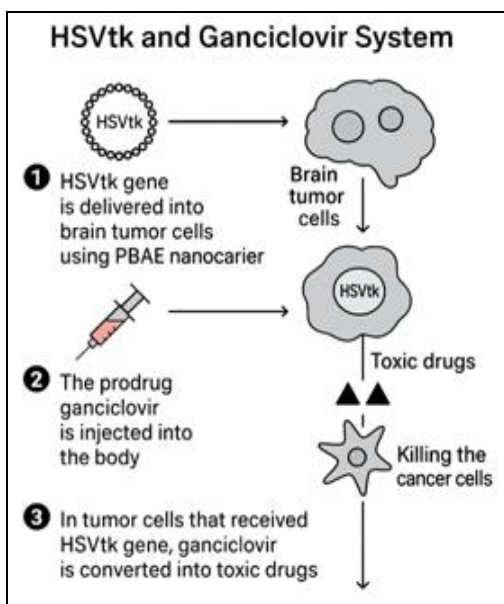


FIG. 9: HSVTK AND GANCICLOVIR SYSTEM

This nanoparticle-mediated suicide gene therapy has demonstrated significant tumor reduction and prolonged survival in preclinical glioblastoma models (rat model), offering a promising and targeted therapeutic avenue for overcoming the inherent challenges of brain cancer treatment¹⁹.

Polymeric Nanoparticle Mediated gene Therapy for Cancer: New cancer therapy target cancer associated gene using molecular tools like DNA and RNA, these tools can regulate specific gene involved in Cancer but Direct delivery of naked nucleic acids is impractical *in-vivo*: unshielded DNA / RNA is rapidly degraded by serum nucleases, opsonised for renal or hepatic clearance, and its strong negative charge repels the anionic cell membrane, culminating in poor cellular uptake and potent innate-immune activation.

Biodegradable cationic polymeric nanoparticles (NPs) elegantly solve these bottlenecks. In aqueous media, the positively charged polymer chains spontaneously condense anionic nucleic acids into compact "polyplexes," and positively charged nanoparticle penetrate the cell membrane in a process called endocytosis, then nanoparticle are trapped in "endocytic vesicle" as vesicles mature into late endosomes their pH drop (more acidic), and polymeric nanoparticles are designed to the react to the acidic conditions inside the endosomes

and they trigger a process called "endosomal escape" to break out and release their cargo into the cytoplasm.

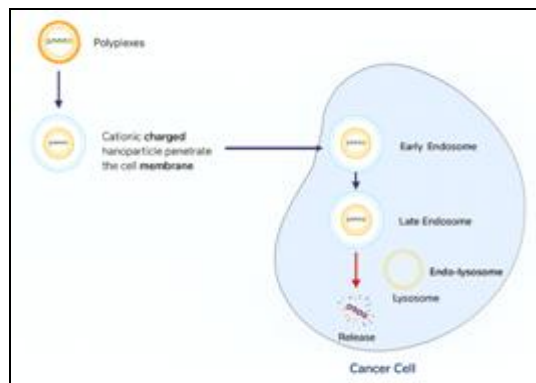


FIG. 10:

Smart polymers rich in protonatable amines or imidazole groups act as proton sponges: they buffer the incoming H^+ ions, induce osmotic swelling, rupture the endosomal membrane, and release the genetic payload into the cytosol before lysosomal degradation can occur²⁰. From there the therapeutic nucleic acid can access its target whether to silence an oncogene, restore a tumour-suppressor, or prime a suicide-gene/prodrug system while any co-encapsulated chemotherapeutic is simultaneously liberated to deliver a synergistic cytotoxic punch. Thus, cationic polymeric NPs provide a single, tunable platform that protects, transports, and precisely releases genetic cargoes, converting fragile molecular tools into clinically viable anticancer agents^{20, 21}.

Design of a Massively Parallel Pooled Screen to Evaluate Nanoparticle Delivery to Cancer Cells Using the PRISM Platform: To systematically evaluate the efficiency and selectivity of nanoparticle (NP) delivery across diverse cancer types, we employed a massively parallel pooled screening approach utilizing the PRISM (Profiling Relative Inhibition Simultaneously in Mixtures) platform. This method enables high-throughput assessment of NP formulations in a mixed population of molecularly barcoded human cancer cell lines²².

Pooled Cell Lines: The screening employs a library of 488 DNA-barcoded human cancer cell lines, pooled together in a single culture. Each cell line in the PRISM collection is uniquely tagged with a short, stable DNA barcode, allowing for

post-treatment identification *via* sequencing. This pooling strategy permits parallel evaluation of NP delivery across hundreds of genetically diverse cancer models within a single experiment²³.

Nanoparticle Formulation Screening: Various NP formulations differing in parameters such as size, charge, composition, targeting ligand, and surface chemistry are synthesized and characterized. These NPs are then administered to the pooled cell population to assess their relative uptake or delivery efficiency.

Time Points: To capture dynamic delivery kinetics, samples are collected at multiple time points post NP exposure (e.g., 1 h, 4 h, 24 h). This temporal profiling helps distinguish early endocytic events from downstream processing and intracellular trafficking of nanoparticles.

Fluorescence-Activated Cell Sorting (FACS)

Post-treatment: Cells are subjected to FACS based on a fluorescence signal linked to NP uptake (e.g., fluorescently labeled NP cargo). Cells are sorted into discrete bins (A–D) reflecting varying levels of fluorescence intensity corresponding to high, medium, low, or no NP uptake.

DNA Barcode Sequencing: Genomic DNA is extracted from cells in each fluorescence bin, and the integrated barcodes are PCR-amplified and sequenced. Quantitative barcode counts indicate the relative abundance of each cell line in each bin, thus enabling reconstruction of NP delivery profiles for each formulation across all cancer cell types.

This approach helps to identify optimal nanoparticles formulations for targetting specific cancer, enabling personalized nanomedicine^{23, 24}.

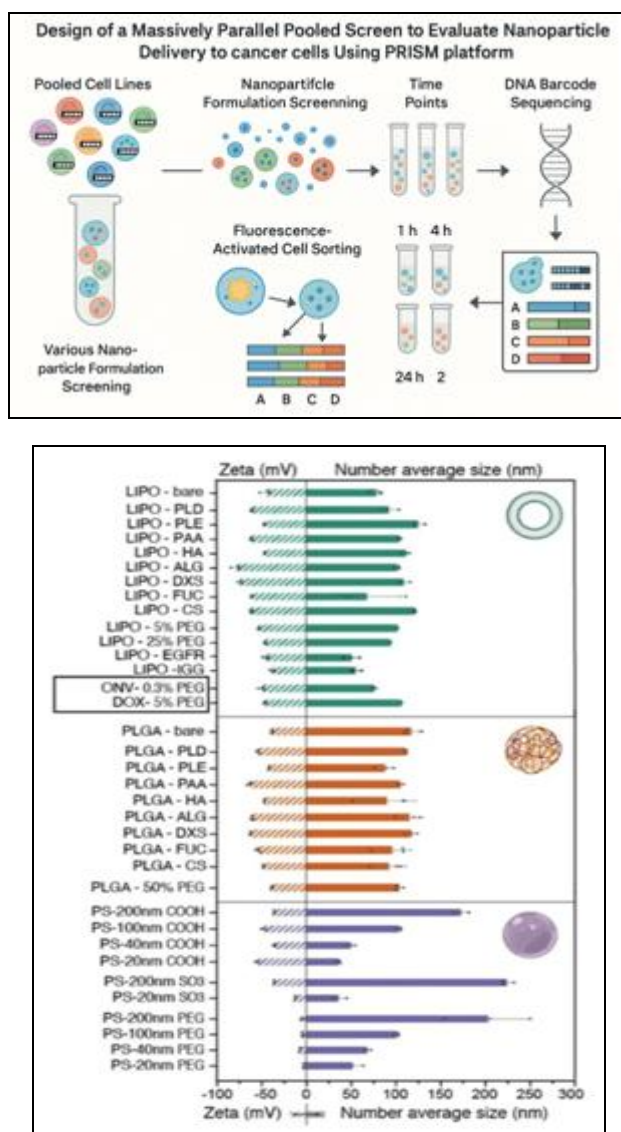


FIG. 11:

CONCLUSION: The paradigm of cancer therapy is rapidly shifting from traditional cytotoxic regimens to targeted, patient-specific interventions enabled by biodegradable nanocarriers. These smart delivery systems offer unprecedented control over drug release, improve therapeutic indices, and reduce systemic toxicity by exploiting tumor-specific cues such as receptor expression, pH, redox environment, and tissue architecture. Whether through passive targeting mechanisms like the EPR effect or active targeting *via* receptor-ligand interactions, nanocarriers provide a modular and tunable platform for precise therapy. Advancements in polymer science have allowed the creation of sophisticated multilayered and stimuli-responsive nanoparticles such as PLGA, poly-L-glutamate, hyaluronic acid, and PBAE-based systems that not only protect sensitive payloads like siRNA and DNA but also ensure controlled intracellular delivery. In hard-to-treat cancers such as glioblastoma and ovarian cancer, these carriers facilitate entry past formidable barriers (e.g., the BBB or peritoneal tumor clusters), achieving therapeutic actions previously unattainable with conventional approaches. Moreover, the integration of high-throughput technologies such as the PRISM platform enables large-scale, multiplexed screening of NP formulations across diverse cancer genotypes, accelerating the identification of optimal nanocarrier designs for precision oncology. In essence, biodegradable nanocarriers are no longer experimental adjuncts they represent the vanguard of next-generation cancer treatment.

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