wjpmr, 2025, 11(10), 143-158

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.wjpmr.com

SJIF Impact Factor: 6.842

Review Article
ISSN (O): 2455-3301
ISSN (P): 3051-2557

HARNESSING MICROBIAL VECTORS: ADVANCES IN BACTERIA- MEDIATED DRUG DELIVERY

Patel Pankti Bhavikkumar*, Dr. Ghanshyam Patel, Patel Poorv, Patel Harsh, Patel Rajkumar, Prajapati Tushar

Dr. Ghanshyam Patel, Patel Poorv, Patel Harsh, Patel Rajkumar, Prajapati Tushar.

***Corresponding Author: Patel Pankti Bhavikkumar**
Dr. Ghanshyam Patel, Patel Poorv, Patel Harsh, Patel Rajkumar, Prajapati Tushar.
DOI: https://doi.org/10.5281/zenodo.17224117

Article Received on 31/07/2025 **Article Revised on 21/08/2025** **Article Accepted on 10/09/2025**

ABSTRACT
Bacteria-mediated drug delivery systems are an emerging frontier in biomedical research, offering a novel method for precise and efficient therapeutic delivery, especially in oncology. These systems exploit the natural motility, chemotaxis, and tumor-targeting abilities of certain bacterial strains—such as Salmonella, Clostridium, and Escherichia coli—which preferentially accumulate in hypoxic and necrotic regions of tumors. By genetically engineering these bacteria or using their derivatives like outer membrane vesicles (OMVs), scientists can load them with anticancer agents, immunomodulators, or gene-editing tools. Once inside the tumor microenvironment, the bacteria can release their payload in response to specific stimuli, such as pH changes or enzymatic activity, ensuring localized treatment and minimizing systemic toxicity. Additionally, some bacteria can stimulate innate and adaptive immune responses, enhancing the therapeutic effect through immunogenic synergy. Despite their promise, challenges remain in ensuring biosafety, controlling bacterial replication, and achieving regulatory approval for clinical use. Nonetheless, this approach holds transformative potential for targeted therapies in hard-to-treat cancers and other localized diseases.

KEYWORDS: Bacteria-mediated delivery, Targeted drug delivery, Tumor-homing bacteria, Hypoxic tumor microenvironment, Bacterial vectors, genetically engineered bacteria, Outer membrane vesicles (OMVs), Microbial therapy, Cancer nanomedicine, Chemotaxis, Tumor penetration.

1. INTRODUCTION
Bacteria-mediated drug delivery is a rapidly evolving field that leverages the unique biological properties of bacteria to transport therapeutic agents directly to diseased tissues, particularly tumors. Unlike conventional drug delivery systems, certain bacterial strains possess natural abilities such as motility, chemotaxis, and selective colonization of hypoxic environments—traits that make them ideal candidates for targeting hard-to-reach tumor regions. Researchers have harnessed these features by engineering bacteria or using bacterial components like outer membrane vesicles to carry drugs, genes, or immune modulators. This approach not only enhances the precision of drug delivery but also reduces systemic toxicity and side effects. Moreover, some bacteria can stimulate the host's immune system, offering a dual therapeutic benefit. As interest grows in combining synthetic biology with microbial therapy, bacteria-mediated drug delivery is emerging as a promising strategy in the fight against cancer and other complex diseases.

The historical development of microbial therapeutics is a fascinating journey that spans centuries, beginning with early speculation and culminating in modern bioengineering. The concept of using microorganisms to treat disease dates back to the Discovery Era (1546–1676), when thinkers like Girolamo Fracastoro proposed that invisible living agents could cause illness. This idea gained traction with Anton van Leeuwenhoek's groundbreaking observations of "animalcules" in 1676, marking the birth of microbiology.

During the Golden Age of Microbiology (1857–1910), pioneers such as Louis Pasteur and Robert Koch established the germ theory of disease and demonstrated that specific microbes were responsible for particular infections. This era also saw the development of vaccines and early antimicrobial treatments, laying the foundation for therapeutic applications of microbes.

In the 20th century, the field expanded dramatically with the discovery of antibiotics like penicillin, and later, the rise of molecular biology enabled genetic manipulation of microbes for therapeutic use. The emergence of probiotics, oncolytic bacteria, and engineered microbial

vectors in recent decades has transformed microbes from mere pathogens into precision tools for drug delivery, cancer therapy, and immune modulation.

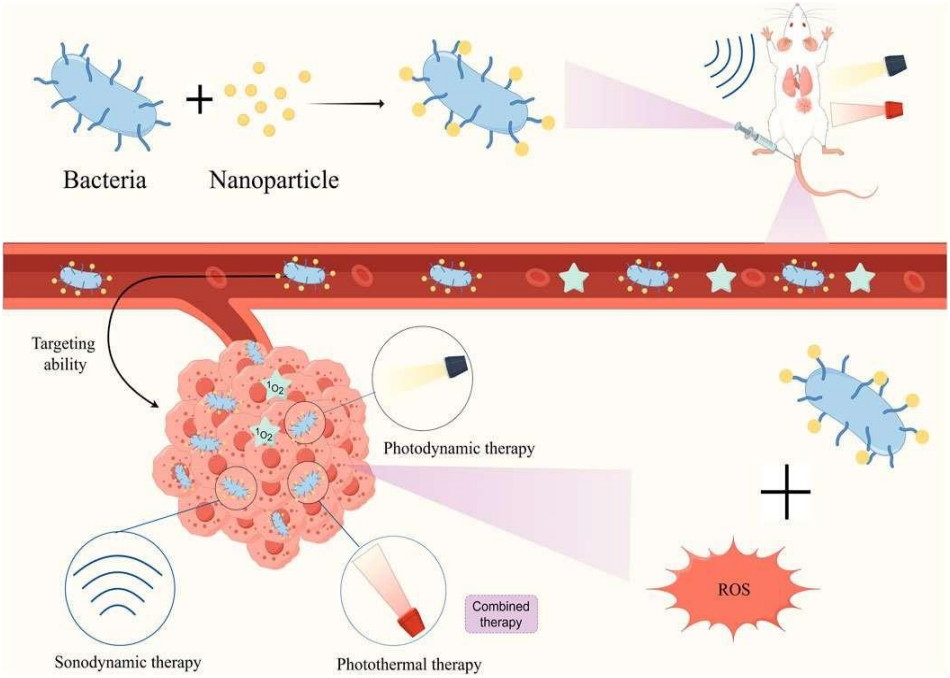
Today, microbial therapeutics are at the cutting edge of biotechnology, with advances in synthetic biology allowing for programmable bacteria that can seek out tumors, deliver drugs, and even self-destruct after completing their mission. This evolution reflects a profound shift—from fearing microbes to harnessing them as allies in medicine.

Bacteria possess a remarkable set of biological traits that make them uniquely suited for drug delivery, especially in targeting complex diseases like cancer. One of their most compelling features is chemotaxis—the ability to move toward specific chemical signals. This allows certain strains, such as Salmonella and Clostridium, to actively migrate into tumor microenvironments, which are often hypoxic and acidic— conditions where traditional drug carriers struggle to penetrate.

Additionally, bacteria can be genetically engineered to express therapeutic proteins, carry plasmids, or release drugs in response to environmental cues like pH or temperature. Their natural ability to colonize tissues, especially necrotic or inflamed regions, gives them an edge in reaching areas that are otherwise inaccessible to conventional therapies. Some bacteria also produce outer membrane vesicles (OMVs), which can be loaded with drugs and used as nano-sized delivery vehicles with high biocompatibility and stability.

Moreover, bacteria can act as immune modulators, stimulating both innate and adaptive immune responses. This dual role—delivering drugs and activating the immune system—makes them powerful tools in immunotherapy and cancer treatment. Advances in synthetic biology have further enhanced their utility, allowing precise control over replication, drug release, and safety profiles.

In short, bacteria are not just passive carriers—they're active, programmable, and biologically intelligent systems that can navigate the body, deliver payloads, and even self-destruct when their job is done.



2. Mechanisms of Targeting and Delivery

Chemotaxis and tumor tropism are two pivotal biological traits that make bacteria exceptionally effective carriers in targeted drug delivery systems, especially for cancer therapy.

Chemotaxis refers to the ability of bacteria to move directionally in response to chemical gradients in their environment. Tumors often release specific metabolites and signalling molecules—such as amino acids, nucleotides, and inflammatory cytokines— that attract motile bacteria. This natural navigation system allows bacteria to actively seek out and infiltrate tumor tissues, unlike passive drug carriers that rely on circulation and diffusion.

Tumor Tropism is the tendency of certain bacterial strains to preferentially colonize tumor microenvironments. Tumors typically exhibit hypoxia (low oxygen), necrosis, and immune suppression—conditions that are hostile to most cells but favorable to anaerobic or facultative anaerobic bacteria like Clostridium, Salmonella, and Bifidobacterium. These bacteria thrive in such niches, allowing them to penetrate

deep into tumor cores where conventional therapies often fail to reach.

Together, chemotaxis and tumor tropism enable bacteria to serve as "living missiles," homing in on cancerous tissues with remarkable precision. This not only enhances the efficacy of drug delivery but also minimizes off-target effects and systemic toxicity. As highlighted in recent studies, these traits are being harnessed through synthetic biology to engineer bacteria with enhanced targeting capabilities and controlled drug release mechanisms.

Hypoxia-targeting behavior is a defining feature that makes certain bacteria highly effective for drug delivery in solid tumors. Most tumors develop regions of chronic hypoxia due to rapid cell proliferation and poor vascularization, which limits the penetration and efficacy of conventional therapies. However, anaerobic and facultative anaerobic bacteria—such as Clostridium, Bifidobacterium, and Salmonella—naturally thrive in these low-oxygen environments, giving them a unique advantage for targeted delivery.

These bacteria can selectively colonize hypoxic tumor cores, where they either directly exert cytotoxic effects or serve as carriers for chemotherapeutic agents. For example, a recent study developed a biohybrid system using Bifidobacterium infantis loaded with doxorubicin nanoparticles (Bif@DOX-NPs), which showed a fourfold increase in drug concentration within hypoxic tumor regions compared to free drug administration. This not only enhanced therapeutic efficacy but also reduced systemic toxicity and prolonged survival in animal models.

By exploiting hypoxia-targeting behavior, researchers are designing smart bacterial systems that can deliver drugs precisely where they're needed most—deep inside tumors that resist traditional treatments. This strategy is paving the way for more effective and less invasive cancer therapies.

Intracellular delivery via bacterial invasion or vesicle fusion is a sophisticated mechanism that enhances the precision and efficacy of bacteria-mediated drug delivery systems. Certain bacteria, such as Salmonella, Listeria, and Shigella, possess the natural ability to invade host cells by manipulating cellular uptake pathways. Once inside, they can release therapeutic payloads directly into the cytoplasm, bypassing extracellular barriers and improving drug bioavailability. This invasive behavior is particularly useful for targeting intracellular pathogens or cancer cells that are shielded from conventional therapies.

In parallel, bacterial membrane vesicles (BMVs)—nano-sized, lipid-bound particles naturally secreted by bacteria—offer a non-invasive alternative. These vesicles can be engineered to encapsulate drugs, proteins, or nucleic acids and deliver them to host cells via membrane fusion or endocytosis. BMVs are biocompatible, stable, and capable of crossing biological membranes, making them ideal for intracellular delivery. Recent studies highlight their potential in anticancer and antimicrobial therapies, with promising results in enhancing drug uptake and reducing systemic toxicity.

Together, bacterial invasion and vesicle fusion represent two complementary strategies for intracellular drug delivery, each with unique advantages in targeting hard-to-reach cellular compartments.

3. TYPES OF BACTERIAL CARRIERS

Bacterial carriers are a fascinating and rapidly evolving class of bio-nanocarriers used in drug delivery, especially for cancer therapy. Their natural ability to target hypoxic tumor environments, penetrate tissues, and stimulate immune responses makes them uniquely suited for this role.

➢ **Types of Bacterial Carriers in Drug Delivery**

Types	Description
Live Attenuated Bacteria	Genetically modified to reduce virulence while retaining tumor-targeting ability
Bacterial Ghosts	Empty bacterial envelopes created by lysis, used to carry drugs or genes
Bacterial (OMVs) Outer Membrane Vesicles	Nano-sized vesicles naturally secreted by bacteria
Engineered Bacteria	Synthetic biology tools used to program bacteria for specific tasks
Bacterial Spores	Dormant forms of bacteria that can survive harsh conditions
Bacterial Minicells	Small, anucleate cells derived from bacteria

❖ **Live attenuated bacteria (Salmonella, Clostridium, E. coli)**

Live attenuated bacteria like Salmonella, Clostridium, and Escherichia coli (E. coli) are genetically or chemically modified strains that have lost their ability to cause disease but still retain the ability to stimulate a strong immune response. These are used in vaccine development and therapeutic delivery systems. Here's how each plays a role:

✓ **Salmonella**

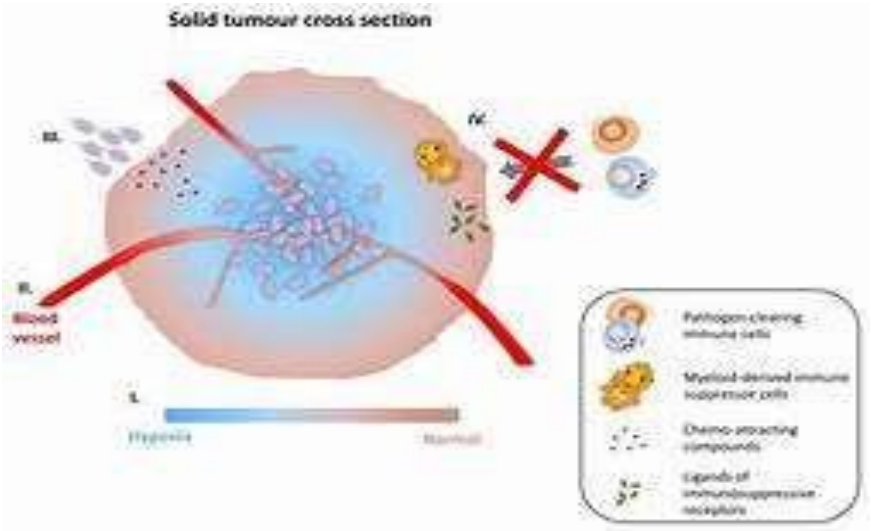
- Vaccine Use: The Ty21a strain is a well-known live

attenuated oral vaccine against typhoid fever.
- Mechanism: It mimics natural infection via the gut, triggering mucosal and systemic immunity.
- Research Applications: Engineered Salmonella strains are used as vectors to deliver DNA vaccines and tumor antigens due to their ability to invade host cells.

✓ **Clostridium**
- Therapeutic Potential: Certain anaerobic Clostridium species naturally target hypoxic tumor environments, making them candidates for cancer immunotherapy.
- Challenges: Safety concerns due to toxin production and potential reversion to virulence require careful genetic attenuation.
- Emerging Use: Research is exploring Clostridium as a delivery system for therapeutic agents in oncology.

✓ **E. coli**
- Vaccine Development: Attenuated E. coli strains are being studied for vaccines against diarrheagenic strains and as carriers for heterologous antigens.
- Advantages: Easy to manipulate genetically and capable of expressing foreign proteins.
- Limitations: Must be carefully attenuated to avoid triggering harmful immune responses or toxin-related effects.

✓ **Why Use Live Attenuated Bacteria?**
- Strong Immunogenicity: They mimic natural infection, leading to robust and long- lasting immunity.
- Mucosal Delivery: Ideal for oral or nasal vaccines, which are less invasive and more accessible.
- Versatility: Can be engineered to carry DNA, RNA, or protein antigens for infectious diseases and cancer.



❖ **Bacterial outer membrane vesicles (OMVs)**

✓ **What Are OMVs?**

OMVs are nanoscale vesicles (typically 50–250 nm in diameter) released from the outer membrane of Gram-negative bacteria. They are formed through a process called blebbing, where parts of the outer membrane pinch off and carry cargo from the bacterial periplasm.

✓ **What Do OMVs Contain?**

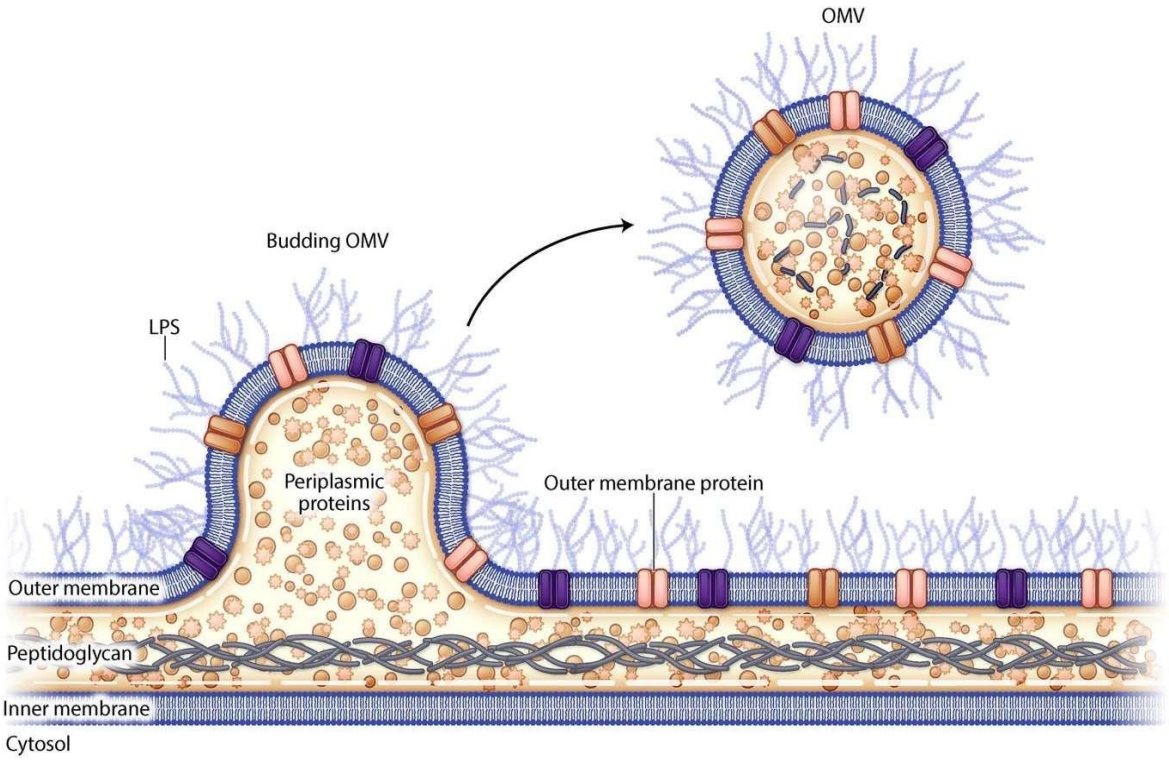
These vesicles are packed with:
- Lipopolysaccharides (LPS) – potent immune stimulators
- Proteins – including enzymes and virulence factors
- DNA and RNA – for genetic exchange
- Peptidoglycan fragments – which can trigger immune responses

✓ **Functions and Roles**

OMVs are not just cellular debris—they're strategic tools. They:
- Facilitate communication between bacteria and host cells
- Deliver toxins and virulence factors during infection
- Modulate immune responses by interacting with host receptors
- Aid in biofilm formation and quorum sensing
- Serve as vehicles for horizontal gene transfer

✓ **Biomedical Applications**

OMVs are being explored for
- Vaccines – e.g., against meningitis and pertussis
- Drug delivery systems – due to their natural targeting abilities
- Diagnostics – as biomarkers for bacterial infection



❖ **Engineered synthetic bacteria and probiotics**

✓ **What Are Engineered Synthetic Bacteria?**

Engineered synthetic bacteria are microorganisms that have been genetically designed or modified using synthetic biology tools. Unlike traditional genetic engineering, synthetic biology allows scientists to build entirely new biological systems or redesign existing ones with precision.

✓ **Applications**

- Disease detection: Bacteria engineered to sense and report biomarkers of cancer, inflammation, or infection.
- Drug delivery: Targeted release of therapeutics at disease sites (e.g., tumors or inflamed tissues).
- Bioremediation: Breakdown of pollutants or toxins in soil and water.
- Agriculture: Boosting plant growth or protecting crops from pathogens.

✓ **What Are Engineered Probiotics?**

Engineered probiotics are beneficial microbes—typically found in the gut—that have been genetically modified to enhance their natural functions or gain new therapeutic capabilities.

- **Natural Role of Probiotics**

- Maintain gut microbiota balance
- Support digestion and nutrient absorption
- Modulate immune responses
- Protect against pathogens

- **Engineered Enhancements**

- Anti-inflammatory effects: Producing molecules that reduce gut inflammation (e.g., in IBD or Crohn's disease).
- Metabolic regulation: Helping manage obesity, diabetes, and cholesterol levels.
- Neurological support: Synthesizing neurotransmitters like GABA or serotonin to influence mood and cognition.
- Antimicrobial action: Secreting peptides that kill harmful bacteria like Clostridium difficile or Staphylococcus aureus.

✓ **Technologies Behind the Engineering**

- CRISPR-Cas systems: Precision gene editing for adding, removing, or modifying DNA sequences.
- Synthetic gene circuits: Logic-based systems that allow bacteria to "decide" when to act.
- Omics integration: Using genomics, proteomics, and metabolomics to design personalized microbial therapies.
- Genome mining: Discovering new biosynthetic pathways from unexplored microbial environments.

✓ **Therapeutic Potential**

According to recent reviews, engineered probiotics are being explored for treating

- Inflammatory bowel disease
- Bacterial infections
- Tumors
- Metabolic disorders

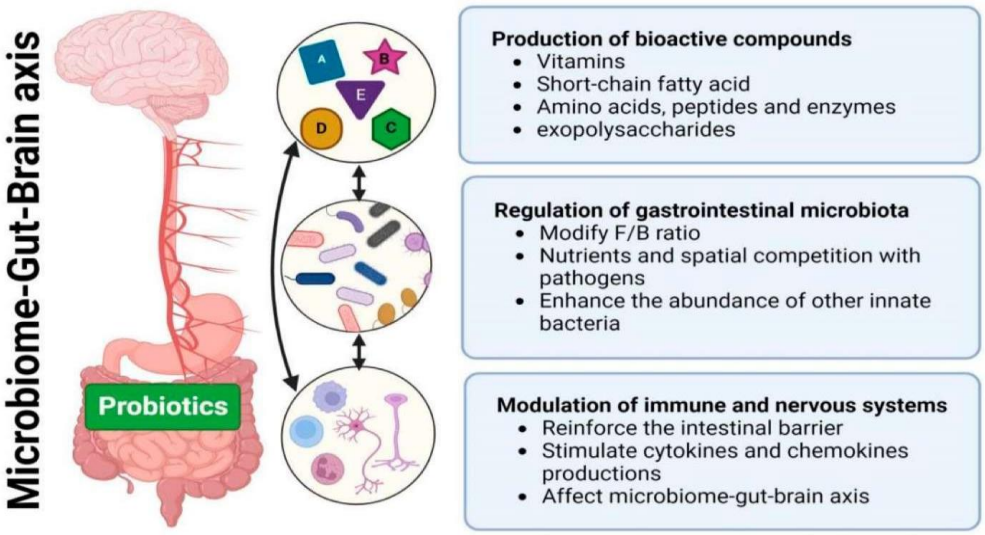
They can also serve as living diagnostics, detecting disease states and reporting them through measurable signals.

✓ **Challenges and Considerations**

- Safety: Ensuring engineered microbes don't cause unintended harm or spread uncontrollably.
- Regulation: Navigating complex approval processes for genetically modified organisms.
- Stability: Maintaining function and viability in the human body or environment.
- Ethics: Addressing concerns around genetic manipulation and synthetic life.

✓ **Future Directions**

- Personalized probiotics tailored to individual microbiomes.
- Smart microbial therapies that adapt to changing conditions in the body.
- Integration with AI to design and optimize microbial functions.
- Global health applications to combat antibiotic resistance and malnutrition.



4. GENETIC ENGINEERING AND SYNTHETIC BIOLOGY

Genetic engineering and synthetic biology are transformative fields that are reshaping science, medicine, and agriculture. Genetic engineering involves directly modifying an organism's DNA to introduce, delete, or alter specific genes. This technique has revolutionized medicine—enabling the production of insulin, gene therapies for inherited disorders, and genetically modified crops that resist pests and drought.

Synthetic biology, meanwhile, goes a step further. It combines biology with engineering and computer science to design and build entirely new biological systems and organisms that don't exist in nature. Scientists use standardized genetic parts to create novel functions, such as biosensors that detect disease, biofuels that reduce carbon emissions, and even artificial organs.

Together, these disciplines are driving innovations like CAR-T cell therapy for cancer, precision agriculture using genetic data and sensors, and personalized medicine tailored to an individual's genetic makeup. As the global population grows and environmental challenges intensify, genetic engineering and synthetic biology offer powerful tools to ensure food security, improve healthcare, and promote sustainability.

CRISPR-based modifications represent a groundbreaking leap in precision genetic engineering, enabling scientists to target and alter specific DNA sequences with remarkable accuracy. At the core of this technology is the CRISPR-Cas9 system, originally derived from bacterial immune defenses, which uses a custom-designed guide RNA (gRNA) to locate a precise genetic sequence within the genome. Once the gRNA binds to its target, the Cas9 enzyme acts as molecular scissors, creating a double- strand break at the exact location. This break activates the cell's natural repair mechanisms, allowing researchers to either disrupt a gene or insert new genetic material through homology-directed repair. The specificity of CRISPR targeting is enhanced by bioinformatics tools that analyze sequence uniqueness and chromatin accessibility, minimizing off-target effects and improving editing efficiency. In therapeutic contexts, CRISPR has been used to correct mutations responsible for diseases like sickle cell anemia and to reprogram immune cells for cancer treatment. Moreover, advanced variants such as CRISPR-Cas12 and CRISPR-Cas13 expand the toolkit to include RNA editing and epigenetic modifications, opening new frontiers in functional genomics and precision medicine. As researchers refine delivery systems—such as lipid nanoparticles and viral vectors—the potential of CRISPR to revolutionize diagnostics, therapeutics, and synthetic biology continues to grow.

Programmable drug release systems represent a cutting-edge advancement in personalized medicine, offering dynamic control over how and when therapeutic agents are delivered within the body. Unlike traditional drug delivery methods that rely on passive diffusion or fixed-release profiles, programmable systems are designed to respond to specific stimuli—such as electrical signals, pH changes, temperature shifts, or biochemical markers—to release drugs in a controlled, on-demand fashion.

One innovative approach involves 3D-printed electro-responsive systems, which integrate conductive polymers with thermoplastic materials to create drug delivery platforms that can be activated by electrical pulses. These systems allow for real-time modulation of drug release, enabling tailored dosing based on patient needs. For example, applying different voltages can alter the release kinetics, offering precise temporal control over medication delivery.

Another promising strategy combines cell-free protein synthesis (CFPS) with vesicle- based delivery platforms, enabling the programmable production and release of therapeutic proteins. These systems can be engineered to respond to environmental cues or genetic circuits, making them highly adaptable for complex treatment scenarios such as cancer or autoimmune diseases.

Additionally, active implantable drug delivery devices are being developed to bypass physiological barriers and deliver drugs directly to targeted tissues. These miniaturized systems can be remotely activated, programmed for sustained or pulsatile release, and are particularly useful for managing chronic conditions or delivering drugs across the blood–brain barrier.

Together, these technologies are paving the way for smart therapeutics that align with the principles of precision medicine—maximizing efficacy while minimizing side effects. If you're curious, I can dive deeper into how these systems are being used in cancer treatment, neurological disorders, or even wearable health tech.

Biosensors and logic gates in bacterial circuits are at the forefront of synthetic biology, enabling microbes to act as intelligent, programmable systems for diagnostics, therapeutics, and environmental monitoring.

- **Biosensors in Bacteria**

Bacterial biosensors are engineered cells that detect specific molecules—such as toxins, disease biomarkers, or metabolic signals—and produce a measurable output like fluorescence, color change, or electrical signal.

These sensors typically consist of

- Input module: A promoter or receptor that responds to a target molecule.
- Signal transduction: Converts the detection into a cellular response.
- Output module: Generates a visible or quantifiable signal.

For example, engineered bacteria can detect inflammation markers in the gut and release anti-inflammatory compounds in response. These "sense-and-respond" systems are being developed for real-time disease monitoring and targeted drug delivery.

- **Logic Gates in Bacterial Circuits**

Logic gates—like AND, OR, and NOT—allow bacteria to process multiple inputs and make decisions based on complex environmental conditions. These gates are built using genetic components such as:

- Promoters and repressors: Control gene expression based on input signals.
- Quorum sensing molecules: Enable communication between bacterial cells.
- CRISPR-based switches: Provide precise control over gene activation or silencing.

A notable example is a bacteria-based AND logic gate that only activates a response when two specific quorum-sensing molecules are present. This allows for highly selective behavior, such as releasing a drug only when both a disease marker and a specific tissue signal are detected.

- **Applications and Future Directions**

- Theranostics: Bacteria that diagnose and treat diseases simultaneously, offering closed-loop control over therapeutic release.
- Environmental sensing: Detect pollutants or heavy metals in water and soil.
- Smart probiotics: Engineered gut bacteria that monitor health and deliver treatments autonomously.

These systems are transforming bacteria into living computers—capable of sensing, processing, and responding to biological information with precision. If you'd like, I can show how these circuits are being used in cancer therapy or even wastewater treatment.

5. THERAPEUTIC PAYLOADS

Therapeutic payloads are the active agents delivered by advanced drug delivery systems to treat diseases with high precision and potency. These payloads can include small molecules, proteins, nucleic acids (like mRNA or siRNA), or cytotoxic agents, depending on the therapeutic goal and delivery platform.

- **Types of Therapeutic Payloads**

- mRNA (Messenger RNA): Used to express therapeutic proteins or antigens. It gained prominence through COVID-19 vaccines and is now being explored for cancer, rare genetic disorders, and infectious diseases. Its transient nature avoids permanent gene integration, and lipid nanoparticles (LNPs) are commonly used to protect and deliver

mRNA efficiently.

- siRNA (Small Interfering RNA): Enables gene silencing by degrading specific mRNA transcripts. siRNA therapies are being developed for conditions like hereditary amyloidosis, cancer, and immune disorders. Like mRNA, siRNA requires protective carriers to avoid degradation and ensure cellular uptake.
- Antibody–Drug Conjugates (ADCs): Combine monoclonal antibodies with potent cytotoxic drugs. These payloads are delivered directly to target cells, minimizing damage to healthy tissue. Recent innovations include dual-payload ADCs, which carry two different drugs to enhance efficacy and reduce resistance in cancer treatment.
- Protein and Peptide Payloads: Used in hormone replacement, enzyme therapy, and immunomodulation. These require stabilization and targeted delivery to remain effective in the body.

- **Delivery Innovations**

- NanoAssemblr™ Technology: A microfluidic platform that improves encapsulation efficiency and reproducibility for mRNA and siRNA-loaded nanoparticles.
- Dual-Payload ADCs: These next-gen ADCs combine two therapeutic agents to target multiple pathways, offering synergistic effects and overcoming drug resistance in tumors.

Therapeutic payloads are the heart of modern biomedicine, transforming how we treat complex diseases with precision and minimal side effects. If you're curious, I can explore how these payloads are being used in personalized medicine or regenerative therapies.

Chemotherapeutic agents are specialized drugs used primarily in the treatment of cancer, designed to target and destroy rapidly dividing cells. These agents interfere with the cell cycle, disrupting DNA replication, RNA transcription, or protein synthesis to inhibit tumor growth. They are broadly classified into categories such as alkylating agents, antimetabolites, antitumor antibiotics, topoisomerase inhibitors, and mitotic inhibitors—each with distinct mechanisms of action. While effective against malignant cells, chemotherapy can also affect healthy fast-growing cells, leading to side effects like hair loss, nausea, and immune suppression. Treatment strategies often involve combination regimens tailored to the cancer type, stage, and patient condition, aiming for curative, palliative, or preventive outcomes.

Immunomodulators and cytokines are integral components of immune-based therapies, offering precise control over immune system activity to treat a wide range of diseases. Immunomodulators function by either enhancing or suppressing immune responses, depending on the clinical need. For example, immunostimulatory agents such as checkpoint inhibitors activate T cells to recognize and destroy cancer cells, while immunosuppressive drugs like corticosteroids or methotrexate are used to dampen excessive immune activity in autoimmune disorders. Cytokines, on the other hand, are small signaling proteins secreted by immune cells that regulate inflammation, cell proliferation, and immune cell communication. Therapeutically, cytokines such as interleukins, interferons, and tumor necrosis factors are administered to boost immune responses in cancer or viral infections, or are targeted by biologic drugs to reduce inflammation in chronic conditions like rheumatoid arthritis. Together, immunomodulators and cytokines form the backbone of many advanced immunotherapies, enabling clinicians to manipulate immune pathways with remarkable specificity and effectiveness, ultimately improving patient outcomes across diverse medical fields.

Gene therapy vectors are specialized delivery systems used to transport therapeutic genetic material into a patient's cells to correct or replace faulty genes. These vectors are essential for the success of gene therapy, as they ensure that the genetic payload reaches the target cells efficiently and safely. Vectors are broadly classified into viral and non-viral types. Viral vectors—such as retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses (AAVs)—are engineered from viruses that naturally infect cells, but are modified to remove disease-causing elements while retaining their ability to deliver genes. Each viral vector has unique properties: for example, retroviruses integrate into the host genome for long-term expression, while AAVs are favored for their safety and ability to target specific tissues without integrating into DNA. Non- viral vectors, including liposomes, nanoparticles, and naked DNA, offer lower immunogenicity and simpler production but often face challenges in efficiency and cell targeting. The choice of vector depends on factors such as the disease being treated, the target tissue, and the desired duration of gene expression. As gene therapy advances, vector design continues to evolve, aiming to improve precision, safety, and therapeutic outcomes.

Nanoparticles and hybrid systems represent a cutting-edge frontier in materials science, offering remarkable versatility and functionality across biomedical, environmental, and industrial applications. Nanoparticles—typically ranging from 1 to 100 nanometers— exhibit unique physical and chemical properties due to their high surface-area-to-volume ratio and quantum effects. These properties enable precise drug delivery, enhanced imaging, and improved catalytic activity. Hybrid systems, which combine nanoparticles with other materials such as polymers, metals, or ceramics, areengineered to synergize the strengths of each component. For example, organic-inorganic nanohybrids can merge the flexibility of polymers with the durability of metal oxides, resulting in materials that are both robust and adaptable. These systems are increasingly used in biosensors, targeted

therapies, and smart coatings, where multifunctionality is essential. By tailoring the composition and structure at the nanoscale, researchers can design hybrid materials with customized electrical, mechanical, and biological characteristics, paving the way for innovations in personalized medicine, environmental monitoring, and next-generation electronics.

6. IMMUNOLOGICAL IMPLICATIONS

Nanoparticles and hybrid systems have profound immunological implications, both beneficial and potentially adverse, depending on their design, composition, and interaction with biological systems. These nanoscale materials can modulate immune responses by acting as carriers for antigens, drugs, or genetic material, thereby enhancing targeted delivery and reducing systemic toxicity. For instance, nanoparticles are increasingly used in vaccine development to improve antigen stability and presentation, leading to stronger and longer-lasting immune responses. Their surface properties—such as charge, size, and functionalization—can be tailored to either stimulate or suppress immune activity, making them valuable tools in immunotherapy and autoimmune disease management.

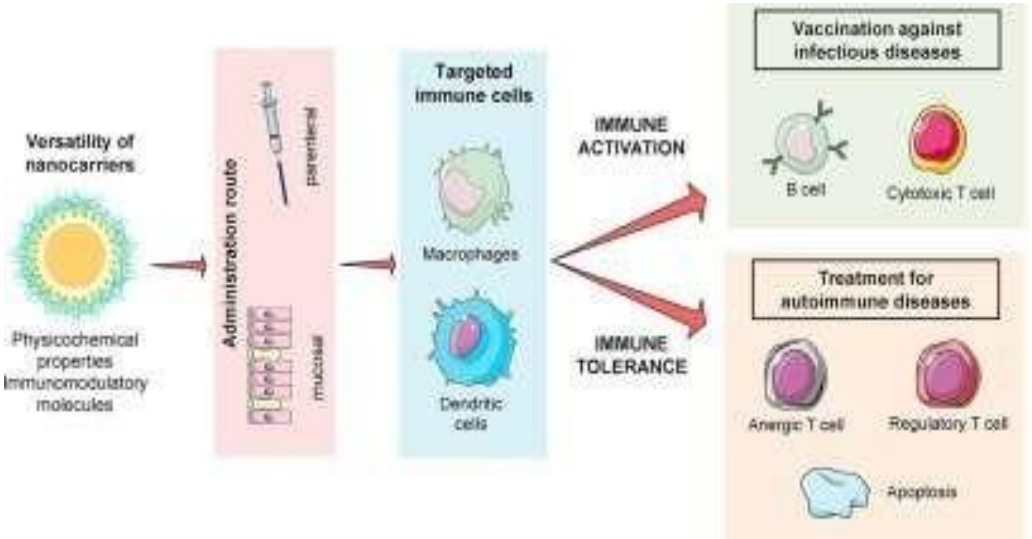
However, their interaction with the immune system is complex. Some nanoparticles may inadvertently trigger inflammatory responses or activate complement pathways, leading to unintended immunogenicity or hypersensitivity reactions. Hybrid systems, which combine organic and inorganic components, offer improved biocompatibility and multifunctionality but also introduce new challenges in predicting immune behavior. The innate immune system, particularly macrophages and dendritic cells, plays a critical role in recognizing and responding to these materials, influencing their clearance, biodistribution, and therapeutic efficacy.

As research advances, understanding the immunological landscape surrounding nanoparticles and hybrid systems is crucial for designing safer and more effective nanomedicines. Ongoing studies aim to refine their properties to minimize adverse effects while maximizing therapeutic potential, especially in areas like cancer immunotherapy, chronic inflammation, and infectious disease control.

Immune system activation and modulation are fundamental processes that maintain the body's defense against pathogens while preserving internal balance. Activation begins when immune cells recognize foreign invaders—such as bacteria, viruses, or abnormal cells—through pattern recognition receptors (PRRs) or antigen presentation. This triggers a cascade of responses involving innate immune cells like macrophages and dendritic cells, which release cytokines and present antigens to adaptive immune cells, notably T and B lymphocytes. These cells then proliferate and differentiate to mount a targeted attack, producing antibodies or cytotoxic responses.

Modulation refers to the fine-tuning of these responses to prevent excessive inflammation or autoimmunity. It involves both stimulatory and suppressive mechanisms. Regulatory T cells (Tregs), anti-inflammatory cytokines like IL-10 and TGF-β, and immune checkpoints such as PD-1 and CTLA-4 help dampen immune activity when necessary. Conversely, in cases of immunodeficiency or cancer, modulation may involve enhancing immune responses through cytokine therapy, checkpoint inhibitors, or vaccines. The spleen and liver also play complementary roles—where the spleen promotes immune activation and the liver contributes to immune tolerance and deactivation.

Understanding and manipulating these pathways is crucial in developing therapies for autoimmune diseases, infections, and cancer, where restoring immune balance can dramatically improve outcomes.



Balancing immunogenicity and safety is a critical challenge in the development of biologics, gene therapies, and nanomedicines. Immunogenicity refers to the ability of a therapeutic agent to provoke an immune response, which can be beneficial in vaccines and cancer immunotherapy but problematic in treatments where unintended immune activation may reduce efficacy or cause adverse effects. Anti-drug antibodies (ADAs), for instance, can neutralize therapeutic proteins or alter their pharmacokinetics, leading to reduced effectiveness or hypersensitivity reactions. To mitigate these risks, developers employ strategies such as protein engineering to reduce foreign epitopes, optimizing formulation components, and selecting appropriate delivery routes. Regulatory frameworks emphasize preclinical screening and immunogenicity risk assessments to anticipate potential immune responses and their clinical consequences. Achieving the right balance requires a nuanced understanding of molecular design, patient-specific factors, and immune system dynamics to ensure that therapies are both effective and safe.

Immunomodulators and cytokines play a transformative role in cancer immunotherapy by enhancing the body's natural ability to detect and destroy malignant cells. These agents work by either stimulating immune responses or removing inhibitory signals that tumors exploit to evade immune surveillance.

✓ **Immunomodulators in Cancer Therapy**

- Checkpoint inhibitors such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies block immune checkpoints—molecular "brakes" that tumors use to suppress T cell activity. By releasing these brakes, checkpoint inhibitors reinvigorate T cells to attack cancer cells effectively.
- Agonists activate co-stimulatory pathways that promote the proliferation and activation of cytotoxic T cells and dendritic cells, enhancing the immune system's ability to mount a robust anti-tumor response.
- Adjuvants are used to stimulate innate immunity and improve the presentation of tumor antigens, thereby boosting adaptive immune responses. These are often included in cancer vaccines.

✓ **Cytokines in Cancer Immunotherapy**

- Interleukins (e.g., IL-2) promote the expansion of T cells and natural killer (NK) cells, increasing their ability to target and destroy cancer cells. IL-2 has been approved for treating melanoma and renal cell carcinoma.
- Interferons (e.g., IFN-α) enhance antigen presentation and activate immune cells like macrophages and NK cells. They can also directly inhibit tumor cell proliferation.
- Colony-stimulating factors (e.g., GM-CSF, G-CSF) not only help recover immune cells after chemotherapy but also boost anti-tumor immunity by increasing the number of functional immune cells.

These therapies are often used alone or in combination with other treatments like chemotherapy, radiation, or targeted therapies to improve outcomes. The strategic modulation of immune pathways has led to durable responses in cancers once considered untreatable, such as metastatic melanoma and non-small cell lung cancer.

7. CHALLENGES AND LIMITATIONS

✓ **Biosafety and regulatory hurdles**

Challenges and Limitationsare critical considerations in the development and deployment of immunotherapies, especially those involving biologics, gene therapies, and nanomedicines. These therapies often interact directly with the immune system, raising concerns about unintended immune responses, long-term safety, and environmental impact.

➢ **Biosafety Challenges**

- Unpredictable Immune Reactions: Therapies like cytokines or monoclonal antibodies can trigger severe responses such as cytokine release syndrome, which is difficult to predict using preclinical models.
- Off-target Effects: Gene therapies and nanoparticles may affect non-target tissues, leading to toxicity or immune activation in unintended areas.
- Environmental Containment: Especially relevant for viral vectors and genetically modified organisms, strict containment protocols are required to prevent accidental release.

➢ **Regulatory Hurdles**

- Immunogenicity Assessment: Regulatory bodies require extensive testing to evaluate the potential for anti-drug antibodies (ADAs), which can compromise efficacy and safety.
- Quality by Design (QbD): Developers must integrate safety considerations from the earliest stages, including molecular design, delivery systems, and manufacturing processes.
- Global Standards: Agencies like the FDA, EMA, and WHO have distinct frameworks for biosafety and biosecurity, which can complicate international trials and approvals.
- Documentation and Transparency: Regulatory submissions must include detailed risk assessments, validated assays, and mitigation strategies to address immune-related risks.

These hurdles are not just bureaucratic—they're essential safeguards to ensure that innovative therapies are both effective and safe for patients and the broader environment.

✓ **Control over bacterial replication and clearance**

Controlling bacterial replication and clearance is essential for maintaining microbial balance and preventing infections. Bacterial replication is tightly regulated through molecular mechanisms that ensure

DNA duplication occurs only once per cell cycle. Central to this process is the initiator protein DnaA, which binds to the origin of replication (oriC) and triggers the assembly of the replication machinery. The activity of DnaA is modulated by ATP binding, autoregulation, and interactions with other proteins like DnaB helicase, ensuring precise timing and fidelity of replication.

Clearance of bacteria, on the other hand, is primarily managed by the host immune system. Innate immune cells such as macrophages and neutrophils recognize bacterial components via pattern recognition receptors (PRRs), leading to phagocytosis and destruction of the pathogens. Complement activation and antimicrobial peptides also contribute to bacterial clearance. In cases of persistent or resistant infections, antibiotics are used to inhibit bacterial replication by targeting key enzymes like DNA gyrase, RNA polymerase, or ribosomal subunits.

Emerging strategies include using engineered bacteriophages, CRISPR-based antimicrobials, and nanoparticle delivery systems to selectively disrupt bacterial replication or enhance immune-mediated clearance. These approaches aim to reduce antibiotic resistance and improve precision in targeting pathogenic bacteria while preserving beneficial microbiota.

✓ **Risk of infection or toxicity**

The risk of infection or toxicity is a major concern in immunotherapy, especially when using agents that modulate the immune system such as checkpoint inhibitors, cytokines, or CAR-T cells. These therapies, while powerful, can disrupt immune balance and lead to unintended consequences:

➢ **Risk of Infection**

- Immune Suppression: Some immunotherapies, particularly high-dose corticosteroids used to manage immune-related adverse events (irAEs), can suppress the immune system and increase vulnerability to bacterial, viral, or fungal infections.
- Opportunistic Pathogens: Patients may develop infections from normally harmless microbes due to weakened immune defenses, especially in the lungs, gut, or bloodstream.
- Delayed Clearance: Immunotherapy can impair the body's ability to clear latent infections, such as tuberculosis or hepatitis B, which may reactivate during treatment.

➢ **Risk of Toxicity**

- Cytokine Release Syndrome (CRS): A potentially life-threatening condition caused by excessive immune activation, leading to fever, hypotension, and organ dysfunction. It's common in CAR-T cell therapy and high-dose cytokine treatments.
- Organ-Specific Toxicities: Checkpoint inhibitors can cause inflammation in various organs—such as colitis, pneumonitis, hepatitis, and myocarditis—due to immune overactivation.
- Neurotoxicity: Some patients experience confusion, seizures, or encephalopathy, particularly with CAR-T therapies or high-grade irAEs.

➢ **Management Strategies**

- Early Detection: Routine monitoring and patient education are essential to catch symptoms early and initiate treatment promptly.
- Immunosuppressive Therapy: Corticosteroids and other agents are used to manage severe irAEs, but must be balanced to avoid compromising anti-tumor immunity.
- Multidisciplinary Care: Severe toxicities often require coordinated care across oncology, infectious disease, and critical care teams.

8. CLINICAL APPLICATIONS AND CASE STUDIES

➢ Preclinical and clinical trial data:

Preclinical and clinical trial data are the backbone of immunotherapy development, guiding the transition from laboratory innovation to safe and effective patient treatments. Here's a breakdown of key insights from recent studies and regulatory perspectives:

✓ **Preclinical Data: Foundation for Safety and Efficacy**

Preclinical studies are designed to evaluate the pharmacology, toxicity, and biological activity of immunotherapies before human trials begin. These include:

- Animal models and in vitro assays to assess immune activation, tumor response, and potential side effects.
- Pharmacokinetics and biodistribution studies to understand how the therapy behaves in the body.
- Toxicology assessments to determine safe starting doses and identify organ-specific risks.

For example, the FDA's guidance on cell-based immunotherapies emphasizes the importance of preclinical data in supporting first-in-human trials, especially for complex products like CAR-T cells and dendritic cell vaccines.

✓ **Clinical Trial Data: Translating Promise into Practice**

Clinical trials progress through phases to evaluate safety, dosing, and efficacy:

- Phase I: Focuses on safety and tolerability in small patient groups.
- Phase II: Explores therapeutic efficacy and optimal dosing.
- Phase III: Confirms effectiveness in larger populations and compares with standard treatments.

Recent clinical and preclinical data from BioNTech highlight the potential of next- generation immunotherapies, such as bispecific antibodies that

combine PD-L1 checkpoint inhibition with VEGF-A neutralization. These showed superior anti-tumor activity in preclinical models and promising results in early clinical trials.

Additionally, studies published in Scientific Reports showcase innovative approaches like combining IFN-γ with chemotherapy to enhance antigen presentation, and using TLR7/8 agonists to boost NK cell-mediated cytotoxicity—offering new dimensions to combination immunotherapy strategies.

➢ **Success stories in cancer therapy**

Cancer immunotherapy has delivered some truly remarkable success stories, transforming grim prognoses into long-term survival and renewed hope. Here are a few standout examples:

✓ **Irisaida's Journey: From Stage IV to No Evidence of Disease**

Originally diagnosed with uterine cancer, Irisaida's disease metastasized to her lungs, placing her in Stage IV with limited options. After chemotherapy failed, she qualified for an immunotherapy trial targeting PD-L1. Within six months, her tumors began shrinking, and two years later, she showed no evidence of disease—a dramatic turnaround that she credits to immunotherapy.

✓ **John's Story: Defying Head and Neck Cancer**

John Dabell was diagnosed with advanced head and neck cancer and underwent extensive surgery, chemotherapy, and radiotherapy. Years later, he faced a second cancer diagnosis. Immunotherapy became his lifeline, helping him manage the disease and regain time with his family. His case highlights how immunotherapy can offer hope even after conventional treatments have been exhausted.

✓ **Alison and Jane: Lung Cancer Survivors**

Alison, diagnosed with advanced non-small cell lung cancer, responded positively to a combination of immunotherapy and chemotherapy. After four years, her scans showed no signs of cancer. Jane, given just six to nine months to live, entered an immunotherapy trial and now shows no active signs of disease. Both stories underscore the life-saving potential of immune-based treatments.

These stories aren't just medical milestones—they're deeply human triumphs. They reflect how harnessing the immune system can rewrite the narrative for patients once considered untreatable.

➢ **Applications in gastrointestinal, neurological, and infectious diseases**

✓ **Gastrointestinal Diseases**

Immunotherapy is increasingly used to treat GI tract cancers and inflammatory conditions:

- Checkpoint inhibitors (e.g., anti-PD-1/PD-L1) are now first-line treatments for advanced gastric, esophageal, and colorectal cancers, especially in patients with MSI- H/dMMR profiles.
- CAR-T cell therapy targeting claudin18.2 is emerging for later-line GI cancers.
- In inflammatory bowel disease (IBD), biologics like anti-TNF agents (infliximab) and IL-12/23 inhibitors (ustekinumab) modulate immune responses to reduce inflammation.

✓ **Neurological Diseases**

Immunotherapy is revolutionizing treatment for neuroimmunological disorders:

- Multiple sclerosis (MS) is managed with agents like interferon-beta, glatiramer acetate, and B-cell depleting therapies (e.g., ocrelizumab).
- Autoimmune encephalitis and paraneoplastic syndromes respond to IVIG, corticosteroids, and rituximab, often restoring neurological function.
- In gliomas, combining immune checkpoint inhibitors with epigenetic modulators and radiotherapy is showing promise in overcoming resistance.

✓ **Infectious Diseases**

Immunotherapy offers novel approaches to combat drug-resistant and chronic infections:

- Monoclonal antibodies like REGN-COV2 for COVID-19 and Inmazeb for Ebola have improved survival and reduced disease severity.
- Therapeutic vaccines are being developed for HIV and hepatitis B to boost immune clearance of persistent viruses.
- Cytokine therapy (e.g., interferons) enhances immune recognition in chronic viral infections like hepatitis C.
- Adoptive T-cell therapy is being explored for latent infections such as CMV and EBV, especially in immunocompromised patients.

These applications reflect a growing shift toward precision immunomodulation, where therapies are tailored to disease mechanisms and individual immune profiles.

9. FUTURE DIRECTIONS

➢ Integration with nanomedicine and AI:

The integration of nanomedicine and artificial intelligence (AI) into immunotherapy is reshaping the future of precision medicine, offering smarter, more targeted, and adaptive treatment strategies across cancer and other complex diseases.

✓ **AI-Driven Personalization**

AI algorithms analyze vast datasets—including genomics, proteomics, and imaging— to predict patient responses to immunotherapy and identify optimal treatment combinations. This enables:

- Biomarker discovery for selecting responsive patients.

- Real-time monitoring of immune activity and tumor evolution.
- Adaptive therapy design, adjusting doses and agents based on dynamic feedback.

For example, AI models are being used to stratify breast cancer patients by immune subtype and predict outcomes with high accuracy.

✓ **Smart Nanomedicine Platforms**

Nanoparticles can be engineered to deliver immunomodulators, cytokines, or antigens directly to target tissues, minimizing systemic toxicity. When combined with AI:

- Design optimization: AI helps fine-tune nanoparticle size, surface charge, and payload for better targeting and immune activation.
- Theranostics: Dual-function nanoparticles can diagnose and treat simultaneously, with AI guiding interpretation of imaging data.
- Controlled release systems: AI models predict release kinetics and immune response timing for synchronized therapy.

In lung cancer, AI-enhanced nanomedicines have shown improved early detection and therapeutic precision.

✓ **Synergistic Feedback Loops**

The convergence of AI and nanomedicine creates a feedback loop

1. Nanodevices collect biological data.
2. AI interprets the data to assess immune status.
3. Therapy is adjusted in real time for maximum efficacy.

This loop is especially promising in autoimmune diseases, infectious disease control, and neuroimmunology, where immune dynamics are complex and variable.

➢ **Personalized bacterial therapies**

Personalized bacterial therapies represent a groundbreaking approach in precision medicine, where treatments are tailored to an individual's unique microbiome composition and immune profile. Unlike traditional antibiotics or generic probiotics, these therapies use specific bacterial strains—either naturally occurring or genetically engineered—to restore microbial balance, modulate immune responses, or deliver therapeutic molecules directly within the body. By analyzing a patient's microbiome through advanced sequencing and integrating data on genetics, diet, and disease history, clinicians can design customized interventions for conditions such as inflammatory bowel disease, metabolic disorders, neurological conditions, and chronic infections. Emerging technologies like pharmabiotics, AI-guided strain selection, and targeted delivery systems are enhancing the safety and efficacy of these therapies, offering a more nuanced and sustainable alternative to conventional treatments.

➢ **Potential in vaccine delivery and regenerative medicine**

The integration of immunotherapy with vaccine delivery and regenerative medicine is opening up transformative possibilities in both disease prevention and tissue repair.

✓ **Vaccine Delivery**

Immunotherapy is enhancing vaccine design by improving antigen presentation, immune activation, and target specificity:

- Advanced adjuvants such as synthetic molecules, bacterial derivatives, and nucleic acid-based agents are being used to boost immune responses while minimizing inflammation.
- Hydrogel-based systems and microneedle patches offer minimally invasive, controlled-release platforms that improve vaccine stability and delivery efficiency.
- Nanoparticle carriers are being engineered to deliver antigens directly to dendritic cells, enhancing both humoral and cellular immunity.

These innovations are especially promising for cancer vaccines, pandemic preparedness, and therapeutic vaccines for chronic infections like HIV and hepatitis.

✓ **Regenerative Medicine**

Immunotherapy is also reshaping regenerative medicine by modulating the immune system to support tissue repair and cell regeneration:

- Immune cells such as macrophages and T cells play key roles in the inflammation, proliferation, and remodeling phases of healing.
- Therapies that reprogram immune cells or alter the immune microenvironment can accelerate wound healing and improve outcomes in degenerative diseases.
- Nanomedicine-based immunotherapy uses nanoparticles to deliver cytokines or immune modulators precisely to damaged tissues, promoting regeneration while reducing scarring.

This approach is being explored in burn recovery, cardiac repair, neuroregeneration, and orthopedic tissue engineering.

Together, these advances reflect a powerful synergy between immune modulation, smart delivery systems, and personalized medicine.

❖ **REFERENCE**

1. Sizuo Yan, Yu Gan, Huizhe Xu, Haozhe Piao, Bacterial Carrier-Mediated Drug Delivery Systems: A Promising Strategy in Cancer Therapy, Frontiers in Bioengineering and Biotechnology, January 2025.
2. Muhammad Ijaz et al., Bacterial Derivatives Mediated Drug Delivery in Cancer Therapy: A New Generation Strategy, Journal of Nanobiotechnology, August 2024.
3. Ali Sahari et al., Directed Transport of Bacteria-

Based Drug Delivery Vehicles: Bacterial Chemotaxis Dominates Particle Shape, Biomedical Microdevices, June 2014.
4. Muhammad Ijaz et al., Bacterial Derivatives Mediated Drug Delivery in Cancer Therapy: A New Generation Strategy, Journal of Nanobiotechnology, 2024.
5. Ali Sahari et al., Directed Transport of Bacteria-Based Drug Delivery Vehicles: Bacterial Chemotaxis Dominates Particle Shape, Biomedical Microdevices, 2014.
6. Nishikawa H, Sato E, Briones G, Chen LM, Matsuo M, Nagata Y, Ritter G, Jäger E, Nomura H, Kondo S, Tawara I. In vivo antigen delivery by a Sal monella typhimurium type III secretion system for therapeutic cancer vaccines. J Clin Investig, 2006; 116(7): 1946–54.
7. Jiang SN, Park SH, Lee HJ, Zheng JH, Kim HS, Bom HS, Hong Y, Szarden ings M, Shin MG, Kim SC, Ntziachristos V. Engineering of bacteria for the visualization of targeted delivery of a cytolytic anticancer agent. Mol Ther., 2013; 21(11): 1985–95.
8. MacDiarmid JA, Langova V, Bailey D, Pattison ST, Pattison SL, Chris tensen N, Armstrong LR, Brahmbhatt VN, Smolarczyk K, Harrison MT, Costa M. Targeted doxorubicin delivery to brain tumors via minicells: proof of principle using dogs with spontaneously occurring tumors as a model. PLoS ONE., 2016; 11(4): e0151832.
9. Solomon BJ, Desai J, Rosenthal M, McArthur GA, Pattison ST, Pattison SL, MacDiarmid J, Brahmbhatt H, Scott AM. A first-time-in-human phase I clinical trial of bispecific antibody-targeted, paclitaxel-packaged bacte rial minicells. PLoS ONE, 2015; 10(12): e0144559.
10. Sagnella SM, Yang L, Stubbs GE, Boslem E, Martino-Echarri E, Smolarc zyk K, Pattison SL, Vanegas N, Clair ES, Clarke S, Boockvar J. Cyto immuno-therapy for cancer: a pathway elicited by tumor-targeted, cytotoxic drug-packaged bacterially derived nanocells. Cancer Cell., 2020; 37(3): 354–70.
11. Sagnella SM, Trieu J, Brahmbhatt H, MacDiarmid JA, MacMillan A, Whan RM, Fife CM, McCarroll JA, Gifford AJ, Ziegler DS, Kavallaris M. Targeted doxorubicin-loaded bacterially derived nano-cells for the treatment of neuroblastoma. Mol Cancer Ther., 2018; 17(5): 1012–23.
12. Whittle JR, Lickliter JD, Gan HK, Scott AM, Simes J, Solomon BJ, MacDi armid JA, Brahmbhatt H, Rosenthal MA. First in human nanotechnology doxorubicin delivery system to target epidermal growth factor recep tors in recurrent glioblastoma. J Clin Neurosci, 2015; 22(12): 1889–94.
13. Gujrati V, Prakash J, Malekzadeh-Najafabadi J, Stiel A, Klemm U, Metten leiter G, Aichler M, Walch A, Ntziachristos V. Bioengineered bacterial vesicles as biological nano-heaters for optoacoustic imaging. Nat Com mun., 2019; 10(1): 1114.
14. Chen W, Wang Y, Qin M, Zhang X, Zhang Z, Sun X, Gu Z. Bacteria-driven hypoxia targeting for combined biotherapy and photothermal therapy. ACS Nano., 2018; 12(6): 5995–6005.
15. Gao J, Su Y, Wang Z. Engineering bacterial membrane nanovesicles for improved therapies in infectious diseases and cancer. Adv Drug Deliv Rev., 2022; 1(186): 114340.
16. Han JW, Choi YJ, Cho S, Zheng S, Ko SY, Park JO, Park S. Active tumor therapeutic liposomal bacteriobot combining a drug (paclitaxel) encapsulated liposome with targeting bacteria (Salmonella Typhimu rium). Sens Actuators B Chem., 2016; 1(224): 217–24.
17. Ektate K, Munteanu MC, Ashar H, Malayer J, Ranjan A. Chemo-immu notherapy of colon cancer with focused ultrasound and Salmonella laden temperature sensitive liposomes (thermobots). Sci Rep., 2018; 8(1): 13062.
18. Park BW, Zhuang J, Yasa O, Sitti M. Multifunctional bacteria-driven microswimmers for targeted active drug delivery. ACS Nano., 2017; 11(9): 8910–23.
19. Alapan Y, Yasa O, Schauer O, Giltinan J, Tabak AF, Sourjik V, Sitti M. Soft erythrocyte- based bacterial microswimmers for cargo delivery. Sci Robot, 2018; 3(17): eaar4423.
20. Quispe-Tintaya W, Chandra D, Jahangir A, Harris M, Casadevall A, Dadachova E, Gravekamp C. Nontoxic radioactive Listeriaat is a highly effective therapy against metastatic pancreatic cancer. Proc Natl Acad Sci., 2013; 110(21): 8668–73.
21. Chandra D, Selvanesan BC, Yuan Z, Libutti SK, Koba W, Beck A, Zhu K, Casadevall A, Dadachova E, Gravekamp C. 32-Phosphorus selectively delivered by listeria to pancreatic cancer demonstrates a strong thera peutic effect. Oncotarget, 2017; 8(13): 20729.
22. Fan JX, Peng MY, Wang H, Zheng HR, Liu ZL, Li CX, Wang XN, Liu XH, Cheng SX, Zhang XZ. Engineered bacterial bioreactor for tumor therapy via Fenton-like reaction with localized H2O2 generation. Adv Mater., 2019; 31(16): 1808278.
23. Priya Tolia, Ghanshyam Patel, Dr. Upendra Patel, Bhavin Bimani, Kirtan Sanghavi, Magnetic Nanoparticles Using For Imaging And Treatment Cancer, International Journal Of Pharmaceutical Research And Bio-Science (Ijprbs), 2014; 3(1): 162-17.1.
24. Shukla Maitri Jayeshkumar, Patel Yashvi, Mr. Bhavin Bhimani, Mr. Ghanshyam Patel, Mr. Jaimin Patel, Dr. Upendra Patel, Nebulization Therapy for Lung Cancer, RJPT, 2019; 12(2): 920-934.
25. Xuemei Zeng, Zhenglin Li, Zhiyao Yi, Shuangqian Yan, Targeting Ion Homeostasis for Cancer Therapy: Mechanisms and Immunomodulatory Effects, Frontiers in Pharmacology (2024).
26. Wenchang Zhou, Jie Zhang, Wankun Chen, Changhong Miao, Prospects of Molecular Hydrogen in Cancer Prevention and Treatment, Journal of Cancer Research and Clinical Oncology (2024).

27. Taufeeque Ali, Daniel Li, Thilini N. F. Ponnamperumage, Alexis K. Peterson, Jatin Pandey, Kulsum Fatima, John Brzezinski, Julia A. R. Jakusz, Hanlun Gao, Gilbert E. Koelsch, Dhivyashree S. Murugan, Xiaohua Peng, Generation of Hydrogen Peroxide in Cancer Cells: Advancing Therapeutic Approaches, Cancers (MDPI) (2024).
28. Sai Li, Rongrong Liao, Xiaoyan Sheng, Xiaojun Luo, Xin Zhang, Xiaomin Wen, Jin Zhou, Kang Peng, Hydrogen Gas in Cancer Treatment, Frontiers in Oncology.
29. Arian Karimi Rouzbehani, Golnaz Mahmoudvand, Zahra Goudarzi, Arshia Fakouri, Simin Farokhi, Saeideh Khorshid Sokhangouy, Elnaz Ghorbani, Amir Avan, Elham Nazari, Majid Khazaei, Hydrogen as a Potential Therapeutic Approach in the Treatment of Cancer: From Bench to Bedside, Molecular Hydrogen in Health and Disease (Springer, 2024).
30. Abbaoui B, Lucas CR, Riedl KM, Clinton SK, Mortazavi A. Cruciferous vegetables, isothiocyanates, and bladder cancer prevention. Mol Nutr Food Res., 2018; 62(18): e1800079.
31. Akagi J, Baba H. Hydrogen gas activates coenzyme Q10 to restore exhausted CD8(+) T cells, especially PD-1(+) Tim3(+) terminal CD8(+) T cells, leading to better nivolumab outcomes in patients with lung cancer. Oncol Lett., 2020; 20(5): 258.
32. Xu C, Chen YP, Du XJ, Liu JQ, Huang CL, Chen L, Zhou GQ, Li WF, Mao YP, Hsu C, Liu Q, Lin AH, Tang LL, Sun Y, Ma J. Comparative safety of immune checkpoint inhibitors in cancer: systematic review and network meta-analysis. BMJ., 2018; 363: k4226.
33. Zhou P, Lin B, Wang P, Pan T, Wang S, Chen W, Cheng S, Liu S. The healing effect of hydrogen-rich water on acute radiation-induced skin injury in rats. J Radiat Res., 2019; 60(1): 17–22.
34. Zhang B, Wang F, Zhou H, Gao D, Yuan Z, Wu C, Zhang X. Polymer dots compartmentalized in liposomes as a photocatalyst for in situ hydrogen therapy. Angew Chem Int Ed Engl., 2019; 58(9): 2744–2748.
35. Zhai S, Zhu L, Qin S, Li L. Effect of lactulose intervention on gut microbiota and short chain fatty acid composition of C57BL/6J mice. Microbiologyopen, 2018; 7(6): e00612.
36. Yu Y, Yang Y, Yang M, Wang C, Xie K, Yu Y. Hydrogen gas reduces HMGB1 release in lung tissues of septic mice in an Nrf2/ HO-1-dependent pathway. Int Immunopharmacol, 2019; 69: 11–18.
37. Yang Y, Li B, Liu C, Chuai Y, Lei J, Gao F, Cui J, Sun D, Cheng Y, Zhou C, Cai J. Hydrogen-rich saline protects immu nocytes from radiation-induced apoptosis. Med Sci Monit, 2012; 18(4): BR144–BR148.
38. Chandel NS, Tuveson DA. The promise and perils of antioxi dants for cancer patients. N Engl J Med., 2014; 371(2): 177–178. Chen J, Vitetta L. Inflammation- modulating effect of butyrate in the prevention of colon cancer by dietary fiber. Clin Colo rectal Cancer, 2018; 17(3): e541–e544.
39. Chen X, Zhai X, Shi J, Liu WW, Tao H, Sun X, Kang Z. Lact ulose mediates suppression of dextran sodium sulfate-induced colon inflammation by increasing hydrogen production. Dig Dis Sci., 2013; 58(6): 1560–1568.
40. Chen H, Xie K, Han H, Li Y, Liu L, Yang T, Yu Y. Molecular hydrogen protects mice against polymicrobial sepsis by ame liorating endothelial dysfunction via an Nrf2/HO-1 signaling pathway. Int Immunopharmacol, 2015; 28(1): 643–654.
41. Chen JB, Kong XF, Qian W, Mu F, Lu TY, Lu YY, Xu KC. Two weeks of hydrogen inhalation can significantly reverse adaptive and innate immune system senescence patients with advanced non-small cell lung cancer: a self-controlled study. Med Gas Res., 2020; 10(4): 149–154.
42. Cheung EC, Vousden KH. The role of ROS in tumour develop ment and progression. Nat Rev Cancer, 2022; 22(5): 280–297.
43. Dole M, Wilson FR, Fife WP Hyperbaric hydrogen therapy: a possible treatment for cancer. Science, 1975; 190(4210): 152–154.
44. Dong A, Yu Y, Wang Y, Li C, Chen H, Bian Y, Zhang P, Zhao Y, Yu Y, Xie K. Protective effects of hydrogen gas against sep sis-induced acute lung injury via regulation of mitochondrial function and dynamics. Int Immunopharmacol, 2018; 65: 366–372.
45. Erny D, Prinz M. How microbiota shape microglial pheno types and epigenetics. Glia., 2020; 68(8): 1655–1672.
46. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol, 2021; 19(1): 55–71.
47. Fang W, Wang G, Tang L, Su H, Chen H, Liao W, Xu J. Hydrogen gas inhalation protects against cutaneous ischaemia/ reperfusion injury in a mouse model of pressure ulcer. J Cell Mol Med., 2018; 22(9): 4243–4252.
48. Flannigan KL, Wallace JL Hydrogen sulfide-based anti inflammatory and chemopreventive therapies: an experimental approach. Curr Pharm Des., 2015; 21(21): 3012–3022.
49. Franchina DG, Dostert C, Brenner D. Reactive oxygen species: involvement in T cell signaling and metabolism. Trends Immunol, 2018; 39(6): 489–502 Franco F, Jaccard A, Romero P, Yu YR, Ho PC Metabolic and epigenetic regulation of T-cell exhaustion. Nat Metab, 2020; 2(10): 1001–1012.
50. Kajiyama S, Hasegawa G, Asano M, Hosoda H, Fukui M, Nakamura N, Kitawaki J, Imai S, Nakano K, Ohta M, Adachi T, Obayashi H, Yoshikawa T. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Nutr Res., 2008; 28(3): 137–143.
51. Kalantar-Zadeh K, Berean KJ, Burgell RE, Muir JG, Gibson PR Intestinal gases: influence on gut

disorders and the role of dietary manipulations. Nat Rev Gastroenterol Hepatol, 2019; 16(12): 733–747.
52. Kamimura N, Nishimaki K, Ohsawa I, Ohta S. Molecular hydro gen improves obesity and diabetes by inducing hepatic FGF21 and stimulating energy metabolism in db/db mice. Obesity (silver Spring), 2011; 19(7): 1396–1403.
53. Kawai D, Takaki A, Nakatsuka A, Wada J, Tamaki N, Yasunaka T, Koike K, Tsuzaki R, Matsumoto K, Miyake Y, Shiraha H, Morita M, Makino H, Yamamoto K. Hydrogen-rich water pre vents progression of nonalcoholic steatohepatitis and accompanying hepatocarcinogenesis in mice. Hepatology, 2012; 56(3): 912–921.
54. Kikkawa YS, Nakagawa T, Taniguchi M, Ito J. Hydrogen pro tects auditory hair cells from cisplatin-induced free radicals. Neurosci Lett., 2014; 579: 125–129.
55. Kong Q, Wang B, Tian P, Li X, Zhao J, Zhang H, Chen W, Wang G. Daily intake of Lactobacillus alleviates autistic-like behaviors by ameliorating the 5- hydroxytryptamine metabolic disorder in VPA-treated rats during weaning and sexual maturation. Food Funct, 2021; 12(6): 2591–2604.