PLANT-BASED MRNA DELIVERY ADJUVANTS: AN OVERVIEW

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Abstract:
Messenger RNA (mRNA) vaccines are a new and powerful type of vaccine that can be quickly designed to protect against infectious diseases and cancer. Even though they are very effective, mRNA vaccines face some challenges, such as instability of mRNA, difficulty in delivering it into cells, and controlling the immune response. To solve these problems, scientists are exploring natural solutions, especially from plants. Plants produce many bioactive compounds such as saponins, polysaccharides, flavonoids, and natural nanoparticles that can help improve mRNA delivery and strengthen immune responses. These plant-derived substances can act as adjuvants, which boost the immune response, or as delivery carriers, which protect mRNA and help it enter cells. Plant-based systems are attractive because they are generally safe, biodegradable, cost-effective, and environmentally friendly. In mRNA vaccines, immune activation can occur in three main ways: through the mRNA itself, through the delivery system such as lipid nanoparticles, and through added immune-stimulating substances. Plant-derived adjuvants can support all these mechanisms by activating immune cells and improving antigen presentation. New plant-based approaches, including plant virus nanoparticles and plant-derived extracellular vesicles, are being studied as alternatives to synthetic delivery systems. Overall, plant-based adjuvants and delivery platforms offer a promising strategy to make mRNA vaccines safer, more stable, and more affordable. With further research and standardization, these natural systems may play an important role in the future development of vaccines and mRNA-based therapies.

Keywords: *mRNA,vaccine, plant derived, immune response etc.*

Introduction:

Messenger RNA (mRNA) vaccines have revolutionized modern immunization, providing rapid and flexible platforms for developing vaccines against infectious diseases and cancers. However, effective delivery of mRNA molecules and activation of a strong immune response remain critical challenges. To overcome these, researchers have turned to nature particularly plants to identify bioactive molecules and nanostructures capable of enhancing delivery efficiency and immune activation. Plant-based adjuvants and delivery systems offer several advantages: they are biocompatible, biodegradable, renewable, and can be produced at large scale using low-cost agricultural systems. The idea of using plant-derived molecules as adjuvants and carriers emerged from traditional medicine, where plant saponins and polysaccharides have long been known to enhance immune responses. Over time, biotechnology enabled the purification, characterization, and adaptation of these compounds for modern vaccines. Adjuvants in mRNA vaccines can be broadly classified into three categories i.e. RNA with self-adjuvant characteristics, compounds of delivery system, exogenous immunostimulants. [1, 2, 3]

1. RNA with self-adjuvant characteristics:

Unmodified or partially modified mRNA can naturally activate innate immune sensors. Key pathways include:

Innate Immune Receptors Activated

- TLR3 (double-stranded RNA recognition)
- TLR7/8 (single-stranded RNA recognition.
- RIG-and MDA5 (cytosolic RNA sensors)

Activation of these pathways induces type I interferons, pro-inflammatory cytokines, and antigen-presenting cell (APC) maturation, enhancing adaptive immune responses.

Role of nucleoside modifications:

Vaccines such as the COVID-19 mRNA vaccines use N1-methyl-pseudouridine to reduce excessive innate sensing for better translation efficiency and tolerability.

However, the RNA still provides some level of innate stimulation.

Key points:

- mRNA itself acts as a self-adjuvant.
- The structure (e.g., dsRNA contaminants, 5' triphosphate) influences intensity of immune activation. [1, 4, 5]

2. Components of the delivery system: (especially lipid nanoparticles)

Modern mRNA vaccines use lipid nanoparticles **(**LNPs**)** to protect mRNA and aid cell entry. These LNPs also have potent adjuvant-like effects**.**

Immunostimulatory components:

- Ionizable cationic lipids (e.g., SM-102, ALC-0315) trigger inflammation, likely through:
  - Inflammasome activation (NLRP3)
  - Induction of IL-1β and IL-6
- PEGylated lipids**,** phospholipids, and cholesterol contribute to stability and delivery, and can indirectly modulate immune responses.

Mechanisms:

- Recruitment of neutrophils and monocytes to the injection site.
- Promotion of APC maturation.
- Enhancement of antigen presentation. [6, 7]

3. Exogenous immunostimulants:

Although most licensed mRNA vaccines do not include classical exogenous adjuvants, next-generation platforms may incorporate additional immune stimulators to shape the immune response.

Examples studied in research:

- TLR agonists **(**e.g. CpGoligodeoxynucleotides**-**TLR9agonists**)**
- STING agonists
- Cytokines (e.g. GM-CSF)
- Pattern-recognition receptor ligands

These can be:

- Mixed with mRNA
- Co-delivered in LNPs
- Encoded as separate mRNA molecules (e.g. cytokine-mRNA)

Use cases:

- Cancer vaccines.
- Therapeutic vaccines requiring strong cellular immunity. [8,9]

Synthesis and modification of mRNA vaccines and drugs:
mRNA used in vaccines and therapeutic drugs is engineered to ensure high translation efficiency**,** stability, and controlled immune activation:

DNA template

⇩

***In vitro* transcription**

⇩

mRNA modification

⇩

Purification

⇩

Formulation (LNP)

⇩

Final vaccine/drug

1. DNA template preparation:
mRNA production begins with a DNA template encoding the antigen (vaccines) or therapeutic protein (drugs).
Steps:
Plasmid DNA is designed containing:
- 5' and 3' untranslated regions (UTRs)
- Coding sequence (CDS)
- Poly(A) tail
- Plasmid is linearized downstream of the poly (A) site.[1, 10]

2. *In-vitro* transcription (IVT) of mRNA:
The primary method for producing synthetic mRNA is T7 RNA polymerase in vitro transcription.
Key elements of IVT:
Linear DNA template + T7, SP6, or T3 polymerase
Ribonucleotides (ATP, CTP, GTP, UTP)
Optional incorporation of modified nucleosides (e.g., N1-methyl-pseudouridine).[11,12]

3. mRNA chemical and structural modifications:
mRNA modifications are designed to increase stability and translation while modulating innate immune sensing.
3.1 Nucleoside modifications:
- Pseudouridine (Ψ)**,** N1-methyl-pseudouridine (m1Ψ)**,** and -5methylcytidine (m5C**)**
- Reduce activation of TLR7/8 and RIG-I
- Improve protein translation and reduce immunogenicity

3.2 5′ Cap structure:
- Cap 0**:** m7GpppN
- Cap 1**:** m7GpppNm (used in most mRNA vaccines)
- Facilitates ribosome recruitment and protects against exonucleases

3.3 Poly (A) tail:
- Typically100-150 nucleotides, promotes translation and mRNA stability

3.4 UTR optimization:
- 5′ UTR enhances ribosome binding
- 3′ UTR improves stability and prevents degradation

3.5 Codon optimization:
- Avoids rare codons and secondary structures
- Prevents cryptic motifs recognized by miRNAs or innate sensors. [13, 14]

4. Purification:
- Fast protein liquid chromatography (FPLC)
- Cellulose purification (to remove dsRNA)

Removal of dsRNA is critical because dsRNA strongly activates TLR3 and MDA5.

5. Formulation and delivery:
mRNA is formulated in lipid nanoparticles (LNPs), which:

- Protect mRNA from degradation
- Facilitate cellular uptake and endosomal escape
- LNP composition: Ionizable lipids, cholesterol, phospholipids (DSPC), and PEG-lipids. [8]

Immunomodulators:
Immunomodulators are substances that can change or control how the immune system works. The immune system protects the body from infections, harmful substances in the environment, and diseases. It also helps the body identify and destroy abnormal cells, such as precancerous and cancerous cells. [6]

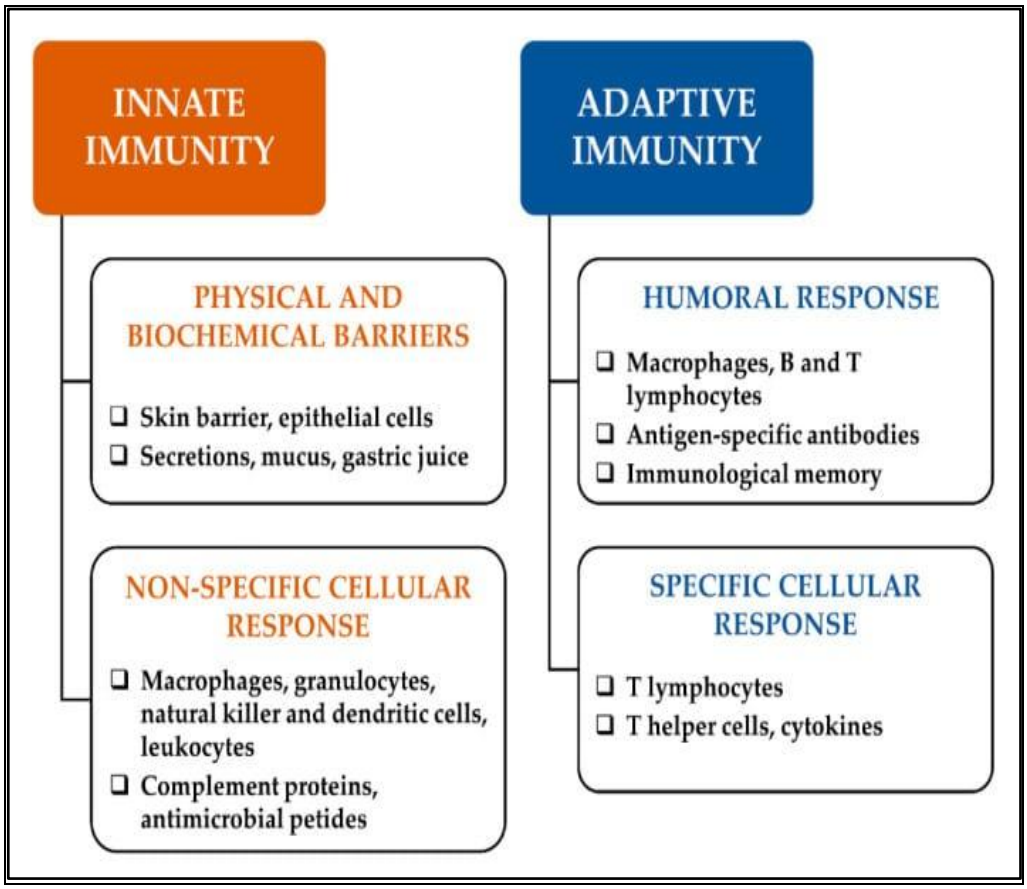

Fig. 1: Innate & adaptive immune responses & their major cellular & biochemical components [15]

The immune system works in two main steps:
The first step is innate immunity, which is the body's immediate defence. It includes physical barriers like the skin, chemical barriers, and general immune cells such as granulocytes, macrophages, and natural killer cells. These work together to quickly fight infections and prevent cancerous changes.
If the infection continues, the body activates adaptive immunity. Here, macrophages show pieces of the pathogen (antigens) to B cells and T cells. B cells then produce specific, high- affinity antibodies and also form memory cells for long-term protection. T cells help by releasing cytokines and supporting cell-mediated immunity.[16,17]

Table 1: Some examples of bioactive constituents obtained from immunomodulatory plants, and their effect on immune function:

Medical plants	Phytochemicals	Effects on immune function/type of immunomodulation	Reference
Acacia catechu Willd.	Flavonoids, phenolic acids, catechins	Anti-inflammatory activity/ Immunoadjuvant	18
Camellia sinensis (L) Kuntze	Polysaccharide	Activation of immunoreactivity through the modulation of gutmicrobiome / Immunostimulant effect	19, 20
Curcuma longa L	Curcuminoids	IL-10-mediated anti-inflammatory and immunosuppressive activity / Immunosuppressor	21

Zingiber officinale Roscoe	Phenolic glucosides	Phenolics (gingerols)	22

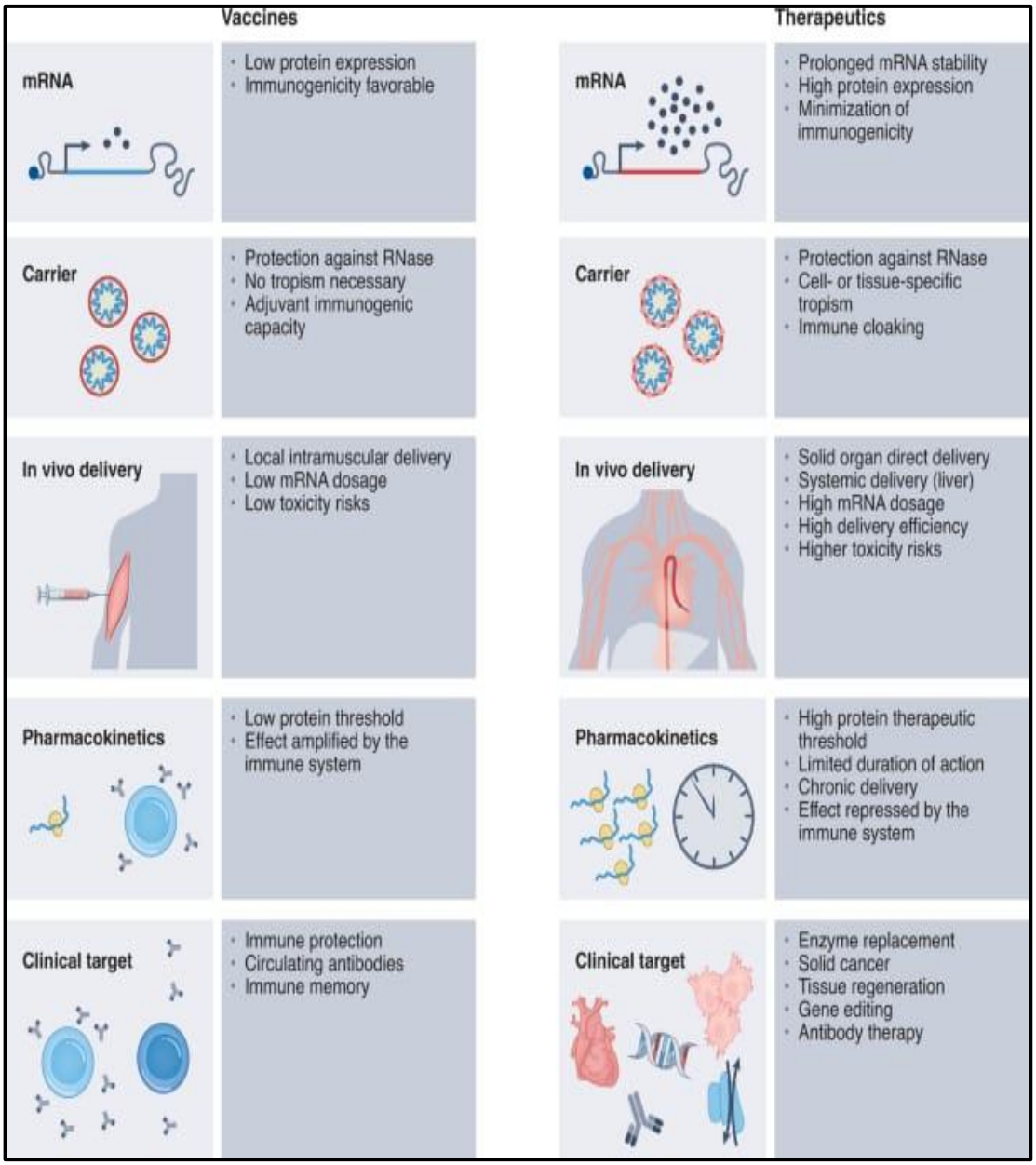

Fig. 2: Design of mRNA therapeutics or vaccines [23]

The figure outlines the stepwise process involved in developing mRNA therapeutics or vaccines. It begins with disease diagnosis, followed by sequence designing to determine the required mRNA code. After this, mRNA synthesis is carried out, and the mRNA is packaged into delivery systems such as lipoplexes, polyplexes, or lipid nanoparticles (LNPs) to protect it and enhances delivery. These formulations then undergo in-vitro and in-vivo safety evaluations in cell cultures and animal models before progressing to manufacturing and clinical trials to ensure efficacy and safety in humans. [1, 7, 24]

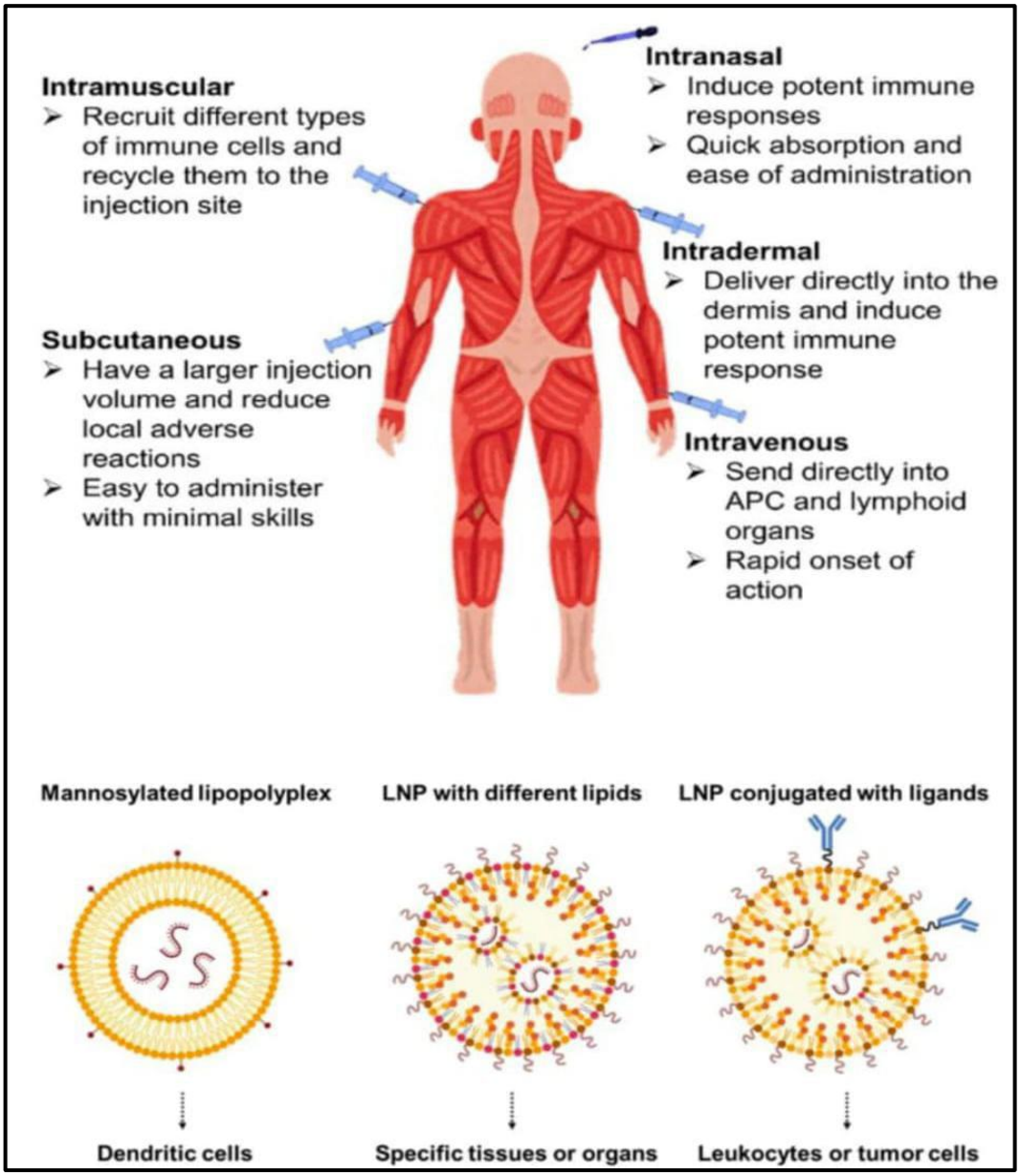

Fig. 3: mRNA drugs or vaccines administration routes [25]

The figure illustrates different administration routes for mRNA drugs or vaccines, each influencing immune response and delivery efficiency:

Intramuscular (IM**):** Injects mRNA into muscle tissue, where immune cells are recruited to enhance antigen presentation. [1]

Subcutaneous (SC)**:** Delivered under the skin, allowing larger doses with fewer local reactions and easy application. [26]

Intranasal: Provides fast absorption through the nasal mucosa and induces strong mucosal and systemic immunity. [27]

Intradermal (ID)**:** Targets the dermis, a region rich in antigen-presenting cells, leading to potent immune stimulation. [28]

Intravenous (IV)**:** Sends mRNA directly into the bloodstream, rapidly reaching lymphoid organs of quick immune activation. [29]

The lower section shows lipid nanoparticle (LNP) types for targeted delivery:

Mannosylated lipopolyplexes: Target dendritic cells.

LNPs with different lipids: Deliver mRNA to specific tissues.

Ligand-conjugated LNPs: Target leukocytes or tumor cells.

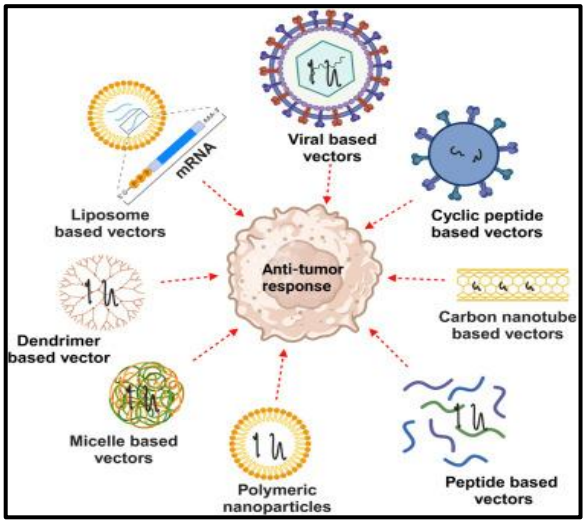

Fig. 4: Drug-delivery systems used in mRNA-based cancer therapy [30]

The diagram illustrates different mRNA delivery vectors used to induce an anti-tumor immune response. These delivery systems protect mRNA, enhance cellular uptake, and stimulate effective immune activation against cancer cells. Various nanocarriers such as liposomes, micelles, polymers, peptides, dendrimers, viral vectors, and carbon-based material are used to transport mRNA into target cells, where it is translated into tumor-antigen proteins that trigger strong anti-tumor immunity.

Labelled components of the diagram:

1. Liposome-based vectors:
Phospholipid vesicles encapsulating mRNA for safe delivery.

2. Dendrimer-based vectors:
Branched polymeric structures that bind and protect nucleic acids.

3. Micelle-based vectors:
Self-assembled amphiphilic nanoparticles carrying mRNA.

4. Polymeric nanoparticles:
Biodegradable polymers enabling controlled mRNA release.

5. Viral-based vectors:
Modified viruses used as efficient gene-delivery carriers.

6. Cyclic peptide-based vectors:
Peptide rings enhancing stability and cellular uptake.

7. Carbon nanotube-based vectors:
Tubular carbon structures allowing high-capacity nucleic acid loading.

8. Peptide-based vectors:
Linear peptides aiding membrane penetration and mRNA delivery.

9. Central: Anti-tumor response:
mRNA delivery leads to antigen expression and activation of immune cells, generating anti-tumor immue cells, generating anti-tumor immunity. [1, 7]

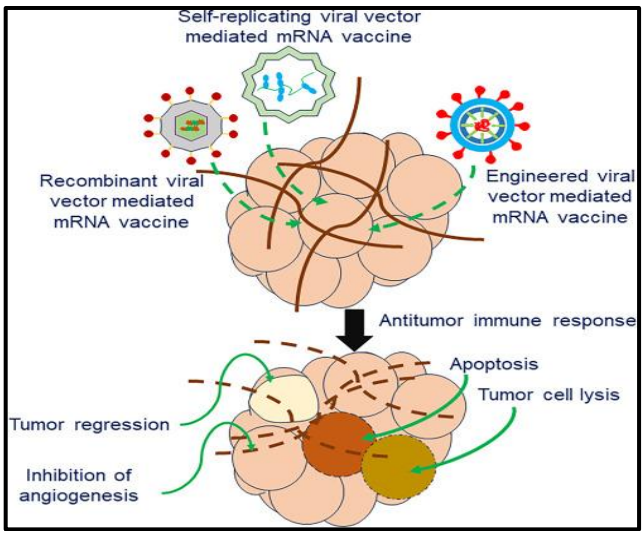

Fig. 5: Mechanisms of viral vector mediated mRNA vaccines in antitumor immunity [30]

The figure illustrates how different viral vector-mediated mRNA vaccine strategies work to produce an antitumor immune response.

1. Types of viral vector-based mRNA vaccines:

The figure shows three major mRNA-delivery systems:

- Recombinant viral vector-mediated mRNA vaccines
- Self-replicating viral vector-mediated mRNA vaccines
- Engineered viral vector-mediated mRNA vaccines. [31, 32, 9]

These viral vectors deliver tumor-related mRNA into the tumor microenvironment. Once inside cells, the mRNA is translated into antigens.

2. Action inside the tumor:

After delivery:
The mRNA is expressed as tumor-specific antigens.
These antigens activate immune cells (T cells, dendritic cells).
This leads to a strong antitumor immune response.

3. Immune outcomes:

The activated immune system produces several effects:
Tumor cell lysis: Immune cells destroy cancer cells.
Apoptosis: Programmed death of tumor cells is triggered.
Inhibition of angiogenesis: Blood-vessel growth to sustain tumors is reduced.
Tumor regression: Overall shrinking of the tumor mass. [33, 8]

Background and early development history:

Adjuvants such as alum and oil emulsions were used to improve vaccine efficacy. In the 1980s and 1990s, researchers began exploring saponins from plants such as Quillajasaponaria (soapbark tree) for their potent immune-stimulatory properties. [2] The QS-21 saponin fraction became a landmark plant-derived adjuvant, forming the basis for several licensed vaccines today.[34] In parallel, advances in nanotechnology introduced the concept of using natural nanoparticles including those derived from plants as antigen carriers or delivery systems. Plant virus nanoparticles (PVNPs) and plant virus-like particles (VLPs) demonstrated that non-infectious plant viruses could serve as stable, immunogenic scaffolds for presenting or carrying vaccine molecules, including mRNA. [34, 35]

Introduction of plant-based mRNA delivery systems:

The introduction of plant-based mRNA delivery adjuvants or immune modulators was driven by the limitations of existing synthetic lipid nanoparticle (LNP) systems. Although LNPs are efficient, they can cause inflammation and are expensive to produce at scale. Researchers sought plant alternatives that could provide biocompatibility and inherent immune modulation. Key research areas included: Plant-derived saponins and polysaccharides used as adjuvants to enhance antigen presentation and T-cell activation. Plant virus nanoparticles (PVNPs) used as delivery scaffolds for mRNA and antigens. [36, 37] Plant extracellular vesicles (EVs) investigated for their natural ability to carry RNAs and bioactive molecules. Hybrid lipid-saponin nanoparticles designed to combine plant-derived immune stimulants with synthetic lipid carriers. [38, 39]

Mechanism of action:

Plant-based adjuvant and carriers modulate both innate and adaptive immunity. Saponins and polysaccharides interact with immune receptors such as TLR2, TLR4, and NLRP3 inflammasomes, leading to cytokine release and activation of antigen-presenting cells. PVNPs and EVs act as particulate delivery systems that facilitate cellular uptake of mRNA and improve antigen presentation. In mRNA delivery, plant-derived vesicles protect the fragile mRNA molecules from enzymatic degradation, assist in membrane fusion, and trigger immune signalling cascades, resulting in a balanced humoral and cellular immune response. [40, 41, 37]

Advantages of plant-based mRNA delivery adjuvant:

1. **Biocompatibility and safety:** Plant-derived compounds are generally non-toxic and well-tolerated, reducing the risk of adverse immune or inflammatory reactions often observed with synthetic lipid or polymer carriers. Many plant bioactives, such as saponins (e.g., QS-21 from Quillaja saponaria), have long histories of safe use as vaccine adjuvant.
2. **Sustainability and cost-effectiveness:** Plants can be cultivated at large scale with low production costs, requiring minimal infrastructure compared to cell culture or synthetic chemistry-based systems. Production is environmentally friendly, aligning with the goals of green biotechnology and sustainable medicine.
3. **Intrinsic immunomodulatory activity:** Numerous plant metabolites (saponins, flavonoids, polysaccharides, alkaloids) naturally activate immune pathways enhancing antigen presentation, cytokine release, and both humoral and cellular responses. These intrinsic immune-stimulating properties can reduce the need for additional adjuvants in mRNA vaccine formulations.
4. **Natural nanocarriers and vesicles:** Plants produce exosome-like nanovesicles capable of encapsulating mRNA, protecting it from degradation, and facilitating uptake by mammalian cells. These vesicles are biodegradable, stable, and can cross biological barriers, offering an attractive alternative to lipid nanoparticles.

5. Oral and mucosal delivery potential: Plant cells can protect mRNA within their cell walls and membranes, enabling oral or mucosal administration routes that are more acceptable and logistically simpler than injections. Edible plants could serve as delivery vehicles for mRNA vaccines, potentially eliminating cold-chain requirements.
6. Scalability and rapid adaptability: Plant-based expression systems allow rapid production and modification of new formulations, which is critical in outbreak scenarios or personalized medicine. Transient expression technologies (e.g., agroinfiltration) enable high-yield production of adjuvant compounds or carrier proteins within days.
7. Reduced cold chain dependence: Certain plant-derived materials offer enhanced stability to encapsulation. [1, 17, 26]
Disadvantages / limitations of plant-based mRNA delivery adjuvant:
1. Variable composition and batch-to-batch inconsistency: The biochemical composition of plant extracts can vary depending on growth conditions, harvest time, and extraction methods, leading to inconsistent efficacy and reproducibility. Standardization and quality control are challenging compared to chemically defined synthetic carriers.
2. Low delivery efficiency and cellular uptake: Although plant-derived vesicles and biopolymers can carry mRNA, their delivery efficiency is often lower than that of optimized lipid nanoparticles (LNPs). Limited endosomal escape and intracellular release of mRNA remain key barriers.
3. Stability and storage challenges: While some plant systems improve mRNA stability, others are sensitive to degradation during purification, drying, or long-term storage. Maintaining consistent RNA integrity through plant processing requires careful formulation optimization. [4,19,32]

Challenges:
Despite their promise, several limitations hinder the full adoption of plant-based systems: Batch-to-batch variation in natural extracts leading to inconsistent potency. Need for detailed toxicological and regulatory evaluation for human use. Limited understanding of long-term stability and biodistribution of plant EVs. Potential ecological concerns in sourcing rare plants such as Quillaja.

Future perspectives future research focuses on:
Hybrid nanoparticle formulations combining synthetic and plant-derived components. Engineering plant virus nanoparticles for targeted mRNA delivery. Using edible plants as oral vaccine delivery platforms. Developing sustainable and reproducible methods for plant adjuvant extraction.

Conclusion:
The introduction of plant-based mRNA delivery adjuvants and immune modulators represents a significant step toward safer, sustainable, and cost-effective vaccine technologies. The synergy between plant biotechnology, nanoscience, and immunology continues to unlock new avenues for next-generation vaccines and therapeutics. While still early in clinical translation, plant-based platforms may soon complement or even replace certain synthetic systems, offering scalable and eco-friendly solutions for global immunization.

Reference:

1. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines- a new era in vaccinology. Nat Rev Drug Discov. 2018; 17: 261-279.
2. Kensil CR, Patel U, Lennick M, Marciani D. Separation and characterization of saponins with adjuvant activity from Quillaja saponaria. J Immunol.1991; 146: 431-437.
3. Mu J, Zhuang X, Zhang L. Plant exosome-like nanoparticles for mRNA and drug delivery to mammalian cells. Mol Ther. 2014; 22: 2110-2120.
4. Mu X, Greenwald E, Kanneganti TD. The role of innate sensing in mRNA vaccine immunogenicity. Nat Rev Immunol. 2022; 22: 75-88.
5. Maggini S, Pierre A, Calder PC. Immune function and micronutrient requirements change over the life course. Nutr. 2018; 10: 1531.
6. Ndeupen S, Qin Z, Jacobsen S. The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory.sci. 2021; 24(12): 103479.
7. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021; 6: 1078-1094.
8. Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy.Nat. 2016; 534 (7607): 396-401.
9. Zhang C, Maruggi G, Shan H, Li J. Advances in self-amplifying mRNA vaccines. Vaccines. 2019; 7(3): 72.
10. Schlake T Thess A, Fotin Mleczek M, Kallen KJ. Developing mRNA vaccine technologies. RNA Biol. 2012; 9(11): 1319-1330.
11. Weissman D. mRNA transcript therapy.Immun. 2015; 43(3): 332-343.
12. Dolgin E. The tangled history of mRNA vaccines. Nat. 2021; 597 (7876): 318-324.
13. Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by toll like receptors the impact of nucleoside modification and the evolutionary origin of RNA. Immun. 2005; 23(2): 165-175.
14. Furuichi Y, Shatkin AJ. Viral and cellular mRNA capping:Past and prospects.Adv Virus Res. 2000; 55: 135-184.
15. Di SottoA, VitaloneA, Di Giacomo S. Plant-derived nutraceuticals and immune system modulation an evidence-based overview. Vaccines. 2020; 8(3): 468.

16. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 9th edition Elsevier. 2018: 45.
17. Chaplin DD. Overview of the immune response. J Allergy Clin Immunol. 2010; 125(2): 3.
18. Stohs SJ, Bagchi D. Antioxidant, anti-inflammatory, and chemoprotective properties of Acacia catechu Heartwood extracts. Phytother Res. 2015; 29: 818-824.
19. Chen D, Chen G, Ding Y, Wan P, Peng Y, Chen C, Ye H, Zeng X, Ran L. Polysaccharides from the flowers of tea (Camellia sinensis L.) modulates gut health and ameliorate Cyclophosphamide-induced immune suppression. J Funct Food. 2019; 61: 103470.
20. Rahayu RP, Prasetyo RA, Purwanto DA, Kresnoadi U, Iskandar RPD, Rubianto M. The immunomodulatory effect of green tea (Camellia Sinensis) leaves extract on immune compromised wistar rats infected by Candida Albicans. Vet. World. 2018; 11: 765-770.
21. Mollazadeh H, Cicero AFG, Blesso CN, Pirro M, Majeed M, Sahebkar A. Immune modulation by curcumin: The role of interleukin-10. Crit Rev Food Sci Nutr. 2019; 59: 89-101.
22. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber Officinale Roscoe): A review of recent research. Food Chem Toxicol. 2008; 46: 409-420.
23. Barbier AJ, Jiang AY, Zhang P, Anderson DG. The clinical progress of mRNA vaccines and immunotherapies. Nat Biotechnol. 2022; 40: 840-854.
24. Sahin U, Karikó K, & Türeci Ö. mRNA-based therapeutics-developing a new class of drugs. Nat Rev Drug Discov. 2014; 13(10): 759-780.
25. Wang YS, Kumari M, Chen GH, Hong MH, Yuan JPY, Tsai JL, Wu HC. mRNA-based vaccines and therapeutics: An in-depth survey of current and upcoming clinical applications. J Biomed Sci. 2023; 30: 84.
26. Zhu M, Zhu L, Wang X, Jin H. Routes of vaccine administration: advantages and challenges. Hum VaccinImmunother. 2021; 17(11): 4226-4234.
27. Lycke N. Recent progress in mucosal vaccine development potential and limitations. Nat Rev Immunol. 2012; 12(8): 592-605.
28. Nicolas JF, Guy B. Intradermal, epidermal and transcutaneous vaccination from immunology to clinical practice. Human Vaccine. 2008; 4(3): 195-205.
29. Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK, Madden TD, Hope MJ, Weissman, D. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. J Control Release. 2015; 217: 345-351.
30. Malla RR, Srilatha M, Farran B, Nagaraju GP. mRNA vaccines and their delivery strategies: A journey from infectious diseases to cancer. Mol Ther. 2024; 32(1): 13-31.
31. Henao-Restrepo AM, Camacho A, Longini IM. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial. The Lancet. 2017; 389(10068): 505-518.
32. Lundstrom K.Viral vectors in vaccine development. Viruses. 2018; 10(1): 15.
33. Sun H, Zhang Y, Wang G, Yang W, Xu Y. mRNA-based therapeutics in cancer treatment. Pharmaceutics. 2023; 15(2): 622.
34. Fernández-Tejada A, Tan DS, Gin DY. Development of improved vaccine adjuvants based on the saponin natural product QS-21 through chemical synthesis. Acc Chem Res. 2016; 49(9): 1741-1756.
35. Lebel M-È, Chartrand K, Leclerc D, Lamarre A. Plant viruses as nanoparticle-based vaccines and adjuvants. Vaccines. 2015; 3(3): 620-637.
36. Magnusson SE, Reimer J, Karlsson KH. Immune enhancing properties of novel saponin-based adjuvants and their potential integration with lipid nanoparticles in vaccine formulations. Vaccine. 2013; 31(12): 173-181.
37. Zhao L, Steinmetz NF. Plant viral nanoparticles for packaging and in vivo delivery of bioactive cargos: platforms for antigen/adjuvant presentation and immunomodulation. Adv Drug Deliv Rev. 2020; 156: 214-227.
38. Pomatto MAC, Gai C, Negro F, Massari L, Deregibus MC. Oral delivery of mRNA vaccine by plant-derived extracellular vesicle carriers. Cells. 2023; 12(14): 1826.
39. Magnusson SE, Natama MH, Somé A. Matrix-M adjuvant: combining plant-derived saponins with lipid moieties to boost immune responses in modern vaccine platforms. Hum VaccinImmunother. 2023; 19(1): 213-223.
40. Tizard IR. Adjuvants and adjuvanticity. Vet Immunol Immunopathol. 2007; 117(3-4): 195-204.
41. Lico C, Mancini C, Italiani P, Betti C, Boraschi D, Benvenuto, E. Plant-produced virus-like particles as vaccines and immunotherapeutics. Hum VaccinImmunother. 2015; 11(12): 2751-2763.