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Review

mRNA Vaccines for Colorectal Cancer: Antigen Design, Nanodelivery, and Clinical Translation

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Abstract

Messenger RNA (mRNA) vaccines offer a flexible approach to colorectal cancer (CRC) immunotherapy, with applications ranging from postoperative residual disease to metastatic cancer. This narrative review examines antigen selection, RNA engineering, nanodelivery, and clinical development, drawing on literature available through 21 October 2026. Tumor-associated antigens, shared driver mutations, and personalized neoantigens offer different advantages in population coverage, specificity, and manufacturing. Their activity also depends on RNA chemistry and carrier design, including lipid composition, endosomal release, APC targeting, tissue distribution, stability, and repeated dosing. Early clinical studies have demonstrated antigen-specific T-cell responses in selected patients, while randomized melanoma data provide a benchmark for combining vaccination with checkpoint blockade. The termination of the CRC adjuvant autogene cevumeran monotherapy trial highlights the difficulty of translating immune priming into recurrence prevention. Antigen heterogeneity, impaired HLA presentation, stromal exclusion, and liver-associated immune suppression remain important barriers in CRC. Advances in neoantigen prediction, self-amplifying RNA, and mechanism-based combinations may improve vaccine design, but their clinical value will depend on disease-specific trials that account for manufacturing feasibility and established oncological endpoints. The available evidence remains heterogeneous, with small CRC cohorts and limited randomized outcomes; the non-systematic search also introduces potential selection bias.

Keywords: colorectal cancer; mRNA vaccine; neoantigen; nanodelivery; lipid nanoparticle; self-amplifying RNA

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1. Introduction

Colorectal cancer (CRC) accounted for approximately 1.9 million new cases and more than 900,000 deaths worldwide in 2022 [1]. Recurrence after apparently curative surgery and resistance in metastatic disease remain major therapeutic challenges. Messenger RNA (mRNA) vaccines offer a programmable means of expressing tumor antigens within host cells, thereby inducing or expanding antigen-specific immunity. Their development integrates three connected decisions: which antigens to encode, how to deliver intact RNA to appropriate cells, and how to evaluate benefit in a biologically defined patient population [2–4].

Mismatch repair (MMR) status and microsatellite instability (MSI) describe related molecular features, whereas an immune-inflamed, immune-excluded, or immune-desert

phenotype describes the spatial and functional organization of antitumor immunity. Proficient MMR (pMMR) and microsatellite-stable (MSS) CRC frequently overlap, as do deficient MMR (dMMR) and MSI-high (MSI-H) CRC, but the respective assays measure different properties. Molecular status is therefore reported alongside, rather than substituted for, immune phenotype. Some pMMR/MSS tumors are infiltrated or hypermutated, including selected polymerase proofreading-defective cancers; conversely, antigen-presentation defects can arise in highly mutated tumors [5,6].

For the colorectal surgeon, the central clinical questions are whether vaccination can eradicate residual disease after resection and improve systemic control in advanced CRC. Answering these questions requires alignment between the antigens expressed by residual or metastatic cells, the immune response induced by the vaccine, and the local conditions in which that response must act. This review examines antigen design, nanodelivery, and clinical translation across CRC subtypes, with particular attention to pMMR/MSS disease. Evidence from melanoma, pancreatic cancer, and non-mRNA CRC vaccines provides context for interpreting the emerging CRC experience.

Literature Search and Scope

This narrative review draws on primary studies of CRC mRNA vaccination, RNA delivery, antigen selection, and tumor immunity, together with clinical studies in other cancers that inform vaccine design and translation. Literature was identified through targeted web searches of PubMed/PMC records and journal websites, with ClinicalTrials.gov records, publicly available protocols, sponsor trial information, and FDA documents used to clarify clinical development. Searches were completed on 21 October 2026 and emphasized studies published since January 2022, alongside earlier foundational work. Search terms combined colorectal, colon, or rectal cancer with mRNA vaccine, self-amplifying RNA, lipid nanoparticle, neoantigen, antigen prediction, endosomal escape, dendritic cell, mucosal immunity, liver metastasis, microbiome, and circulating tumor DNA. Individual products, trial identifiers, and DOIs were searched to resolve study-specific details.

Studies were selected for their relevance to antigen-directed vaccination, delivery, immune resistance, or clinical translation. Primary reports were prioritized; non-CRC studies were included for a defined mechanistic or clinical comparison, and computational studies were distinguished from experimental validation. Publications reporting follow-up of the same cohort were considered together. Trial registrations and sponsor announcements were used for design and development status, with their source identified alongside published outcomes. This was a non-systematic, English-language search without preregistration, independent duplicate screening, or meta-analysis. The synthesis is therefore subject to selection bias, variable access to full reports, and differences in study populations, platforms, and follow-up.

2. RNA Design and the Biological Basis of Vaccination

Conventional vaccine mRNA contains an antigen-coding open reading frame, 5′ and 3′ untranslated regions, a cap, and a poly(A) tail. Sequence engineering, capping, purification, and nucleoside chemistry influence translation, stability, and innate sensing [7]. Karikó and colleagues demonstrated reduced Toll-like receptor recognition with modified nucleosides; subsequent experiments with N1-methylpseudouridine showed increased protein expression and reduced innate immunostimulation in tested systems [8,9]. Some cancer vaccine platforms instead use unmodified uridine RNA to couple antigen expression with innate activation [4]. These represent different product design choices rather than a universal ranking of RNA chemistries.

Conventional mRNA undergoes translation without RNA replication. Self-amplifying RNA (saRNA) additionally encodes replication machinery, usually derived from an alphavirus replicon, to generate intracellular RNA copies and antigen transcripts. This creates opportunities for lower input doses or sustained expression, alongside longer constructs, replication-associated inflammatory signals, and additional integrity and potency measurements. Both conventional mRNA and saRNA can be formulated in lipid nanoparticles (LNPs), while saRNA can also be packaged in virus-like particles (VLPs). Their biological and manufacturing features depend on the combination of RNA format and carrier (Table 1) [10,11].

Following cytoplasmic delivery to antigen-presenting cells (APCs), translated proteins can enter proteasomal processing and MHC class I presentation to CD8+ T cells. MHC class II presentation to CD4+ T cells generally uses endosomal/lysosomal processing; RNA-encoded products can reach this pathway through secretion and uptake, autophagy, or engineered trafficking. Cross-presentation describes presentation of an externally acquired antigen on MHC class I. The relevant substrates are processed peptides bound to MHC/HLA molecules. APC maturation, costimulation, and cytokines then shape priming, expansion, and memory. Figure 1 outlines this sequence from RNA delivery to immune memory [4,8,12].

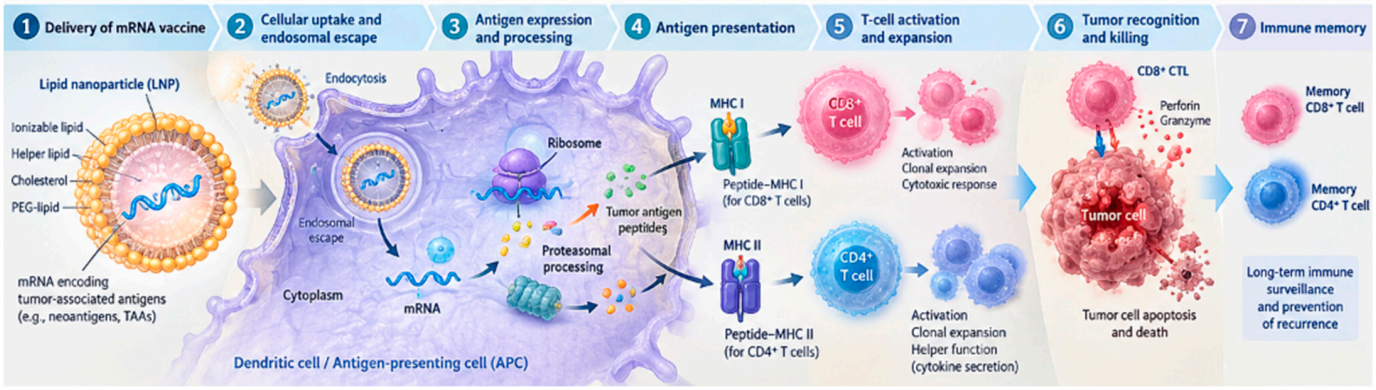


Figure 1. Antitumor immune responses induced by nanodelivered mRNA vaccines. Following cellular uptake and endosomal escape, mRNA is translated in APCs and processed for peptide presentation to CD8+ and CD4+ T cells. MHC class II presentation involves endosomal trafficking, secretion and reuptake, or autophagy. Effector T cells subsequently recognize matching peptide–HLA complexes on tumor cells, while memory populations may support longer-term surveillance. Neoantigens and tumor-associated self-antigens are distinct target classes. Recurrence prevention represents a therapeutic objective. APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte; PEG, polyethylene glycol [4,6,8,12]. Prepared using chatGPT.

Vaccine priming and tumor recognition occur in different cells. An APC expressing a vaccine construct can efficiently display an engineered antigen fragment and activate a T-cell clone even when a malignant cell expresses little of the endogenous protein, processes it differently, or lacks the restricting HLA allele. Tumor killing requires that the same T-cell receptor recognize an appropriate peptide–HLA complex on the malignant cell at a functional density. Peptide-loaded target cells test the recognition of a supplied peptide; autologous tumor cells or organoids expressing the endogenous antigen address the additional requirement for natural processing and presentation. This distinction explains why immunogenicity, intratumoral function, and clinical outcomes are assessed separately [6,13,14].

Table 1. RNA formats and delivery platforms.

RNA Format or Formulation	Cargo and Amplification	Carrier Architecture	Administration Route	Evidence Relevant to CRC	Practical Strengths and Constraints
Conventional linear mRNA	Antigen ORF; no RNA replicase; modified or unmodified nucleosides	Naked RNA or a carrier	Product-specific	Underlying RNA format of several clinical programs [14,15]	Shorter than replicons; translation depends on sequence, purity and delivery. Conventional RNA is the reference RNA format, not a separate carrier.
Conventional mRNA–LNP	Non-self-amplifying antigen RNA	Ionizable lipid, helper lipid, cholesterol and PEG-lipid	Often IM	CRC mouse studies; clinical lipid-formulated products [12,14,16]	RNA protection and controlled formulation; escape, stability and repeat dosing remain product-specific.
Conventional RNA–LPX	Uridine RNA encoding shared or individualized antigens	Electrostatic RNA–lipid complexes	IV in cited programs	Autogene cevumeran: mixed-tumor clinical and CRC-only phase II [15,17]	Lymphoid APC delivery and innate activation; architecture, charge ratio and route differ from common IM LNP designs.
saRNA–LNP	Antigen plus replicase; intracellular amplification	Lipid nanoparticle containing a replicon	IM boost in GRANITE	RNA component of heterologous clinical regimens [10,18]	Potential dose sparing; longer RNA, integrity and replication-associated sensing. Independent contribution not isolated in combination trials.
saRNA–VLP	Replicon encoding mutant APC-gene sequences	Chimeric virus-like nanoparticle	IM in mouse study [11]	In vitro and mouse proof of concept [11]	Particle assembly and anti-particle immunity add variables; patient benefit and direct superiority remain untested.

IM, intramuscular; IV, intravenous; LPX, lipoplex; VLP, virus-like particle. Conventional mRNA refers to non-self-amplifying linear RNA. RNA format and delivery vehicle can be combined in different ways.

3. Antigen Selection and Coordinated Immune Responses

3.1. Shared Self-Antigens and the Therapeutic Window

Tumor-associated antigens (TAAs) originate from self-proteins with aberrant, increased, or lineage-associated expression. Neoantigens originate from tumor-associated sequence alterations and may be shared across patients or individualized [19]. Antigen origin is independent of the product model: both shared TAAs and recurrent mutant neoantigens can support off-the-shelf manufacture, whereas personalized products select targets from an individual tumor [18,20,21]. Table 2 relates these choices to population coverage and safety.

Table 2. Antigen strategies and target validation.

Target Strategy	Population and Coverage	Potential Value	Principal Validation and Safety Questions
CEA/CEACAM5 [22]	CRC with relevant tumor expression and HLA presentation	Shared target and advance manufacturing	Matched normal mucosa; peptide–HLA abundance; T-cell cross-reactivity; colitis risk. Serum CEA is not a stand-alone selection assay.
GUCY2C [23]	Tumors retaining intestinal lineage antigen	Shared target with human adenoviral-vaccine immunogenicity	Normal tissue and tumor processing; HLA; platform-specific reassessment for mRNA delivery.

Table 2. *Cont.*

Target Strategy	Population and Coverage	Potential Value	Principal Validation and Safety Questions
MAGE-A3/other cancer–testis antigens [24]	Expression-positive subgroups	Shared targeting in a selected population	Transcript prevalence versus protein/presentation; normal tissue; cross-reactivity.
Shared KRAS mutations [13,18,25]	Matching mutation, expression, and HLA context	Off-the-shelf neoantigen targeting	Natural peptide presentation; mutation/HLA population coverage; escape through HLA loss.
Personalized neoantigens [14,15]	Adequate tumor/normal material and feasible manufacture	Patient-specific clonal and complementary targets	Multi-lesion coverage, residual-clone persistence, candidate quality and manufacturing attrition.
Multi-epitope/hybrid constructs [12,26]	Patients with complementary validated candidates	Breadth of helper and cytotoxic recognition	Expression imbalance, junctional peptides, immunodominance; simulations separated from biological validation.

TAA, tumor-associated antigen; CEA, carcinoembryonic antigen; GUCY2C, guanylyl cyclase C. Shared or personalized manufacture is distinct from the biological origin of the antigen.

Carcinoembryonic antigen (CEA), primarily CEACAM5, illustrates the balance between shared tumor expression and normal-tissue risk. Serum concentration reflects shedding and tumor burden as well as other biological factors, whereas tissue staining measures protein abundance and localization. Neither directly measures the abundance of a target peptide–HLA complex. Normal intestinal mucosal expression creates a potential route for on-target, off-tumor recognition. Although membrane polarity can affect antibody access to CEA, T cells recognize processed peptides on HLA; accessibility of a surface protein and accessibility of its HLA-presented peptides are different considerations. Heterogeneous tumor expression and inflammatory changes in normal mucosa further complicate the therapeutic window [22].

CEA-directed adoptive T-cell treatment produced tumor regression together with severe transient colitis in a small metastatic CRC study [22]. Although adoptive cell therapy differs substantially from vaccination, this experience makes the normal intestinal epithelium a central consideration in CEA-directed vaccine development. A CEA vaccine assessment can therefore combine tumor and matched normal-mucosa expression, HLA typing, immunopeptidomics when feasible, and functional testing against tumor and normal epithelial targets. Affinity and specificity of induced T cells, cross-reactivity with related peptides, baseline intestinal inflammation, and gastrointestinal toxicity are relevant to dose escalation and monitoring. There is currently no single serum CEA threshold that establishes suitability for CEA-directed mRNA vaccination.

Guanylyl cyclase C (GUCY2C) is an intestinal lineage antigen. An early clinical study of Ad5-GUCY2C-PADRE in patients with resected colon cancer detected vaccine-induced T-cell responses, supporting the biological feasibility of this target [23]. Ad5 denotes adenovirus type 5, and PADRE denotes a pan-HLA-DR helper epitope. However, findings with an adenoviral vaccine do not establish the efficacy of an mRNA formulation. Antigen expression, processing, dose, and toxicity must be reassessed after a change in delivery platform.

Melanoma-associated antigen A3 (MAGE-A3), New York esophageal squamous cell carcinoma 1 (NY-ESO-1), and other cancer–testis antigens generally have restricted expression in most adult somatic tissues but may be re-expressed in tumors. Membership in this class does not itself establish safety; each target requires assessment of normal-tissue expression, cross-reactivity, and tumor expression. Expression in CRC is subgroup-specific, making biomarker-positive populations the appropriate candidates for target-directed products [20,24].

In an analysis of 82 paired CRC and normal-tissue samples, approximately 28% of tumors met the study-defined threshold for positive MAGE-A3 mRNA expression [24]. This finding constrains the potentially eligible population and indicates that transcript-level screening should be followed by confirmation of protein expression and peptide presentation. Frequencies reported in other malignancies should not be extrapolated to CRC.

3.2. Shared and Personalized Neoantigens

Recurrent KRAS mutations provide shared neoantigens with potential for advance manufacturing. Immune recognition depends on mutation-specific peptides, HLA compatibility, and natural processing. Clinical recognition of KRAS G12D by transferred T cells in CRC established target feasibility and also illustrated escape through loss of the relevant HLA haplotype [13]. Small-molecule KRAS inhibition and mutant KRAS vaccination act through different mechanisms. The population reachable by a shared vaccine depends jointly on mutation prevalence, antigen expression, HLA frequencies, and validated epitope coverage; the number of mutations encoded alone gives an incomplete estimate.

Personalized workflows generally begin with sequencing of tumor and matched normal tissue, RNA sequencing, HLA typing, and bioinformatic prioritization. Normal samples help exclude germline variants, while RNA evidence supports transcription of mutant sequences [14,21]. Prioritization may additionally consider protein processing, HLA binding, peptide stability, immunopeptidomics, and patient-derived T-cell assays [27–29]. Whole-exome sequencing provides variant information but cannot independently deliver an effective vaccine design.

Personalized selection therefore considers both the quality of an individual candidate and its relevance to the tumor being treated [21,29]. In postoperative CRC, the resected specimen provides material for vaccine design, while the residual disease burden may differ from the sampled tumor. Reports should document specimen adequacy, manufacturing success, and progression before vaccination so that the treated population can be interpreted in relation to all patients screened [14,30,31]. ctDNA can complement tissue analysis by identifying residual disease, but it does not establish protein expression or peptide–HLA presentation [32,33].

Noncanonical transcripts, frameshifts, and splice-derived sequences may expand candidate repertoires, particularly when conventional mutation-derived targets are scarce. Their value depends on tumor-restricted expression, translation, presentation, and normal-tissue specificity. Computational identification of a differentially expressed gene is considered a target-discovery hypothesis. For example, the multi-epitope construct reported by Wang and colleagues was evaluated through computational design and immune simulation, rather than tumor-bearing animals or patients [26].

3.3. AI-Assisted Prioritization and Multi-Epitope Constructs

AI-assisted selection involves several prediction tasks: peptide–HLA binding, cell-surface presentation, and T-cell immunogenicity. Table 3 separates these tasks and the associated validation needs. NetMHCpan/NetMHCIIpan, MHCflurry, and BigMHC use different data and outputs; scores are therefore interpreted within the specific model, HLA allele, and application [27,28,34]. Consortium benchmarking by TESLA supports evaluation against experimentally tested candidates rather than treating binding predictions as immunogenicity labels [29]. Class II helper-epitope assessment requires suitable class II data and models [35].

Table 3. AI-assisted antigen prioritization and experimental validation.

Tool or task/Inputs and Output	Evidence Category	Use in a CRC Workflow	Limitations and Validation
NetMHCpan-4.1/NetMHCIIpan-4.0 [27] Peptide and HLA; binding/presentation-related ranks for class I or II	Computational, experimentally benchmarked model publication	Rank HLA-compatible candidates for CD8+/CD4+ testing	Allele representation and score type matter; rank is not a probability of patient response.
MHCflurry 2.0 [28] Peptide/HLA and sequence context; binding, processing and integrated class I presentation scores	Computational and held-out experimental-ligand benchmarking	Complementary class I prioritization	Presentation scores are not direct immunogenicity measurements; external patient-separated validation needed.
BigMHC [34] Peptide/HLA; learned presentation features with transfer learning for immunogenicity	Computational with experimental benchmarking	Prioritize candidates for tumor-specific T-cell experiments	Model-specific training distribution, rare alleles and peptide similarity influence generalization.
Class II immunogenicity analysis [35] Class II candidate features and experimental response data	Computational and human tumor immunology research	Identify complementary helper targets	Class I scores do not replace class II processing and CD4+ recognition measurements.
TESLA benchmark [29] Shared sequencing/HLA inputs, ranked candidate lists and patient-matched assays	Consortium computational/experimental benchmark	Evaluate top-ranked yield and reproducibility	Benchmark performance is dataset-specific; candidate testing does not establish vaccine efficacy.
CRC multi-epitope and UTR design [26] Predicted epitopes, sequence optimization, RNA-FM/CNN-assisted UTR screening and immune simulation	CRC-focused computational study	Generate candidate constructs for subsequent laboratory work	No demonstrated tumor-cell killing or patient outcome; validate expression, processing, normal-tissue specificity and function.

AI, artificial intelligence; UTR, untranslated region; CNN, convolutional neural network. Model versions correspond to the cited studies. Validation progresses from peptide–HLA presentation to T-cell recognition of endogenous tumor antigens.

Consortium benchmarking supports evaluating ranked candidates against patient-matched experimental responses rather than assuming that predicted binding establishes immunogenicity [29]. For CRC applications, useful reporting would include patient-separated validation, HLA representation, candidate-testing denominators, and precision among the candidates selected for manufacture. Model versions, input-quality criteria, ranking thresholds, and manual exclusions should also be documented. These are proposed reporting considerations; the clinical value of AI-assisted prioritization still requires prospective assessment of verified target yield, induced responses, manufacturing feasibility, and patient outcomes.

Multi-epitope constructs concatenate antigen fragments or combine separate RNAs. A single transcript can favor delivery to the same cell, whereas multiple RNAs permit adjustment of component ratios. Expression imbalance, immunodominance, processing competition, and unintended junctional peptides can influence the resulting repertoire. CD4+ helper responses can support APC licensing and CD8+ expansion and persistence. Antibody responses require additional attention to antigen conformation and extracellular accessibility; antibodies against intracellular mutant sequences are not direct measures of viable tumor-cell recognition. Co-administration of MHC-I- and MHC-II-restricted neoantigens enhanced antigen-specific CD8+ responses and antitumor activity in mouse CRC models, including postoperative experiments [12]. These experiments support coordinated epitope design at a preclinical evidence level.

The contribution of CD4+ cells extends beyond adding another set of epitopes. In foundational cross-priming experiments, CD40 signaling on APCs could substitute for helper-cell activity, establishing a mechanism by which helper recognition supports cytotoxic T-cell priming [36]. For CRC constructs, this supports selecting helper and cytotoxic epitopes that can be presented by the same APC, while testing whether the resulting CD8+ cells

recognize naturally processed tumor antigens. Adding predicted class II binders alone does not establish effective help; APC activation, functional CD4+ responses, and sustained tumor-reactive CD8+ responses provide more direct evidence [12,35,36].

4. Nanodelivery from Formulation to Functional Antigen Presentation

4.1. LNP Composition and RNA–Lipoplex Architecture

A common LNP formulation combines an ionizable lipid, a helper phospholipid, cholesterol, and a PEG-lipid. During acidic mixing, protonated lipids associate with negatively charged RNA. At physiological pH, reduced surface charge can moderate nonspecific interactions; protonation during endosomal acidification promotes membrane interactions relevant to RNA release. Helper phospholipids affect particle structure and fusion behavior, cholesterol influences packing and membrane properties, and PEG-lipids help control particle assembly and colloidal interactions. These roles depend on chemical identity, component ratios, RNA-to-lipid ratio, and manufacturing conditions. In cancer models, Oberli and colleagues linked LNP delivery to APC transfection and cytotoxic responses; phospholipid optimization studies further showed effects on membrane fusion, escape, and organ tropism [37,38].

RNA–lipoplexes (RNA–LPX) and ionizable-lipid LNPs are overlapping descriptions of lipid-based nanomedicines, with different architectures and formulation conventions in the cited programs. The systemic RNA–LPX approach reported by Kranz and colleagues used electrostatic complexation with cationic lipid-containing carriers and adjustment of the net negative charge to favor lymphoid APC delivery. This platform underlies intravenous uridine-RNA cancer vaccination. Four-component ionizable LNPs typically embed RNA within lipid assemblies created by controlled mixing and are also used for intramuscular vaccination. Autogene cevumeran is reported as RNA–LPX, while mRNA-4650 and V940 are described as lipid-formulated mRNA products. Carrier structure, RNA chemistry, charge ratio, route, and measured biodistribution are more informative than labeling every preparation simply “LNP” [4,14,15].

These formulation differences help explain why spleen-associated DC targeting after intravenous RNA–LPX administration may differ from the distribution of an intramuscular ionizable-LNP vaccine [4,37]. The identity and molar ratio of the lipids further influence particle behavior [38–40]. Comparisons of clinical products should therefore distinguish measured distribution from assumptions based on a carrier label.

4.2. Particle Characteristics and Endosomal Release

Particle diameter and distribution, surface properties, RNA encapsulation, and RNA integrity are useful formulation measurements. Their interpretation benefits from complementary methods that assess particle structure, expression, and intracellular trafficking [37,38,41]. A narrow size distribution and high encapsulation do not by themselves establish productive cytoplasmic delivery [41]. For CRC vaccine comparisons, matched antigen constructs, RNA chemistry and dose, administration route, and sampling times would help separate carrier effects from other product differences.

Endosomal escape separates internalization from productive transfection. Paramasivam and colleagues visualized release from early/recycling endosomal structures and found that uptake and expression could diverge across formulations and cell types [41]. Ionizable-lipid protonation, lipid packing, fusogenic helper lipids, and intracellular trafficking jointly influence membrane destabilization and RNA release. Increasing membrane disruption can also increase cellular injury, making expression, viability, and inflammatory readouts complementary design endpoints. Reporter uptake, punctate fluorescence, or lysosomal

colocalization is therefore interpreted alongside cytosolic RNA or protein expression and antigen-specific presentation assays.

For active vaccination, useful potency assays link particle attributes to expression and peptide presentation in relevant APC populations. A tumor-cell reporter assay may be appropriate for intratumoral immunomodulator delivery, but it answers a different question from systemic DC priming. Where formulations are compared, RNA mass and, for constructs of different lengths, molecule number help interpret apparent dose sparing. Measurements of expression duration, CD4+/CD8+ response breadth, and tolerability are especially informative when comparing conventional mRNA with saRNA.

4.3. Biodistribution and CRC-Relevant Targeting

Administration route determines the initial biological compartment. Studies of systemic RNA–LPX and local LNP vaccination illustrate how route and carrier design can influence APC exposure and lymphoid delivery [4,37,42]. SORT experiments further demonstrate formulation-dependent organ expression [39,40]. Whole-organ RNA or lipid accumulation, antigen expression in a specific cell type, and functional antigen presentation are distinct measurements. Hepatic accumulation should therefore not be interpreted as evidence of delivery to a CRC metastasis.

In mouse studies, selective organ targeting (SORT) altered organ expression by adding a fifth lipid component; mechanistic work implicated lipid chemistry, PEG-lipid desorption, protein corona formation, and receptor-associated uptake [39,40]. These results demonstrate formulation-dependent tropism rather than validated targeting of human CRC metastases. Chen and colleagues reported a lymph node-targeting 113-O12B LNP with immune activity in melanoma models, providing another transferable preclinical delivery concept [42]. Organ targeting is thus followed by cell-type and presentation measurements when applied to vaccination.

CRC additionally raises the question of effector-cell homing to intestinal or metastatic sites after priming. An ATRA-adjuvanted mRNA-LNP study reported increased gut-homing responses and antitumor activity in mouse CRC, supporting co-design of antigen delivery and mucosal immune trafficking [16]. This is different from selective nanoparticle binding to a human intestinal tumor. The distribution of the carrier, the site of priming, and the destination of activated T cells are three related but separable pharmacological events. Table 4 summarizes CRC-specific and transferable preclinical delivery studies, including the evidence level of saRNA-VLP research [11,12,16,37–42].

Table 4. Preclinical nanodelivery and design evidence.

Study and Cargo	Carrier or Intervention	Disease/Model Category	Observed Result and Evidentiary Scope
Li et al. [16]; antigen-encoding mRNA with ATRA	ATRA-adjuvanted LNP	CRC preclinical, including orthotopic models	Gut-homing/mucosal immune responses and antitumor activity; no human CRC outcome.
Cho et al. [12]; MHC-I/II neoantigens	mRNA–LNP co-administration	CRC preclinical	Enhanced antigen-specific CD8+ responses and antitumor effects, including postoperative mouse experiments.
Simmons et al. [11]; mutant APC-gene sequences	saRNA-containing chimeric VLP	CRC-relevant in vitro/mouse proof of concept	T-cell activation and antigen-specific antibodies; no clinical efficacy estimate or carrier superiority comparison.
Oberli et al. [37]; gp100/TRP2 and model antigens	LNP; APC transfection	Other-cancer preclinical, melanoma	Cytotoxic response and mouse tumor control; transferable delivery mechanism.

Table 4. *Cont.*

Study and Cargo	Carrier or Intervention	Disease/Model Category	Observed Result and Evidentiary Scope
Cheng and Dilliard et al. [39,40]; reporter/editing cargo	SORT LNP composition and protein-corona engineering	General delivery preclinical	Formulation-dependent organ expression; not human CRC lesion targeting.
Álvarez-Benedicto et al. [38]; reporter mRNA	Phospholipid variation in LNPs	General delivery preclinical	Membrane fusion, escape and organ tropism depend on phospholipid chemistry.
Paramasivam et al. [41]; reporter mRNA	LNPs evaluated by subcellular imaging	Mechanistic cell study	Escape linked to early/recycling compartments; uptake differs from productive expression.
Chen et al. [42]; antigen mRNA	113-O12B LNP	Other-cancer preclinical, melanoma	Lymph node targeting and CD8+ responses; relevance to CRC requires disease-specific testing.

APC, antigen-presenting cell; ATRA, all-trans-retinoic acid; SORT, selective organ targeting. CRC-specific experiments are distinguished from general delivery and other-cancer models.

4.4. Stability and Repeat Administration

RNA is susceptible to chemical and enzymatic degradation, making storage conditions and formulation important determinants of product stability [7]. Freeze–thaw handling also affects the carrier: Cheng and colleagues showed that freezing-induced incorporation of betaine into LNPs could preserve particle stability and alter subsequent mRNA delivery in experimental systems [43]. This finding supports evaluating handling conditions together with biological activity; it does not establish a storage formulation for CRC vaccines. Storage limits and shelf life need to be defined for each product.

Repeated cancer vaccination creates a longer exposure schedule than a single-dose experiment. Anti-carrier responses, PEG-reactive antibodies, complement activation, inflammatory reactions, and changes in tissue disposition may influence subsequent doses. Complement-mediated disruption of PEGylated lipid particles has been demonstrated experimentally [44]. In contrast, a human repeated COVID-19 vaccination cohort did not find a corresponding impairment of antigen-specific antibody responses or serious adverse effects attributable to measured anti-PEG antibodies [45]. These findings support product- and route-specific monitoring rather than a general conclusion that PEG immunity prevents boosting. Interpretation in oncology also considers concomitant chemotherapy, checkpoint blockade, baseline liver function, and prior exposure to PEG-containing products.

For saRNA-containing VLPs, particle assembly, replicon integrity, residual production components, and immune recognition of particle proteins introduce additional comparability questions. The mutant APC-gene saRNA-VLP study by Simmons and colleagues reported in vitro T-cell activation and antigen-specific antibody responses in mice [11]. This is CRC-relevant platform proof of concept; therapeutic efficacy in patients and comparative superiority over mRNA-LNP remain open questions. In that context, APC gene refers to adenomatous polyposis coli, whereas APC elsewhere refers to an antigen-presenting cell.

5. Clinical Evidence by Disease and Vaccine Platform

5.1. Direct CRC and Mixed-Cohort RNA Vaccine Studies

Clinical development encompasses postoperative CRC trials, early studies in metastatic gastrointestinal cancer, and mixed-tumor programs targeting shared mutations (Table 5). In the published mRNA-4650 study, four patients with metastatic gastrointestinal cancers received individualized vaccines; mutation-specific responses were observed without objective responses [14]. The NCI protocol identifies a lipid-formulated personalized mRNA product manufactured by Moderna and a phase I/II design [30]. The study established

the feasibility of individualized vaccination in heavily pretreated gastrointestinal cancer, although tumor regression was not observed.

Table 5. CRC-related RNA vaccine clinical programs.

Program and Sponsor/Population and Phase	Encoded Antigen and Delivery	Regimen and Major Endpoints	Evidence Category and Available Outcome
mRNA-4650; NCT03480152 [14,30] NCI; Moderna manufacture Metastatic GI cancers; phase I/II; published treated cohort $n = 4$	Personalized mutation-derived fragments; lipid-formulated conventional mRNA; IM; full lipid recipe not publicly specified	Vaccine without checkpoint blockade in published cohort; safety, feasibility and immune responses; clinical activity exploratory	Mixed-GI clinical, including CRC-related context. Mutation-specific immunity; no objective response in four treated patients. Not a CRC-only efficacy estimate.
GRANITE; NCT03639714 [10,31] Gritstone Oncology/bio in cited protocol Advanced solid tumors including MSS CRC; phase I/II; phase I interim publication	Individualized neoantigens; ChAd68 prime plus saRNA nanoparticle boost; IM; complete lipid composition not publicly specified	Nivolumab + ipilimumab; primary safety/tolerability/RP2D; immune responses, manufacture and OS secondary	Mixed-tumor clinical with MSS CRC observations. Durable responses and exploratory ctDNA/OS associations; nonrandomized, multicomponent.
GRANITE maintenance; NCT05141721 [46] Gritstone bio in 2022 trial-in-progress report First-line metastatic MSS/pMMR CRC; phase II/III design as originally reported	Patient-specific neoantigens; ChAd68 GRT-C901 + saRNA GRT-R902	Fluoropyrimidine/leucovorin + bevacizumab; vaccine arm adds atezolizumab/ipilimumab; ctDNA/PFS development endpoints	CRC-only randomized trial design. The trial-in-progress report describes the maintenance regimen; mature randomized outcomes remain necessary to assess clinical benefit.
SLATE; NCT03953235 [18] Gritstone bio Advanced solid tumors; phase I/II; interim publication $n = 19$	Twenty shared HLA-matched neoantigens; ChAd68 + saRNA regimen	Nivolumab + ipilimumab; primary safety/tolerability; immunogenicity, ORR, PFS and OS secondary	Mixed-tumor clinical, largely KRAS-mutant. Antigen-specific responses; no randomized CRC treatment effect.
Autogene cevumeran; NCT03289962 [15,47] Genentech; BioNTech collaboration Advanced solid tumors including MSS CRC; phase I; 30 monotherapy + 183 combination in the published cohort	Up to 20 personalized neoantigens; uridine RNA–LPX; IV; full product lipid ratio not stated	Alone or + atezolizumab; primary safety/tolerability; exploratory immune and antitumor activity	Mixed-tumor clinical. MSS CRC ex vivo immune responses in 10/11 assessed patients, not 10/11 clinical responses. Registry completed, updated 8 June 2026.
BNT122-01; NCT04486378 [17,48] BioNTech SE; Genentech collaboration Resected high-risk stage II/stage III, ctDNA-positive CRC; randomized phase II	Autogene cevumeran personalized uridine RNA–LPX; IV	Adjuvant vaccine monotherapy versus observation; DFS efficacy objective; other survival and safety assessments	CRC-only trial; sponsor update, 28 August 2026: termination after monitoring review/futility and numerical OS imbalance. No arm-level survival estimates supplied; no new safety signal reported.
mRNA-5671/V941; NCT03948763 [49] Merck Sharp & Dohme; ModernaTX collaboration KRAS-mutant advanced NSCLC, CRC or pancreatic cancer; phase I; total actual $n = 70$	KRAS G12D/G12V/G13D/G12C; lipid-formulated mRNA; IM; complete lipid composition not publicly specified	Alone or + pembrolizumab; safety/DLT and dose selection; activity assessments	Mixed-tumor clinical study; terminated for business reasons. Registry results posted 28 January 2025; a CRC-specific treatment effect has not been established.
ABO2102; NCT06577532 [50] Ruijin Hospital; Suzhou Abogen collaborator Advanced pancreatic and other solid tumors; early phase I; planned $n = 56$; CRC enrollment unconfirmed	KRAS-neoantigen mRNA nanoparticles; IM; lipid identities/ratios not specified in the registry	Alone or + toripalimab; part I DLT/TEAEs; part II ORR	Registered mixed-solid-tumor program relevant to shared-KRAS development, not direct CRC outcome evidence. Recruiting in record updated 21 May 2025; no results posted.

IM, intramuscular; IV, intravenous; DLT, dose-limiting toxicity; RP2D, recommended phase II dose; TEAE, treatment-emergent adverse event; GI, gastrointestinal; NSCLC, non-small-cell lung cancer. Planned enrollment and immune-assessed subsets are identified separately from treated populations. Complete proprietary lipid compositions are unavailable for several products.

The GRANITE phase I report evaluated individualized ChAd68 adenoviral priming and saRNA boosting with nivolumab and ipilimumab. The primary objectives concerned

safety, tolerability, and recommended phase II dose; immune and ctDNA findings in MSS CRC were exploratory [10,31]. A related randomized maintenance program, NCT05141721, was described with fluoropyrimidine/leucovorin and bevacizumab plus vaccination, atezolizumab, and ipilimumab [46]. The trial-in-progress report described the rationale and treatment design; mature randomized outcomes are needed to assess the contribution of this maintenance strategy.

The shared-neoantigen SLATE phase I interim report broadened the RNA-containing clinical experience. Nineteen patients with advanced solid tumors received ChAd68/saRNA vaccination and nivolumab/ipilimumab; most carried a KRAS mutation. The report assessed safety and immunogenicity of a shared, HLA-matched antigen strategy [18]. It complements personalized GRANITE development but remains a mixed-tumor, multicomponent regimen.

For autogene cevumeran, a 2025 phase I report described 30 monotherapy and 183 combination-treated patients with advanced solid tumors. In the exploratory ex vivo biomarker analysis, 10 of 11 evaluable patients with MSS CRC showed neoantigen-specific responses. That denominator represents an immune-assessed subset, not the whole enrolled CRC population or an objective response rate [15]. The trial is listed as completed [47].

The CRC-only randomized phase II BNT122-01 study (NCT04486378) compared autogene cevumeran monotherapy with observation in ctDNA-positive patients after resection of high-risk stage II or stage III disease [48]. On 28 August 2026, the sponsor announced termination after an independent monitoring-board review: the futility boundary had previously been crossed, and the later review identified a numerical overall-survival imbalance and a low likelihood that continuation would alter the efficacy outcome. The statement reported no new safety signal [17]. Arm-level survival estimates and a peer-reviewed analysis were not provided in that announcement. A full analysis will be important for interpreting patient selection, treatment exposure, and the survival findings.

The mRNA-5671/V941 shared-KRAS trial (NCT03948763) enrolled 70 participants across advanced solid tumors and was terminated for business reasons, with registry results posted in January 2025 [49]. Its nonrandomized design and mixed population provide limited grounds for CRC-specific efficacy inference. Emerging Chinese development includes the ABO2102 KRAS-neoantigen mRNA nanoparticle study, sponsored by Ruijin Hospital with Abogen as collaborator, alone or with toripalimab [50]. The registered study focuses on safety and preliminary activity in pancreatic and other advanced solid tumors, with CRC-specific enrollment and outcomes yet to be reported.

5.2. Clinical Benchmarks from Other Cancers

Studies in melanoma and pancreatic cancer address questions that remain unresolved in CRC: whether vaccination adds benefit to checkpoint blockade, whether individualized manufacture fits a postoperative treatment pathway, and whether induced T cells persist. Their relevance lies in these clinical and analytical methods. CRC-specific antigen presentation, immune exclusion, and liver-associated suppression still require direct testing before adopting the same treatment strategy [6,51–54].

The randomized phase IIb KEYNOTE-942 trial in resected high-risk melanoma compared V940 plus pembrolizumab with pembrolizumab alone in 157 patients. The initial recurrence-or-death hazard ratio was 0.561 (95% CI, 0.309–1.017) [51]. The descriptive five-year update reported recurrence-free and distant metastasis-free survival hazard ratios of 0.510 (95% CI, 0.294–0.887) and 0.411 (95% CI, 0.200–0.843), respectively [55]. These observations support a clinical benchmark for individualized vaccination combined with checkpoint blockade. They derive from the same randomized cohort, with overall survival still requiring cautious interpretation.

In resected pancreatic ductal adenocarcinoma, autogene cevumeran was studied with atezolizumab and modified FOLFIRINOX. Eight of 16 vaccinated patients developed high-magnitude neoantigen-specific responses, and follow-up demonstrated persistence of selected CD8+ clones for approximately three years [52,56]. This is a small nonrandomized cohort. Immune-responder comparisons are susceptible to baseline differences and selection effects and are not randomized estimates of vaccine benefit. BNT111 in melanoma additionally illustrates clinical development of an off-the-shelf RNA–LPX encoding shared TAAs [20]. Table 6 separates these other-cancer benchmarks from CRC outcomes.

Table 6. Clinical RNA vaccine benchmarks from other cancers.

Program and Sponsor/Institution/Disease and Design	Antigen and Carrier	Treatment And Endpoints	Evidence Category and Outcome
KEYNOTE-942; NCT03897881 [51,55] Moderna; Merck collaboration Resected high-risk melanoma; randomized phase IIb; $n = 157$	V940/intismeran autogene; individualized neoantigens; mRNA–LNP, IM; full lipid recipe not publicly specified	V940 + pembrolizumab versus pembrolizumab; primary RFS; DMFS/OS and safety	Other-cancer randomized clinical. Initial RFS HR 0.561 (95% CI 0.309–1.017); descriptive five-year RFS HR 0.510 (0.294–0.887). Same cohort, not independent confirmation.
Autogene cevumeran; NCT04161755 [52,56] Memorial Sloan Kettering; BioNTech/Genentech collaboration Resected PDAC; single-arm phase I; $n = 16$ vaccinated	Up to 20 individualized neoantigens; uridine RNA–LPX, IV	Atezolizumab + vaccine + modified FOLFIRINOX; feasibility/safety and immune responses; recurrence exploratory	Other-cancer clinical. Eight of 16 immune responders; selected clones persisted about three years. Responder–RFS association is nonrandomized.
BNT111; Lipo-MERIT, NCT02410733 [20] BioNTech Advanced melanoma; phase I	Shared NY-ESO-1, MAGE-A3, tyrosinase and TPTE; uridine RNA–LPX, IV	Vaccine alone or with PD-1 blockade; safety, immune responses and exploratory activity	Other-cancer early clinical. Shared-TAA immune activity; provides product-model context, not a CRC benefit estimate.

DMFS, distant metastasis-free survival; HR, hazard ratio; PDAC, pancreatic ductal adenocarcinoma. Initial reports and extended follow-up from the same study describe one cohort.

Cross-cancer interpretation depends on the antigen landscape and treatment context. Ultraviolet-associated cutaneous melanoma often offers a larger mutation-derived candidate pool, whereas CRC includes both non-hypermutated and hypermutated subtypes [5,57]. Immune exclusion, antigen-presentation defects, and organ-specific suppression can occur in both diseases. Manufacturing approaches, clonotype tracking, and trial designs are transferable methods; numerical treatment effects are estimated within each disease and regimen.

For CRC combination trials, KEYNOTE-942 provides a useful design principle: retain the same background treatment in both groups so that randomization estimates the incremental contribution of vaccination [51]. In pMMR/MSS disease, where checkpoint blockade alone often has limited activity, the background regimen must first be justified for the selected population [2]. In postoperative studies, stage and ctDNA status may inform eligibility or stratification; recurrence endpoints require prespecified definitions [32,58]. In metastatic studies, liver involvement is a relevant candidate stratification factor, alongside treatment line and disease burden [54]. MMR/MSI status should be incorporated according to the population: it may define eligibility in an MSS/pMMR-only trial or support stratification when molecular subgroups are enrolled together [5,6]. These proposed design choices require CRC-specific validation.

The pancreatic studies offer a complementary monitoring approach: serial neoantigen-specific assays and T-cell receptor tracking can distinguish transient expansion from persistent vaccine-induced clones [52,56]. Applied to CRC, paired blood and tumor samples could test whether those clones enter lesions and recognize endogenous targets [6,14]. Serial ctDNA could provide a separate measure of residual disease [32,33]. Manufacturing

success, time to vaccination, and progression before dosing should be reported for all enrolled patients. BNT111 similarly informs shared-antigen product development [20], while a CRC product would need CRC-specific expression and HLA-presentation criteria [6,24]. These measurements can help explain a trial result; immune persistence or ctDNA clearance alone cannot establish clinical benefit [33,58].

5.3. Non-mRNA CRC Vaccines and Target-Level Clinical Context

Non-mRNA vaccination provides complementary information about target recognizability and immune delivery. AMPLIFY-201 evaluated the lymph node-targeted amphiphile peptide product ELI-002 2P, encoding no RNA. The phase I cohort comprised 20 pancreatic cancer and five CRC patients; mutant KRAS-specific T-cell responses were detected in 21 of the 25 participants. The 2024 report and 2025 follow-up describe the same cohort [25,59]. The vaccine combines KRAS G12D/G12R peptides with an amphiphile CpG adjuvant. Biomarker reductions and responder–outcome associations are exploratory mixed-cohort observations.

The Ad5-GUCY2C-PADRE phase I study in resected colon cancer demonstrated that an intestinal lineage antigen can elicit T-cell responses using an adenoviral carrier [23,60]. This is direct CRC clinical evidence for that vector and target, rather than an mRNA delivery result. Likewise, CEA- and KRAS-directed adoptive T-cell studies inform target safety and HLA-dependent recognition but are not vaccine trials [13,22]. Table 7 identifies the non-mRNA vaccine studies separately to preserve these distinctions.

Table 7. Non-mRNA CRC vaccine studies.

Trial and Sponsor/Disease and Phase	Antigen and Non-mRNA Platform	Regimen and Endpoints	Evidence Category and Findings
AMPLIFY-201; NCT04853017 [25,59] Elicio Therapeutics MRD-positive after local treatment; phase I; 20 PDAC + 5 CRC	ELI-002 2P; KRAS G12D/G12R amphiphile peptides + amphiphile CpG; SC; no encoded RNA	Vaccine after standard locoregional treatment; safety/RP2D; biomarker change, immunity and RFS	Mixed-tumor non-mRNA clinical including CRC. T-cell responses in 21/25 overall. Exploratory biomarker and responder associations; 2024/2025 reports are one cohort.
Ad5-GUCY2C-PADRE; NCT01972737 [23,60] Thomas Jefferson University cancer center Resected stage I/II colon cancer; phase I; $n = 10$	GUCY2C plus PADRE; adenoviral vector; IM; no mRNA nanoparticle	Single-dose target-directed vaccination; safety and immunogenicity	Direct CRC non-mRNA clinical. T-cell responses supported target feasibility; study was not powered to estimate recurrence benefit.

SC, subcutaneous; MRD, molecular/minimal residual disease according to the trial definition. Adoptive T-cell studies inform target biology and are discussed separately in the text.

6. Translational Barriers and Combination Strategies

6.1. Intratumoral Recognition and Liver-Associated Immune Suppression

Antigen-specific expansion in blood is one stage in a longer mechanism of action. Paired tissues can determine whether vaccine-induced clones reach malignant-cell neighborhoods, retain cytotoxic function, and encounter the expected peptide–HLA complexes. TGF-β-associated stroma and suppressive myeloid populations can limit this transition in CRC models [53]. Grasso and colleagues identified antigen-presentation gene alterations and links between WNT-pathway activity and T-cell exclusion in human CRC [6]. Antigen heterogeneity, target loss, HLA haplotype loss, and B2M-associated defects therefore create different forms of resistance. Increasing epitope breadth addresses some target heterogeneity, whereas global presentation failure affects all epitopes using the impaired pathway.

Liver metastases are especially relevant because the liver is a common metastatic site in CRC and can alter systemic immunity. Yu and colleagues combined clinical associations with mechanistic mouse experiments, including CRC-related models. Liver lesions recruited

or engaged activated antigen-specific CD8+ T cells, which underwent apoptosis after interaction with Fas-ligand-expressing monocyte-derived macrophages. This produced loss of effector cells beyond the liver and reduced checkpoint-treatment activity [54]. The Fas–FasL elimination mechanism was demonstrated experimentally; the human observations do not establish its frequency in every patient with CRC.

These findings distinguish a systemic loss of activated T cells from simple failure to prime or infiltrate a single lesion. They motivate analysis of liver disease burden, paired hepatic and extrahepatic samples, macrophage phenotype, T-cell apoptosis, and vaccine-clone persistence. Liver-directed radiotherapy and myeloid interventions improved immunity in experimental settings [54]; their use with CRC mRNA vaccines remains a proposed combination strategy requiring prospective assessment. Nonspecific hepatic uptake of an RNA carrier is also biologically different from control of the suppressive metastatic niche.

6.2. Combining Vaccination with Treatment of the Immune Microenvironment

Checkpoint blockade can support vaccine-expanded T cells when inhibitory signaling limits effector function. Randomized melanoma data provide a clinical example of this combination [51,55], while GRANITE and SLATE provide early human experience with multicomponent regimens involving CRC [10,18]. For CRC, the biological rationale depends on retained target presentation and the location and state of induced cells. A matched control retaining the same standard therapy and checkpoint background helps estimate the additional effect of vaccination.

Targeted therapy, vascular modulation, stromal interventions, chemotherapy, and radiotherapy may reduce burden or change immune access, but can also affect lymphocyte numbers, antigen expression, and clonal selection. TGF-β-associated exclusion supports investigation of stromal modulation in appropriately characterized lesions [53]. Proposed combinations with KRAS/BRAF-directed therapy, anti-VEGF treatment, myeloid modulation, or immunogenic-cell-death approaches are most informative when linked to a measurable resistance mechanism. Antigen release or cell-death markers describe one component of that mechanism; functional recognition and clinical control remain separate endpoints. Table 8 outlines how these biological barriers can inform patient selection and the evaluation of combination treatments.

Table 8. Translational barriers and proposed measurements.

Barrier or Opportunity	Current Evidence Category	Proposed Measurement or Trial Approach	Interpretation
Molecular versus immune phenotype [5,6,53]	CRC genomics and preclinical mechanisms	MMR/MSI with spatial CD8 distribution, stromal activity and presentation machinery	MSS/pMMR alone does not describe an individual lesion's immune state.
Antigen heterogeneity or HLA loss [6,13]	CRC target-level clinical and genomic evidence	Clonality, multi-lesion sampling, endogenous tumor recognition, HLA/B2M assessment	Multi-antigen coverage and global presentation failure require different approaches.
Liver-associated T-cell deletion [54]	Mechanistic mouse studies with human clinical associations	Hepatic burden; paired lesions and blood; macrophage FasL and T-cell persistence	Myeloid modulation or liver-directed therapy with vaccination remains prospective research.
Immune activation versus lesion control	Early clinical and mechanistic evidence	Vaccine-specific clonotypes, parenchymal localization, killing assays, randomized outcomes	Figure 2 distinguishes stages; no single immune readout is a validated clinical surrogate.
Manufacturing attrition [30,31]	Clinical protocols and early trials	Time from tissue to dose; manufacturing success; progression before treatment; intention-to-treat analysis	Delivery failures and delays are clinically relevant outcomes.

Table 8. *Cont.*

Barrier or Opportunity	Current Evidence Category	Proposed Measurement or Trial Approach	Interpretation
MRD enrichment and ctDNA [17,32,33]	CRC randomized chemotherapy study, FDA guidance and sponsor vaccine update	Standardized assays, prespecified times, randomized DFS/RFS and OS	Risk enrichment and pharmacodynamic change differ from prediction or surrogate validation.
Combination and microbiome opportunities [53,61,62]	CRC/other-cancer mechanisms and observational data; proposed future strategies	Matched background therapy; mechanism-specific pharmacodynamics; controlled interventions	Experimental directions, with efficacy and safety to be tested prospectively.

MMR, mismatch repair; MSI, microsatellite instability; B2M, beta-2-microglobulin; RFS, recurrence-free survival; DFS, disease-free survival; OS, overall survival. Proposed combinations require prospective clinical evaluation.

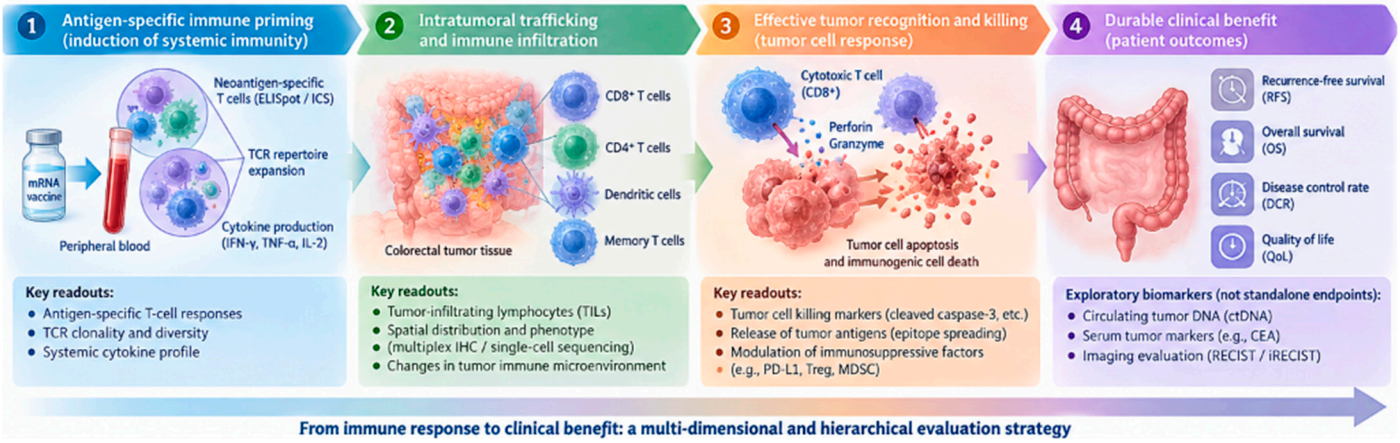


Figure 2. From vaccine-induced immune priming to clinical benefit in pMMR/MSS CRC. Evaluation follows antigen-specific T-cell expansion, intratumoral trafficking, tumor-cell recognition, and patient outcomes. MMR/MSI status describes molecular features, whereas immune phenotype reflects the distribution and function of immune cells. ctDNA and tissue assays complement clinical endpoints; cell-death markers alone do not demonstrate antigen-specific killing or immunogenic cell death. Disease control rate measures tumor control rather than durable survival. ELISpot, enzyme-linked immunospot; ICS, intracellular cytokine staining; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; IHC, immunohistochemistry; Treg, regulatory T cell; MDSC, myeloid-derived suppressor cell; QoL, quality of life; RECIST, Response Evaluation Criteria in Solid Tumours. Prepared using chatGPT.

Microbiome findings add another exploratory dimension. Dietary fiber and probiotic-associated observations in melanoma provide other-cancer context [61]. The CRC-associated Fusobacterium nucleatum subspecies animalis C2 clade illustrates the importance of strain-level biology [62]. Neither finding identifies a validated microbiome intervention for CRC mRNA vaccination. Proposed studies can combine longitudinal metagenomics, tissue microbial localization, metabolites, and matched immune samples, accounting for antibiotics, bowel preparation, diet, stoma status, and concomitant treatment. These factors are currently exploratory biomarkers or experimental intervention targets rather than established vaccine-selection criteria.

6.3. Manufacturing Feasibility and Clinically Meaningful Endpoints

Personalized turnaround encompasses specimen acquisition, sequencing, analysis, sequence selection, synthesis, purification, formulation, release testing, and delivery. Each interval can affect eligibility and treatment timing. The original mRNA-4650 protocol described an approximately eight-week manufacturing process [30], illustrating why a universal three-to-four-week claim is inappropriate. Reports are most interpretable when

they include the screened, sequenced, successfully manufactured, vaccinated, and evaluable populations, with reasons for attrition. Intention-to-treat analyses preserve randomized comparisons when progression or manufacturing failure prevents treatment.

For CRC vaccine development, product characterization should connect RNA quality and formulation consistency with biological activity. RNA purification and expression are established platform considerations [7], while delivery studies illustrate the complementary value of particle measurements and functional assays [37,38,41]. A practical development program would assess sequence identity, RNA integrity and impurities, formulation attributes, sterility, potency, and stability. Changes in RNA format, carrier, or antigen-selection workflow would require an assessment of their effects on product performance. These are development considerations rather than a uniform set of release specifications for all personalized vaccines.

Endpoint interpretation depends on disease setting. Objective response rate (ORR), disease control rate, progression-free survival (PFS), recurrence-free survival (RFS), disease-free survival (DFS), distant metastasis-free survival, and overall survival (OS) retain their trial-specific definitions and analysis populations. RFS and DFS are not assumed to be identical across protocols. Safety and dose selection dominate early-phase development, while randomized recurrence or survival endpoints address clinical benefit. Prespecified immune assays, tissue sampling, response thresholds, multiplicity procedures, and follow-up improve the relation between mechanism and outcome [58].

ctDNA can enrich postoperative risk and provide a longitudinal pharmacodynamic measure. DYNAMIC addressed ctDNA-guided chemotherapy in stage II colon cancer [32]; vaccine responsiveness was outside that trial's question. The BNT122-01 termination further illustrates that selection for residual disease does not itself establish responsiveness to vaccine monotherapy [17]. Prognostic enrichment, the prediction of an incremental treatment effect, and the validation of a surrogate endpoint are distinct roles. FDA guidance discusses assay standardization and ctDNA use in curative-intent development [33], while vaccine-specific surrogate validation remains a separate empirical question.

Potential predictive biomarkers include functional antigen presentation, HLA genotype and integrity, clonal target coverage, and immune spatial state. Their predictive value is assessed through treatment-by-biomarker interactions within controlled studies rather than outcome associations in vaccine recipients alone. In US development, an IND and, ultimately, a BLA require a product-specific benefit–risk assessment; cancer vaccine guidance offers development considerations rather than an automatic approval pathway [58]. Other jurisdictions require their own regulatory assessment.

7. Conclusions

CRC mRNA vaccine development links antigen quality to productive nanodelivery and sustained recognition of tumor cells. Shared and personalized antigens offer different coverage and manufacturing tradeoffs, while RNA chemistry, carrier architecture, endosomal escape, biodistribution, stability, and repeated administration jointly determine biological exposure. Clinical studies demonstrate feasibility and immune activity in selected settings, with evidence varying substantially by platform and population. The contrasting clinical experience in CRC monotherapy and melanoma combination treatment underscores the influence of disease biology and treatment context. CRC-specific randomized outcomes, supported by tissue-based mechanistic studies and transparent manufacturing denominators, remain central to determining which patients benefit. AI-guided selection, saRNA-VLP delivery, microbiome modulation, and mechanism-matched combinations provide research directions whose clinical value is still being defined.

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Abbreviations

The following abbreviations are used in this manuscript:

AI	Artificial intelligence
APC	Antigen-presenting cell
ATRA	All-trans-retinoic acid
BLA	Biologics license application
CEA	Carcinoembryonic antigen
CI	Confidence interval
CRC	Colorectal cancer
ctDNA	Circulating tumor DNA
CTL	Cytotoxic T lymphocyte
dMMR	Mismatch repair deficient
FDA	Food and Drug Administration
HLA	Human leukocyte antigen
ICI	Immune checkpoint inhibitor
IND	Investigational new drug
LNP	Lipid nanoparticle
MHC	Major histocompatibility complex
MMR	Mismatch repair
mRNA	Messenger RNA
MRD	Minimal residual disease
MSI-H	Microsatellite instability high
MSS	Microsatellite stable
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PDAC	Pancreatic ductal adenocarcinoma
PEG	Polyethylene glycol
pMMR	Mismatch repair proficient
saRNA	Self-amplifying RNA
TAA	Tumor-associated antigen
TGF-β	Transforming growth factor beta
TMB	Tumor mutational burden
TME	Tumor microenvironment
VEGF	Vascular endothelial growth factor

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