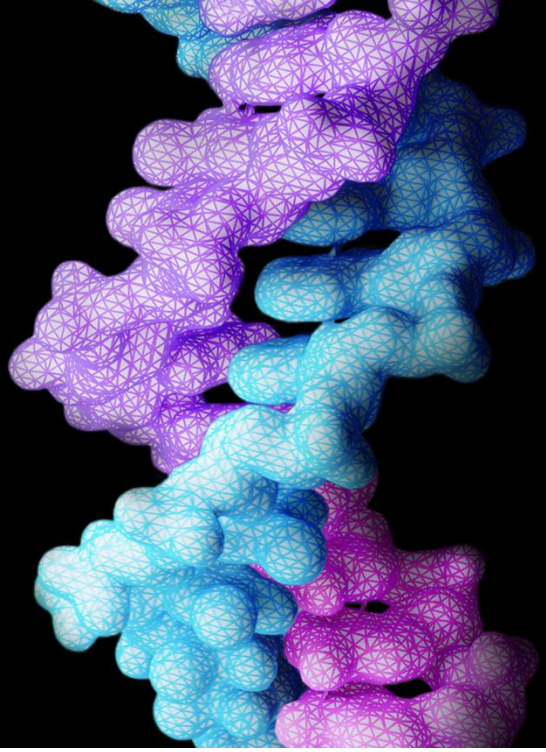
4basebio



Redefining Biomanufacturing for Personalized Cancer Vaccines: A Synthetic DNA Approach for Speed, Scale, and Flexibility

Overview

The advent of personalized cancer immunotherapy marks a transformative shift in oncology, designed to harness the body's immune system to target and eliminate tumor-specific antigens, known as neoantigens. The first early phase clinical trials for mRNA-based cancer immunotherapies have shown encouraging safety and efficacy, attracting significant investment, including the UK government's recent £129 million commitment to support BioNTech's mRNA cancer immunotherapy R&D programs.

However, manufacturing and regulatory hurdles remain before these therapies can reach their full potential. These therapies must be developed within a 6–8 week window from tumor biopsy to treatment and, require small, individualized batch manufacturing. Central to their advancement is the challenge of rapid and reliable production of DNA or mRNA that encodes the neoantigens. Plasmid DNA (pDNA), typically used as a DNA vector or, as critical starting material for mRNA production, is poorly suited for the modest scale, speed, and flexibility demanded by these personalized therapies.

Synthetic DNA addresses these challenges through an entirely cell-free enzymatic approach designed to accelerate DNA and mRNA manufacturing while providing flexibility of scale and maintaining quality. This whitepaper explores how 4basebio's technology combined with Neomatrix's neoantigen platform is enabling the next generation of personalized cancer immunotherapies.

A personalized approach to the manufacturing of therapeutics

Personalized neoantigen immunotherapies are based on the identification of patient-specific neoantigens from patient biopsies by next-generation sequencing. These neoantigens are encoded into mRNA, which is delivered back to the patient, typically via lipid nanoparticles (LNPs), to stimulate a targeted immune response. Although mRNA-LNPs are the most well-established technologies in this space, other approaches such as DNA-based immunotherapies can also be considered.

Fast and faithful synthesis of mRNA is critical to clinical success. For cancer patients, the rapid turnaround of these immunotherapies between tumor biopsy and vaccine administration is paramount to clinical outcome. The rise of such personalized approaches poses significant manufacturing challenges. The development of the mRNA-LNP COVID-19 vaccines pushed the industry to focus on scaling-up, providing large batch sizes to meet the needs of a global pandemic. This overcapacity is not suited for nimble, high throughput innovative medicines manufacture and instead, manufacturers must now adopt a scale-out model, producing multiple small batches in parallel, on an individual patient level.

A critical bottleneck in this process is the production of high-quality, scalable DNA templates, which serve as critical starting material for in vitro transcription (IVT) mRNA synthesis. Currently, industry relies upon pDNA, which is produced by bacterial fermentation in large-scale bioreactors with lead times stretching from 9 to 12 months.

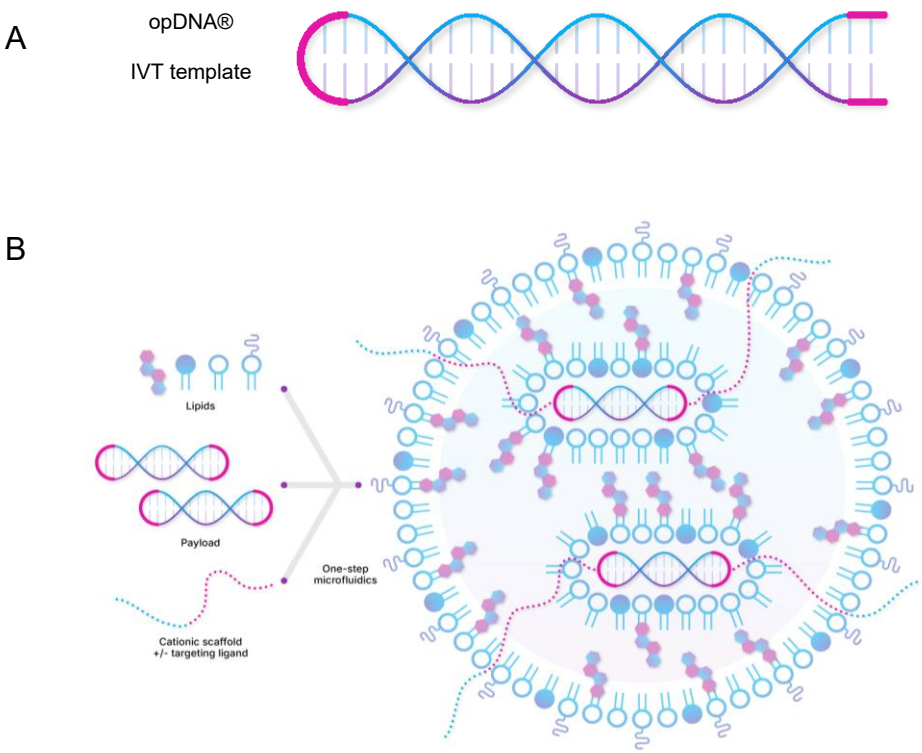


Figure 1: A) opDNA® IVT templates are linear double stranded DNA molecules, closed at the 5' end with a single stranded hairpin, but open on the 3' end, allowing use directly in the IVT reaction without linearisation. B) 4Basebio's Hermes® nanoparticle platform includes a 5th cationic scaffold component +/- targeting ligand, as well as lipids used for nucleic acid payload encapsulation.

This approach involves the preparation and maintenance of working and master cell banks, a lengthy process that doesn't align with personalized therapy timelines. Moreover, the inflexibility of bioreactor workflows forces manufacturers to produce large minimum batch sizes, typically hundreds of milligrams, when only a few milligrams may be needed for a single patient. Alternatively, PCR based methods can be used but this approach is limited by the fidelity of the enzymes used and the requirement for polyA tailing. Clearly, new processes are needed to control costs, accelerate timelines and facilitate multiple parallel batches for individual patients though clinical trials and commercialization.

A synthetic solution: 4basebio's DNA platform

4basebio has pioneered a fully enzymatic, cell-free approach to DNA manufacturing that is ideally suited to enable personalized cancer immunotherapies. This synthetic DNA platform produces linear double-stranded DNA free from bacterial sequences and antibiotic resistance genes. It contains only the therapeutic sequence, enhancing both safety and regulatory compliance.

The enzymatic process allows for production of GMP-grade DNA in weeks rather than months, aligning with the rapid timelines required between biopsy and therapeutic delivery. The cell-free system's small footprint, supports scale-out manufacturing, allowing small, individualized batches that eliminate inefficiencies associated with minimum batch sizes and cuts production costs connected to plasmid DNA manufacture.

For IVT mRNA applications, opDNA® serves as a ready-to-use DNA template. With a linear double-stranded DNA with an open 3' end, the construct can be configured as either blunt or overhang-ended (Figure 1A). The open end includes a template encoded homopolymeric A tract, enabling direct use in IVT reactions and eliminating enzymatic linearization. This simplifies the mRNA manufacturing workflow through cost reductions, processing times, and enhanced quality of DNA template input.

This platform is underpinned by 4basebio's proprietary Trueprime® amplification technology; an enzymatic, primer-free DNA amplification

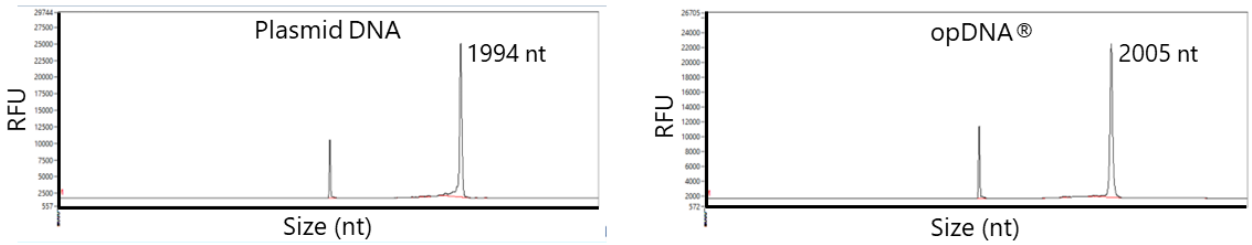


Figure 2: Capillary electrophoresis (Fragment Analyzer) of neoantigen encoding mRNA synthesised by IVT from either a linearized plasmid DNA template or an opDNA template.

method that delivers high-fidelity DNA with an error rate of 3.75×10^{-9}. This fidelity surpasses conventional PCR by approximately 200,000-fold and operates without sequence bias. This capability is critical for maintaining stability of challenging sequences such as polyA tails, which are prone to instability and recombination in bacterial systems. By encoding long polyA sequences directly into the DNA template, the need for enzymatic tailing processing steps is removed, saving crucial time. Further, risk of batch failure due to polyA recombination is reduced, ensuring reliability and consistency when timelines are so tight.

Case study: opDNA® for neoantigen mRNA vaccines with Neomatrix

To validate opDNA® as a robust IVT template for neoantigen vaccines, 4basebio collaborated with Neomatrix Biotech, a leader in neoantigen identification, vaccine design, and immuno-oncology delivery platforms. Neomatrix combines tumor-specific neoantigen discovery with 4basebio's fully synthetic DNA technology and immune checkpoint inhibitors (IHI) to potentiate anti-tumor immunity. A key success factor in mRNA therapeutics is delivery. 4basebio and Neomatrix collaborated to optimize and validate Hermes®, 4basebio's nanoparticle platform, for delivering opDNA®-derived mRNA in pre-clinical cancer models. In addition to traditional LNP lipids Hermes® formulations incorporate a cationic scaffold to confer stability. The optimized microfluidics method uses a third channel to incorporate the scaffold component in one-step through self-assembly (Figure 1B). The scaffold component also provides the option to include additional components, such as targeting ligands, without any additional conjugation steps.

In this study, a vaccine against the MC38 murine cancer model was evaluated. Using their MARciso

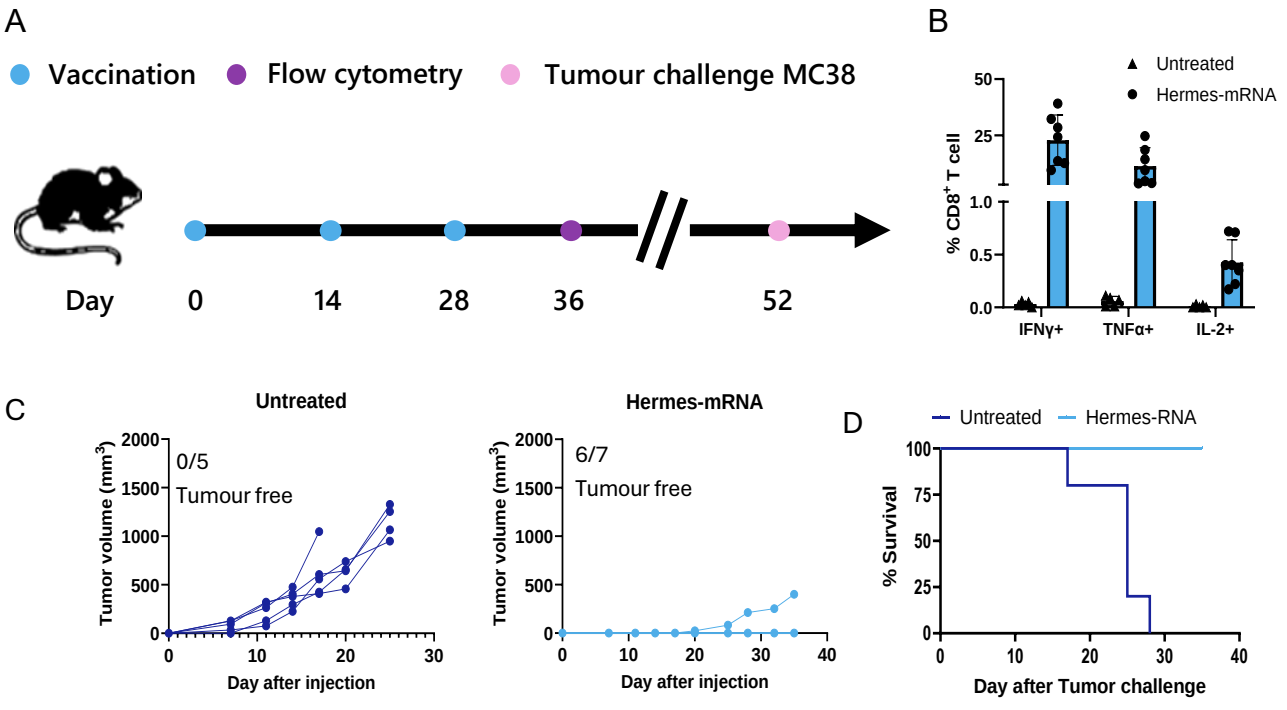


Figure 3: A) Experimental design of the prophylactic vaccination strategy with 6.7 µg Hermes®-mRNA nanoparticles encoding 20 different MC38 neoantigens. B) neoantigen-specific CD8+ T cells producing IFNγ, TNFα and IL-2 36 days following initial vaccination. C) Tumor growth and D) survival of animals and following subcutaneously delivery of MC38 cells. n = 5-7.

algorithm, Neomatrix designed a vaccine encoding 20 different literature identified antigens. opDNA® encoding these neoantigens was synthesized by 4basebio, incorporating a long 120 base pair (bp) polyA tail encoded directly into the template. mRNA was synthesized via IVT using standard capping reagents and N1-Methylpseudouridine UTPs, using either pDNA or opDNA® as the IVT template. Capillary gel electrophoresis analysis demonstrated a homogeneous mRNA population from both DNA templates of the expected 2.05 kb length (+/- 5%). Smear analysis detected minimal secondary peaks, with opDNA® demonstrating greater mRNA integrity than plasmid DNA of 88.1%, compared to 82.7%, validating opDNA® as a template to produce high purity mRNA (Figure 2).

Immunogenicity was assessed, after intramuscular dosing at 6.7 µg in a prime-boost regimen of three biweekly injections (Figure 3A). 36 days following the initial vaccination, blood was collected and the percentage of neoantigen-specific CD8+ T cells was assessed in PBMCs by intracellular cytokine staining (ICS). In vaccinated animals, CD8+ T cells producing IFNγ, TNFα and IL-2 were significantly elevated compared to the untreated group, demonstrating the ability of Hermes®-mRNA to induce a neoantigen-specific immune response (Figure 3B).

Next, mice were subcutaneously injected with MC38 tumor cells, 52 days after initial vaccination to assess protective immunity against tumor challenge. All untreated animals developed tumors, while 6 of 7 in the Hermes®-mRNA vaccinated group remained tumor free and the remaining animal demonstrating reduced tumor growth (Figure 3C). Therefore, survival rate was significantly greater in the Hermes®-mRNA group compared to the untreated (Figure 3D).

Finally, the Hermes®-mRNA formulation was compared to a clinical COVID-19 LNP formulation (Moderna vaccine) encapsulating the same neoantigen mRNA payload. In a therapeutic protocol, mice were challenged with MC38 tumor cells before vaccination on days 2, 8, and 16 alongside the ICI, anti-PD L1(Figure 4A). The percentage of INFγ and TNFα neoantigen-specific CD8+ T cells on day 14 showed comparable induction of neoantigen-specific CD8+ T cells between Hermes® and LNP groups (Figure 4B-C). Further, vaccination with either Hermes® or the LNP formulation reduced the rate of tumor growth to a comparable extent, validating Hermes® as an effective delivery platform (Figure 4D).

Conclusion

The transition to personalized cancer immunotherapies represents a new era in oncology, demanding a fundamental rethinking of biomanufacturing strategies. 4basebio's fully synthetic, enzymatic DNA platform overcomes critical bottlenecks in mRNA vaccine production,

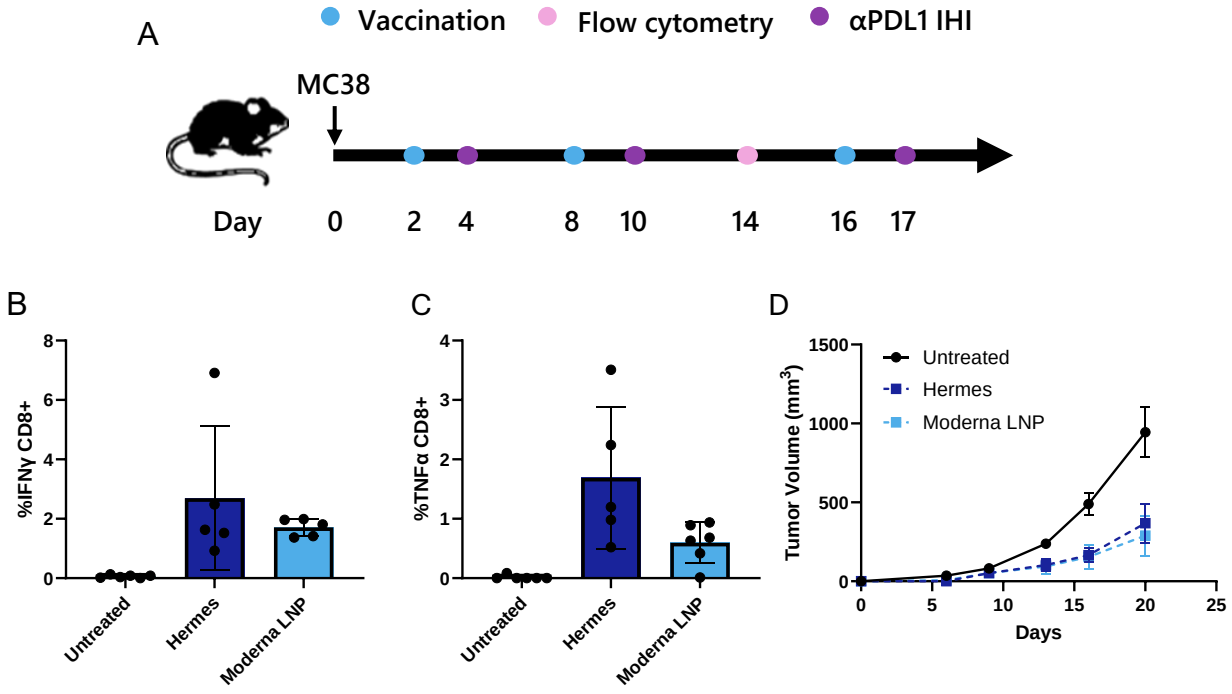


Figure 4: A) Experimental design of an early therapeutic vaccination strategy with 6.7 µg mRNA encoding 20 different MC38 neoantigens. B-C) Neoantigen-specific CD8+ T cell responses and D) tumor progression following vaccination with either Hermes® or LNP formulations. *n* = 5.

offering a faster more flexible alternative to conventional plasmid-based methods. With its opDNA® technology and proprietary Trueprime® amplification, 4basebio delivers high quality template DNA at speed aligned with personalized therapy timelines.

The collaboration with Neomatrix, and application of opDNA® in neoantigen vaccine development has shown that mRNA derived from synthetic DNA provides effective immunogenicity and tumor protection in preclinical models when combined with the Hermes® delivery system. Centralizing manufacture from DNA to mRNA to LNP will be essential in delivering on the 6-8 week promise without wasted shipping time.

Together, these innovations not only reduce the time and cost associated with therapeutic development but also open the door to more agile, patient-centric treatments in oncology. The future of cancer care is increasingly personalized and novel manufacturing approaches must be adopted now to facilitate their journey to clinic.