A pathway toward clinically-oriented polymeric nanovectors for cancer immunotherapy

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Genetic modification of immune cells has ushered in a new era of immunotherapies, offering a transformative therapeutic approach. Within this context, nanotechnology-based nanovectors (NVs) represent a safer and more clinically translatable alternative to viral vectors, with the potential to expand nucleic acid delivery for immune cell engineering. Polymer-based nanotechnologies are central to this effort, thanks to their structural versatility that allows the use of both natural and synthetic polymers, many of which are biodegradable and FDA/EMA-approved, thus facilitating clinical translation. Their tunable physicochemical features, including size, charge, hydrophilicity/hydrophobicity balance, and introduction of stimuli-responsive moieties, enable rational design of nanostructures capable of precise cell targeting, controlled release, and reduced toxicity. This adaptability makes polymer-based nanovectors (pNVs) strong candidates for encapsulating and delivering genetic material and therapeutic agents. Our strategy relies on an iterative design process in which pNVs are systematically synthesized, characterized, and tested, with each stage informing the next. The nanoformulations are based on pharmaceutical-grade, FDA/EMA-approved polymers, biopolymers, and excipients, with raw material traceability and qualification. Nanoparticle synthesis is then performed using an automated microfluidic-based approach, which allows precise regulation of mixing, reaction conditions, and nanoparticle formation. Unlike bulk mixing, which often leads to heterogeneity, microfluidic systems enable rapid and reproducible mixing of reagents under laminar flow, ensuring fine control over reaction kinetics. This enables accurate tuning of critical parameters such as nanoparticle size, polydispersity, surface charge, and encapsulation efficiency, and contamination risks that are strictly controlled for each batch of pNVs in order to establish critical quality attributes and to identify critical process parameters requiring strict control to consistently meet quality attributes.

Additional assessments include nucleic acid encapsulation, release kinetics, transfection efficiency, and biocompatibility in primary human immune cells. Insights from these evaluations guide structural refinements, adjusting the physical and chemical properties of the polymeric building blocks to enhance performance while minimizing toxicity. Crucially, every round of testing integrates feedback on cell viability, immune functionality, and nanovector efficacy, reinforcing biocompatibility as a non-negotiable requirement.

Sterility and contamination control are also integral to this process. Validated decontamination procedures, microbial and pyrogenic monitoring, and low-bioburden processing are applied to mitigate cross-contamination risks. These operations are continuously improved to establish closed aseptic processes for the synthesis of biologically relevant pNVs that are not suitable for sterile filtration, such as when particle diameter approaches or exceeds the 0.22 µm cutoff. Through this continuous cycle of design, evaluation, and optimization, polymer-based NVs can be progressively refined into potentially clinically relevant tools. This approach successfully assembled polymeric nanovectors (pNVs) from functionalized polyesters and tailored cationic biopolymers, achieving efficient nucleic acid loading alongside optimized physicochemical and biological properties. Ongoing in-depth evaluations aim to uncover design principles that will further enhance targeting precision and control intracellular trafficking, paving the way for next-generation nanovector platforms. The iterative nature of this process not only enhances transfection efficacy and safety but also lays the foundation for more robust and predictable immunotherapy outcomes enabled by modern non-viral nanovectors (**Figure 1**).

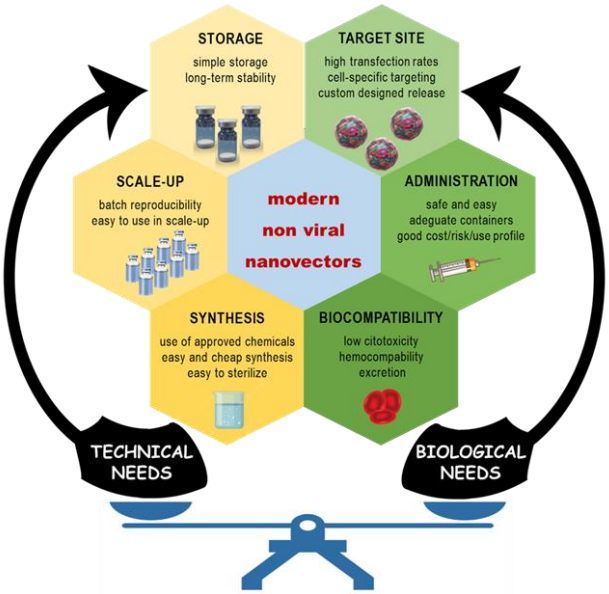

Figure 1. Critical design challenges for modern non-viral nanovectors in clinical applications

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