NON-VIRAL VECTORS:
A PROMISING TOOL FOR CELL TARGETING THERAPIES

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INTRODUCTION

Non-viral vectors (NVVs) are nanocontainers for encapsulating, transporting and introducing genetic material or drugs into target cells. They combine functional components in a single nanoparticle made of synthetic polymers or biomaterials [1].

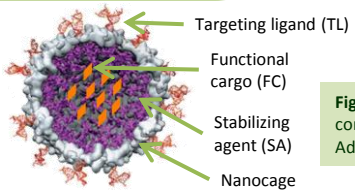

Figure 1. Basic components of a NVV. Adapted from [1].

AN ALTERNATIVE TO VIRAL VECTORS (VVs)

	VIRAL VECTORS	NON-VIRAL VECTORS
Efficiency and stability	● ●	●
Risks	Immunogenicity, oncogenicity, virulence reversion	Immunogenicity
Production cost and scalability	●	●
Flexibility of modification	●	● ●
Applications	Gene therapy	Gene therapy, drug delivery, diagnosis

Table I. Comparison of VVs and NVVs.
● Positive attribute
● Ambiguous attribute
● Negative attribute

DIFFERENT APPROACHES TO ENGINEER NVVs

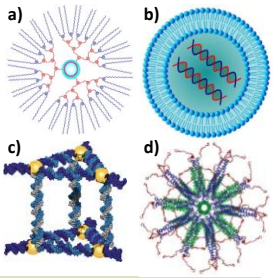

Figure 2. NVVs' architectures. **(a)** Amphiphilic block copolymer. Hydrophilic segments (blue) are covalently attached to hydrophobic segments (red). **(b)** Liposome. **(c)** DNA triangular prism encapsulating gold particles (yellow). **(d)** Coiled coil self-assembling protein nanoparticle functionalized with malaria epitopes (red). Reproduced from [5] and [6].

Table II. Comparison of different examples of existing NVVs. GT, Gene Therapy; DD, Drug Delivery.

	EXAMPLE	INDICATION	COMPONENTS	MECHANISM OF ACTION	STATUS
DENDRIMERS AND SYNTHETIC POLYMERS	CALAA-01 [2]	Cancer (GT)	▪ Cyclodextrin polymer ▪ FC: anti-R2 siRNA ▪ TL: Human transferrin protein (Tf) ▪ SA: Polyethylene glycol (PEG)	The nanocomplex protects siRNA from degradation and releases it in tumor cells expressing Tf receptors. siRNA inhibits tumor growth by reducing the expression of the M2 subunit of ribonucleotide reductase (R2).	Clinical phase I
LIPOSOMES	Doxil® [3]	Cancer (DD)	▪ Phospholipid bilayer vesicle ▪ FC: Anthracycline topoisomerase inhibitor (doxorubicin) ▪ SA: PEG coating	Liposomes increase doxorubicin circulation time and allow it to penetrate the vasculature of tumors. The drug binds to DNA and inhibits nucleic acid synthesis and mitosis, killing tumor cells.	Approved
NUCLEIC ACID NANOASSEMBLIES	Folate-chitosan-DNA nanoparticles [4]	Rheumatoid arthritis (GT)	▪ FC: IL-1 receptor antagonist (IL-1Ra) gene ▪ TL: Folic acid ▪ SA: Chitosan	The conjugate binds to folic acid receptors on activated synovial macrophages and internalizes. IL-1Ra is synthesized and inhibits the inflammatory effects of IL-1.	Preclinical assays
PROTEIN NANOASSEMBLIES	M13 VLPs [5]	Cancer imaging and DD	▪ M13 phage VLPs ▪ FC: Fluorescent dye ▪ TL: Folic acid	VLPs bind to folic acid receptors overexpressed in cancer cells and internalize, thus creating a fluorescent image of the tumor.	Preclinical assays
MODULAR PROTEINS	Multifunctional DNA Carrier (MDC) [6]	Melanoma (GT)	▪ FC:Reporter gene ▪ TL: Melanocyte stimulating hormone ▪ Sperm protamine ▪ Fusogenic domain of Diphteria Toxin (DT) ▪ T antigen NLS	MDC with a transgene bonded to protamines is internalized in nondividing melanoma cells and translocated from the endosome thanks to the DT domain. NLS interacts with importins, the reporter gene reaches the nucleus and is expressed.	Preclinical assays

VIRUS LIKE PARTICLES (VLPs)

VLPs are one of the most studied NVVs for cell targeting therapies. They are composed of viral capsids devoid of their genetic material, thus taking advantage of viruses' natural infection abilities but being non-pathogenic. They are biocompatible, stable and have a defined and uniform supramolecular structure based on subunit self-assembling. The degree of similarity between VLPs and viruses depend on the number of proteins incorporated in the particle. In 1983, mouse polyomavirus became the first described VLPs [7].

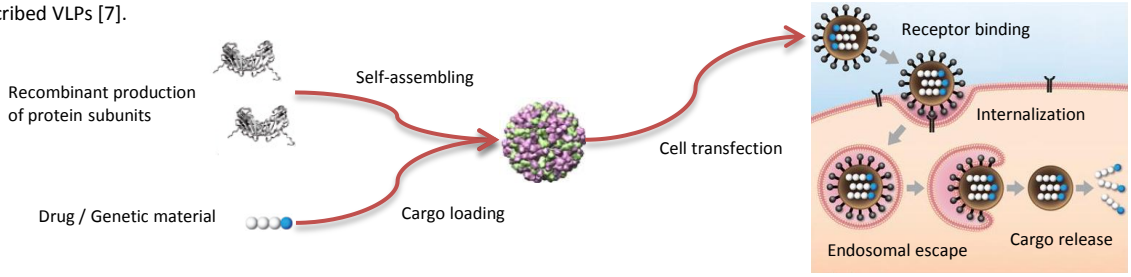

Figure 3. Production process of a VLP and its application to cell targeting therapies. Capsids can be modified and functionalized in order to overcome biological barriers and reach a specific target. Adapted from [1] and [8].

CELL TARGETING APPLICATIONS: THEIR CURRENT STATUS

Gene therapy involves the genetic manipulation of cells by introducing functional genes that express a new desired function or correct a defective function in the cell by silencing a deregulated gene. The first gene therapy treatment was approved in Europe last October and uses VVs, although in China there have been gene therapy treatments for cancer available since 2003 [9].

Drug delivery is based in the encapsulation of drugs or proteins in vectors that carry them specifically to target tissues, which reduces side effects of conventional pharmacological treatments. Nowadays half of the clinical trials study antibodies as biological therapeutics [10].

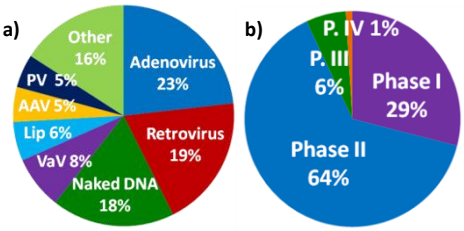

Figure 4. **(a)** Vectors used in the almost 1850 gene therapy clinical trials to date. Though 2/3 are VVs, NVVs are gaining importance [9]. PV, Poxvirus; AAV, adeno-associated virus; Lip, Lipofection; VaV, Vaccinia virus. **(b)** Distribution of clinical phases of nanoparticles used in drug delivery [10]. Most of the trials are still at initial stages, but liposome-encapsulated drugs are already in clinical use for cancer treatment [5].

CONCLUSIONS

- To date, many different types of NVVs have been studied and have shown to possess several advantages as opposed to VVs.
- Their high specificity, versatility, biosafety and low production costs make VLPs an exceptionally promising tool in nanomedicine.
- Cell targeting improves classical treatments and enables new therapies for pathologies with just symptomatic or palliative medical approaches.
- This field develops slowly, but NVVs have already raised many expectations. Currently, there are important investments in clinical trials and products are starting to appear in the market.

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