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The double-edged cytokine sword of non-viral gene targeting to tumors ▼

Therapeutic gene therapy faces formidable barriers. DNA must be protected from degradation, delivered to the proper cellular target and intracellular site for (optimum) expression, and must evade innate and adaptive immune mechanisms that have evolved over millions of years to block the introduction of foreign DNA. In a recent review published in *Drug Discovery Today*, Ogris and Wagner outlined new approaches to overcome these challenges in the treatment of tumors [1]. Several points raised by this excellent review merit highlighting and additional discussion.

Over the past decade, most efforts have focused on the use of viral vectors to deliver genes to correct a genetic defect. However, viral components often induce a limiting immune response. Attempts to develop non-viral delivery alternatives require formulations that compact and protect the DNA from degradation. In spite of many clever solutions, these systems are limited by low, transient expression, in part because of an immune response to the unmethylated CpG sequences contained within bacterial plasmid DNA. Tumor therapies based on DNA delivery could turn some of these problems into a virtue. Vaccinations might not require high, prolonged expression and the cytokine response to plasmid DNA might suppress tumor growth. Nevertheless, several major challenges still remain.

The cytokine response to plasmid DNA, and particularly to certain lipid–DNA formulations, offers the opportunity to develop novel adjuvants to supplement anti-tumor therapies. Adjuvants, the 'dirty little secret of immunology', are substances that enhance the immunogenicity of an antigen. The classic adjuvant, Freund's complete adjuvant (CFA), is a mixture of heat-killed *Mycobacteria* in an oil and water emulsion. The unmethylated bacterial DNA sequences and muramyl dipeptide derived from the mycobacterial cell wall in CFA aid the activation of the innate immune system. This in turn increases cytokine responses and antigen presentation, augmenting the specific immune response to the antigen co-administered with the adjuvant. The combination of DNA with a lipid-condensing agent resembles traditional adjuvants, and induces a potent cytokine response. These cytokines can suppress tumor growth.

Huang and colleagues demonstrated that plasmid DNA inhibited tumor growth, even if the DNA did not encode a functional gene [2]. The systemic delivery of lipid–DNA complexes activated natural killer (NK) cells, increased interferon-γ levels, and suppressed tumor growth in animal models of cancer [3,4].

The systemic administration of lipid–DNA should be undertaken with caution. Few tumors are readily accessible to direct injection, but intravenous administration can permit targeting by trapping of encapsulated DNA in the microvasculature of a growing tumor. However, this also increases the risk of DNA trapping in the lungs. Furthermore, the strategies that cause this trapping could inhibit internalization and expression of the complexed DNA.

Improved pharmacokinetics and tissue targeting does not always produce improved expression. Although the cytokine storm induced by plasmid DNA might have potent anti-tumor effects (suppression of tumor cell proliferation, angiostasis, increased antigen processing and presentation), this could be a double-edged sword. Over-stimulation of cytokine release can lead to fever, nausea, and in severe cases, pulmonary edema and death. Therefore, anyone contemplating systemic administration of DNA should carefully monitor the levels of systemic cytokine responses. Formulations that might be excellent adjuvants when administered locally could be toxic when administered systemically. Intravenous administration of lipid–DNA can induce leukopenia, thrombocytopenia, and hepatic necrosis [5].

Whereas a systemic cytokine response can invoke serious adverse effects, a cytokine response localized within a tumor could prove beneficial. However, the increasingly complex systems developed recently to improve pharmacokinetics and targeting could generate new problems. For example, systems that protect and deliver plasmid DNA can also interfere with uptake or

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nuclear localization. Combinations of targeting ligands, polyethylene glycol, and compacting lipids with plasmid DNA could present considerable manufacturing challenges, including characterization and reproducibility. In other words, systems that are effective at a laboratory scale are not necessarily practical at a production or commercial scale.

One other lesson from immunology might be worth noting when considering the commercial prospects for gene delivery. In 1975, Georges Kohler and Cesar Milstein devised a strategy for the production of monoclonal antibodies. The enthusiastic rush to clinic was stalled by barriers of immunogenicity – the mouse sequences in the first generation of monoclonal antibodies induced a neutralizing immune response. Therapeutic applications had to wait for the development of new techniques of humanization, deimmunization and immunoglobulin-transgenic mice. With one exception, the first monoclonal antibodies were approved nearly 25 years after the initial scientific breakthrough.

Currently, immune barriers to DNA delivery vectors have limited the application of gene therapies. Perhaps the development pathway of gene therapy will parallel the development of monoclonal antibodies, with a renaissance of clinical applications following new solutions to the barriers of immunogenicity. In the meantime, there might be opportunities to harness the immunostimulatory properties of lipid–DNA formulations to develop novel cancer therapies if applied with caution.

References

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The importance of predictive ADME simulation ▼

In their article on computational modelling and prediction of ADME properties that recently appeared in *Drug Discovery Today* [1], Beresford *et al*. compared the design of aircraft with the design of new drugs. However, the simulation process for new aircraft is far more advanced than that of drug design – we still have to synthesize a large number of drugs and investigate their ADME properties before identifying a marketable drug. We do not have sufficient confidence in current *in silico* models to back our predictions against experimental measurements. Fortunately, the authors of the ADME simulation article all come from an experimental background, and so they recognize the limitations of the *in silico* methodology.

In silico systems are most useful in library design (before compounds are synthesized) to filter out compounds with clear-cut undesirable properties. At this stage the accuracy of the models (currently ~80%) is sufficient to provide a cut-off for lead molecules to pass through the computational screens. In a few cases before the project starts (i.e. before compound synthesis), undesirable properties based on physicochemical characteristics [2] could become apparent using these *in silico* methods. In these cases, project teams could prioritize their approaches to check these undesirable ADME properties.

However, for molecules that have already been synthesized, the chemists like to see some effort expended in the laboratory rather than rely totally on these *in silico* predictions. This usually entails some *in vitro* experimentation using human material (e.g. for inhibition of cytochrome P450s) or animal models (e.g for absorption, clearance, bioavailability, and so on). The *in silico* simulations can be useful in prioritizing this *in vivo* work using a funnel approach to reduce the numbers of compounds, as described in Figure 5 of Beresford *et al*. [1]. The chemists would usually prefer to use an *in silico* approach after *in vitro* data has been generated, so that they gain confidence in the predictions on the specific series of their interest. However, if there is an ADME issue based on experimental data, then the chemists are quite happy to use these *in silico* approaches as guidance for lead optimization.

In silico models for all aspects governing ADME are needed [3], for example, the concerted efforts of CYP3A4 and P-glycoprotein (P-gp) need to be addressed as a primary barrier to absorption in the gut, and P-gp plays an important role in protecting the brain. Computational models might have an important role to play in terms of generating binding affinity data, but it should be recognized that most approaches fail to predict activity within a single order of magnitude.

It should also be acknowledged that *in silico* technology has only been in existence for a relatively short time and

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