EXPERT OPINION

Pharmacogenomics-based RNA interference nanodelivery: focus on solid malignant tumors

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Introduction: RNA interference represents one of the most promising strategies in fighting disease. However, small RNA interference faces substantial challenges for *in vivo* application due to the inherent instability of the RNA interference molecule. Among the nonviral gene delivery carriers, nanoparticles have attracted interest due to their success in various model systems. Nanomaterials have unique properties compared to conventional bulk materials that may be applicable in this setting. The nanoparticle complex carrying small interference RNA can undergo surface modification to achieve targeted modification for tissue-specific delivery. However, toxicity issues of the delivery systems need to be addressed and they require a pharmacogenomic profile of their own.

Areas covered: The authors review pharmacogenomics, toxicogenomics, nanoparticle-based drug delivery, and small interference RNA, with a focus on how logically engineered nanoparticle delivery systems can be used for personalized medicine in malignant tumors.

Expert opinion: Pharmacogenomics may be helpful in addressing possible individualized drug response for both the gene silencing capability of the delivered siRNA and the nanoparticle drug delivery system as both complete and distinct units. This may be done by assessing variations in gene expressions and single nucleotide polymorphisms. Patient profiling may be key as patient noncompliance due to toxicity plays a major role in treatment failure.

Keywords: delivery, functionalization, nanoparticle, pharmacogenomics, targeted

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1. Introduction

A key role of pharmacogenomics is to elaborate the role of individual genetic variation on drug response. Gene expression variations and single nucleotide polymorphisms are typically analyzed. Pharmacogenomics holds great promise for the next generation of pharmacological therapies by facilitating a personalized approach to fight disease. Recent advances seen in sequencing the human genome has led many to envision a future of personalized medicine as genes responsible for conferring susceptibility to various chronic medical conditions and sensitivity to pharmacologic agents are being identified. Pharmacogenomics represents the intersection of identifying genetic properties of disease and uniting this with more rationally designed pharmacologic agents. The field has undergone vast growth recently as increasingly genetic predispositions and genetic properties of various degenerative and oncologic diseases are being identified. The rational design of therapeutics has therefore been informed by this progress. The knowledge gained from sequencing data has led to several possible implications for therapeutics: (i) identification of genes responsible for causing or predisposing to a particular condition, (ii) identification of metabolic variations between patients that may predict an individual's response to treatment. When discussing drug delivery systems,

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Article highlights.

- A key role of pharmacogenomics is to elaborate the role of individual genetic variation on drug response by analyzing gene expression variations and single nucleotide polymorphisms.
- Targeted delivery of RNAi therapy is the most elusive milestone to achieve simply because every therapy requires a different approach in terms of payload, biocompatibility, bioabsorption, biodegradation, toxicity, targetability, and most importantly selective cell uptake.
- An important advantage of the nanoparticle delivery system is the ability for surface modification by undergoing functionalization, the process of adding functional groups on the surface of the complex to enhance their specificity.
- Drug delivery will be key in mitigating the toxicological risks.
- Pharmacogenomics may facilitate assessment of individualized drug response for both the gene silencing capability of the delivered siRNA and the nanoparticle drug delivery system as both complete and distinct units.

This box summarizes key points contained in the article.

pharmacogenomics can have an important role in both efficacy of profiling of the delivered entity and toxicity profiling that can better assist patient stratification and tailored therapy [1-14].

RNA interference (RNAi) mechanism represents one of the most promising strategies in fighting disease. Introducing double-stranded RNA (dsRNA) into cells expressing a homologous gene triggers RNA interference (RNAi). This allows an RNA-designated gene silencing. The dsRNA degrades the designated host messenger RNA into small interfering RNAs (siRNAs) by a protein complex which has Dicer. The siRNAs are then incorporated into the RNA-induced silencing complex (RISC). This complex has helicase, RecA, and exo- and endo-nucleases and other relevant proteins involved in the process. The RISC then guides the RNA degradation system to the target RNAs and cleaves the cognate target RNA. The cleavage occurs in a sequence-specific, siRNA-dependent manner [5,10,15,16].

Already several companies have been formed to commercialize RNAi-based therapies and currently Phase I/II trials have commenced. In spite of the progress toward developing RNAi-based therapies, as well as other forms of personalized treatment, development of a targeted delivery remains one of the most difficult bottlenecks. Targeted delivery of RNAi therapy is the most elusive milestone to achieve simply because every therapy requires a different approach in terms of payload, biocompatibility, bioabsorption, biodegradation, toxicity, targetability, and most importantly selective cell uptake [1,4-11,17-24].

As small interference RNA (siRNA) and many other therapies are being integrated with nanocarrier systems, the use of pharmacogenomics has more a pronounced dual role for treatment response prediction. Predicting treatment response will involve both the siRNA and the nanocarrier as a unit. In addition, assessing functionalized nanocarriers using pharmacogenomics will be particularly important [4-11,13,14,16-21,25-35,37-41].

This review provides an examination of pharmacogenomic approaches to nanocarrier-based personalized siRNA therapies for malignant tumors. We begin with a review of siRNA, followed by specific delivery systems that can potentially make a therapeutic impact for RNAi, including nanoparticles and viral vectors. We include forthcoming data from human trials of efficacy and toxicity. We discuss the use of toxicogenomic profiling for genomic signatures that may allow for personalized tailoring of therapy. Finally, we present potential applications in oncology, including identification of oncogenes and specific therapies for such approaches. We conclude with a brief description of current challenges for pharmacogenomics as it relates to development of therapies.

1.1 siRNA challenges

siRNA faces substantial challenges to *in vivo* application due to the inherent instability of the RNAi molecule [1-10,17-20]. This instability is due to the susceptibility of RNA molecules to serum nucleases, renal clearance, and nonspecific biodistribution. In addition, siRNA molecules have net negative charge and are hydrophilic, two characteristics which prevent their access to extracellular and intracellular sites. siRNA therefore requires a delivery system that is both effective and ideally tissue-specific
[1-9,17-20]. Due to immunological and chromosomal toxicities, viral vectors have had limited success. Among the nonviral gene delivery carriers, nanoparticles, also termed polyplexes, have attracted interest due to their success in a variety of model systems [1-12,15,17-25,42-74].

Any delivery systems must achieve several important objectives. First, an increase in residence time in the circulatory system is required which can be achieved by reducing the rate of renal clearance. Second, siRNAs also require protection from serum nucleases to prolong their stability. Third, an effective biodistribution is required. Fourth, targeted delivery and uptake of the siRNAs by targeted cells are needed. Finally, trafficking to the cytoplasm and uptake into the multi-subunit RNA-induced silencing complex (RISC) are needed [1-10,17-25,43-50].

2. Delivery systems

2.1 Nanocarrier systems

Nanomaterials have unique and valuable properties, compared to conventional bulk materials. The advantages accompany these materials at the nanoscale, which can range from tens to hundreds of nanometers [1-4,6,17,19]. They are similar in size to subcellular organelles, biomacromolecules, and viruses [1-4,10,11,13-15,21-25,42-47,49-53,56,72-75].

It has been shown that nanoparticles with diameters less than 200 nm can readily penetrate the capillary wall thereby enabling delivery to the liver, spleen, and solid tumors. However, it should be noted that nanoparticles smaller than 200 nm enter through the fenestrated and sinusoids; furthermore, there are tissues which have tighter capillary walls wherein particles as small as 50 nm cannot go through [1,10,11,72-75]. The positive charged nanoparticle complex encapsulating the siRNA facilitates uptake across negatively charged cell membrane [48]. The nanoparticle complex, with the siRNA, is picked up by the targeted cells and released from the endosome into the cytoplasm to enter the RNA interference pathway [1,21,75].

There are two rudimentary ways to prepare nanomaterials. The top-down approach involves a final nanostructure being carved into a desired shape and size from a larger block of matter without atomic-level control. This can be done using external radiation and/or chemical technique. For example, lithography may be used for this purpose. The bottom-up approach involves building the nanostructure from self-assembled or self-organizing molecular building blocks thereby allowing atomic- or molecular-level control. Self-assembly relies on noncovalent interactions or weak covalent bonds such as van der Waal's interactions, the hydrophobic effect, electrostatic interactions, hydrogen bonding, and coordination bonding.

Current nanoparticle delivery systems can be categorized into organic and inorganic systems. Organic systems include lipids and cationic polymers. Cationic polymers are particularly promising as they have been found to have favorable properties in terms of *in vivo* stability, biocompatibility, and minimized toxicity. In addition, poly-lactic-co-glycolic acid (PLGA), chitosan, polyester, block copolymer, and polydithiamine are biodegradable polymers, making them attractive delivery systems [1,11,24,43,47]. Inorganic systems include magnetic nanoparticles, quantum dots, carbon nanotubes, and gold nanoparticles or nanorods [1-10,15,17-20,22-24,49-53,68-74] (see **Table 1**).

2.2 Surface modification of nanoparticles for targeted delivery

Surface modification has several important objectives. One is the enhanced permeability and retention which can be important for tumor sites. Another is for an increased binding affinity and specificity. This can be done by coating the nanomaterials with multiple bioactive ligands thereby taking advantage of the multivalent effect [2,25,42-47].

An important advantage of the nanoparticle delivery system is the ability for surface modification by undergoing functionalization, the process of adding functional groups on the surface of the complex to enhance their specificity. These functional groups can facilitate crossing barriers such as negatively charged cell membranes, short residence times in the blood, and nonspecific delivery [1-11,17-21,25,42-48,75]. In addition, surface modification allows further stability of the delivery complex in order to achieve adequate RNA interference *in vivo* [1-11,17-21]. Functionalization with polyethylene glycol (PEG), a hydrophilic conjugate, allows overcoming a key barrier to delivery. The negatively charged cell membrane and serum proteins which can slow or even prevent the siRNA complex from reaching its targeted sites cannot be overcome with the nonfunctionalized siRNA system, which has a net positive charge. These PEGylated delivery systems can be used to adjust the particle size and prevent aggregation as well as to facilitate the steric stability of the siRNA complex and uptake [1-4,22,23,61,68-74]. Another advantage of adding PEG to the surface of the nanoparticles is the increased hydrophilicity of the conjugate, which further stabilizes the nanoparticle complex. Such modification can extend residence time in the blood circulation. Furthermore, activated base groups at the end of the PEG chain can be modified in such a way to allow conjugation with the delivered siRNA [1-4,23,53,59,76].

Increased biocompatibility and stability can be implemented by adding hyaluronic acid (HA) to the nanoparticle delivery system. HA can protect the siRNA system from nuclease degradation and improve the stability of the nanoparticles *in vivo*. The negative charge of HA can neutralize the positive charge of cationic liposome nanoparticles and cationic polymer nanoparticles thereby stabilizing the entire delivery system and reducing cytotoxicity. Interestingly, the liver, spleen and some tumors express HA receptors which may address a new receptor targeted approach for tumors [1,24].

The nanoparticle complex carrying siRNA can undergo surface modification to achieve targeted modification to facilitate tissue-specific delivery. Targeted modification can include adding a cell penetration peptide/peptide transduction domain, aptamers, antibodies and their fragments; moreover, bispecific antibodies and single-chain variable fragment antibodies can be added as well [1,49-52]. Target modification can produce immunoliposomes which can be formed by linking specific monoclonal antibodies or single-chain antibody fragments to PEGylated cationic nanoparticles [1-4,15,23,53,59].

Since cancer cells upregulate the expression of some surface receptors, ligands specific for these receptors can be functionalized to the surface of the delivery system thereby increasing its targeting ability thereby enhancing efficacy, and minimizing systemic toxicity and immunogenicity. Commonly used ligands are used as target moieties such as those related to integrin and transferrin (Tf) [1,54,55]. Folic acid can be functionalized to the delivery complex to target the delivery complex to the folic receptor, an important target found in a variety of tumors [1,56].

3. Recent noted ongoing human clinical trials

Several trials with nanoparticle-based drug delivery for siRNA for malignant solid tumors have been registered according to the registry of ClinicalTrials.gov. Presently, two key trials are in the recruiting stage. In addition, a successful Phase I trial using a nanoparticle-based delivery for siRNA has been demonstrated for metastatic melanoma.

Table 1. Various nanoparticles and their evidence of siRNA delivery.

Nanoparticle	Type	Evidence of siRNA delivery
Stable nucleic acid liposome nanoparticles (SNALP)	Lipid/complex/liposomes	Cynomolgus monkeys intravenously injected with Apo B-specific siRNAs encapsulated in SNALP showed dose-dependent silencing of Apo B expression of more than 90% [1,65]
Chitosan	Cationic natural polymer/organic	Knowdown efficiency of 44% when anti-TNF-α-1-Dicer-substrate siRNA-specific chitosan nanoparticles were intraperitoneally injected into mice [1,66]
Atelocollagen	Cationic natural polymer/organic	Full demonstration of atellocollagen–siRNA complex to silence expression of targeted anti-apoptotic factor, Bcl-xL via intravenous administration in mice [1,12]
Cyclodextrin	Cationic natural polymer/organic	Reduce expression of the ribonucleotide reductase M2 (RRM2) protein levels in melanoma cells administered systemically in humans [1,6]
Polyethyleneimine (PEI)	Cationic natural polymer/organic	PEI complex modified with stearic acid reduced signal and transducer and activator of transcription 3 (STAT3) by 40% with significant reduction in both VEGF level and tumor volume (38%) in a mouse model [1,67]
Block copolymers	Cationic synthetic polymer/organic	Silenced expression of VEGF by co-delivery of VEGF, siRNA, and paclitaxel thereby allowing co-delivery of a combination of chemotherapy [1,68]
Dendrimer	Cationic synthetic polymer/organic	Marked reduction of expression of lipoprotein Apo-B in C57BL/6 mice, without hepatotoxicity, and reduction in serum low-density lipoprotein Apo-E-deficient mice [1,69]
Poly(lactic-co-glycolic acid) (PLGA)	Cationic synthetic polymer/organic	Local application to vaginal mucous membrane, enhanced gene silencing activity observed for up to 14 days [1,70]
Magnetic nanoparticles (including superparamagnetic particles of iron oxide and magnetic iron tetroxide particles	Inorganic	Nuclear magnetic crystals wrapped in SiRNA nanoparticles to allow MRI imaging to directly track dynamics of drug distribution and half-life [1,71]
Quantum dots [Qdots] semiconductor (includes cadmium selenide/zinc sulfide, cadmium telluride and silicon)	Inorganic	Observation of accumulation of Qdots-labeled nanoparticles with anti-VEGF siRNA/PEI-HA within tumor tissue [1,72]
Silica nanoparticles (includes mesoporous silica nanoparticles and hollow silica nanoparticles	Inorganic	Suggested for RNAi; showed delivery capabilities in various applications [1,101]
Carbon nanotubes (includes single-wall and multiple-wall carbon nanotubes)	Inorganic	Single-wall carbon nanotubes loaded with SiRNA successfully inhibited suppressor of cytokine signaling 1 expression both *in vitro* and *in vivo* experiments with B16 tumor bearing mice [1,73]
Gold nanoparticles/nanorods	Inorganic	*In vitro* blood–brain barrier penetration with significant reduction of dopaminergic neuron cell expression of DARPP-32 [1,74]

Davis *et al.* conducted the first in-human Phase I clinical trial involving the systemic administration of siRNA to patients with solid cancers using the targeted, nanoparticle delivery system, CALAA-01. Patients with melanoma tumors refractory to standard of care therapies were administered doses of the targeted nanoparticle-based system therapy on days 1, 3, 8, and 10 of the 21-day cycle with a 30-min intravenous infusion. Their nanoparticle system consisted of several key components. A linear cylodextrin-based polymer was used as the nanocarrier. A human transferrin (Tf) protein was used to engage transferring receptors on the surface of the cancer cells. The hydrophilic polymer component, polyethylene glycol (PEG), was used to promote nanoparticle stability in biological fluids. siRNA was used to reduce expression of the ribonucleotide reductase M2 (RRM2) protein levels.

The trial (NCT00689065), in the recruiting stage, involves the use of CALAA-01, with active ingredient being anti-ribonucleotide reductase (R2) siRNA. The siRNA may inhibit tumor growth by reducing the expression of the M2 subunit of ribonucleotide reductase (R2). The complete nanocomplex formulation consists of four key parts: a duplex of synthetic, nonchemically-modified siRNA (C05C), cyclodextrin-containing polymer (CAL101), the stabilizing agent (AD-PEG), and the targeting agent (AD-PEG-Tf). The targeting component, the human transferrin protein (Tf), binds to overexpressed surface Tf receptors in targeted tumor cells. In addition, the cationic polymer component interacts electrostatically with anionic siRNA to assemble into nanocomplexes. These can assemble below approximately 100 nm in diameter which protect the siRNA from nuclease degradation in the serum [55,77-81].

The second recruiting Phase I trial involves a dose escalation study for hepatic arterial infusion (HAI) with a TKM-080301 compound to patients with unresectable and/or life-threatening primary liver cancer or liver metastases. This is a Phase I dose escalation study of hepatic intra-arterial administration. Solid tumors include colorectal, pancreas, gastric, breast, ovarian, and esophageal with hepatic metastasis. TKM-080301 is a lipid nanoparticle (LNP) also referred to as SNALP (stable nucleic acid lipid particles) formulation containing siRNA against the PLK1 (polo-like kinase-1) gene product. PLK1 has been validated as a molecular target and a prognostic factor in a variety of solid tumors. Inhibition of PLK1 activity in proliferating cancer cells rapidly induces mitotic arrest and apoptosis [77,82-84].

Tumor biopsies from melanoma patients obtained after treatment show the presence of intracellularly localized nanoparticles in amounts that correlated with dose levels of the nanoparticles administered. Furthermore, the authors have demonstrated a reduction was found in both the specific messenger RNA (M2 subunit of ribonucleotide reductase (RRM2)) and the protein (RRM2) levels when compared to pre-dosing tissue. The authors also showed a detectable presence of an mRNA fragment demonstrating that siRNA-mediated mRNA cleavage occurs specifically at the site predicted for an RNAi mechanism from a patient who received the highest dose of the nanoparticles [10].

4. Toxicity

Toxicity remains a concern and more extensive data are required before large conclusions can be drawn about siRNA safety. Cytotoxic, immunogenic, and hemocompatiblity concerns are among those which have been raised. Surface modification, structural changes, lower molecular weight, and chemical modification have been some of the strategies to minimize toxicity [1-4,63,64,85,86].

4.1 Blood compatibility

Intravenous administration of elevated doses of liposome/siRNA complexes in mice has led to severe neutropenia, thrombocytopenia, and increased transaminase levels which indicate hepatic damage. In addition, erythrocytolysis and erythrocyte fusion has also been observed [1,57-59]. PEI has been shown to induce platelet aggregation [1,60].

Several systems have shown relatively good blood compatibility. In particular, folate-coated, PEG-coated gadolinium nanoparticles did not cause platelet aggregation and neutrophil activation [59]. In addition, low molecular weight chitosan gene vectors did not cause hemolysis [1,61].

4.2 Immunogenicity

Although nonviral gene vectors have a low immunogenicity, there is the possibility of activating the innate immune system. Cationic liposomes can activate complement, induce production of factors such as tumor necrosis factor-α, and interleukin-6 and -12 [1,62,63]. Liposome/siRNA complexes can induce type 1 and 2 interferon responses and activation of transcription activator protein STAT1 [1,53,64]. PEI has shown to activate the complement system [1,60].

4.3 Cytotoxicity

Cytotoxicity has been demonstrated in a variety of nanoparticles. Hong *et al.* have indicated that the stability of nanoparticles in the cell culture medium may be one of the most crucial factors in evaluating toxicity and elucidating the toxicity mechanism. It has been also proposed that the functional groups and sizes of superparamagnetic iron oxide nanoparticles are critical determinants of cellular responses, degrees of cytotoxicity and genotoxicity, and potential mechanisms of toxicity [86]. This has been demonstrated particularly in L-929 cells treated with each superparamagnetic iron oxide nanoparticles having functional groups which included groups – hydroxyl (–OH), carboxylic (–COOH), and amine ($-NH_2$) groups – by coating their surfaces with tetraethyl orthosilicate (TEOS), (3-aminopropyl)trimethoxysilane (APTMS), TEOS-APTMS, or citrate, which induced different surface charges and overall sizes to the particles studied. For example, the integrity of cell membrane was shown to be particularly and severely damaged with intracellular

Table 2. Classification of oncogenes.

Group	Example	Oncologic association	Therapeutics
Growth factors	c-Sis	Sporadic meningioma [102]	
Receptor tyrosine kinases	EGFR, PDGFR, VEGFR, Her2/neu	Nonsmall cell lung cancer (EGFR)	Small molecule EGFR inhibitors [103]
Cytoplasm tyrosine kinase	Abl gene, SRC-family, BTK family, Syk-ZAP-70 family	B-cell malignancies and X-linked agammaglobulinemia (BTK family) [104,76]	Small molecule BTK inhibitors [4]
Cytoplasm serine/threonine kinases	Raf kinase, cyclin-dependent kinase	Non-Hodgkins lymphoma, colorectal cancer, malignant melanoma (B-Raf) [105]	B-Raf inhibitors
Regulatory GTPases	Ras protein	Glioblastoma multiforme, neurofibromatosis 1 [26,28]	Ras inhibitor trans-farnesylthiosalicylic acid [27,28]
Transcription factors	myc	Burkitt's lymphoma [29-32] breast, colon and cervical carcinomas, small cell lung carcinomas, osteosarcomas, glioblastomas, myeloid leukemias [33]	Retinoic acid + IFN-γ, [90] Myc inhibitors

vesicles containing more concentrated superparamagnetic iron oxide nanoparticles in the cell exposed to (3-aminopropyl) trimethoxysilane (APTMS) [1-4,85,86].

For example, studies of the cytotoxicity of silver nanoparticles (AgNPs) in three different characteristic sizes (~ 10, 50, and 100 nm) against several cell lines including MC3T3-E1 and PC12 have been demonstrated. For example, the smallest sized AgNPs (10 nm size) showed a greater ability to induce apoptosis in the MC3T3-E1 cells when compared to the other two sizes [85].

4.4 Genocompatiblity and toxicogenomic profiling

Drug delivery systems may induce undesirable genomic changes. The introduction of nanocarriers for therapeutic delivery requires a mapping of their potential effects. Toxicogenomic profiling may allow genomic signatures for potential toxicity for the nanocarrier alone and with its delivered entities. This would allow a better tailoring of treatment for stratified patient population. It has been suggested by Akhtar *et al.* that, alternatively, it may be possible to engineer a delivery system that may enhance the genomic silencing effect along with the delivered siRNA and therefore a synergistic response [87,88].

It has been demonstrated that direct intratumoral administration of polyethyleneimine (PEI) to epithelial tumor xenografts in nude mice showed that the branched PEI had a greater propensity than its linear counterpart form to induce gene changes *in vivo*. Furthermore, this was correlated with a higher toxicity as well. PEI has been used in various methodologies for siRNA delivery and therefore genocompatibility is particularly relevant here [1,67,72,87,88].

siRNA themselves may also have potential genotoxicity such as inducing genomic changes at off-targets and induce toxic phenotypes. Fedorov *et al.* demonstrated a strong correlation between the toxicity and the presence of a 4-base-pair motif (UGGC) in the RISC-entering strand of toxic siRNA. However, this may be mitigated by the addition of chemical modifications to the siRNA [89].

5. Oncogenes

Identification of genes that directly cause cancer development or merely predispose patients to cancers has been one focus in biomedical research over the last few decades. Efforts toward characterization of oncogenes have led to their classification into several distinct groups: growth factors or mitogens, receptor tyrosine kinases, cytoplasmic tyrosine kinases, GTPases, and transcription factors (**Table 2**). These oncogenes represent mutations or translocations of genes that are required for normal cell cycle regulation. Identification of oncogenes, such as the transcription factor Myc, has led to a deeper understanding of the genetics of cancer.

Examination of Myc protein's role in tumorigenesis highlights the potential in the field of pharmacogenomics and its logical translation into therapeutics. Myc protein is a transcription factor that can activate expression of multiple genes through binding on enhancer box sequences (E-boxes) and recruiting histone acetyltransferases (HATs). Alternatively, it can act as a transcriptional repressor. Myc has been shown to displace the p300 co-activator by binding to the Miz-1 transcription factor, thus inhibiting expression of Miz-1 target genes [31,32,90]. Myc activation falls under the province of several regulatory and mitogenic signals such as EGF, SHH, and Wnt that result in increased cell proliferation. Several groups have demonstrated the complex and varied Myc target genes and Myc's role in upregulating cyclins (thereby promoting cell proliferation), upregulating rRNA (promoting cell growth) and downregulating Bcl-2 (inhibiting apoptosis) [31,90,91]. Given

its redundant and vast reach, overexpression of the Myc gene can easily result in amplified cell proliferation. Not surprisingly, Myc dysregulation has been tied to various cancers including Burkitt's lymphoma, breast, colon and cervical carcinomas, small cell lung carcinomas, osteosarcomas, glioblastomas, myeloid leukemias, and prostate adenocarcinomas [30-34,90] It is therefore conceivable that manipulation of the expression of the Myc gene should add to armamentarium of treating many of these cancers. The most apparent way to thwart Myc-dependent oncogenesis is to induce downregulation or inactivation in tumors [92]. Investigators have shown that transgenic mice expressing Myc develop tumors that typically remain dependent on the artificially deregulated, elevated levels of Myc [93,94]. While these data make the "turning off" of Myc expression an attractive tumor target, groups have reported that subpopulation of tumor cells ultimately becomes resistant to Myc downregulation, suggesting that this treatment alone would not be adequate to completely halt tumor growth [95]. However, while Myc-targeted treatments alone may not be enough, there may exist a role for their use in slowing the growth of any tumors that ultimately proliferate using Myc-driven genes, irrespective of the role of Myc in tumorigenesis. Efforts toward this goal have resulted in the investigative combination IFN-γ and retinoic acid in the treatment of Myc-driven cancers, particularly neuroblastoma [91]. While attention has been paid on identifying the genetics underlying oncogenesis, there has been a body of work focusing on other chronic medical conditions and neurodegenerative disorders. The work that has focused on identifying Myc-targeted therapies in the treatment of cancers is illustrative of the potential work to be done in any conditions where the underlying genetics have been identified.

6. Pharmacogenomics-based drug response

While uncovering the genetics underlying tumorigenesis highlights the special role pharmacogenomics may play in elucidating new therapeutics, smaller victories may been seen in the field, particularly in the prediction of a patient's response to treatments. The recent identification of a polymorphism that is predictive of the effectiveness of an interferon treatment for hepatitis C virus (HCV) is one such example. For HCV treated with interferon-α-2a or interferon-α-2b combined with ribavirin, it has been shown that genetic polymorphisms near the human IL28B gene are associated with significant differences in response to the treatment. This finding showed that patients with this genetic variation not only predicted treatment response in patients but was also associated with the natural clearance of HCV. Such examples highlight the vast reach of identification of genetic variability in patients. Finally, it should be noted that a pegylated interferon alfa-2b formulation is also being used for melanoma and therefore warrants additional pharmacogenomics drug response profiling, particularly if nanoparticle drug delivery systems will be used [13,96,97].

An important example of pharmacogenomics drug response profiling for a drug delivery system for malignant tumors involved the assessment of an etoposide-loaded poly (lactide-*co*-glycolide) nanoparticles for a retinoblastoma cell line (Y-79). An upregulation of apoptotic gene activity was demonstrated with these nanoparticles when compared to native etoposide which was shown using microarray analysis. These nanoparticles were also shown to have a greater (G1/S phase) blocking and decreased mitochondrial membrane potential which was indicated using flow cytometry. Finally, a greater anti-proliferative activity, an estimated 100 times greater, was observed with these nanoparticles (IC_{50} = 0.002 μg/ml) than that of native etoposide (IC_{50} = 0.2 μg/ml) [13,96,98].

Based on a similar pharmacogenomic concept for drug response, a genomic profile that could predict RNAi as a function of lowered levels of targeted proteins could be used to assess drug response prediction. Moreover, this can be correlated with a specific targeted functionalized molecule placed on the delivery system.

7. Patient clinical considerations for tailored therapy

Malignant tumors may initiate a plethora of mechanisms to exacerbate the overall condition for patient with malignant tumors. Many chemotherapeutic compounds can induce renal and liver failure in addition to the existing metastatic side-effects of the tumors themselves. Immunosuppression can also be a side-effect of various chemotherapeutic agents [13,14].

Hypercoagulability is often seen in patients with solid malignant tumors. This may be partly due to the various circulating procoagulant factors. Furthermore, there is a relationship between venous thromboembolism and malignant solid tumors wherein circulating microparticles (MP) originating from different cells which express tissue factor are involved [13,14]. For example, it has been shown in glioblastoma multiforme, a malignant and devastating brain tumor, that patients may have an increase in MP-associated procoagulant activity that could contribute to prothrombotic states and increases the likelihood of venous thromboembolism complications; however, this procoagulant activity drops when the disease is controlled [99].

Genetic profiling such as assessing single nucleotide polymorphisms (SNPs), a parameter commonly used in pharmacogenomics, may assist drug response as well. For example, the single nucleotide polymorphisms of CYP19A1 has been shown to predict clinical outcomes and adverse events associated with letrozole in patients with metastatic breast cancer [36]. Additional SNPs and their relation to treatment response can be assessed for nanoparticles themselves, functionalized nanoparticles, the delivered entity, and the entire delivery system [98].

Such side-effects warrant a tailored treatment that takes into account these potential causes of patient incompliance

and treatment failures. That is, a treatment that focuses on common toxicity and pre-existing comorbid conditions often seen with malignant tumors such as hypercoagulability must be tailored. Furthermore, using pharmaco- and toxicogenomic profiling, such potential complications along with exacerbations of co-morbid conditions may be able to be further monitored [13,14,99].

As noted, monitoring for potential off-target genomic effects specific for designated siRNA may also be considered as a possibility for implementation for tailored therapy. This may assist in engineering a more suitable siRNA [89].

8. Expert opinion

In cases where the genetics underlying predisposition to oncologic conditions or other chronic medical conditions have been identified, the natural progression of thought is to translation into rationally-designed therapeutic targets [97,100]. While some success has been achieved in the field, there remain barriers to the progress in the element of pharmacogenomics. Several factors have limited the progress in this field including the complexity of identifying gene variations that predict drug response or contribute to disease onset, prohibitive costs associated with drug development for small, targeted patient populations, and challenges in drug delivery.

The growing understanding of genetic predispositions or underlying single nucleotide polymorphisms (SNPs) has identified new therapeutic targets. These genetic targets may be addressed at the RNA level using RNAi emerging technology. As mentioned, further work is necessary to improve the biocompatibility and stability of siRNA platforms before progress is realized in this area of therapeutics.

Single nucleotide polymorphisms (SNPs) have been found to occur every 100 – 300 bases along the 3-billion-base human genome, resulting in millions of SNPs to be identified and analyzed for their involvement in drug response [100,13,14]. This presents a cumbersome computational challenge. When such polymorphisms are identified, further challenges exist with how to use this information. In many cases, only one or two approved drugs may be available for treatment for a given condition. For the patient with a polymorphism that renders a treatment less efficacious, the clinician is left with few, if any, alternative therapeutics. Cost remains another barrier in pharmacogenomics. Much resources are needed to bring a new drug through the development, testing, and the regulatory process [100]. Finally, a significant challenge is the ability to deliver these therapies in a targeted fashion. In the case of Myc-targeted cancers, for example, the Myc transcription factor has downstream targets that assist in normal cellular function as well as tumor cell function and replication. To deliver any such treatment therefore requires cell-specific, selective drug delivery with acceptable toxicity and pharmacological profiles.

Drug delivery will be key in mitigating the toxicological risks. With that being said, drug delivery itself will have to be analyzed for its own toxicological risks. Pharmacogenomics may be helpful in addressing possible drug response for both the gene silencing and the drug delivery system as both complete and distinct units. In addition, the area of toxicogenomics, the genomic profiling of possible toxicity, may be used for the same purpose. In assessing the validity of such systems in malignant tumors, patient profiling may be key as much patient noncompliance due to toxicity play a major role in treatment failure.

Declaration of interest

Y Rosen is the Chief Executive Officer (CEO) and principal founder of Superior NanoBioSystems LLC.

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