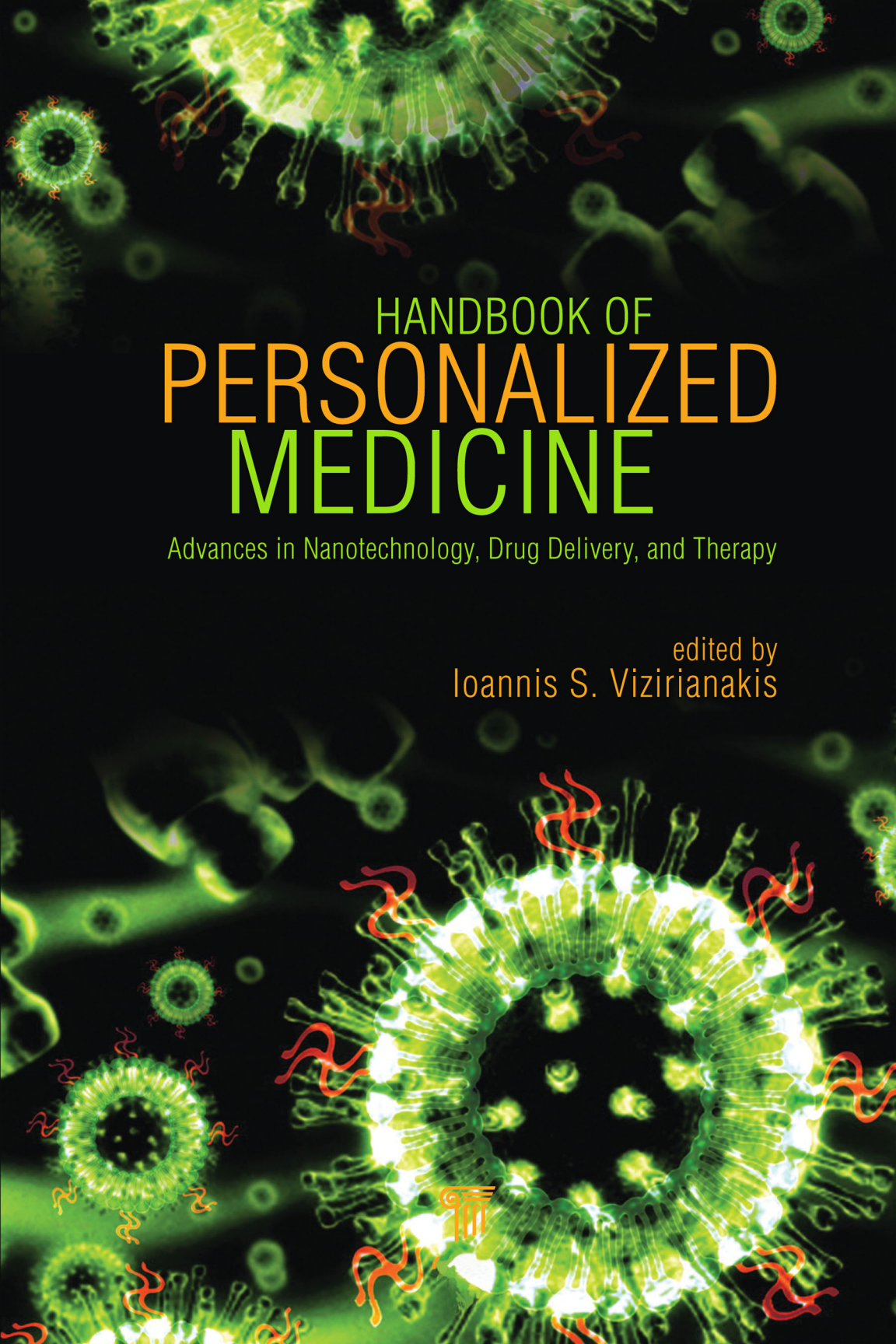
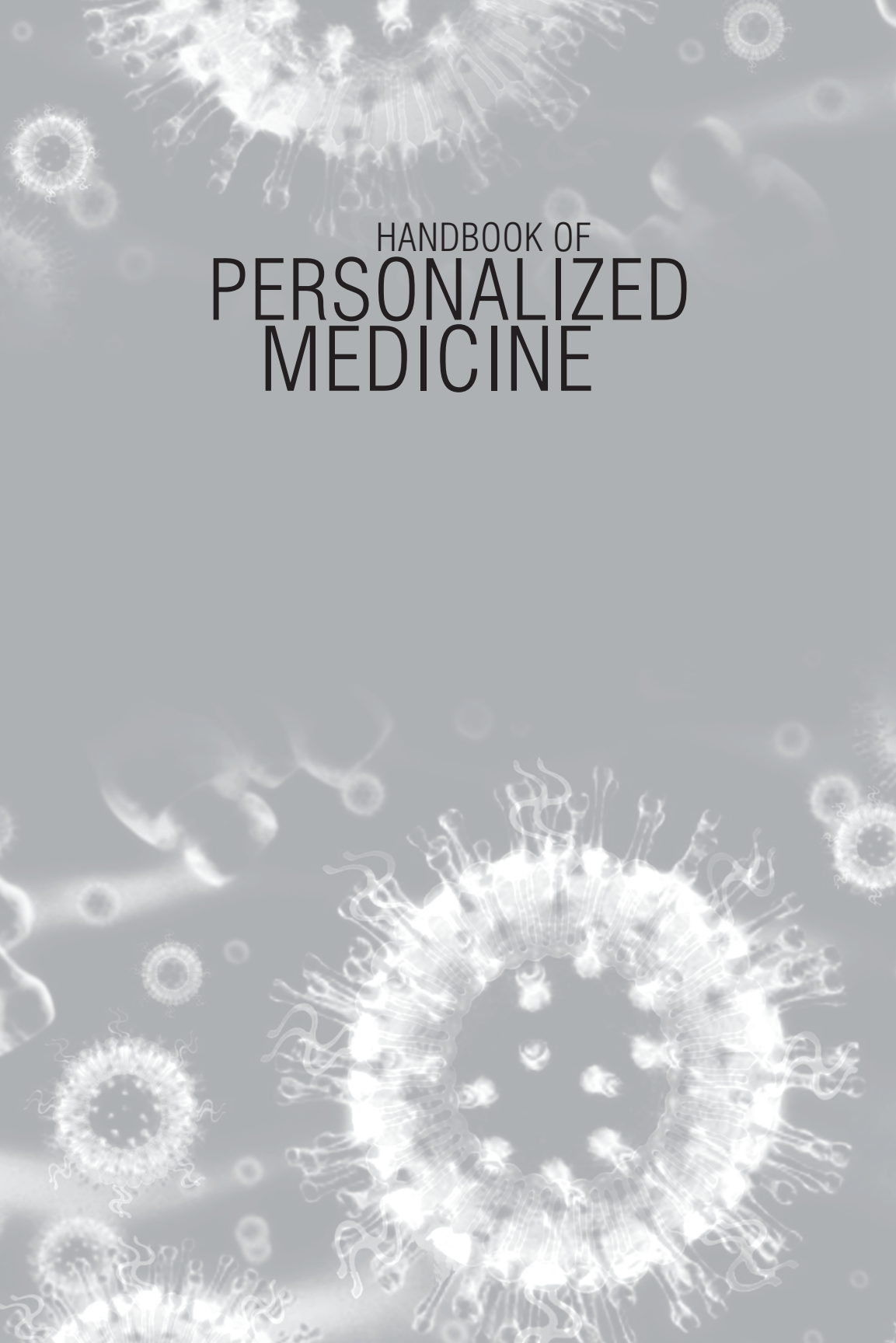

HANDBOOK OF
PERSONALIZED
MEDICINE
Advances in Nanotechnology, Drug Delivery, and Therapy
edited by
Ioannis S. Vizirianakis



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This volume is dedicated to

my parents, Aimilia and Spiros, whose perspective on life, work, and behavior shows me the way to grow, improve, and progress as a human being by putting tasks and achieving targets, as well as keeping dreams alive, both as a person and in academia

my wife, Lila, and my kids, Emily and Spiros, whose continuous contact, devotion, and love show me the way to behave and better handle issues related to community and society, thus permitting me to follow dreams and run toward realistic targets

my students and colleagues for their trust, collaboration, and contribution that give me an opportunity to become a better teacher as well as to enrich knowledge, skills, and expertise, thus making research projects a reality and finally a success

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Contents

Preface

The response of an organism to drugs has been challenging scientists through the years, and it must be considered as one aspect of the overall responses that living species exert to different environmental impacts and stressors within an ever-changing environment. To this regard, our knowledge of illness etiology and drug actions in the body goes in parallel with the scientific advances focusing to elucidate mechanisms and processes that contribute to the existence of life itself. In this way, understanding the pathophysiology of disease phenotypes as well as deciphering the underlying pharmacological mechanisms have long been set as the primary goals to be achieved, maximizing benefits in medical and pharmacy practice. Moreover, maximum efficacy and safety upon drug delivery, implying the improvement of pharmacotherapy profiles, is a long-desirable target for drug administration and coincides chronologically with the establishment of pharmacology as a basic and clinical discipline. Especially, over the past 80 years, medical and pharmaceutical specialties were given the capacity to suitably adopt scientific advancements coming from various research areas, thus providing health care practitioners with the suitable skills and expertise to improve disease prognosis and diagnosis as well as drug delivery clinical outcomes. As an example, if the scientific achievements will be considered over this period for the drug discovery and development era, one can easily came to the conclusion that it has been mainly influenced by fundamental advances in chemistry, physiology, and pharmacology, whereas specific contributions occurred at various decades from disciplines as these were being expanded through the years. Such examples refer to breakthroughs from microbiology in the 1930s and 1940s, from biochemistry and enzymology in the 1950s and 1960s, and

from molecular biology and recombinant DNA (rDNA) technology from the middle of 1970s and onward.

Nowadays, advances in nanotechnology, genomic technologies, informatics, molecular biology and pharmacology have long held out the promise of transforming medical practice, drug development and delivery from a matter of serendipity to a rational pursuit grounded in a fundamental understanding of the mechanisms of life. As far as the drug-related research and clinical environment is concerned, pharmacogenomics revived pharmacogenetics and pharmacology research boundaries to keep pace with fastevolving life-imposed scientific advances. The application of pharmacogenomics focuses on the clinical translation of genomics data to predict and evaluate disease risk and progression, as well as the pharmacological response to drugs in individual patients or groups of patients. As a matter of fact, the clinically validated genomic knowledge of target receptors, ion channels, enzymes, or transporters could be an additional clinical factor in guiding personalized prescription of most, if not all, currently in practice, orally delivered drugs to achieve the best-possible efficacy and safety profiles. By definition, personalized medicine implies the management of a patient's disease in terms of prognosis, diagnosis, and drug delivery to achieve therapy with the best-possible medical outcome for that individual. To this end, the concept of personalized medicine has emerged as the way by which a suitable infrastructure setting in research, clinics, education and regulation could be built to hasten the translational efficiency of genomic, molecular and technological advancements into the practice of medicine and pharmacy. The latter means that both clinical and research efforts focusing on those concepts might formulate and broaden the era of personalized medicine and could facilitate as well as accelerate its practical utility in the clinical settings. This is considered a very important aspect toward achieving major benefits for personalized medicine worldwide. Such an approach was further supported by the notion that the possibility of focusing on the development of "personalized medicines" for specific individual patients could hardly be attained in practice, since it represents a very difficult task to be affordably achieved in terms of existing regulatory drug development issues, world-broad clinical utility, and therapy costs.

Personalized medicine, although in its infancy, represents already the next evolutionary step in medicine and pharmacy by gaining acceptance as an independent area of research to join the gap as well as connect experimentally the interfaces between the clinical settings with health-related basic disciplines. Through the application in everyday clinical practice of personalized medicine concepts, the improvement of prognosis, diagnosis, and therapy outcomes can be achieved in an affordable way as well in real time by permitting the stratification of patients suffering the same complex illness (e.g., cancer, cardiovascular disorders). It is expected to revolutionize the whole health and pharmaceutical care environment by focusing on the individualization approach both in research and in everyday clinical practice. This refers, among others, to disease risk assessment, diagnosis profiles, and new drug development approaches in order for the clinical translation of genomics information to be more efficiently achieved, thus maximizing drug delivery and prescription worldwide.

Having this in mind, the organization of a multidisciplinary approach toward serving better the clinical exploitation of the knowledge achieved thus far from cutting-edge genomics, innovative bioinformatics, and frontline nanotechnological advancements seems reasonable and attainable. Furthermore, this direction might more affordably permit the application of personalized medicine concepts in routine health care as well as cultivate the functional merger and unification of these core research directions into a common ground of "communication research language" to achieve the desirable personalized medicine targets. For example, by strengthening the clinical benefits of genomic knowledge as well as applying informatics methodologies and nanotechnological procedures and putting in perspective their advancements that contribute to personalized medicine, such an idea is gaining practical utility in clinical practice and drug delivery in a way that it connects the outcomes with specific markers and gene expression signatures of prognostic, diagnostic, and even therapeutic value. To this end, practical clinical utility worldwide could be faster and more efficiently achieved. And more importantly, by fulfilling the needs of broader clinical utility for personalized medicine, this also coincides with the active participation of health care educators in

the advancements happening both in research and at the clinical level in order then to transfer their expertise and experience into future professionals through the creation of suitable education programs in medicine and pharmacy. Such direction is crucial, since the implementation of the curricula has to take into consideration the scientific approaches with practical clinical consequences in the profiles of individual patients for diagnosis and drug delivery outcomes.

Handbook of Personalized Medicine represents an effort to critically shape the era in which various advancements contributing to health care disciplines merge to formulate the structure needed for allowing personalized medicine concepts to emerge in everyday clinical practice. The latter implies that these advancements are clinically validated, getting practical utility and broad use, and meeting regulatory requirements, as well as receiving a final approval to enter health care. To achieve this goal, leading scientists in their areas of expertise with various scientific backgrounds have been invited to contribute. To this end, recent advancements in genomics and nanotechnology will be presented that create a fertile ground for pharmacogenomics and personalized medicine to advance prognosis and diagnosis profiles for specific groups or individual patients and move toward pharmacotyping in drug prescription, that is, the individualized specific drug and dosage scheme selection based on the patient's clinical and genetic data. Within this frame, this book is unique in its structure by including issues related to nanosystems and nanodevices, innovative drug formulations and nanotheranostics, molecular imaging and signatures, translational nanomedicine and informatics, predictability of drug effect behavior, genetic etiology of drug response heterogeneity, pharmacogenetics-guided drug prescription, pharmacovigilance and regulatory aspects, ethical and cost-effectiveness consequences, personal genome analysis, pharmacogenomics knowledgebase, education issues, and information-based medicine, as well as, last but not least, a framework and infrastructure to support personalized medicine utility for everyday clinical practice. This multidisciplinary *Handbook of Personalized Medicine* is also unique in its concept by including and presenting selective cutting-edge technological advancements from genomics, pharmacology, nanotechnology, informatics, and statistics

that focus on pharmacogenomics and personalized medicine and allow the practical utility of clinically relevant genomic knowledge to enter health and pharmacy care. The idea to present various topics addressing the practical utility of personalized medicine and pharmacogenomics in a feasible and cost-affordable manner for routine health care is also innovative for this book volume. The text, although organized in such a way that each chapter represents an independent area of research, simultaneously allows an easy manner for the reader to intercorrelate various subjects covered in separate chapters. I sincerely hope that the book will assist readers in understanding the multidisciplinary nature of the changes happening in health and pharmaceutical care sectors and also to enrich their knowledge and their own perspectives on how genomics, informatics, pharmacology, and nanotechnology affect health-related professions to better adjust themselves in the new setting.

From the beginning and upon completion of this volume, new scientific achievements have stressed toward the empowerment of personalized medicine decisions by working and building a more multidisciplinary infrastructure in research and clinics. It is, for example, very interesting to note the vast load of human and other complex genomes functional data published in September 2012 from the ENCODE Project Consortium (*The Encyclopedia of DNA Elements*; *ENCODE*) that provides new insights into genetic variability patterns seen in individuals and populations. As is pointed out, many previously clinically validated DNA variants are located within or very near to intergenic regions and other noncoding functional DNA elements, thus providing new ways to clinically translate genomic information by linking specific genetic polymorphisms and disease etiology and progression profiles. Such new genetic information impinges on the regulation of complex mechanisms involved in human genome function, which, in turn, may contribute to molecular pathophysiology mechanisms. The latter stressfully points toward a more multidisciplinary effort for a practical clinical utility infrastructure in the era of personalised medicine for the benefit of society and individual patients worldwide. And more importantly, as recently published, the application of an integrative personal “omics” profile analysis

that combines genomic, transcriptomic, proteomic, metabolomic, and autoantibody profiles from a single individual has revealed the dynamics of this approach toward achieving personalized medicine decisions in clinical practice.

Last, but not least, the dynamic scientific environment that already exists in the era of nanotechnology and genomics with the potential to affect health care and drug delivery decisions needs more collaborative multidisciplinary efforts to make practical clinical utility of personalized medicine a maximum success. As a matter of fact, by crossing the borderlines of genomics with nanotechnology a fertile ground can be created to lead to the advent of "personalised nanomedicine" as a new discipline to enforce individualized therapeutic decisions with maximum safety and efficacy. To this end, a theme issue on "personalized nanomedicine" in the journal *Advanced Drug Delivery Reviews* has been recently coedited (October 2012) to define and exemplify that necessity in both research and clinical settings. The interested reader can follow such referred theme issues for further information and consideration.

I feel so deeply grateful, and I express my sincere thanks to all authors who contributed to this volume by taking time from their busy schedule, as well as presented their work and provided their personal perspectives on the concept of personalized medicine, thus making the initial multidisciplinary approach a reality and get its sense in the book.

Special thanks are also expressed to the Pan Stanford Publishing staff for their kind help as well as their work to see this volume being completed.

I cordially express my gratitude to my family members for their patience in all stages of this project, as well as for their continuous encouragement and the creation of such a supportive and creative environment that make this work finalized and complete.

Ioannis S. Vizirianakis
Thessaloniki, Autumn 2013

Chapter 1

Nanotechnology toward Advancing Personalized Medicine

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1.1 Introduction

We are living in an era of mass marketing and big business—a strategy that favors retail giants such as Walmart®, Costco®, and Best Buy® in attempts to satisfy the insatiable commercial needs of a growing population. Current economic drivers instinctively motivate fiscally conscious consumers to flock to warehouse-style retailers to purchase mass-produced generic products; rather than

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paying a premium at privately owned and operated boutique shops that sell unique goods marketed toward specific subgroups of customers. Unfortunately, this trend superficially appears to have been adopted with vigor by large pharmaceutical companies, "Big Pharma" as they are commonly referred to, as they develop and market blockbuster drugs to treat the masses. As a result, an individual patient's clinical needs have been blurred in efforts to accommodate entire populations of patients. But before we lump Big Pharma into the likes of retail giants that mass-produce products to lower costs and boost margins, one must understand the harsh realities of drug development. On average, it is estimated that a single new drug compound costs over $1 billion and 10–15 years to develop [1]. And shockingly, only one out of five new drug compounds actually generates revenue equal or greater to its inherent developmental costs [1]! It is obvious that to disrupt this drug development trend, a "perfect storm" of novel emerging technologies, nonconventional regulatory approaches, Big Pharma support, and health insurance reform must converge to initiate the shift toward developing personalized therapies (Fig. 1.1).

Personalized medicine is the collection and analysis of clinically relevant patient data (e.g., genomic, proteomics, metabolomics, etc.) to determine the most effective, tailor-made treatment strategy possible. The transition to individualized therapy is a palatable idea to embrace since its application is deeply rooted in the logical evolution of clinical medicine; however, its ubiquitous implementation will require an unprecedented synchronized integration of effort from the pharmaceutical industry, the Food and Drug Administration (FDA), and medical insurance companies—pushed by scientific advancements and pulled by clinical demand from physicians and patients. Nanotechnology has been hailed by many as the enabler of individualized therapy since nano-based medicine, or nanomedicine, allows us to interact with disease at the scale of biology. We are now able to bring the battle to the level where a war is being waged. Nanotechnology provides scientists and clinicians with access to disease pathways, mechanisms to exploit minuscule pathologic changes in anatomy, strategies to augment imaging modalities, and tools to collect near-overwhelming amounts of patient information to reveal new approaches to identifying

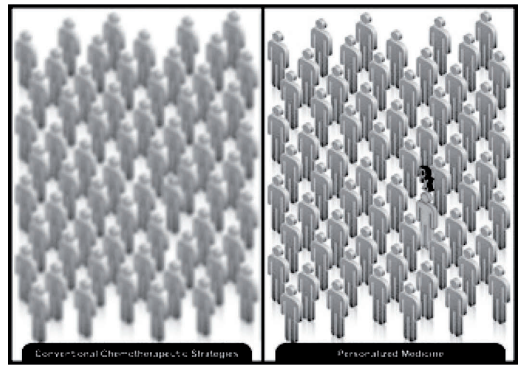

Figure 1.1 (A) Conventional chemotherapeutic strategies are often administered as standard protocols for patients with cancer. This practice "blurs" patient-to-patient distinction and approaches the treatment of cancer in the context of populations of the disease. (B) Personalized medicine is a developing clinical approach that "focuses" upon the needs of individual patients and is predicated upon the assembly and analysis of patient-specific information made possible through emerging technologies such as nanotechnology.

vulnerabilities of complex ailments such as cancer, heart disease, and other clinical challenges.

This chapter will provide a snapshot of nano-based strategies that have reached the clinic in the context of cancer and those that remain in the process of translation. In addition to this summary of nanotechnology and personalized medicine for the treatment of cancer, this chapter will also feature multiple perspectives regarding the enabling of individualized therapy from several key vantage points ranging from a practicing surgeon to a biotech chief executive officer (CEO) to an FDA consultant to a patient advocate.

1.2 Conventional Cancer Chemotherapeutics

1.2.1 *A Brief History*

The dawn of modern cancer chemotherapy may have risen from the aftermath of the smoke and twisted metal of Allied warships stationed in Bari Harbor, Italy, on December 2, 1943. A German

strategic air raid sought and destroyed 17 vessels as they unloaded supplies and cargo intended to support the final Allied push into Italy [2]. Unknowing to the squadron of German Luftwaffe, the strike left a US Liberty ship in wreckage, detonating its deadly payload of mustard gas bombs and subsequently exposing the weaponized chemical agent into the harbor and surrounding city. This tragic event, which killed over 2,000 Allied servicemen and Italian citizens, enabled two Yale pharmacology professors to validate their research focused upon the leukopenic effects of nitrogen mustard [3]. Drs. Alfred Gilman and Louis Goodman began their research in 1941 under a program funded by the US government's Office of Scientific Research and Development [4]. The attack of 1943 provided a grim opportunity for these scientists to document how β-chloroethylamines destroyed lymphatic tissue and bone marrow in human subjects. Their clinical findings were published in a landmark article titled "Nitrogen Mustard Therapy: Use of Methyl-bis(Beta-Chloroethyl)Amine Hydrochloride and tris(Beta-Chloroethyl)Amine Hydrochloride for Hodgkin's Disease, Lymphosarcoma, Leukemia, and Certain Allied and Miscellaneous Disorders" in the *Journal of the American Medical Association* (*JAMA*) in 1946 [5].

Over 60 years have passed since the tragedy in Bali Harbor, and modern medicine is still employing extremely toxic, nonspecific agents to combat disease. In fact, cyclophosphamide, a nitrogen mustard alkylating agent and direct descendant from the therapeutic discovery of the US Liberty ship disaster, is still being actively used today as adjuvant therapy or as part of first-line treatments for various cancers. At the time of this chapter, a search of ClinicalTrials.gov found 2,269 studies that involved cyclophosphamide for clinical indications ranging from early-stage breast cancer to multiple myeloma. The results of such a query can be viewed as strong evidence of the clinical importance of the discovery of nitrogen mustard alkylating agents or perhaps an indication to the lack of significant therapeutic innovation achieved over the past half-century in cancer therapeutics. Statistics remain the most powerful metric to measure the progress of our fight against cancer, and the American Cancer Society recently published

its latest figures tabulating trends in the five-year relative survival rate in the United States from 1975 to 2005. The findings indicate a promising 18% increase of the five-year relative survival rate when comparing 24 cancer types over the 30-year period [6]. We have achieved major improvements in several cancers such as prostate cancer (increase of 31%), non-Hodgkin lymphoma (increase of 21%), and breast cancer (increase of 15%). However in certain cancers we have attained minimal improvement; the five-year relative survival rate for pancreatic cancer remains in the single digits (6%) and has only improved by 3% since 1975 [6].

1.2.2 *A Summary of Conventional Anticancer Drugs*

Most conventional anticancer agents can be categorized into one of three therapeutic mechanisms: 1) to damage the deoxyribonucleic acid (DNA) of the affected cancer cells, 2) to inhibit the synthesis of new DNA strands to halt cell replication, and 3) to stop mitosis, thereby inhibiting uncontrolled cell division [7]. Table 1.1 summarizes the hallmarks of several drug classes commonly employed as anticancer drugs. Scientific journals are filled with case studies demonstrating the clinical merits of exploiting cancer cell vulnerabilities associated with these mechanisms; however, decreasing the rate of cell division is not a ubiquitously welcomed therapeutic consequence. Many bodily systems naturally depend upon the healthy rapid turnover of cells, including the skin, gastrointestinal lining, bone marrow, and hair follicles. Herein lies the most fundamental conundrum of cancer therapeutics—how to kill cancer cells, sparing as many healthy cells as possible (the patient must survive the rigors of his or her therapy to be cured of the cancer). It is alarming that conventional clinical wisdom still prescribes treatments where often times only 1 out of 100,000 drug molecules actually reach the intended site of disease [8]. A simple calculation reveals that 99.99% of the injected dose is nonspecifically distributed throughout the body, subjecting healthy organ systems to the brutal consequences of cytotoxic adverse side effects associated with most cancer therapeutics.

Table 1.1 Conventional cancer chemotherapeutics

Drug class	Mode of action	Cancer chemotherapies
Alkylating agents	Damages DNA to prevent cancer cell proliferation	**Nitrogent mustards:** Mechlorethamine, chlorambucil, cyclosphamide **Nitrosoureas:** Streptozocin, carmustine, lomustine **Alkyl sulfonates:** Busulfan **Traizines**: Dacarbazine, temozolomide **Ethylenimines:** Thiotepa, altretamine
Antimetabolites	Interferes with DNA synthesis	5-fluorouracil, capecitabine, 6-mercaptopurine, methotrexate, gemcitabine, cytarabine, fludarabine, pemetrexed
Antitumor antibiotics	Interferes with enzymes involved in DNA replication	**Anthracyclines**: Daunorubicin, doxorubicin, epirubicin, idarubicin **Others:** Actinomycin-D, bleomycin, mitomycin-C, mitoxantrone
Topoisomerase inhibitors	Interferes with topoisomerases, which help separate the strands of DNA for replication	**Topoisomerase I inhibitors:** Topotecan, irinotecan **Topoisomerase II inhibitors:** Etposide, teniposide, mitoxantrone
Mitotic inhibitors	Stops mitosis by disrupting normal function of mitotic spindles, thereby halting cell division	**Taxanes:** Paclitaxel, docetaxel **Epothilones:** Ixabepilone **Vinca alkaloids**: Vinblastine, vincristine, vinorelbine **Others:** Estramustine

Immunotherapy	Enhances, suppresses, or induces immune responses	**Monoclonal antibodies:** Rituximab, alemtuzumab, trastuzumab **Nonspecific immunotherapies and adjuvants:** BCG, interleukin-2, interferon-alpha **Immunomodulating drugs:** Thalidomide, lenalidomide
Hormone therapy	Alters action or production of female or male hormones	**Antiestrogens:** Fulvestrant, tamoxifen, toremifene **Aromatase inhibitors:** Anastrozole, exemestane, letrozole **Progestins:** Megestrol acetate **Estrogens** **Antiandrogens:** Bicalutamide, flutamide, nilutamide **Gonadotropin-releasing hormone:** Leuprolide, goserelin
Miscellaneous		L-asparaginase, proteosome inhibitor bortezomib, cisplatin, carboplatin

Abbreviation: BCG, bacillus Calmette–Guérin.

1.3 Concept of Personalized Medicine

Personalized medicine is the next evolutionary stage of development for traditional health care, building upon the strong foundations of evidence-based observation, symptomatic analysis, and pathologic expression/presentation [9]. Scientific advances in several emerging fields, such as bioinformatics, systems biology, and nanomedicine, are providing scientists and clinicians with extraordinary access to a wealth of information with tremendous clinical value. As new tools are invented to integrate and process this collection of patient data, the clinical boundaries that distinguish patients as individuals become less blurred, providing clarity to resolve a patient's specific needs. Treating the masses with standardized "one-size-fits-all" therapies become less acceptable, and ethical, as new clinical evidence becomes accessible with the promise of more efficacious courses of action. Current applications of personalized medicine integrates such information as a patient's molecular profile or genetic map to supplement conventionally acquired patient information, such as mammogram images and/or histological pathology, prior to determining the final treatment strategy; however, this just represents the "tip of the iceberg" of the wealth of potentially applicable clinical knowledge that new emerging technologies can provide access to [9].

1.4 Nanotechnology in Medicine

The day Drs. Smalley, Curl, and Kroto published their discovery of the carbon-60 fullerene in *Nature* (1985), they effectively established a new field called "nanotechnology" and introduced to the scientific community a world that exists at a minute scale where material behavior can no longer be predicted by conventional wisdom and theory [10]. Unlike biotechnology, which presented clinical medicine with innovative therapeutically "active" agents that necessitated the creation of a new class of drugs, for example, monoclonal antibodies, molecular targeted inhibitors, and recombinant proteins, among others, nanotechnology offers the ability to exploit a "toolbox" of novel material properties that may be

applied to offer new approaches to fight human disease. Nanomedicine facilitates interaction with disease processes at the cellular and molecular scale with the objective of disrupting, abating, or terminating pathologic progression. Furthermore, nanotechnology provides unique access to biologically relevant information and means to control drug release profiles pending on device integration and design.

1.5 Injectable Therapeutics

1.5.1 *Personalization by Design of Nanovectors with Lesion-Specific Transport Properties*

The fundamental basis for the administration of drugs or imaging agents is to achieve a favorable therapeutic/diagnostic outcome with minimal detrimental adverse reactions. When referring to any systemically injectable therapeutic, diagnostic, or theranostic agent, the set of obstacles preventing the mass transport among various compartments/systems of the body (e.g., circulation, tumor tissue, interactions with various cells on the cell membrane level and in subcellular compartments) should be clearly understood. These barriers, also termed biobarriers, can be of biological, physical, chemical, or combined (biophysical, biochemical, physicochemical) nature. Sequentially, from the point of intravenous administration, biobarriers can involve enzymatic degradation of the active agent, inefficient margination in the bloodstream, inability to overcome vascular endothelium, and insufficient delivery into affected cells [11, 12]. To demonstrate the effectiveness of these combined biobarriers, it has been calculated that only 1 out of 100,000 molecules of a drug successfully reaches the intended pathological site. Thus, to achieve therapeutic efficacy, unreasonably high doses of the active agents should be administered with 99.99% distributing to unintended sites, causing unwanted side effects. As an example, studies in Kaposi's sarcoma models demonstrated that ~0.001% of doxorubicin accumulated at tumor sites in patients [13].

Solid tumors, as well as several other pathologies (e.g., cardiovascular, inflammatory, and infectious diseases), can be generally

considered as diseases of biobarrier dysregulation ranging from the molecular to whole-body scale. As such, novel strategies must be conceived to circumnavigate or, if possible, overcome these barriers to drug delivery. Initially postulated as the "magic bullet" theory by Paul Erhlich early in the 20th century [14], and initially considered too outlandish, the idea of getting the right amount of drug to the right place at the right time is now a possibility, thanks to advancements in nanotechnology. Such is the immense potential of nanotechnology to surmount these biobarriers that the field of nanomedicine, a science that enables the clinical use of existing agents through the utilization of nanoscale (1–1,000 nm) constructs [11], has yielded several drug-containing platforms currently used in clinics. This is best exemplified by Doxil®, a polyethylene glycol (PEG)-ylated liposomal formulation of doxorubicin, approved by the US FDA in 1995 for the treatment of Kaposi's sarcoma [15]. Liposomal doxorubicin was able to collect more efficiently in tumors by taking advantage of the impaired endothelial barrier integrity in cancer lesions, essentially using the tumors' own biology against them. The enlarged fenestrations in tumor-associated angiogenic endothelia, and the resulting hyperpermeability of the neovasculature in the tumor microenvironment, explain why systemically injected nanoparticles tend to accumulate more in tumor sites. The proposed mechanism, called the enhanced permeation and retention (EPR) effect, was initially described by Maeda et al. [16–19] and is considered the main reason underlying the therapeutic index advantages stemming from the use of nanoparticles for drug delivery. Moreover, the addition of PEG to the surface of doxorubicin liposomes significantly increased the blood circulation time from 10 minutes to 50 hours [20], effectively overcoming the barrier sequestration by the mononuclear phagocyte system (MPS), a system of monocytes and macrophages that effectively scavenge foreign particulates. Last but not least, the encapsulation and packaging of the drug within the core of the liposome help protect the drug from enzymatic degradation, all the while preventing release until its arrival at the site of action. This not only maximizes efficacy at the site of action but also decreases harmful side effects, such as cardiotoxicity in the case of doxorubicin. These and many other advantages afforded by nanoparticulate systems for drug

delivery are the reason for the hundreds of clinical trials currently underway, making nanomedicine a significant player in the current therapeutic/diagnostic options in oncology for the past two decades.

A recent boom has occurred in the field of nanomedicine, with several novel nanoscale platforms generated for drug delivery purposes. However, the most ubiquitous platforms, either in clinics or in clinical trials, remain liposomes and micelles. Liposomes were among the first nanoparticle platforms approved for clinical use, helping pave the way for future generations of nanoparticles. Liposomes are phospholipid-based bilayered membrane structures with sizes approximating ~100 nm in diameter [21]. The advantages of liposomal doxorubicin have been detailed earlier, with their impact on patient survival proving highly impressive. In one study, 53 patients with advanced Kaposi's sarcoma underwent liposomal doxorubicin administration every three weeks. Of these patients, 19 showed a partial response, while 1 patient experienced a clinically complete response [22]. Since then, several other liposomal formulations have found their way into the clinical arena. One such example, LErafAON, is a liposomal formulation of the *raf* antisense oligonucleotide. These liposomes are meant as an adjuvant therapy, as the oligonucleotide acts on c-*raf*, a protein that enables tumors to become resistant to radiation or chemotherapy [23]. In a phase I trial, patients undergoing radiation therapy were administered LErafAON twice a week, with 4 of 12 patients presenting stable disease and 4 of 12 showing a partial response [23, 24].

Polymer micelles represent an emerging nanomedicine platform, currently undergoing various phases of clinical trials in several countries. These spherical nanostructures, ranging from 10 to 100 nm, were first developed for drug delivery by Ringsdorf et al. in the early 1980s [25]. Polymer micelles are composed of amphiphilic-block copolymers and form spherical structures through self-assembly in aqueous environments [26]. The unique chemistry of polymer micelles proves highly advantageous for chemotherapy. Firstly, the hydrophobic core that results from their self-assembly provides an ideal compartment for the encapsulation and solubilization of water-insoluble drugs, which most anticancer agents prove to be, given their polycyclic nature [27]. Secondly, the hydrophilic

outer shell made of PEG, arising also from the self-assembly process, naturally provides protection from aggregation and opsonization, resulting in increased circulation times [28]. Other advantages include the inclusion of novel polymers that allow for chemical attachment of drugs or for incorporation of functionalities for controlled release [29] and the outfitting of micelles to include targeting moieties for enhanced tumor accumulation strategies [30].

As mentioned previously, polymer micelles are emerging as nanoplatforms with immense potential for chemotherapy. NK911, a micellar formulation of doxorubicin (∼40 nm in size), showed long circulation times and resulted in a partial response in a patient with metastatic pancreatic cancer in a phase I clinical trial [31]. A cisplatin formulation of polymer micelles, NC-6004, recently entered clinical phase I trials. Seven of seventeen patients treated had a stable disease response, with much less toxic side effects and associated treatment morbidity [32].

The goal of personalized therapy is to have nanovectors serve as tools for exploring biobarriers, as well as instruments designed to overcome or take advantage of these barriers to efficiently deliver therapeutics to tumor sites. This especially holds true while considering that biobarriers largely vary from one type of disease to another, from patient to patient, and from lesion to lesion, changing also over time in the course of therapy. It should also be kept in mind that inefficient negotiation of sequential biobarreirs can prevent, for example, molecular recognition, at the disease site. If the agent is not delivered in close (submicron) proximity to the specific cell population (e.g., tumor cells) that expresses the antigen, it cannot create a close-enough contact for receptor–substrate interaction governed by Michaelis–Menten kinetics. In this case, the specificity in receptor recognition observed in vitro will not be confirmed in in vivo studies. Thus, the increased molecular selectivity and resolution of the problems associated with biobarriers have in many cases proven largely to be reciprocally exclusive processes.

Recently, a paradigm shift in the design of nanovectors occurred with the emergence of logic-embedded vectors [9, 12]. These multifunctional constructs comprised of several nanoelements were designed to act in a synergistic fashion to sequentially avoid/overcome biobarriers and efficiently codeliver multiple payloads to the disease

site [33–37]. The emblematic system in this subcategory is the multistage system designed to perform a time sequence of functions that involve cooperative coordination of multiple nanoparticles and/or nanocomponents. The system, recently reviewed in [38], is based on the nanoporous silicon particles (first stage) that utilize their unique nonspherical geometry in concert with active tumor biological targeting moieties to efficiently deliver payloads of second-stage nanoparticles (S2NPs) to the disease site. First-stage nanoporous silicon particles are specifically designed through mathematical modeling to exhibit superior margination and adhesion properties during their negotiation through systemic blood flow en route to the affected site [39–41]. Particle characteristics such as size, shape, porosity, and charge can be exquisitely controlled with precise reproducibility through semiconductor fabrication techniques [34, 42, 43]. In addition to its favorable physical characteristics, the stage 1 particle can be surface-treated with such modifications as PEG for MPS avoidance [34] and equipped with biologically active targeting moieties (e.g., aptamers, peptides, phage, antibodies) [44–46] to enhance the specificity of tumor targeting and imaging [36]. This approach decouples the challenges of 1) transporting therapeutic agents to the tumor-associated vasculature and 2) delivering therapeutic agents to cancer cells. Within the nanoporous structure of stage 1 particles, S2NPs can be safely delivered into the intended vascular target. S2NPs generically represent any nanoparticle construct within the approximate diameter range of 5–100 nm. Various nanoparticle payloads were investigated, including liposomes [47], carbon nanotubes [37, 48], iron oxide [9] and gold nanoparticles [44], fullerenes [48], polymeric micelles, and others. The ability to load multiple payloads in a single multistage particle was also demonstrated [37]. It is important to emphasize that unlike its nonporous counterpart, porous silicon is biodegradable, with the degradation product being harmless orthtosilicic acid [34]. This has been shown in various studies in vitro in cell cultures of immune and endothelial cells as well as in vivo in healthy animals that the system is biocompatible [34, 36, 47, 49].

As predicted by mathematical modeling, the nonspherical geometry of the first-stage particles contributes to accumulation of up to five times higher concentrations of the nanovectors in the tumor

microenvironment as compared to their spherical counterparts [50]. These findings support the proposed mechanism of action for the multistage system, where each stage performs part of the journey from the site of administration toward the target lesion, negotiating one or more biological barriers and adding a degree of targeting selectivity in the process. Following arrival to the tumor microenvironment, second-stage particles are released, permeating into the tumor mass, further reaching the target cells with biological specificity. It was shown that single or multiple payloads can be specifically released at different subcellular locations, with potentially different time release profiles. Personalization in this case is based on the optimal/rational design of the nanovector geometry for specific accumulation into the tumor site, porosity of the carrier suitable for loading specific S2NPs and attaining the desired release kinetics, and surface properties for recognition of the inflamed endothelium in the neovasculature.

Recent advances in molecular oncology enabled a better understanding of the pathological pathways involved in tumor formation and maturation. Elucidation of some of the molecular mechanisms brought about new potent drug candidates. Small interfering ribonucleic acid (RNA) (siRNA) therapeutics belong to one important class of new potent agents. These double-stranded RNA molecules, able to specifically silence gene activity [41], were discovered a decade ago by Fire et al. [51]. The main obstacle in the clinical translation of siRNA therapeutics is the delivery overcoming some of the above-mentioned biobarriers, including an extremely prompt degradation in physiological conditions and an inability to cross membranes. While siRNA liposomes have shown some efficacy in animal studies [52, 53], frequent intravenous doses seem to be unfeasible and are not cost effective in the clinical setting. The antitumor efficacy of multistage silicon vectors (MSVs) loaded with neutral dioleoyl phosphatidylcholine (DOPC) nanoliposomes containing EphA2-specific siRNA was tested in animals with two independent orthotopic mouse models of ovarian cancer [47]. EphA2 is an oncoprotein overexpressed in most malignancies, including ovarian tumors. Interestingly, after a single treatment with EphA2-targeted MSV and without concurrent chemotherapy, gene silencing, and a decrease in tumor burden, evaluated through cell proliferation

(Ki-67) and angiogenesis (CD31), were observed. To achieve a similar effect with siRNA-DOPC, six administration doses were required with a twice-higher total administered amount of siRNA. The mechanism of action of sustained liposomal siRNA delivery was likely to rely on surface modification, tissue distribution, and slow biodegradation of the first-stage mesoporous particle (S1MP). S1MP not only served as storage for liposomal siRNA but also shielded siRNA oligos from degradation by enzymes inside the body. This novel approach opens new avenues in personalization of siRNA therapeutics through controlled delivery of synergistic payloads in a time-controllable fashion.

Another example of a nanovector with emerging imaging properties is an agent based on magnetic resonance imaging (MRI) contrast agents loaded into the porous structure of the first-stage particle [48]. The MRI resolution determines the efficiency of early diagnosis, treatment monitoring, and prognosis and can be highly enhanced by using contrast agents based on paramagnetic materials. The most commonly used contrast agents in the clinical setting are gadolinium (Gd) chelates. Gd^{3+} ions are highly toxic in free form and, thus, have to be chelated to minimize toxicity. However, chelation also significantly reduces the number of coordination sites, resulting in low relaxivities of less than 4 $mM^{-1}s^{-1}$ at 1.41 T. The multistage approach was used in the design of a new category of MRI contrast-enhancing agents. Gd-based contrast agents, such as a clinically used chelate (Magnevist®, MAG) and Gd^{3+}-loaded carbon nanoparticles (carbon nanotubes, gadolinium nanotubes (GDNTs), and fullerenes, gadolinium fullerenes GFs) were loaded within the nanoporous structure of discoidal (D) or hemispherical (HS) S1MP [48]. The resulting MSV constructs showed a significant boost in longitudinal relaxivity, resulting in up to 40 times higher values than clinically used MAG. The proposed mechanism of the prominent enhancement in the MRI contrast is based on the geometrical confinement of Gd-based contrast agents within the porous silicon S1MP, which affects the paramagnetic behavior of the Gd^{3+} ions by enhancing interactions between neighboring contrast agents through reduction of the mobility of water molecules and the ability of contrast agents to rotate [48].

To conclude, this section described how impairment in the transport phenomena in the disease tissue can be utilized for personalization or even individualization of the injectable therapeutics. Ideally, the individualization of therapy is consequently built in the carrier vector, which enables direct imaging observation of the lesion, and is present regardless of the drug delivered, though obviously optimal when molecularly targeted drugs are delivered. The time dynamics of the evolution of the lesion do not essentially necessitate an adjustment in therapeutic payload, since the response to the evolution of the lesion and its microenvironment may be built in the individualization of nanovectors.

1.6 Molecular Imaging

1.6.1 *Collection of Patient-Specific Data for Tailoring Treatments*

Clinical imaging is experiencing a major paradigm shift, moving away from structural-based diagnostics to dynamic molecular imaging. The purpose of molecular imaging is to facilitate the noninvasive detection and visualization of morphological and biochemical changes that influence disease and/or its response to therapy. Progress in this field has been driven largely by applications in oncology, from the identification of specific molecular pathways associated with tumor growth and progression to the clinical monitoring of cancer biomarkers before and after treatment [54]. With the advent of molecular-specific chemotherapies, it is becoming increasingly important to collect patient-specific data for tailoring treatment regimens.

Molecular imaging is already in clinical practice today. Positron emission tomography (PET), single-photon-emission computed tomography (SPECT), and MRI are some of the first clinical imaging modalities capable of generating images with molecular specificity. These technologies monitor the localization of different, exogenously administered contrast agents to collect information about tissue anatomy, physiology, and metabolism. New contrast

agents for these and other imaging modalities are continually being introduced to enhance clinical care.

Nanoparticles have been proposed as an enabling technology for molecular imaging. Advantages of nanoparticles include high contrast, tunable physical properties, long circulation times, and ease of integrating multiple functionalities [55, 56]. A variety of nanoparticle-based contrast agents are currently under development for a range of clinical indications, including superparamagnetic agents, metal nanoparticles, liposomes, and more. Each of these platforms differs in bioavailability, pharmacokinetics, toxicity, immunogenicity, and specificity. It is likely that a variety of different and specialized nanoparticle platforms will be required for targeting different disease processes. Several nanoparticle-based contrast agents have entered the market, and additional products are currently undergoing clinical testing or entering the pipeline. The integration of molecular imaging with nanoparticle-based contrast agents is expected to have a major impact on the detection, diagnosis, and decision making for personalized treatment.

Much of the innovation in nanoparticle-based contrast agents is driven by the quest for personalized medicine. Many nanoparticles under development contain active targeting ligands. These ligands are used to enhance the specificity of contrast agents, resulting in the localized accumulation of contrast agents at the molecular target of interest. Targets include cancer biomarkers (e.g., human epidermal growth factor receptor 2 [HER2], epidermal growth factor receptor [EGFR], integrin $\alpha v\beta 3$, prostate-specific membrane antigen [PSMA], CD20), inflammatory biomarkers (e.g., E-selectin, intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1]), apoptosis markers, and many others. An early example of molecule-specific targeting for in vivo imaging was provided by Weissleder et al., who used monocrystalline iron oxide functionalized with antimyosin F_{ab} fragments to detect myocardial infarcts in rats [57]. More recently, effort has been directed toward the rational design of ligand attachment [58]. It has been demonstrated, for example, that nanoparticles that present multiple small ligands have increased target affinity over monovalent particles [59]. Mathematical models that consider parameters such as ligand density, ligand accessibility, and receptor

distribution have been used to successfully improve nanoparticle specificity in vivo [60, 61].

Molecular-specific, nanoparticle-based contrast agents have the ability to provide information that is not readily available using conventional diagnostics. In the simplest case, an intravenously injected contrast agent could be used to noninvasively detect the expression of biomarkers important for disease diagnosis and treatment selection, without the need for biopsy. The design of nanoparticles with long circulation times, or the repeat administration of nanoparticles, would facilitate dynamic monitoring of how biomarker expression changes with time, which is important for determining disease progression and response to therapy. More complex nanoparticle-based contrast agents, known as "smart" bioprobes, could be used to collect functional information from specific molecular targets. In cancer, for example, elevated telomerase activity is associated with poor prognosis and increased risk of recurrence [62–65]. Measurement of telomerase activity and other prognostic proteins could be used for the smarter selection of personalized therapy.

1.7 Early Detection

1.7.1 *The -Omic Technologies and Systems Biology: Resolving the "Portrait of Health"*

One of the most recognized leaders and visionaries of personalized medicine is Leroy Hood, MD, PhD, president and cofounder of the Institute for Systems Biology in Seattle, Washington. Dr. Hood states, "Over the next 5–20 years, medicine will move from being largely reactive, to being predictive, personalized, preventive, and participatory (P4)" [66]. The movement can be simplified through the analogy of comparing a disease to a digital image. Each pixel of the photo adds its enabling contribution to resolve an image on a digital canvas. No single pixel has the ability to reveal the complete image—just as information regarding the metabolism of sugar can provide complete evidence and cause of disease. Technological advancements have given the scientific community a new resource of information contained in the study of "-omic technologies"

(e.g., genomics, proteomics, metabolomics, transcriptomics, etc.); however, this wealth of information represents only a fragment of data that contributes to the complete understanding of the state of health. A systems biology approach respects each "pixel" of clinical data and offers a strategy that compiles and integrates all available information to form a more complete "image" or "portrait of health" that best represents the entire system or organism.

The inherent nature of nanotechnology offers the ability to interact with the scale of biology through a vast spectrum of nanoconstructs and devices. In the context of early detection, nanotechnology provides an enabling role for the utilization of -omic approaches, and furthermore, the nanoscale offers the intrinsic ability to multiplex procedures in a high-throughput nature and to analyze minute quantities. Here is a summary of a few examples in the fields of high-throughput technology, nanodiagnostics, and nanofluidics.

1.7.1.1 Microarray technology

Since first demonstrated as an analytical device by Schena et al. in 1995 [67], the microarray has been extensively developed to be a multiplex lab-on-a-chip for high-throughput screening. The general production of microarrays consists of printing and immobilizing a series of chemical molecules, nucleic acids, proteins or lipids on a functionalized substrate. Detection with a fluorescent probe and imaging capture are carried out after incubating the analyte on the array surface [68]. Thanks to their miniaturized size and large amount of genetic information, DNA microarray-based technologies have exhibited tremendous promise for unraveling complex gene expression profiles of cancer clinical diagnosis [69]. The formation and progression of cancer involve mutation in various genes, including the change of both gene structure and gene expression. DNA microarrays are capable of determining alterations in tens of thousands of genes simultaneously. Emerging results suggest that the use of DNA microarrays can distinguish between tumors of similar morphology and predict response/resistance to anticancer therapies [70, 71]. Another member in the family of microarray technologies, protein microarrays, has also been explored as a

promising method for a wide range of applications, including the identification of protein–protein interactions and quantification of proteins present in samples, protein–phospholipid interactions, small-molecule targets (like identification of drug activity), and substrates of proteins kinases [72]. Depending on different functions to study the biochemical activities of proteins, protein microarrays can be classified into three types: analytical microarrays, functional microarrays, and reverse-phase microarrays. To date, the most sensitive method for protein microarray processing is the "sandwich assay" based on the enzyme-linked immunosorbent assay (ELISA) technique. It uses two antibodies that bind with the same antigen simultaneously with dual the function of immobilization and detection. Biomarker concentration in the analyte is demonstrated by the intensity of the fluorescent signal. Through investigating proteomic information in a single pattern, protein microarrays enable us to accelerate and improve clinical diagnostics. For example, Joos's team is developing sandwich immunoassays for the detection of the well-established prognostic indicators and predictive factors involved in tumor proliferation, tumor vascularization and metastatic potential, for example, the cell surface receptors HER2 and EGFR and hormone receptors ERα and PR [73]. It has been confirmed that microarray technology will undoubtedly improve diagnosis and management of patients with specific cancers. However, it is impossible that microarray technology will fully replace current existing methods. A more individualized approach to cancer patient management could be achieved by efficiently combining old and new technologies.

1.7.1.2 Nanodiagnostics

A variety of nanodiagnostic platforms are under development for the detection and monitoring of cancer [74, 75]. These diagnostics rely on the use of nanoscale particles or nanotextured surfaces to selectively capture and identify molecules of interest. Progress in this field has largely been driven by the need to detect clinically relevant biomarkers in a rapid, sensitive, and cost-effective manner. This is especially important for the early detection of cancer, in which patients may not show any overt symptoms before diagnosis.

Particle-based nanodiagnostics generally rely on the binding between nanoparticles and target molecules of interest to produce a measurable signal. Optically active nanoparticles, such as quantum dots and gold nanoparticles, can be readily engineered to self-assemble in recognizable patterns in the presence of specific targets. Magnetic nanoparticles may be used to pull targets out of a large mix of analytes for increased sampling sensitivity. An elegant example of particle-based nanodiagnostics for early detection is the polyvalent gold assay developed by the Mirkin group [76, 77]. Here, monodisperse gold nanoparticles functionalized with oligonucleotides have been used to detect prostate-specific antigen (PSA) from serum with femtomolar sensitivity [76]. Similar technologies, designed to test genetic sensitivity to warfarin and genetic predisposition to blood clots, have already been commercialized and FDA approved. Further refinements to this scheme, such as the addition of pH-sensitive chemotherapeutics [78], have the potential to expand the utility of polyvalent gold assays beyond the in vitro setting.

Nanowire biosensors provide an alternative approach for detecting known biomarkers with high sensitivity. Semiconductor nanowires patterned in two dimensions and three dimensions, for example, take advantage of field effects to produce a change in conductance upon the binding of target molecules. The high surface-to-volume ratio of nanowires allows molecules in solution to be detected with high sensitivity. A major advantage of this approach is that genetic alterations or the presence of rare molecular biomarkers can be detected without additional amplification. A two-dimensional (2D) silicon nanowire platform functionalized with single-stranded DNA has been used by Wu et al. to detect a cancer BRAF gene mutation [79], a common mutation associated with a variety of human cancers. Nanowires functionalized with antibodies or aptamers have demonstrated multifold increased sensitivity over ELISA assays, facilitating the detection of cancer biomarkers such as VEGF [80] and CA125 [81]. Multiple biomarkers may also be assayed simultaneously. Zheng et al. have described the multiplexed detection of PSA, PSA-alpha1-antichymotrypsin, carcinoembryonic antigen (CEA) and mucin-1 in serum with pg/ml sensitivity [82]. In the future, it is likely that nanowire biosensors will be constructed as

large microfluidic circuits for sampling a variety of genes or proteins from clinical samples [83].

Nanocantilever systems are another category of nanodiagnostics for highly sensitive molecular detection. Like nanowires, they have no intrinsic chemical selectivity and are coated with self-assembled monolayers, nucleic acids, antibodies, or peptides. When a target molecule binds, surface stresses cause the lever to undergo nanomechanical bending that can be measured using a variety of techniques. Multiplexed DNA and RNA hybridization to nucleic acids immobilized on cantilever tips has shown sensitivity in the nanomolar [84] to picomolar range [85]. Cantilever nanosensors have been successfully used to detect alpha-fetoprotein (AFP), a potential prognostic and diagnostic marker of hepatocarcinoma, by sensing resonance changes in cantilever movement in response to AFP adhesion to immobilized antibodies [86]. The dynamic range of such a system can be varied by using an array of cantilevers with different tip sizes, as demonstrated by Wu et al. for the detection of PSA (from 0.2 ng/ml to 60 g/ml) [87]. Additional enhancements, such as the use of antibody-presenting silica beads as extra weight transducers, can improve the lower sensitivity to the pg/ml range [88]. Future advances in efficient immobilization techniques, nanoscale motion detection, and microfluidics integration are expected to make such chips a clinical reality.

1.7.1.3 Nanofluidics

Leveraging the technological advances of the integrated circuit, scientists applied novel semiconductor fabrication techniques to transition from solid-state microfluidic microelectromechanical (MEM) devices [89] to bring forth the next evolutionary embodiment that feature the integration of nano-channeled structures [90, 91]. The ability to achieve nanoconfinement through silicon nanofabrication techniques has enabled such achievements as increased sensitivity and specificity of biomolecular detection and the ability to manipulate DNA to screen for infectious disease. An important feature of nanofluidic devices is the inherent ability to employ miniscule amounts of a sample with the reproducibility and reliability necessary for use as clinical diagnostic/screening

tools. This benefit can be attributed to the increased surface-area-to-volume ratios and subsequent improvement of the surface interactions between the nanochannel wall and target molecules unique to the scale of nanotechnology, relative to their microfluidic counterparts [92].

In the context of early detection of disease, the Kitamori laboratory has engineered a μ-ELISA system with integrated nanoscaled features for the detection of AFP [93, 94]. This device achieves single-molecule detection by employing a one-dimensional nanochannel (500 nm deep, 100 μm wide, and 70 mm long) to create an environment that offers an increased bound-analyte-to-volume ratio to improve device sensitivity when used in conjunction with a fluorescence microscope [93]. The Kitamori device provides evidence of the benefit of the nanoscale to increase sensitivity; however, Wang et al. leverage nanoconfinement to more efficiently aggregate metal nanoparticles and target molecules to improve surface-enhanced Raman scattering (SERS) [95]. This device features microchannel to nanochannel transitions that effectively accumulate, or "trap," molecules and nanoparticles at the junction to create "SERS-active clusters" that allow the detection of trace molecules when excited by a laser source [95]. Another embodiment of the utilization of nanofluidics has been used to develop a novel DNA diagnostic device. Hashioka et al. engineered a device with 50 nm gap arrays that allow DNA to be "stretched, denatured, hybridized, and detected" [96]. This approach allows the analysis and detection of viral DNA to be applied to the diagnosis of influenza, human immunodeficiency virus (HIV), and other infectious diseases.

1.7.1.4 Biomarker discovery

Advances in early detection will be heavily impacted by the discovery and validation of new biomarkers of disease. Even after the release of the entire human genome sequence nearly a decade ago, diagnosis and prognosis of many diseases still rely on the conventional biochemical and clinical methods. These methods do not reflect the vast heterogeneity and complexity of the disease, and they poorly predict clinical outcomes and response to therapy. Additionally, diseases like cancers are often

detected only in advanced stages and that too by a combination of physical examination, X-rays, needle biopsy, and, in some cases, blood tests. For example, breast mammograms are capable of detecting the tumors only when they have grown to a critical size, and mammogram sensitivity can be as low as 34% in some of the subtypes [97]. Similarly, the common method of detecting cardiovascular disease is to measure lipid profiles and perform electrocardiography (ECG) only when symptoms such as chest pain are present. Therefore, novel and reliable biomarkers are needed not only to assess the response to therapy and progression of disease but also to detect disease early enough to increase survival rates [98].

With the recent advancements in -omic technologies, it is now possible to identify a panel of biomarkers for a disease rather than the traditional single gene-/protein-based markers. Identification of such a panel of markers has facilitated avenues for personalized medicine. Gene expression profiles have been reported to be useful for the classification of cancers and cancer subtypes [99, 100]. Progress in the field of mass spectrometry and sample processing methods made proteomics a promising -omic science in identifying reliable and patient-specific biomarkers. Besides other clinical samples such as tissue specimens, the highly complex body fluids plasma and serum have been suggested as major sources of biomarkers [101, 102]. Since biologically important fluids can be acquired using minimally invasive techniques, they can be sampled at any stage of disease. Despite the application of several protein separation and mass spectrometry methods [9, 101, 102], due to the vast concentration range of their proteins and variability, serum and plasma remain challenging for the identification of low-abundance, clinically important biomarkers. Nanotechnology promises significant advances in molecular detection by improving the sensitivity and specificity over current technologies and accelerating novel biomarker discovery for individualized therapy [103].

Blood contains a treasure trove of previously unstudied biomarkers that could reflect the ongoing physiologic state of all tissues [104]. These are the low-molecular-weight (LMW) proteins and peptides that result from degradation and enzymatic cleavage of larger proteins secreted or released into the bloodstream. Although proteins entering the blood from the surrounding tissue

are much less abundant, this treasure trove could consist of all classes of proteins whose diagnostic information has been largely unknown until now [104, 105]. To overcome the interference of large and high-abundance proteins and to enrich the level of the clinically important LMW proteins, the Ferrari laboratory has developed a novel nanotechnology-based silica chip [106, 107]. These mesoporous silica chips are made through a process involving self-assembly of a mixture of triblock copolymers and hydrolyzed silicate precursors [108, 109]. Evaporation of solvents after spin-coating drives the self-assembly, and thin silica films with uniform nanoscale pore size and thickness are subsequently formed.

Nanoporous silica chips (NSCs) are convenient and easy to use as their use involves only three simple steps: sample loading, washing, and elution [106] (Fig. 1.2). Serum or plasma samples can be studied with just a few microliters, and the washing step ensures removal of high-molecular-weight proteins excluded by the nanopores. Thus, NSCs help for 1) the selective removal of high-molecular-weight and abundant proteins, such as albumins, and 2) the enrichment of low-abundance, LMW proteins from complex samples, such as serum and plasma. The LMW proteins eluted from the chips are subsequently spotted on to a matrix-assisted laser desorption/ionization (MALDI) plate, along with a suitable matrix (α-cyano-4-hydroxycinnamic acid [CHCA]), and protein profiles are obtained [107]. By comparing the MALDI profiles for control and test serum/plasma samples (Fig. 1.3), biomarkers that are specific for diseases such as cancer can be identified at an early stage.

Nanotechnology-based NSCs not only rely on the ease and convenience of sample processing, they allow controllable pore size and surface chemistry, which facilitates selective enrichment of LMW peptides and proteins with a specific molecular weight range and physico-chemical properties, and post-translational modifications [106, 107, 110]. Very recently, mesoporous thin silica films with precisely engineered pore sizes that sterically select for molecular size combined with chemically selective surface modifications (i.e., Ga^{3+}, Ti^{4+}, and Zr^{4+}) that target phosphoroproteins are demonstrated [110]. As the NSCs could selectively exclude high-molecular-weight proteins such as trypsin, the trapped LMW peptides and proteins are also reported to be protected from enzymatic degradations [107,

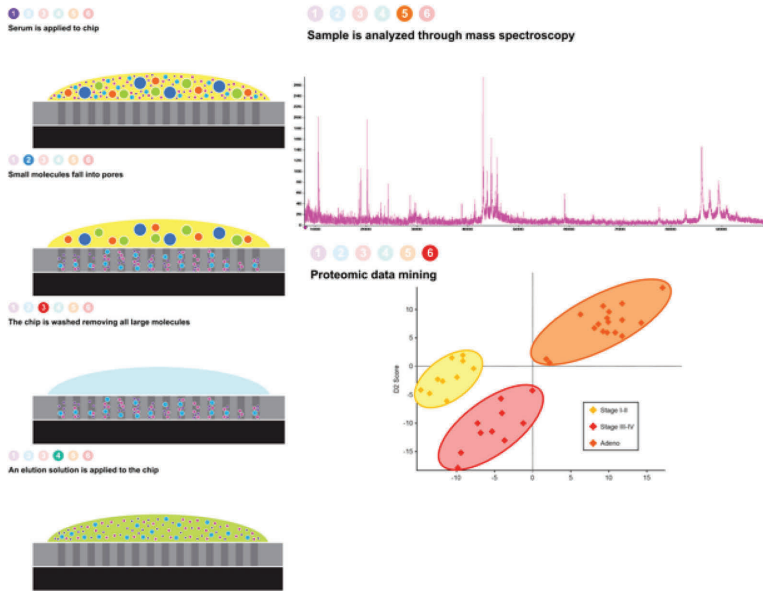

Figure 1.2 Sample fractionation using mesoporous silica chips. The schematic shows the four primary steps in sample processing, which results in the removal of high-molecular-weight, high-abundance proteins and enrichment of low-abundance, LMW proteins. The elution sample is then analyzed via mass spectroscopy, and the data can be subsequently mined and analyzed.

110]. There are other research efforts that are also attempting to tap into the potential of LMW proteins. Luchini et al. demonstrated the use of hydrogel particles for harvesting and protecting LMW peptides and proteins from biofluids [111]. This technology involves introduction of an affinity bait molecule into *N*-isopropylacrylamide for the capture and protection of LMW peptides and proteins.

1.8 Regenerative Medicine and Tissue Engineering

Cancer can be an incredibly disruptive and destructive disease both physically and mentally for patients to endure. For some, the disease eats away at one's most fundamental structure—bone. And for others, invasive disfiguring surgeries are performed to contain

spreading and to protect the remaining healthy tissues from being invaded. For too many, cancer leaves an indelible mark, which now scientists and clinicians are trying erase with new regenerative medicine approaches and tissue engineering technologies aimed to rebuild that which is destroyed and to offer patients solutions to address their needs.

1.8.1 *Stem Cells for Regenerative Medicine*

As the primitive and most potent cell source available, stem cells naturally regenerate and heal damaged tissues in the body. Stem cells come in many different forms, depending on the age of the donor and the harvest site. Embryonic stem (ES) cells are derived from human embryos created through in vitro fertilization. These cells and their early progeny are termed either totipotent (capable of becoming any human tissue) or pluriponent (capable of differentiating into tissues of the three germ layers: endoderm, mesoderm, and ectoderm) [112–114]. However, ethical and political questions surround the research and clinical application of ES cells [115, 116]. Additionally, ES cells will inherently be from a different donor with unique genetics, which may require permanent immune suppression to prevent the rejection of implanted cells and secondary complications [117]. To avoid this dilemma and in the spirit of "personalized medicine," significant research has been conducted in the area of mesenchymal stem cells (MSCs). MSCs are derived from adult tissues, including bone marrow, adipose, and other mesoderm-related systems. These cells have demonstrated the ability to differentiate into bone, cartilage, fat, muscle, and even nerve and cardiovascular tissues in vitro and in vivo [118–123]. For nearly 30 years, MSCs have purified and expanded in vitro to attain great numbers of potent cells for tissue regeneration purposes [124, 125]. MSCs secrete factors or cytokines essential for signaling the host system and stimulating necessary functions for cell maintenance and tissue growth. By using a patient's own cells, the donor-specific levels and secretion rates of these cytokines are exactly matched [126, 127]. With detectable variations in metabolic signaling cascades from person to person, personalized therapeutic strategies, including autologous MSC delivery, appear more efficient

and likely more effective for long-term clinical success than the use of donor ES cells or MSCs.

To purify MSCs from bulk cell populations taken through procedures, including marrow aspiration and liposuction, cell-sorting technologies were developed based upon the protein markers displayed on the surface of different cell types. Traditional fluorescence-assisted cell sorting, or FACS, employs fluorescence-labeled antibodies against proteins that can definitively distinguish a cell to be a "stem cell" or not [128–130]. However, this process requires expensive equipment, a time-consuming preparation, and a significant loss of viable cells. New strategies have focused on nanotechnology for sorting stem cells. Nanoparticles labeled with appropriate antibodies are fabricated from materials such as iron oxide, silicon, or aptamers [131–133]. These particles possess a tunable magnetic character that can separate linked MSC-nanoparticle conjugates by exposure to magnets or electric fields. This method allows for point-of-care prospective isolation of autologous MSCs in an economical and time-efficient manner.

1.8.2 *Controlled Drug Release*

Regenerative medicine requires the delivery of growth factors to stimulate cell growth and migration, angiogenesis or blood vessel formation, and differentiation agents to direct MSCs toward their final fate. Inductive factors are necessary to promote MSC differentiation toward destined tissue lineages such as bone, cartilage, muscle, or fat [134–138]. Scientists have endeavored to deliver these types of molecules or growth factors in a controlled and sustained manner [139, 140]. Anticancer drugs and antibiotics to treat tumors and prevent primary and secondary infections, respectively, have also been used with these technologies for sustained release over days, weeks, or even months. Nanosized particles, or microparticles with nanofeatures, such as pores or targeting moieties, have been employed in these controlled release strategies for improved sensitivity and bioavailability. This includes particles comprised of polysaccharides, polyesters, silicon, lipids and liposomes, and composites of these materials [141–145]. The combination of materials used, methodology of particle synthesis,

mechanism of drug loading, and pretreatment conditions all affect the release kinetics of the bioactive factors. Combinations of materials, including coatings or surface modifications (electrostatic charge, antibody tethering, etc.), allow for enhanced drug targeting and temporally appropriate release of the particles' biomolecular payload [146–148]. Other manipulations trigger the release of molecules based on environment cues such as temperature or pH [149, 150]. Using different particles or functionalization strategies in concert for the simultaneous delivery of multiple growth factors is sometimes necessary as the molecules' biological functions are dose dependent and may be sequential in the cascade of tissue development. Nanoparticles offer distinct advantages over other drug delivery systems in that they have a significantly greater surface area per mass or volume for expanded release and the ability to travel through a patient's vascular network directly to a targeted site, and they may transverse through cell and tissue membranes.

1.8.3 *Nanotechnology and Biomaterials*

Tissue engineering and regenerative medicine require a combination of three essential elements: cells (either implanted or recruited), locally released growth factors to induce cell activity toward tissue formation, and biocompatible scaffolds to direct and support tissue growth [151]. These scaffolds may be composed of ceramics, metals, resorbable polymers, or proteins. Recently, scaffolds have incorporated nanomaterials and nanofeatures to provide additional beneficial properties to the material. Carbon nanotubes, porous silicon, mineral apatite crystals, alumoxane, and other nanoparticles have been integrated into polymer or cement matrices for mechanical reinforcement [152–155]. Strengthening of biomaterials is crucial for replacement of load-bearing tissues like bone and cartilage. Magnetic nanomaterials have also been incorporated into scaffolds, allowing for their suspension during in vitro culture in the presence of a electric field for alteration of gravitational effects, for triggering of the release of embedded biomolecules and growth factors, or for enhanced in vivo magnetic resonance imaging [156, 157]. Nanoscale features of the material surface influence biological behavior of local cells (including

induced differentiation) and tissues and include nanofibers and nanoroughness [158, 159]. Nanofibers possess superior surface-area-to-volume ratios to minimize the amount of synthetic scaffold material that is implanted and provide unique dimensions for extracellular matrix deposition. The size of the fibers can also preferentially regulate the infiltration of cells and blood vessels.

As regenerative medicine and nanotechnology evolve as sciences, their futures will no doubt be intertwined. The advantages of nanomaterials for mechanical reinforcement, drug delivery, imaging, and separations will continue to be incorporated into biomaterials, stem cell therapies, and tissue engineering platforms. Nanotechnology provides scientists, engineers, and clinicians with new tools to mimic tissue features in three-dimensional (3D) environments on the nanoscale. Obstacles such as the body's interaction with nanomaterials require further study, but the implementation of these strategies will further enhance the capabilities of tissue engineering and play a pivotal role in nanotechnology-based personalized medicine.

1.9 The Role of Nanotechnology and Personalized Medicine

The journey to recovery for a typical cancer patient begins with detection and treatment and then moves to coping with indelible marks that the disease forever leaves on a survivor. Nanomedicine "levels the playing field" when it comes to fighting disease, providing scientists and clinicians with the tools necessary to battle cancer at the scale of biology. This chapter has provided a brief commentary on how nanotechnology is being applied to the different facets of cancer with an emphasis on patient specificity. New developments in early detection and imaging have been discussed, which provide a wealth of information on how to exploit the vulnerabilities of the disease and to detect its presence. The discussion then continued to inform how this new patient information assists in the design of innovative nanodrugs and novel nano-based drug delivery systems that are predicated upon a patient's own clinical data to optimize timing, accumulation, and effectiveness of the therapy.

The technology review then concluded with nanotechnology's r ole in rebuilding through advances in tissue regeneration and tissue regeneration to help patients fight and/or cope with the trauma of cancer. Cancer manifests uniquely to every patient, and therefore it is intuitive to employ strategies that address and exploit opportunities that render cancer susceptible to treatment—nanotechnology provides the access and tools that may eventually lead to the eradication of cancer suffering.

1.10 Vantage Points: Nanomedicine Advancing Personalized Medicine

1.10.1 *The Evolutionary Process of Personalized Medicine: The Real Drivers of Innovation*

The implementation of personalized medicine will be a gradual evolution of standard medical care that may ultimately take a few decades, if not longer, to be successfully achieved. Where computing power has been, and continues to be, predicted by Moore's law, individualized therapy will not be solely driven by technology innovation. There are too many critical factors that must be addressed, and matured, to inspire acceptance and adoption. These factors refer to (1) the willingness of physicians to embrace and utilize emerging technologies over gold standard procedures, (2) the current regulatory process, (3) the existing corporate philosophy regarding pharmaceutical business models and availability of capital, and (4) the patients' demand for change.

Since a definitive solution or plan is impossible to provide, an assembly of key stakeholders has been asked to provide insight from their unique vantage points.

1.10.2 *A Physician's Perspective*

Current tools used in medicine are inadequate for thoroughly characterizing cellular function at the molecular level. Biological systems are made up of individual molecules operating on a nanoscale, and therefore physiological and pathological processes

at the cell level occur on a nanoscale. Personalized medicine offers tremendous potential to deliver timely, appropriate prevention and care. However, it also adds complexity to the decision-making process, and as every advancement in medical technology, it will play a major role in the costs of health care. In the United States many legislative and government initiatives have been introduced for the support of personalized medicine, such as the passage in 2008 of the Genetic Information Non-Discriminating Act (GINA) and the Personalized Health Care Initiative launched by the Human Health Services (HSS). In Europe the European Personalized Medicine Diagnostic (EPEMED) was created in 2009. The ability to classify and treat diseases by their molecular profiles, avoiding passing the expense and risks of unnecessary medical treatments on to the patient, is the ultimate realization of policy makers, diagnostic manufacturers, and of course clinicians. Physicians must play a role in this fundamental shift in the delivery of health care that will, eventually, involve the population as a whole. The challenges are immense and include regulatory, technological, reimbursement, legal, and ethical issues, to name the most important ones. Realization of personalized medicine is dependent on the ability to collect, disseminate, and process information in the context of clinical care and this will require an electronic health record (EHR) infrastructure to provide access to key clinical data with clinical decision support (CDS) capabilities. In the United States, President's Obama goal is to have an EHR for everyone by 2014. The use of molecular markers to signal the risk of disease or its presence before the disease becomes clinically manifest is the base of personalized medicine, but currently, not all the existing tests have therapeutic options, and sometimes despite the proven value of risk assessment tools, they have not been largely embraced as part of the formal patient evaluation because of both the lack of standards for the clinical data required and the algorithms used. For physicians the constraints and demands of current clinical practice often times discourage the acquisition of this knowledge. The complete application of genomic and personalized medicine in health care will require dramatic changes in reimbursement policies as currently Medicare does not contemplate reimbursement for tests

that are performed in the absence of signs, symptoms, complaints, or personal history of disease [160].

Furthermore new genetic tests are undervalued under present policies. There are suggestions, though, that payment policies are beginning to shift toward the implementation of personalized medicine as several large US insurers have initiated coverage plans that pay for genetic tests that either identify high-risk populations or steer toward optimal therapy. Physicians will have to discern between biomarkers used in diagnostics, therapeutics, and drug development. While advocates of personalized medicine envision the sequencing of the full genome at birth, physicians and all the other stakeholders should not underscore the enormous ethical implication of gene-based tests, as these will blur the boundaries between the healthy and the diseased that are so well defined across the Western world. Where will the "presymptomatic" patients' category fall? The necessary reclassification of health versus disease is going to have a significant social impact as one only thinks about the different social entitlements to health care once a genetic predisposition to any disease is found. How will the knowledge of having any cancer-causing mutations or a copy of the Alzheimer's predisposing apolipoprotein E4 allele impact an individual's life and his or her working life remains to be seen. It is unlikely that the average human being will have the same curious approach of the Nobel Prize winner and codiscoverer of the structure of DNA, James Watson [161]. In the past few years several different direct-to-consumer (DTC) companies have started offering DNA tests design to provide insights into our personal genetic predisposition for certain disease risks. A 2009 comparison from two DTC companies showed that for some diseases, only 50% or less of the predictions agreed between the two companies. The discrepancies arise from the fact that each company has its own criteria on which a set of markers are used in the relative risk calculation [162]. Much needs to be done to move from reactive to predictive and preventive personalized medicine and to incorporate these technologies in clinical practice. It has been hypothesized that physicians are going to be "followers rather than leaders in the clinical translation of pharmacogenetics" because of liability concerns [162]. Physicians will find themselves facing potential lawsuits until the tracking

for the clinical utility of diagnostic and treatment intervention is examined and becomes a standard of care. Physicians should use the full potential of pharmacogenomic data to stratify and hence enrich the population of a clinical trial in order to select patients who are more likely to be responsive to that specific drug so as to reduce the required time and sample size. Last but not least, there are very few medical schools that have included courses on the practice of genomic medicine as there are only a small number of hospitals that have embraced early clinical adoption of personalized medicine. This must be an imperative as the clinician will be the one ultimately providing care and counsel for patients.

1.10.3 *A Regulatory Consultant's Perspective*

The first scientific publications using the term "nanomedicine" are from the year 2000 (Science Citation Index, Institute for Scientific Information, Thompson, Philadelphia, PA, USA), making this an extremely new and challenging field of medicine. It did not take very long for the ingenuity and futuristic thinking behind US pharmaceutical and device companies to see the potential of these exciting new particles. In October 2006, the United States was reported to be leading the field in the use of nanomedicine research, with 32% of the publications and 54% of the patent filings [163]. As in other innovative medical developments, science leads the way, and the regulatory processes required to ensure their safety and effectiveness are compelled to follow.

As scientists discovered more and more uses for nanomaterials, some feared that they could cause more harm than good, and even before these statistics were reported in October 2006, the US FDA was being asked to better regulate nanotechnology. The FDA was "petitioned" in May 2006 by a coalition of consumer groups and environmental groups to increase its regulation of some nanoparticle-containing products and to even recall others [164]. Soon thereafter, in an article for the *Chicago-Kent Law Review*, Jessica K. Fender (2008) claimed that this new "tiny technology" was going to cause "big problems" for the FDA, including stretching the agency's already "extremely thin" resources even further, claiming

that the FDA would not have the necessary funding to provide adequate oversight [165].

The FDA was not blind to what was developing rapidly around it and has not let nanotechnology slip past its radar. Even before these criticisms arose, using the description of nanomedicine as "a technology that allows scientists to create, explore, and manipulate materials that are measured in nanometers", the FDA has assumed oversight for many nanomedicine products, including foods, cosmetics, drugs, devices, in vivo imaging products and in vitro diagnostics, veterinary products, and tobacco products. To ensure that oversight for these products *remained with the FDA*, Congress passed the Food and Drug Administration Amendment Act of 2007. While the main focus of this amendment was to revise and extend the user fee programs for prescription drugs and medical devices and to enhance postmarketing authorities of the FDA with respect to the safety of drugs, language was added in which "promising technologies," such as "nanotechnology," are specifically named. By adding this language, nanomedicine was placed clearly under the auspices of FDA governance. Shortly before the enactment of this amendment, the FDA, under the direction of the then acting xommissioner Andrew C. Von Eschenbach, MD, formed a Nanotechnology Task Force (August 2006) and outlined the scientific and regulatory needs for regulation of the new products being designed and developed on a nanoscale. FDA representation in this task force included members from many of the major offices of the FDA, including the Office of Policy, Planning & Budget, the Office of Special Medical Programs, the Office of the Chief Counsel, the Office of the Chief Scientist, the Office of Regulatory Affairs (ORA), the Center for Drug Evaluation & Research (CDER), the Center for Devices & Radiological Health (CDRH), the Center for Biologics Evaluation & Research (CBER), the National Center for Toxicological Research and Offices for International and External Affairs, the Center for Veterinary Medicine (CVM), and Food Safety and Tobacco Products. The diversity of members from many divisions in the FDA clearly shows that nanomedicine was expected to have wide-ranging applicability and bring broad challenges to the agency.

The report from this task force clearly shows that the FDA will continue to have the authority to regulate these new nanomedicines and nanomaterials. It also showed that the FDA realized that having the necessary authority for oversight did not lessen the burden that these products would create upon the agency. At a conference for the Food & Drug Law Institute (February 2008) Norris E. Alderson, associate commissioner for science at the FDA, reported the findings of the task force and outlined the "myriad scientific and legal issues facing the agency in trying to regulate products that use nanomaterials." Norris was quoted as saying that the FDA did not know "if the changing properties of these materials [would or could] change the toxicity" and that the FDA did not, at that time, "have standards for measuring or detecting these materials" (6). Alderson and other FDA representatives noted that nanomaterials could have different toxicity characteristics than the same materials in a larger amount and that the surface area of the particles, the particles' surface charge, and, in some cases, their solubility could affect their toxicity [166]. They also noted that the FDA historically has relied on bioassays as a means of determining if a product was safe and that it is unclear if the in vivo and in vitro tests available to the FDA will be able to determine biocompatibility of nanomaterials. The possibility that nanoparticles may readily cross the blood–brain barrier could bring immense benefit to many patients with neurological illnesses, but the rarity of this therapeutic mechanism brings with it previously undefined side effects. Clearly the diversity of nanomedical products will require the FDA to approach each drug or device with an individual approach. This will require a delicate balance between strictly controlling the new science and facilitating the approval of potentially life-saving products.

The FDA is vividly aware of the complex challenges nanomedicine presents, and it does not plan to take on these challenges in a vacuum. In a recent update to its website (www.fda.gov), the FDA acknowledged that it was its goal to "promote and to participate in regulatory science research and other efforts to increase scientific understanding, to facilitate assessment of data needs for regulated products." It states that these activities should, where appropriate, "be coordinated with and leveraged against activities supported by other federal agencies, the private sector

and other international regulatory counterparts," and it suggested four areas of regulatory science research that were of interest to it in regard to nanotechnology—physico-chemical characterization in FDA-regulated products, nonclinical modeling of nanomaterials in FDA-regulated products, risk characterization information, and risk assessment. The FDA has also recently announced that draft guidance documents are being developed for the industry by the CVM on nanotechnology in CVM-regulated products. While guidance documents for veterinary products generally precede those for products used in human applications, the FDA is currently working on a guidance document for manufacturers of clinical products also.

The FDA is aware that many of the new products that will encompass nanotechnology will indeed be "combination products." These are products that comprise characteristics of both a drug and a medical device. In nanomedicine combination products could have a third characteristic included as well. Nanoparticles could be combination of drug products, medical device products, and biologic products also. The safety and effectiveness of combination products have been a challenge to the FDA in the past and led to the formation of the Office of Combination Products (OCP). This group works closely with other FDA agencies, including the CDER, CBER, and CDRH. The OCP will evaluate a product with combination attributes and assign it to one of these centers, depending on the product's primary mode of action (PMOA). If the product is entirely new to the FDA in regard to its safety and effectiveness profile, the product will be assigned to the center with the most expertise in the safety and effectiveness issues that may arise from the product. The OCP takes the recommendation of the manufacturer into account when assigning a product to a center for evaluation. The FDA believes the manufacturer can provide valuable insight into the planned actions of the drug or device and that it has critical history in the development process of the drug or device. The FDA encourages manufacturers to invoke early communications with the agency and to include the OCP or the designated center in the planning phases of drug/device development.

The FDA has taken other steps to ensure that nanomedicine is provided with adequate regulatory oversight. While the *Nanotechnology Task Force Report* of 2007 states that most of the laws

and regulations that the FDA operates under were in effect before the advent of nanotechnology, they were written with sufficient generality to allow them to be adapted to new technologies that arise. In the "Mission Statement for the Task Force" it was made clear that input from the public would be encouraged. The FDA also facilitated collaboration with other regulatory agencies in an effort to broaden its base of knowledge about nanotechnology. These agencies included the National Institutes of Health (NIH), the National Institute of Standards and Technology (NIST), and the Environmental Protection Agency (EPA). The FDA and these collaborative agencies have partnered in a "Memorandum of Understanding" to form the Nanotechnology Characterization Laboratory (NCL). This laboratory will use the expertise of the coalition to develop characterization assay cascades for nanoparticles and develop standard approaches for evaluating these tiny particles.

The FDA also values input from the scientific arena and has recommended that collaboration, collation, and interpretation of scientific data will be key to the successful transmission into the future of nanomedicine. The FDA has partnered with the NIH, the National Cancer Institute (NCI), and Johns Hopkins University and has formed collaborations with the Houston-based Alliance for NanoHealth (ANH). The ANH has an eight-member coalition of medical and scientific institutes based in Houston, Texas, known as the FDA-ANH Nanotechnology Initiative (FANTI) [167]. This initiative is tasked with the goals of collaborating to develop strategic plans, set priorities, and leverage resources and expertise from multiple sources, facilitating the development of nanotechnologies that constitute novel research tools. The FDA's interest and perspective for participating in this type of collaboration is to provide safer, more effective therapies by establishing a framework for effective risk identification, assessment, and evaluation of emerging products based on nanotechnology.

On the basis of input from these many sources, the FDA has developed a plan for evaluation of products such as drugs, devices, and biologics that are used in products subject to premarket authorization. The current testing required for these products may be revised to include an individualized approach based on the specific characteristics of the nanoparticles used in the product

development. Long- and short-term toxicity will be evaluated, and if the FDA believes the nanoparticles could affect these toxicities the manufacturer will be required to submit additional in vitro and in vivo test results that demonstrate that a nanoparticle's size does not change its toxicity profile. The FDA's approach to nanomedicine will mimic its standard practice in new product evaluations and will be based on risk management. The FDA launched its Critical Path Initiative in 2004, a plan that modernized the scientific process through which FDA-regulated products are developed, evaluated, and manufactured. This initiative will be key in the evaluation of nanoproducts.

The FDA regulates products, not technology. Product manufacturers of nanoproducts will also be subject to FDA requirements of meeting current good manufacturing practices (cGMPs), and the FDA will conduct audits of facilities prior to market release. Most manufacturers conducting clinical trials under FDA jurisdiction are inspected an average of four times prior to premarket approval. Inspections will focus on compliance and enforcement actions, review of deviation reports, and assessment of risk and response. The FDA is also expected to utilize process analytical technology (PAT) in its evaluation of the pharmaceutical development of nanoproducts and their manufacturing and quality assurance. A "Guidance for PAT" was published by the FDA in September 2004, which outlines the FDA's expectations. PAT is a system of designing, analyzing, and controlling manufacturing through timely measurements of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. Questions asked when using this process include the following: What are the mechanisms of degradation, drug release, and absorption? What are the effects of product components on quality? What sources of variability are critical? How does the process manage variability? The FDA believes that using PAT will also enhance communications between the FDA and manufacturers throughout the life cycle of the products being reviewed.

Clinical sites where the studies are being run will also expect frequent inspections from the FDA, generally involving a cyclic review of every principal investigator every four years. Investiga-

tional sites will be held to tight standards for current good clinical practices (cGCPs). Annual reports will be scrutinized for adverse events and progress in the trial. Clinical trials are expected to begin with small feasibility studies, involving small numbers of patients, and stopping rules focusing on adverse events and failure to meet expected outcomes will be part of the protocol design.

By working with industry and scientific investigators in the design and development of nanomedicine products, the FDA will be able to contribute to the advancement of personalized medicine. By reconfirming that they *regulate products, not technology*, the FDA will be able to successfully evaluate clinical trials based on individual patient results. Looking at nanotechnology from the perspective of the individual patient and the accomplishment of expected individual outcomes rather than shying away from a new and complex technology fits with the longstanding mission of the FDA. It will evaluate safety and effectiveness on the basis of the results produced in carefully planned and executed clinical trials.

1.10.4 *A Biotech Startup CEO's Perspective*

Personalized medicine is being viewed as a potential panacea by the health care industry as a means to lower the overall cost of therapy, improve individual patient outcomes, and, in the case of the pharmaceutical and biotech industries, revive research and development (R&D) productivity that have been falling at an unsustainable rate over the past decade or more.

Physicians know that while patients may display the same signs or symptoms, the underlying cause may vary. Take the simple example of high blood pressure. The elevation may be the same from patient to patient, but the causes differ. Because of this, the commonly used medications only work in 50–60% of patients. Additionally, side effects will occur unpredictably in both responders and nonresponders. While certain attributes may help guide therapy (for instance, Caucasians are statistically more likely to respond to angiotensin-converting-enzyme [ACE] inhibitors than people of African origin [168]), finding the right regimen for the individual patient is largely a matter of trial and error in general practice. This inability to precisely predict which patients will respond safely to

which antihypertensive at the time of initial diagnosis adds waste (cost) and inconvenience to the health care system and can even lead to tragedy in rare cases, such as when a fatal drug reaction occurs.

But usually in hypertension the result of our inability to individualize therapy is just waste and inconvenience, and eventually an effective and well-tolerated treatment regimen is found. Contrast that to the situation in cancer. Tumors may have the same size, shape, distribution of metastases, and histology (microscopic appearance) and yet respond dramatically differently to therapy. Approved cancer therapies can have response rates as low as 10% in a given cancer type. In most cancers, knowing which patients will respond is the central question. Increasingly, by conducting special studies on tumors removed or biopsied from an individual patient, treatment can be individualized. Trastuzumab (Herceptin®) is prescribed in ~20% of patients with breast cancer expressing a high concentration of human EGFR-2 on the surface of their tumors [169]. In a more recent example, the experimental agent crizotinib has been shown to lead to dramatic increases in response and survival in approximately 2–7% of patients with non-small-cell lung cancer expressing a certain mutation of the anaplastic lymphoma kinase gene [170].

From a strictly commercial perspective, regulatory authorities, and most especially the US FDA, have relentlessly raised the bar on efficacy and safety to the point that R&D productivity has declined dramatically at major pharmaceutical companies and it has been predicted that some may actually fail over the next 10 years. In addition, governments increasingly don't want to pay for treatment failures, especially as the price for medications has escalated to compensate for loss of revenue as patents expire and regulatory approvals becoming too rare to compensate. By allowing the a priori identification of a patient subset dramatically more likely to respond to therapy and perhaps in the future also unlikely to have unacceptable side effects, it is expected that personalized medicine will lead to a higher likelihood of regulatory approval (improved R&D efficiency), while reducing system costs associated with treatment failures and adverse effects. As such, personalized medicine could simultaneously meet the needs of companies, regulators, payors, and consumers—groups that often

find themselves with competing interests in today's constrained health care environment.

This chapter has documented some of the ways that nanotechnology is contributing to the toolset of personalized medicine. Over the coming years, it is anticipated that driven in some cases by nanotechnology, we will increasingly determine an individual's (or his or her tumor's in the case of cancer) genetics and/or proteomics before initiating therapy and use such techniques as molecular imaging and biomarker analysis to rapidly assess therapeutic response and, in the case of tumors, emergence of mutations and resistance. Such a revolution in the practice of medicine raises a number of practical issues to be overcome for this vision to be achieved.

For example, some clinical trials now include the routine genetic analysis of resected tumor samples to determine eligibility for trial inclusion or for stratification in randomization schemes. The logistics of collecting specimens from distant sites, processing and transporting them properly to avoid tissue degradation, and then providing a timely analysis is challenging in the clinical trial setting. Doing so outside the clinical trial setting is a more daunting task. In a future where much cancer therapy is personalized, it would be anticipated that such testing would best be conducted locally, for instance, as pathology examination normally has been. A broadly utilized test will almost certainly require FDA approval, a long, laborious, and expensive process. To highlight the challenge, the FDA has recently refused to approve what would be the first premorbid diagnostic test for Alzheimer's disease over concerns about the ability of community-based physicians to properly interpret the test results.

Similarly, if a new drug's efficacy has been proven in a specific patient population (take crizotinib, for example), then the drug will only be approved for use in those patients where it has been proven effective. This means that often the diagnostic test must be approved ahead of (unlikely), or more commonly, alongside the new drug. This means that the pharmaceutical company will be seeking independent approvals for the drug and a diagnostic test from different FDA divisions. While the author is not aware of such a case, the future may see the unfortunate scenario of a very promising

drug being kept from the market because something has happened in the development of the diagnostic test that prevents its approval. The FDA and the industry are currently grappling with how to make such codevelopments of drugs and diagnostics straightforward and less prone to error [171].

This is by no means an exhaustive list of the challenges that personalized medicine will face; rather the above represent some of the more important issues already at hand. Fortunately, nanotechnology does not present particularly unique issues. As described in this chapter, nanotechnology offers a means to solve some of the more difficult challenges posed by personalized medicine. As with all innovations, there will be hurdles, both expected and unforeseen, but history says that they will be overcome. Innovation comes more slowly than we would like in commercial medicine, in no small part due to the need to satisfy regulatory systems put in place to ensure safety for consumers. But nanotechnology and personalized medicine will cooperate to offer a future of hope for previously untreatable disease.

1.10.5 *A Patient Advocate's Perspective*

When one survives breast cancer for 22 years, one thankfully sees much progress toward curing the disease. As learned over time, and with much research, the cancer of any organ site is actually a compellation of numerous subtypes, breast cancer being no exception. Trite as the phrase is, it does seem that the more we learn, the more there is to learn. Although a drug regiment may be successful for one subtype, unfortunately it may very well not translate into shared success with other subtypes. As stated in the introduction, there will not be one answer to all, which means bucking the current drug development trend and most notably addressing the novel emerging technologies, in respect to drug delivery systems, drug treatments, and drug development to target cell-surface receptors, to identify potential for metastasis and resistance to therapeutics.

As a patient with an aggressive form of breast cancer, over a 10-month period I was treated with two different regiments of chemotherapy combinations: the standard cocktail or the big-

box approach at the time, fluorouracil (5FU), adriamycin, and cytoxan (FAC) and a clinical trial of methotrexate and vinblastine. The expected side effects, hair loss, fatigue, and nausea being the most prominent, were experienced. The regimen was delivered by infusion—two given immediately and one with a pump over a three-day period, which could arranged to include a weekend, so that only one day of work each month was missed. It cannot be said that life was normal during this period, but with some adaptations, a "near or new normal."

Fortunately a comprehensive cancer center was available with the most current treatments of the day; in addition, clinical trials were accessible. Thankfully the cancer center was in close proximity. This is a success story for me, but it cannot be said for thousands of others who were diagnosed and treated during the same period or in subsequent years. Many were diagnosed with later-stage disease or developed metastasis, and some patients did not respond to any treatments. Certainly some could have been saved today with the discovery of HER2 and Herceptin, but even today lives are still lost. Therefore delivery of new and current drugs needs to reach the target, whether it is a primary or a metastatic site, to destroy cancer cells.

If the identification of the cancer and its particular cell types and pathways could be determined using small amounts of blood to test, this would certainly be a cheaper, more efficient method to establish the target pathways. With the development of molecule-specific contrast agents, it is now possible to facilitate the noninvasive detection and visualization of morphological and biochemical changes that influence disease and predict response to therapy. Physicians are better able to understand the molecular signatures of cancer cells, enabling them to target abnormally activated pathways. When combined with more conventional diagnostic imaging, one could expect to have a major impact on the detection, diagnosis, and decision making for personalized treatment. One attainable goal is to discover new biomarkers to verify cell types and specific pathways to tailor treatments to the individual and to prevent unwarranted treatment. The times of one shot for all, big box, are over.

Using nanotechnology, nanoporous silicon particles, to deliver the drugs in a multistage approach allows these MSVs to carry their

specific payload to the target, increasing their therapeutic efficacy. This multistage approach, along with implantable miniature devices to release the drugs, further provides a controlled drug delivery of the predetermined, effective drug over a prescribed period of time. Rather than using a cumbersome pump, this miniature device could be implanted, relieving the patient of the burden of returning to a clinic for multiple infusions. The device might handle the drug delivery for months. This would save time and money for the patient and caregivers. The benefit to rural patients would be incredible. The side effects of the drugs should be significantly reduced, and therefore this system should allow the patient to lead a more normal life during treatment, and hopefully reach the target with limited invasiveness and without effecting surrounding organs and tissue.

These new technologies provide much hope for patients. Through research and clinical trials many lives should be saved and the quality of life should be improved during treatment.

1.11 Summary

The solution for the enablement of personalized medicine will eventually be realized; however, the activation energy required for clinical acceptance and implementation will necessitate a significant overhaul of current practices. This chapter briefly reviewed the nanotechnologies that are providing the catalysis to this movement, but it also addressed the challenges impeding progress through the insights from several critical vantage points. The incentive of personalized medicine is too great to be ignored; the immediate question remains: Who will lead us into the next clinical evolution: scientists/clinicians, regulatory agencies, the industry, or the patient population?

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Chapter 2

RNAi Nanomedicines toward Advancing Personalized Medicine: Challenges and Opportunities for Targeted Therapy in the Immune System

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Utilizing RNAi as a novel therapeutic modality has an enormous potential to bring the era of personalized medicine from a notion to reality. However, targeting of RNAi molecules into specific tissues and cells is still a hurdle. Major attempts are done for developing carriers that could overcome systemic, local, and cellular barriers. This chapter will present the recent progress in this emerging field, focusing on strategies of systemic, active cellular targeting, which is considered a major challenge for drug delivery.

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2.1 Introduction

Ribonucleic acid interference (RNAi) is a natural cellular mechanism for RNA-guided regulation of gene expression. This regulation is carried out by double-stranded RNA (dsRNA) that suppress the expression of specific genes with complementary nucleotide sequences either by degrading specific messenger RNA (mRNA) or by blocking mRNA translation. RNAi can be activated exogenously by expressing short hairpin RNA (shRNA) with viral vectors or by incorporating synthetic small interfering RNAs (siRNAs) directly into the cell cytoplasm [1, 2].

siRNA is a chemically synthesized dsRNA of 19–23 base pairs with two nucleotides unpaired in the 5′-phosphorylated ends and unphosphorylated 3 –ends [3, 4]. Inside the cell cytoplasm, siRNAs are incorporated into an RNA-induced silencing complex (RISC), a protein-RNA complex that separates the strands of the RNA duplex and discards the sense strand. The antisense RNA strand then guides the RISC to anneal and cleave the target mRNA or block its translation [2]. By recycling the target mRNA, the RISC complex incorporates the antisense strand may show a therapeutic effect for up to seven days in dividing cells and for several weeks in nondividing cells. Furthermore, repeated administration of siRNA can result in stable silencing of its target [5].

The combination of knocking down any gene of interest and the ability to treat various diseases by addressing otherwise "undruggable" targets (i.e., molecules without ligand-binding domains or those that have a structural homology with other important molecules in the cell), the elimination of clinical safety concerns associated with viral vectors, and the reduced likelihood for interference to the endogenous microRNA machinery (which could occur due to saturation of enzymes or transport proteins) emphasizes the potential of siRNAs to serve as a new platform for therapy in personalized medicine.

Despite this promise, utilizing siRNA as therapeutics is not a trivial task. For example, due to the large molecular weight (∼13 kD) and the net negative charge, the efficiency with which naked molecules of siRNA cross the plasma membrane and enter the cell cytoplasm is very low [2, 6]. When injected intravenously, in addition

to rapid renal clearance and susceptibility to degradation by RNAses, unmodified naked siRNAs are recognized by Toll-like receptors (TLRs). This often stimulates the immune system and provoking interferon response, complement activation, cytokine induction, and coagulation cascades. Besides the undesired immune activation, these effects can globally suppress gene expression and generate off-target effects and misinterpreted outcomes [6, 7]. Therefore, there is a clear need for appropriate delivery systems for siRNAs, all of which have to utilize cellular mechanisms for internalization, release (from the carriers), and escape (from the endosomes), in addition to accumulation of siRNAs in the cell cytoplasm and RISC activation. This chapter will present the recent progress in this emerging field, focusing mostly on the in vivo applications with special emphasize on the strategies for RNAi delivery to leukocytes in an era of personalized medicine, where complete sequencing of the transcriptome of a diseased individual becomes a reality and so the option to design sequence-specific molecules that can interfere with translation of any given protein and can be used to manipulate cellular functions is not a dream anymore and might soon become a reality [8].

2.1.1 *Cellular Delivery Strategies of RNAi*

Most of the methods commonly used for in vitro or ex vivo delivery of siRNAs are conventional transfection methods. Studies with purely physical methods such as microinjection and electroporation [9–12], as well as studies using calcium coprecipitation [13], commercial cationic polymers and lipids [3, 14–19], and cell-penetrating peptides [20–24], have demonstrated an effective knockdown of desired genes. Except for the physical methods (in which the cell is subjected to an injection of small volumes of siRNAs directly into the cell cytoplasm or to a burst of electricity that causes pores in the membrane, hence elevating the ability of extracellular material to enter into the cell), all the methods share a main feature—a positive (cationic) charge that enables complexation of the siRNAs and interacts with the negatively charged plasma membrane. In this manner, it is important to note that there is evidence of toxicities of the commercial cationic lipids and polymers [25], reviewed in [26].

This emphasizes the promise of the cell-penetrating proteins, which are much less toxic and have the potential ability to target specific cells.

2.1.2 *Translation of siRNA into Clinical Practice*

Silencing of gene expression in vitro is a great tool for functional and validation studies. Nevertheless, understanding gene expression in a disease model by validating specific genes' roles in vivo, along with the potential to induce therapeutic gene silencing, opens new avenues for utilizing RNAi as a novel therapeutic modality and brings the era of theranostics and personalized medicine a step further from a vision to a potential reality.

Despite the large diversity of available methods for in vitro siRNA delivery shortly represented above, there are additional hurdles to translate these methods into clinical therapeutic tools. As detailed below, the biggest hurdle facing the translation of siRNAs' therapeutic potential into the clinic is their delivery.

2.1.2.1 In vivo delivery of siRNA

Local delivery of siRNAs has been demonstrated in various animal models [23, 27–29] and is employed in several ongoing clinical trials. On the basis of local injections of naked or cationic lipid-/polymer-formulated siRNAs, this method of treatment, although having demonstrated very promising outcomes, is suitable only for mucosal diseases or subcutaneous tissues.

Systemic delivery of siRNAs is the most challenging and daunting task in this field. While cellular and local delivery strategies have to deal with the need for internalization, release, and accumulation of the siRNAs in the cell cytoplasm, delivery strategies for systemic treatment of an entire animal enforces additionally to deal with the siRNAs' interaction with blood components, entrapment within capillaries, uptake by the reticuloendothelial cells, degradation by RNAses, renal clearance, anatomical barriers (such as the liver), immune stimulation, extravasation from blood vessels to target tissues, and permeation within the tissue.

Systemic delivery of naked siRNAs may occur by the hydrodynamic method. This method, whose precise mechanism is unsolved yet, involves rapid injection of a large volume of siRNAs in physiologic solutions (about 10% of the body weight administered within 5–10 seconds) [30, 31]. Hepatocytes in the liver are the main target of this approach. Different studies were done with this method, demonstrating functionally a knockdown of specific genes in the animals' liver [30–33]. Nevertheless, due to volume overload side effects, the hydrodynamic method is not appropriate for therapeutic use.

Naked siRNAs could also be utilized for targeting the kidney. When systematically administrated, a large amount of naked siRNAs are excreted by the glomerulus (which excretes any molecule with a molecular weight less than 40 KDa) and reabsorbed in the proximal tubule. The accumulation of free siRNA in the kidney is 40 times higher than in any other organ, an ideal propriety for selective gene therapy. Studies in rat models for renal injury indicated functional silencing of p53, a major proapoptotic gene, and renal protection, both in single and multiple injections administration [34, 35]. A product based on these studies, QPI-1002, is being developed by Quark Pharmaceuticals for systemic delivery of p53-siRNA in acute renal injury and delayed graft function [35].

Because of the rapid renal clearance, utilizing naked siRNAs systematically is relevant only when the target organ is the kidney. Otherwise, strategies for systemic delivery of siRNA must rely on carriers. These carriers should be made from fully degradable materials (to avoid undesired and probably toxic accumulation of the delivery system components in the body) and should act on specific cells or tissues, while avoid damaging others.

The systemic siRNA delivery strategies are divided into two major categories, passive and active (cellular-targeted) delivery. Passive delivery exploits the inherited tendency of nanoparticles to accumulate in organs of the reticuloendothelial system (RES), also known as the mononuclear phagocytic system (MPS). The RES, part of the immune system, consists of phagocytic cells located in reticular connective tissue, primarily monocytes and macrophages. These cells accumulate in lymph nodes, the spleen, and Kupffer cells in the liver and uptake foreign particles believed to be intruders in

the body, such as viruses, bacteria, and parasites of different types, sizes, shapes, and charges. Hence, it is not surprising that major attempts have been made to develop siRNA delivery systems for treating different liver diseases. Active (cellular-targeted) delivery is based on specific antibodies, ligands, or ligand mimetics that direct the nanocarriers to specific target cells and tissues.

2.1.2.2 Passive systemic siRNA delivery

A stable nucleic acid–lipid particle (SNALP) is a ~100 nm nontargeted liposome with low cationic lipid content that encapsulates siRNAs and is coated with a diffusible polyethylene glycol (PEG)-lipid conjugate [36, 37]. The PEG-lipid coat stabilizes the particle during formation and provides a neutral and hydrophilic exterior that prevents rapid systemic clearance. The lipid bilayer containing a mixture of cationic and fusogenic lipids enables the internalization of the SNALP and an endosomal escape while releasing the siRNA payload. A biodistribution study indicates that most (28%) of the siRNAs carried by the SNALPs were accumulated in the liver (and only 0.3% in the lungs). A functional study of SNALP-encapsulated apolipoprotein B (ApoB)-siRNA has shown significant reduction in ApoB mRNA levels. Despite the presence of cationic lipids known to trigger toxicities [26], mice and non-human primates did not reveal any adverse effects except for liver enzyme release. On the basis of these results, several clinical trials are conducted these days to test the ability of SNALPs to deliver siRNAs for liver cancer treatment and for lowering cholesterol levels [2, 35]. SNALPs encapsulating siRNA against the polymerase gene of the Zaire strain has been shown to protect guinea pigs from the lethal challenge of the Ebola virus [38]. Other formulations of cationic liposmes, with larger cationic lipid content than SNALPs, have induced effective gene silencing but also cytokine induction and toxicities and thus cannot be used for clinical evaluation.

Significant toxicities have been associated with cationic liposomes [25, 39]; therefore, liposomes neutral in charge are very promising carriers for systemic delivery of siRNAs. 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine (DOPC) non-PEGylated liposomes encapsulating siRNA against different molecules express

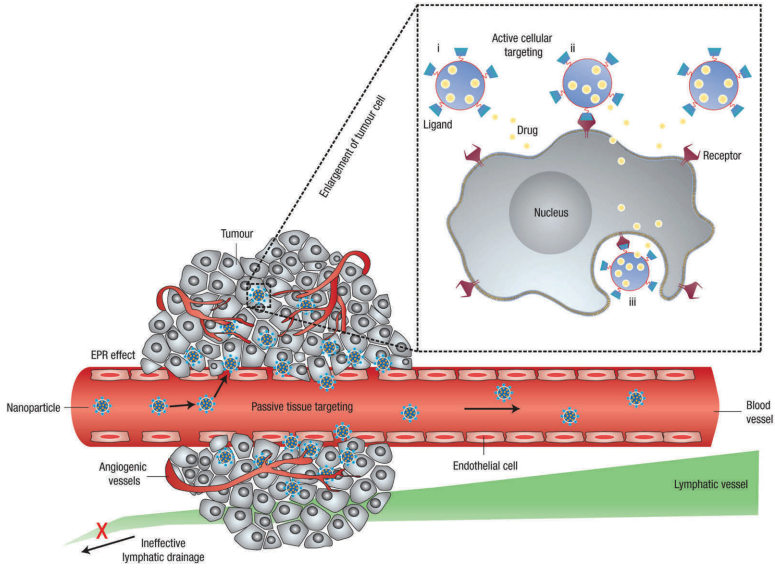

Figure 2.1 Passive and active tumor targeting. Passive tissue targeting is achieved by extravasation of nanoparticles through increased permeability of the tumor vasculature and ineffective lymphatic drainage (the EPR effect). Active cellular targeting (inset) can be achieved by functionalizing the surface of nanoparticles with ligands that promote cell-specific recognition and binding. The nanoparticles can (i) release their contents in close proximity to the target cells; (ii) attach to the membrane of the cell and act as an extracellular sustained-release drug depot; or (iii) internalize into the cell. (Reprinted with permission from Ref. 42. Copyright 2007 *Nat. Nanotechnol.*)

on melanomas and ovarian cancers inhibited tumor growth in human xenograft models [40, 41]. The accumulation of these liposomes in the cancerous tissues on the basis of the enhanced permeability and retention (EPR) effect increased permeability of the blood vessels in tumors caused by rapid and defective angiogenesis and dysfunctional lymphatic drainage that retains the accumulated liposomes [42] (see Fig. 2.1).

Cationic lipidoid (synthetic lipid-like molecules)-containing liposomes is another siRNA delivery system that has been shown to induce effective gene silencing (80% reduction in ApoB and factor VII mice's mRNA levels) in the liver. A single intravenous injection

of cationic lipidoid-containing liposomes encapsulating ApoB-siRNA resulted in a 50% decrease in the protein level in three days and up to two weeks after treatment. Although no immune response was indicated, increases in the levels of two liver enzymes suggest liver toxicity [43, 44].

HK peptides are another effective delivery system for siRNAs. This system is based on the addition of histidines into polylysine peptides. While lysine is important for binding the siRNAs, histidines stabilize the particles and have an important role in buffering acidic endosomes, thereby leading to endosomal disruption and payload release. Specific ratios and patterns of histidine and lysine have been found to augment the siRNA delivery, while carriers with a higher ratio of histidine to lysine content seemed to be more effective [45]. HK peptides carrying Raf-1-siRNA or human rhomboid family-1-siRNA induced significant silencing of target genes and growth inhibition of tumor xenografts [46, 47].

Atelocollagen is a biomaterial consists of a low-immunogenic fraction of pepsin-digested collagen type I from calf dermis. Rich in positively charged residues (lysine and hydroxylysine), it complexes the negatively charged siRNAs and interacts with the plasma membrane and hence helps incorporate the siRNAs into the cells. Although these particles have not been modified to target tumors, passive targeting due to the EPR effect causes selective accumulation within the cancerous tissues, as shown in several studies with different tumor xenografts [22, 48–50]. Initial studies indicated that atelocollagen particles could be administered safely without induction of cytokines or observed toxicity to the tissues.

2.1.2.3 Active (cellular-targeted) systemic siRNA delivery

siRNAs conjugated to other molecules is a common strategy for active delivery. A cholesterol-siRNA conjugate is one example. The specificity of this delivery system is determined by the lipoprotein to which the cholesterol-siRNA conjugates are attached in the circulation. When the conjugates bind low-density lipoprotein (LDL), the particles are mainly taken up by the liver due to its LDL receptors' expression, whereas when they bind high-density lipoprotein (HDL), they accumulate in the liver, the gut, the kidneys,

and steroidogenic organs, all of which express scavenger receptor class B, type I (SR-BI) receptors, which bind HDL [51]. The cholesterol-ApoB-siRNA conjugate as well as α-tocopherol [52] and lithocholic acid or lauric acid conjugated to ApoB-siRNA [53] reduced serum cholesterol and ApoB mRNA levels in the liver. Another example of this strategy is the dynamic polyconjugates [54]. This system includes membrane-active polymers whose activity is masked until reaching the acidic environment of the endosomes. Thanks to their employment of *N*-acetylgalactosamine, which binds to the asialoglycoprotein receptor, they are target hepatocytes. Like the SNALPs, these particles, when carrying ApoB-siRNAs, decreased ApoB mRNA levels in the liver.

Polyethylenimine (PEI) nanoplexes carrying siRNAs have also induced functional silencing in subcutaneously transplanted tumors in nude mice. These particles composed of Arg-Gly-Asp (RGD) peptide coupled via PEG (that is required for greater specificity, longer half-life, and reduced immunogenicity) to PEI (a cationic polymer that in addition to its ability to condense nucleic acids, its pH-buffering property, disrupts endosomes, thus enabling to reach the cytoplasm). When complexed with siRNAs, some RGD-PEG-PEI molecules form a polyplex, with the positively charged RGD-PEG components exposed on its surface. The targeting ability of this particle is based on the overexpression of a_v integrins, whose RGD peptides bind in certain cancers and in tumor vasculature [55]. Like the two last examples, cyclodextrin-containing polycation (CDP) particles have been successfully used for siRNAs delivery into mice's subcutaneous tumors [56]. CDP is a polymer with a cyclic oligomeric glucose backbone that when complexed with siRNAs assembles into a colloidal 50–70 nm particle. To achieve targeting, transferin-coupled PEG is attached to the surface of the particles, exploiting the upregulation of transcription factor (Tf) receptors in cancers. However, despite being considered less toxic than conventional cationic polymers (such as PEI), safety experiments on nonhuman primates revealed that in high-concentration tests, injection of these particles induced elevation in blood urea (that might indicate kidney toxicity), a mild increase in liver enzyme levels, and a mild increase in interluekin 6 (IL-6) levels. Multiple injections of the particles induced antibodies to human-Tf. Despite these disadvantages, the

efficacy of Tf-coupled CDP containing siRNAs for ovarian cancer treatment is evaluated nowadays in clinical trials [57].

Antibody-protamine fusion carriers are a promising system for systemic siRNA delivery. Protamines are relatively small (5–8 KDa) and highly basic proteins composed of 55–79% arginine residues [58]. Positively charged protamine interacts with the negatively charged siRNAs and hence stabilizes, neutralizes, and condenses the siRNAs. The ErbB2-protamine fusion protein in a complex with siRNA significantly inhibited growth of breast cancer cells [59].

Aptamer-siRNA chimeras are completely RNA-based particles for specific delivery of siRNAs. This approach relies only on the fact that structured RNAs are capable of binding a variety of proteins with high affinity and specificity. The chimera includes a targeting moiety, an aptamer, and an RNA-silencing moiety, the siRNA. The aptamer-siRNA chimeras have demonstrated specific binding and delivery of siRNAs into a xenograft model of prostate cancer. The aptamer portion of the chimeras mediates binding to prostate-specific membrane antigen (PSMA), a cell surface receptor overexpressed in prostate cancer cells and tumor vascular endothelium, whereas the siRNAs reduce the expression of survival genes [60]. This approach eliminates various side effects; hence aptamers and siRNAs have low immunogenicity. Additional advantages are the possibility to synthesize large quantities at a relatively low cost and the smaller size of aptamers compared with that of antibodies ($<$15 kDa vs. 150 kDa), which promotes better tissue penetration.

Different formulations of targeted cationic liposomes served for selective targeting of hepatic stellate cells (which are major cell populations involved in the formation of scar tissue in response to liver damage, named fibrosis) or solid tumors. Stellate cells express receptors for retinol-binding proteins, which efficiently uptake vitamin A. On the basis of these, injection of cationic liposomes coupled to vitamin A and complexed with siRNA to a murine key fibrogenesis factor (gp46) into cirrhotic mice silenced the specific gene in the mice's liver and resolved fibrosis [61]. 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) liposomes encapsulating HER2-siRNAs and containing histidine-lysine peptides (to enhance escape from the endosomes) and a single-chain antibody fragment targeting transferrin receptors (elevated on the membranes of

tumor cells) on their surface have been targeted to a tumor xenograft and inhibited its growth [62]. Anisamid-PEG-liposomes-polycation–deoxyribonuclein acid (DNA) (anisamid-PEG-LPDs) are unilamellar cationic liposomes coated with PEG-linked anisamide (a small-molecule compound binds sigma receptors) on their surface and a protamine-condensed mixture of siRNAs and a carrier calf thymus DNA in their core. Encapsulating EGFR-siRNA, anismaide-PEG-LPDs injected intravenously into tumor-bearing mice has been shown to increase the mice's sensitivity to chemotherapy [63]. Unfortunately, these particles induced a significant increase in serum cytokines levels and hence weakened the potential for clinical therapeutic use. However, it is important to note that cytokine response is not always deleterious with therapy, and there are cases when immune activation could enhance the therapeutic effects.

2.1.2.4 Targeted delivery systems for leukocytes

Utilizing siRNAs to manipulate gene expression in leukocytes holds great promise for the drug discovery field, as well as for facilitating the development of new therapies' platforms for leukocyte-implicated diseases such as inflammation, blood cancers, and leukocyte-tropic viral infections. However, due to their resistance to conventional transfection methods and to their dispersing in the body, systemic delivery to leukocytes is even more challenging than systemic delivery to other organs and tissues.

Kortylewski et al. [64] used siRNAs synthetically linked to a CpG oligonucleotide agonist of TLR9 for targeting myeloid cells and B-cells (both are key components of the tumor microenvironment) that express this receptor. These particles simultaneously silenced stat3 by siRNA and activated TLRs' responses by their agonists. Consequently, they effectively shifted the tumor microenvironment from pro-oncogenic to antioncogenic (by causing activation of tumor-associated immune cells and potent antitumor immune responses).

Two studies from the same group presented newly developed siRNA delivery systems for treating viral infections. scFvCD7Cys is a single-chain antibody against CD7 (a surface antigen present on the majority of human T-cells) that was modified to include

a Cys residue for conjugation to a 9-Arg peptide. This conjugate was used for targeted delivery of CCR5 (a chemokine receptor that functions as a coreceptor for human immunodeficiency virus [HIV]) and Vif/Tat (HIV replication proteins)-siRNA payloads into T-cells and has been demonstrated to suppress HIV infection in humanized mice without inducing toxicity in their target cells [65, 66]. A similar approach for treating dengue virus–infected cells employed DC3 (a 12-mer peptide that targeting dendritic cells)-9dR for targeting, with tumor necrosis factor (TNF)-α (which plays a major role in dengue pathogenesis) or specific highly conserved sequence in the viral envelope-siRNAs. These complexes significantly reduced virus-induced production of TNF-α and succeeded in suppressing the viral replication in monocyte-derived dendritic cells and macrophages in vitro. In vivo, treatment of mice with intravenous injection of DC3-9dR-complexes carrying TNF-α-siRNAs effectively suppressed this cytokine production by dendritic cells [67].

Our approach for targeting leukocytes is based on leukocytes' integrins, which are cell adhesion molecules mediating cell–cell and cell–matrix interactions [68]. We have developed antibody-protamine fusion proteins utilizing the lymphocyte function–associated antigen-1 (LFA-1) integrin, which is expressed in all leukocytes' subtypes, for selective targeting. The use of LFA-1 for targeting leukocytes is supported by its exclusive expression on leukocytes, its constitutive internalization and recycling activity, and its ability to undergo activation-dependent conformational changes. Using those antibody-protamine fusion proteins we have demonstrated selective delivery of siRNAs into leukocytes, both in vitro and in vivo. Importantly, neither lymphocytes' activation nor interferon response induction was indicated. Furthermore, by targeting these fusion proteins to the high-affinity conformation of LFA-1 that characterizes activated lymphocytes, we demonstrated even more selective gene silencing, which, unlike most immunosuppressive therapies, could provide a way to overcome the unwanted immune stimulation without global immunosuppressive effects on bystander immune cells. Additionally, due to the prevalence of aberrant affinity modulation of integrins in a variety of leukocyte-implication diseases [69, 70], targeting the high-affinity conformation of LFA-1 seems to be a very promising therapeutic tool [71].

Next, to increase the payload and achieve more robust targeted gene silencing, we have generated integrin-targeted stabilized nanoparticles (I-tsNPs) that successfully deliver siRNAs into a specific leukocyte subset involved in gut inflammation. Using this system, we identified cyclin D1, a regulator protein of the entry into, and the progression throughout, the cell cycle, as a potential new target for treating inflammation. The I-tsNPs have been developed as ~80 nm neutral liposomes that were loaded with siRNAs condensed with protamine. The particles have been coated with hyaluronan (HA), a naturally accruing glycosaminoglycan, for stabilization during siRNA entrapment and prolonged circulation time in vivo. The targeting ability of the particles has been achieved by attaching a monoclonal antibody against β_7 integrin (which is highly expressed in gut mononuclear leukocytes) to the HA [72]. Made from natural biomaterials, these nanoparticles offer a safe platform for siRNAs delivery, avoiding cytokine induction and liver damage. Enabling the usage of low doses of siRNAs (2.5 mg/kg), this system, in addition to advantages such as high payload capacity (~4,000 siRNA molecules per particle) and low off-target effects and toxicities, is economically worthy. We also used the I-tsNP platform with an LFA-1 integrin-targeted antibody for delivery of CCR5-siRNAs to human lymphocytes and monocytes. This system has been shown to protect mice from the HIV challenge [73]. LFA-1 I-tsNPs with CCR5-siRNAs did not induce an interferon response or TNF-α (inflammatory cytokine) secretion and hence strengthened the potential for clinical relevance.

In summary, although there is no clinically approved siRNA delivery system yet, we are convinced that in the coming years this situation will change. We base this assumption on one of the major advantages of siRNA delivery systems—the relative ease of alternating them for purposes other than the origins by changing either the payloads inside the nanoparticles (by using different sequences of siRNAs or other drugs) or, in active delivery systems, the targeting agent (by replacing the antibody or the ligand decorating the nanoparticle's surface). This opens new avenues for treating a wide diversity of diseases as well as adjusting the

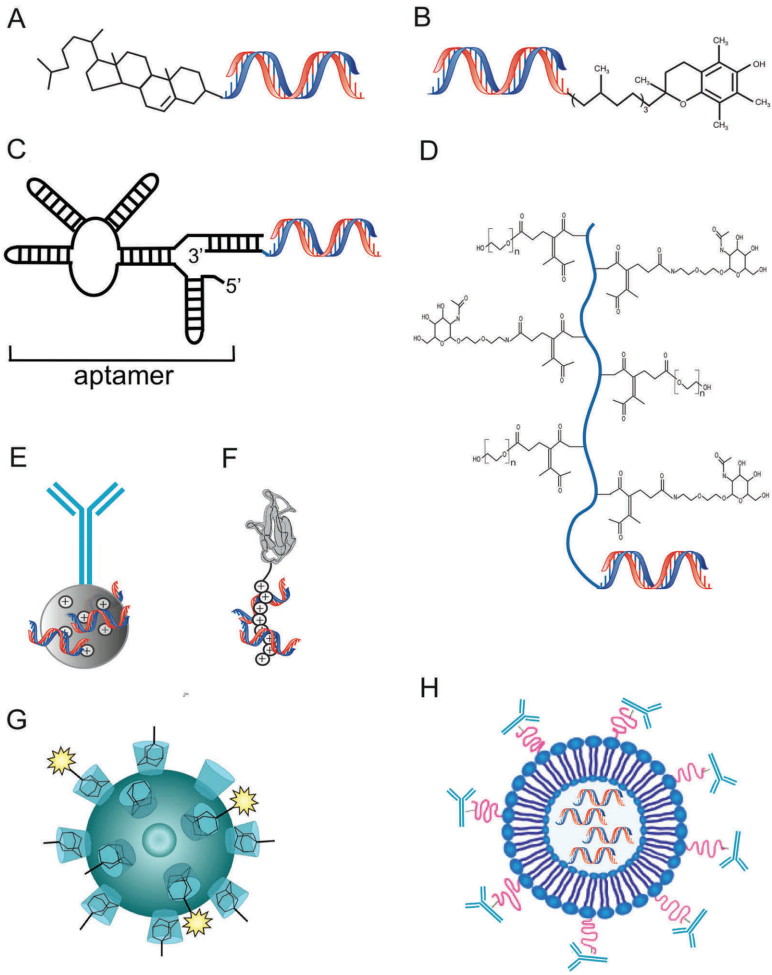

Figure 2.2 siRNA delivery strategies under development include siRNAs that are directly conjugated to cholesterol (A) or other small targeting molecules (B), joined to an aptamer that binds to a cell surface receptor (C), conjugated to membrane-penetrating polymers linked to targeting small molecules (D), complexed with fusion proteins composed of an antibody fragment or targeting peptide linked to an RNA-binding domain that is either protamine (E) or polyarginine (F), or encapsulated within nanoparticles (G) or liposomes (H) bearing targeting moieties. (Reprinted with permission from Ref. 35. Copyright 2011 *Gene Therapy*.)

treatment to the unique molecular abnormalities of a specific patient in a personalized medicine era.

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Chapter 3

Impact of Current Medical Imaging Technologies on Individualized Patient-Specific Cancer Management: A Clinical Perspective

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Modern molecular imaging techniques are increasingly playing an important role in individualized diagnosis and therapy in a wide array of disorders. The impact is most evident in the field of cancer, where patient-specific and tumor-specific information can be obtained both at diagnosis and during the subsequent disease course (viz., following initiation and completion of a particular therapeutic intervention and in post-therapy disease surveillance). The radionuclide-based PET-CT and SPEC) techniques have taken the lead role in this arena. In recent years, there have been varying degrees of success of novel methodologies being applied to cross-sectional imaging as well. Examples include application of (a) targeted microbubble techniques in the domain of ultrasound (US), (b) hyperpolarized magnetic resonance imaging (MRI) (e.g.,

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metabolic MRI employing hyperpolarized 13C-labeled pyruvate molecules), (c) diffusion-weighted MRI, (d) and magnetic resonance spectroscopy (MRS). Such endeavors are likely to help in molecular characterization of tumors and may have potential implications for personalized cancer medicine. For the purpose of discussion, the available imaging modalities, thus, have been broadly classified into two broad groups, (A) the radionuclide-based methods (e.g., PET-CT, SPECT, and planar technologies) and (B) nonradioactive molecular imaging modalities. The latter could be subclassified into (1) MRI, (2) US, and (3) optical imaging (near-infrared fluorescence and bioluminescence). In the recent literature on molecular imaging–based personalized medicine, particular emphasis has been given on radionuclide-based PET-CT and SPECT imaging, which could provide tumor-specific information in an individual (e.g., tumor metabolism, cell proliferation, hypoxia, receptor status, and other pathway activities). It has a very high impact on revolutionizing and materializing the concept of personalized medicine in the field of oncology. The potential of nonradioactive molecular imaging modalities is also being examined at present for defining their precise clinical role in the future. In the present chapter, the current status and future potentials of these promising medical imaging modalities in advancing personalized cancer medicine have been reviewed from a clinical perspective with an emphasis on how they can influence clinical management decisions in cancer.

3.1 Reasons for an Individualized Approach in an Era of Evidence-Based Medicine in Oncology

Despite the enormous popularity of “evidence-based medicine” in the medical community, different outcomes are encountered in different individuals belonging to the same cohort. This is most evident in the field of oncology, where it has a significant bearing on mortality and morbidity and has a very high impact on health care cost management issues as well. Such heterogeneity and unpredictability thus challenge the traditional “one-size-fits-all” approach and underscores the value of early assessment of therapeutic effectiveness (especially when multiple salvage

regimens and approaches are being increasingly available for a given setting), disease monitoring, and appropriate staging of the disease in patients with cancer. Two previous communications by the author discuss the subject in detail and put forward the concept of incorporation of a positron emission tomography (PET)-based personalized approach in oncology that can further strengthen the evidence-based approach in oncology [1, 2].

3.2 Reasons Molecular Imaging Is at the Forefront of Personalized Cancer Medicine

In recent times, the importance of histopathological data and in vitro diagnostics has been typically highlighted in personalized medicine in oncology and other clinical disciplines. In vivo molecular imaging, both by radionuclide and nonradioactive imaging technologies, on the other hand, can address some of the practical shortcomings of these in vitro biomarker tests (that assesses the unique variables of individuals' genetic material, proteins, and other biological molecules). In vivo molecular imaging can be helpful in the following ways:

(a) Obtaining a biopsy may not be an option in all disease states or sites.
(b) Furthermore, significant intra- and intertumoral heterogeneity in cellular characteristics can be observed during the disease course, leading to varying degrees of response amongst the different primary and metastatic sites or even within the same lesion in the same individual.
(c) The final outcome of a therapeutic approach is the result of complex interactions between a number of host and tumor characteristics that the in vitro parameters alone will not be able to predict.

The aforementioned factors lead to significant spatial and temporal heterogeneity in a given malignancy that could be successfully predicted and determined by the in vivo molecular imaging methodologies. This has been termed by some authorities as "regional proteomics" [3], which makes the in vivo molecular

imaging approach attractive, not only as a reliable and objective parameter, but also as a practically feasible technique that can be employed and interpreted in a robust manner.

3.3 Medical Imaging Modalities with Significant Potential toward Advancing Personalized Cancer Medicine

Molecular imaging technologies encompass a number of modalities that have been and are being explored for their potential toward individualized diagnosis and treatment in cancer and other disorders. It is perceivable that their role will be complementary.

The imaging modalities (Fig. 3.1) can be broadly classified into two groups, (A) radionuclide-based methods (e.g., positron emission tomography–computed tomography [PET-CT] and single-photon-emission computed tomography [SPECT] technologies), which have demonstrated the greatest clinical success till date, and (B) nonradioactive molecular imaging modalities like magnetic resonance imaging (MRI), ultrasound (US), and optical imaging (near-infrared [NIR] fluorescence and bioluminescence).

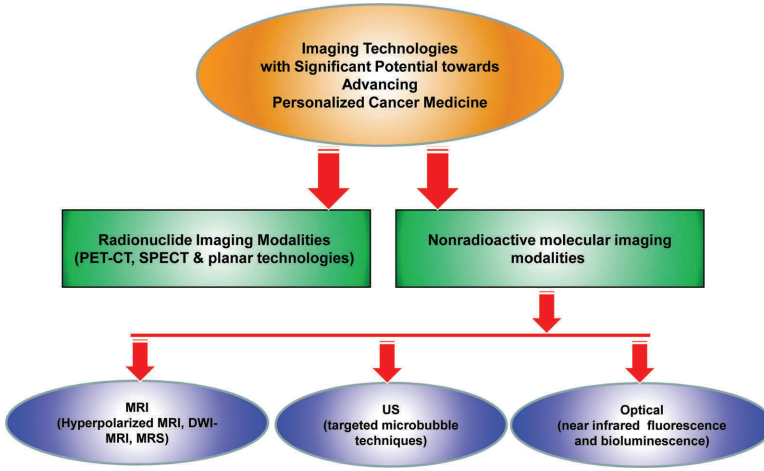

Figure 3.1 Molecular imaging technologies with significant potential towards advancing personalized cancer medicine and their classification.

3.4 Radionuclide Functional Imaging vs. Conventional Morphological Imaging Methodologies: Advantages of the Former with Regard to Materializing the Concept of Personalized Cancer Medicine

In the current clinical practice, the radionuclide imaging modalities have emerged as the most successful approach that has made the concept of "personalized cancer medicine" a clinical reality rather than a theoretical hypothesis. Hence, the radionuclide imaging techniques, especially PET and SPECT, as well as planar methods, are considered the best performers toward this end [1, 2]. This success is mainly due to two reasons:

(a) The ability to image the different biological characteristics with different tracers. This is particularly true with PET imaging.
(b) The superior sensitivity achieved by the radiolabeled biomarkers (in the range of femto- to picomolar concentrations of radiolabeled compounds) as compared to the millimolar-level detection with conventional structural methods (e.g., CT iodinated contrast or the gadolinium agents of MRI).

3.4.1 *The Functional Radionuclide Modalities*

3.4.1.1 Targets in functional radionuclide imaging that have a bearing on a personalized approach in oncology

As mentioned previously, the functional radionuclide techniques, especially PET, have been at the forefront of the current molecular imaging modalities and have revolutionized the concept of personalized cancer medicine. The feasibility to study and image various tumor functional characteristics with various tracers has been the prime reason for their success. The different targets that have been utilized are depicted in Fig. 3.2. The common targets that have been utilized in various studies include (i) glucose metabolism, (ii) cell proliferation, (iii) tumor hypoxia, (iv) amino acid metabolism, (v) cell membrane synthesis, and (vi) cell surface peptide receptors and hormonal receptors.

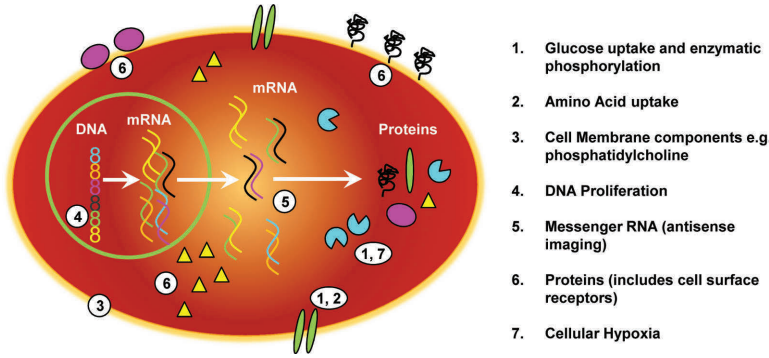

Figure 3.2 Clinically important targets in the domain of functional radionuclide imaging. 1. Glucose uptake and enzymatic phosphorylation; 2. amino acid uptake; 3. cell membrane components, e.g., phosphatidylcholine; 4. DNA proliferation; 5. mRNA (antisense imaging); 6. proteins (includes cell surface receptors); 7. cellular hypoxia. *Abbreviation*: mRNA, messenger ribonucleic acid.

The various radiotracers have been primarily developed toward assessing these targets, which will influence the individual decision-making process. An example in the arena of brain tumor imaging is depicted below, where the various tumor characteristics are pivotal for optimal therapeutic decision making. This has led to the exploration of the various PET tracers that would characterize the tumor biology (Table 3.1) in this malignancy.

Table 3.1 PET tracers investigated for brain tumor imaging

	Principal class of PET tracer and molecular mechanism involved	Name of tracer
A	Glucose metabolism	Fluorodeoxyglucose (FDG)
B	Amino acid analogues	[^{11}C]Methionine (MET), fluoroethyl-L-tyrosine (FET) and L-3, 4-dihydroxy-6-[^{18}F]fluoroprieny-lalanirie(FDOPA), L-1-[^{11}C]tyrosine (TYRl, and L-3-[^{18}F]fluoro- -methyltyrosirie (FMT)
C	Radiolabeled cell membrane components	[^{11}C]Choline PET (CHO)
D	Radiolabeled nucleosides	[^{18}F]Fluorothymidine(FLT)
E	Hypoxia Imaging Tracers	[^{18}F]Fljoromisoriidazole, [^{18}F]EF5
F	Somatostatin receptor imaging tracers	[^{68}Ga]DOTA-TOCPET

Reprinted with permission from Ref. [4].

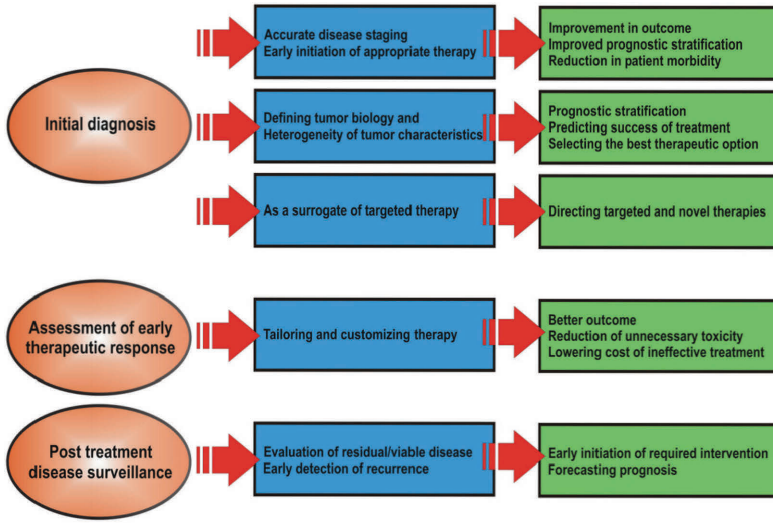

Figure 3.3 The key components of personalized cancer management (blue boxes in the middle) based upon radionuclide molecular imaging in each decision-making step (brown at the extreme left) and their implications for improving patient care (green at the extreme right).

3.4.1.2 Management of individualization in various decision-making steps in cancer with functional radionuclide modalities

The impact of the functional imaging modalities is not limited to a single point of decision making; rather it influences multiple steps in the decision-making process of cancer management. Significant data has been generated in the last two decades by examining each of the decision-making steps in different malignancies in large- and small-scale clinical trials.

For the purpose of easy understanding of the readers, the clinical utilities are categorized into three broad settings (Fig. 3.3). Under each of these broad settings, the specific clinical aspects and advantages are highlighted in the middle column and the expected outcome parameters are on the right column of the figure.

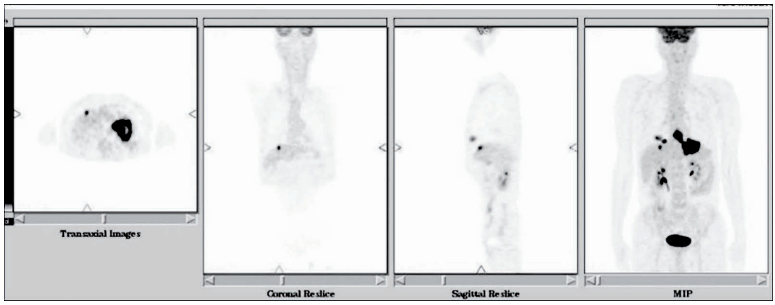

Figure 3.4 A 62-year-old man, a known patient of squamous cell carcinoma of the middle-third of the esophagus with contiguous nodal extension. After FGD-PET imaging, unsuspected lung and liver metastases are detected. The staging changes from M0 to M1b. (Reprinted with permission from Ref. 10.)

3.4.1.2.1 The varying aspects and advantages of individualization of management strategy at initial diagnosis

At initial diagnosis, the information from radionuclide functional imaging helps personalization of disease management in three possible ways:

a. Better and appropriate initial disease staging: Appropriate disease staging has been a major advantage of PET-CT with 18F-fluorodeoxyglucose (FDG) and hence is extensively employed in a wide array of malignancies [6–11]. This has the following implications:

- Better triaging of cancer patients (Figs. 3.4 and 3.5)
- Initiation of appropriate therapy for an individual at the earliest
- Reduction of patient morbidity (due to an inappropriate strategy)
- Redefining of prognosis and survival in patients at all stages of the disease (the Will Rogers effect) [6]

b. Defining of heterogeneity of tumor biology and characteristics: PET-CT depicts intra- and intertumor heterogeneity in a global fashion [12] (see Table 3.2). This aspect has been relatively less explored but can potentially aid in personalized decision making in the following ways:

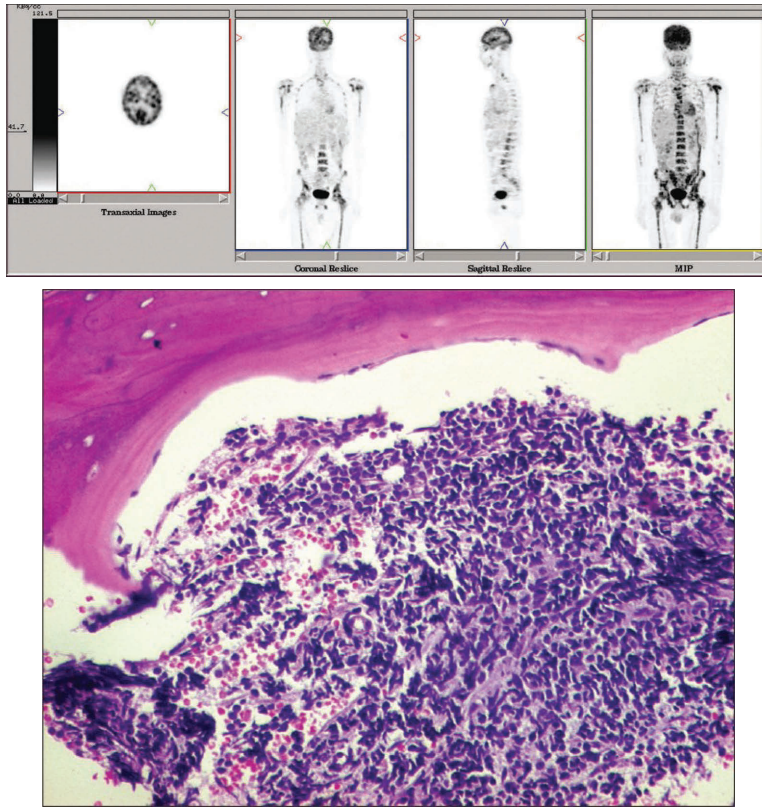

Figure 3.5 (a) Whole-body 18F-FDG-PET scan, done 60 min after the intravenous injection of 300MBq of 18F-FDG, shows diffuse and patchy FDG uptake in the entire axial skeleton and bilateral humerii and femora-consistent bone marrow involvement (reproduced with permission from Basu et al. [11]). Bone marrow involvement by the disease is a distinct advantage of FDG-PET. In this patient of cerebellar medulloblastoma the extensive bone marrow involvement was unsuspected, which was subsequently proven by bone marrow biopsy. (b) Section from metastases demonstrating high-grade round cell tumor involving bone (H and E, ×20) (reproduced with permission from Basu et al. [11]).

- Prognostic stratification due to FDG uptake in a tumor [13–17]
- Prediction of the probability of success and directing of the optimal therapeutic strategy and dose of various treatments

Table 3.2 Spectrum of tumor heterogeneity observed in the parlance of clinical PET

A. Intertumor Heterogeneity
• Using various radiotracers: Different tumors may have differential amounts of uptake of two or more radiotracers. For example, neuroendocrine tumors may have varying degrees of uptake of [I124]-octreotide and FDG on PET imaging, and endocrine tumors such as thyroid carcinoma may have varying degrees of [I124] and FDG uptake on PET imaging.
• Using FDG: Different tumors may have differential SUVs (based on PET imaging) and rates of increase in SUVs over time (based on multiple time points or dynamic PET imaging).
B. Intratumor Heterogeneity
• Using various radiotracers: Individual tumors may have differential amounts and patterns of uptake of two or more radiotracers. For example, a particular brain tumor may have differential amounts of FDG, amino acid, or hypoxia agent on PET imaging.
• Using FDG: Decreased FDG uptake on PET within the same lesion may represent regional necrosis, but variability of tumor metabolism within the same lesion could also explain the reason for this observation and should be investigated in the future.

Reproduced with permission from Ref. [12]. *Abbreviation*: SUV, standardized uptake value.

in an individual (based upon the findings of multitracer PET imaging).

Classic example: The role of PET hypoxia imaging in optimizing external radiotherapy

c. Predicting and directing of targeted and other novel therapies: This helps in determining the suitability of appropriate targeted therapies (both radionuclide therapies and other targeted therapies) [2].

Clinical examples:

As a surrogate for radionuclide therapies:

(i) Radioiodine scan for ^{131}I therapy in patients of thyroid cancer
(ii) ^{123}I–metaiodobenzyl guanidine (MIBG) scintigraphy for deciding upon the possibility of radioiodine-labeled MIBG (^{131}I-MIBG) therapy in neural crest tumors
(iii) Radioimmunoscintigraphy for deciding upon the suitability of radioimmunotherapy in patients of lymphoma
(iv) Somatostatin receptor (SSTR)based SPECT and PET studies, for example, ^{111}In-pentetreotide scintigraphy, (68)Ga-[1, 4, 7, 10-tetraazacyclododecane-1, 4, 7, 10-tetraacetic acid]-1-NaI^{3}-octreotide (^{68}Ga-DOTA-NOC) to determine the suitability of

yttrium- or lutetiumlabeled octerotide analog treatment in patients of neuroendocrine tumors

As a surrogate for other targeted novel therapies:

(i) Imaging of epidermal growth factor receptor (EGFR) and EGFR tyrosine kinase (EGFR-TKI) overexpression in tumors by PET and SPECT modalities allowing in vivo a priori determination of EGFR-targeted drug efficacy [18]
(ii) Imaging of estrogen receptor status and function and HER2 receptors in patients of metastatic breast carcinoma allowing decision making of therapy with various estrogen receptor–targeted agents and trastuzumab respectively [19, 20]

3.4.1.2.2 Assessment of early therapeutic response

The role of functional imaging with PET, SPECT, and planar radionuclide imaging technologies has been pivotal in assessing early therapeutic response [1, 2, 16, 21–43]. The advantages of this have been in the following ways:

- Assessing the efficacy of a particular therapeutic approach (e.g., systemic chemotherapy, radiotherapy or the newer targeted therapies) early in its course enabling changes being made in case of ineffective therapy at the earliest
- Substantial reduction of unnecessary toxicity
- Reduction of the cost of ineffective treatment

Some clinical examples of this can be seen in the following scenario:

(i) The revolutionary impact of early interim FDG-PET in the decision making of lymphoma treatment (Hodgkin and non-Hodgkin and FDG avid variants of lymphomas at both nodal and primary extranodal types) (Fig. 3.6)
(ii) Proven benefit of FDG-PET in early treatment monitoring of patients with gastrointestinal stromal tumors (GISTs) once therapy with imatinib mesylate is initiated and in its subsequent course (Fig. 3.7)

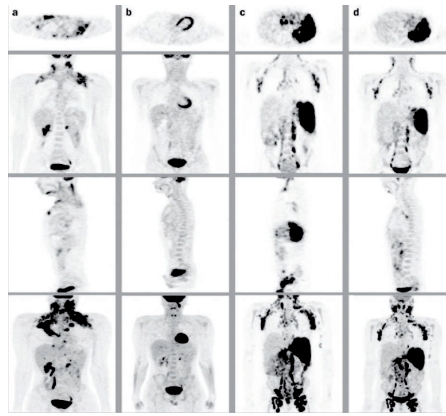

Figure 3.6 A 30-year-old male with Hodgkin's lymphoma, demonstrating avid FDG uptake in the bulky mediastinal disease that shows CMR after the third cycle of chemotherapy. (a) FDG-PET images at diagnosis. (b) FDG-PET images following three cycles of chemotherapy. A 68-year-old male with diffuse large B-cell lymphoma, demonstrating significant persistent disease following the third cycle of chemotherapy. These images indicate that a salvage schedule should be considered. (c) FDG-PET images at diagnosis. (d) FDG-PET images following three cycles of chemotherapy. (Reprinted with permission from Basu [1].) *Abbreviation*: CMR, complete metabolic response.

(iii) The role of early monitoring of therapeutic response by FDG-PET routinely employed in neoadjuvant therapy in various tumors (Figs. 3.8 and 3.9)

(iv) Ability of FDG PET/CT to monitor the disease before and one week after administration of a molecule-targeted agent called EGFR-TKI reported in a recent study

(v) (11)C-labeled 4-N-(3-bromoanilino)-6,7-dimethoxy-quinazoline ((11)C-PD153035), an imaging biomarker of EGFR, proposed to be a noninvasive and rapid method for identifying patients with refractory advanced non-small-cell lung cancer (NSCLC) of adenocarcinoma or squamous histology likely to respond to the EGFR-TKI erlotinib [41]

(vi) ^{68}Ga-DOTATATE PET-CT now being regularly examined and utilized to evaluate the effectiveness of peptide receptor radionuclide treatment [43]

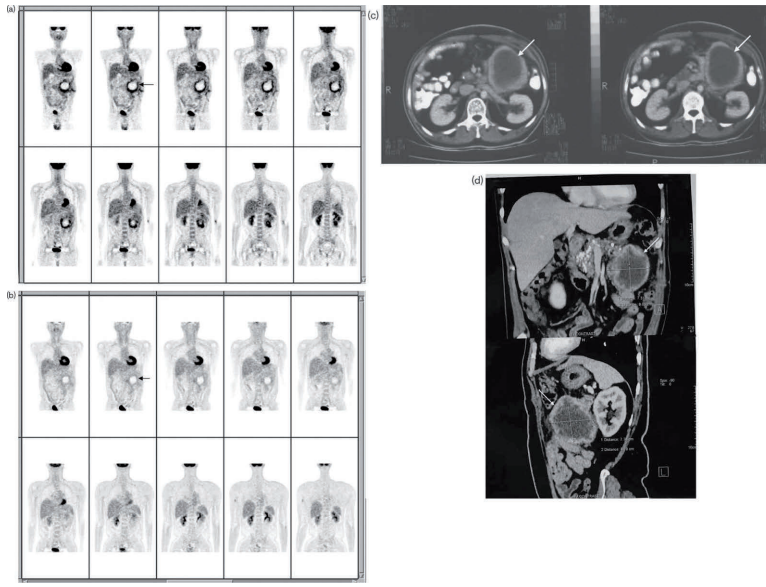

Figure 3.7 (a) Coronal views of FDG-PET at the baseline, demonstrating avid FDG uptake at the periphery of the described abdominal mass. Note the uptake pattern commonly observed in a majority of active GISTs and their metastases, the center of many of which is predominantly cystic or necrotic. (b) Coronal views of FDG-PET at one month post-treatment (with imatinib), demonstrating disappearance of FDG uptake from the periphery of the mass, depicting a CMR. (c) One month post-imatinib treatment CT scan of the abdomen (axial view), showing a persistent, thick-walled, peripherally enhancing cystic lesion at the same site as described in the pretherapy scan. (d) CT of the abdomen at three months, demonstrating a persistent, peripherally enhancing mass in the left anterior pararenal space close to the body and tail of the pancreas. The lesion shows a decrease in size, whereas FDG-PET at this time continued to show a CMR. (Reproduced with permission from Basu et al. [33].)

3.4.1.3 Post-treatment disease surveillance

FDG-PET/CT and other radionuclide methods have been utilized in this area for two definitive benefits:

(i) Utilized in a number of major malignancies where there is clinical or radiographic evidence of mass following completion

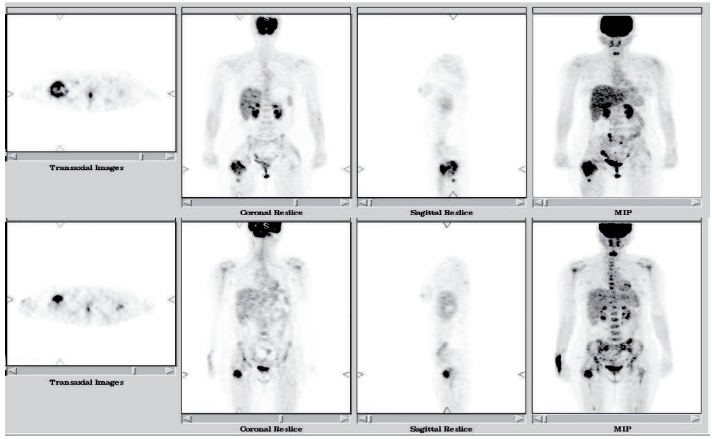

Figure 3.8 (A) (upper panel): 25-year-old female, PNET of right proximal femur. Prechemotherapy FDG-PET showing FDG uptake at the primary site with SUV_{max}: 8.68. (B) (lower panel): Postchemotherapy SUV_{max}: 7.44. Percentage change in SUV_{max}: 14.28%; HPE: 34% necrosis. (Reproduced with permission from Ref. [16].) *Abbreviations*: PNET, primitive neuroectodermal tumor; HPE, histopathological examination.

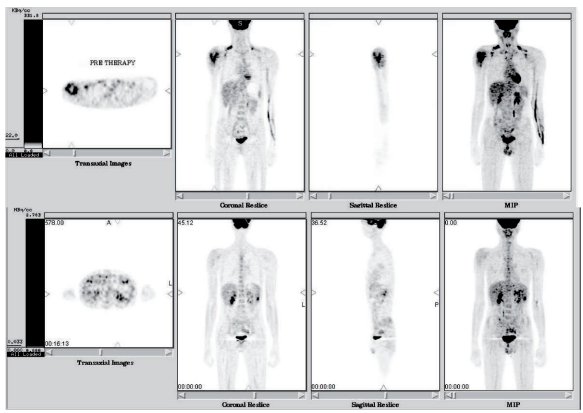

Figure 3.9 (A) (upper panel): 20-year-old male, PNET of right proximal humerus. Whole-body FDG-PET at the baseline, showing uptake at the site of primary tumor. Prechemotherapy SUV_{max} of primary tumor: 4.71. (B) (lower panel): Whole-body FDG-PET after NACT. Postchemotherapy SUV_{max}: 1.02. Percentage change in SUV_{max}: 78.04%; HPE: 95% tumor necrosis. (Reproduced with permission from Ref. [16].) *Abbreviation*: NACT, neoadjuvant chemotherapy.

of therapy with the goal of evaluation of residual/viable disease in the tumor

(ii) Utilized for early detection of recurrence

In both aforementioned scenarios, the findings will influence patient management at an individual level.

3.4.1.4 Other advantages of whole-body FDG-PET imaging

Synchronous or metachronous second primaries unrelated to the primary have been increasingly detected with the increasing use of FDG-PET/CT in oncology practice. This has been reported in the literature and involves a wide range of malignancies. It is also emphasized that disease may be detected when it is confined to the site of primary [44, 45]. This allows disease management at the individual level at the earliest opportunity (Fig. 3.10).

Thus, as noted above, adopting a decision model based upon functional radionuclide imaging into clinical practice has the potential to address all the key objectives of personalized medicine [2], that is, better diagnosis and accurate disease staging, earlier and accurate therapeutic intervention and optimizing of the correct dose in an individual, reduction of patient morbidity related to adverse effects of ineffective therapies and a decrease of health care costs, and overall initiation and facilitation of novel therapies (drug development).

3.5 Functional Molecular Imaging with US: The Potentials toward Personalization

3.5.1 *Basic Principle*

Functional molecular imaging with US has been primarily dependent upon the development of microbubble contrast agents. This promising method has been the focus of translational research on molecular imaging and disease characterization with US, as well as for delivering targeted drug or gene therapy. The multiple applications of this technique using targeted microbubbles include

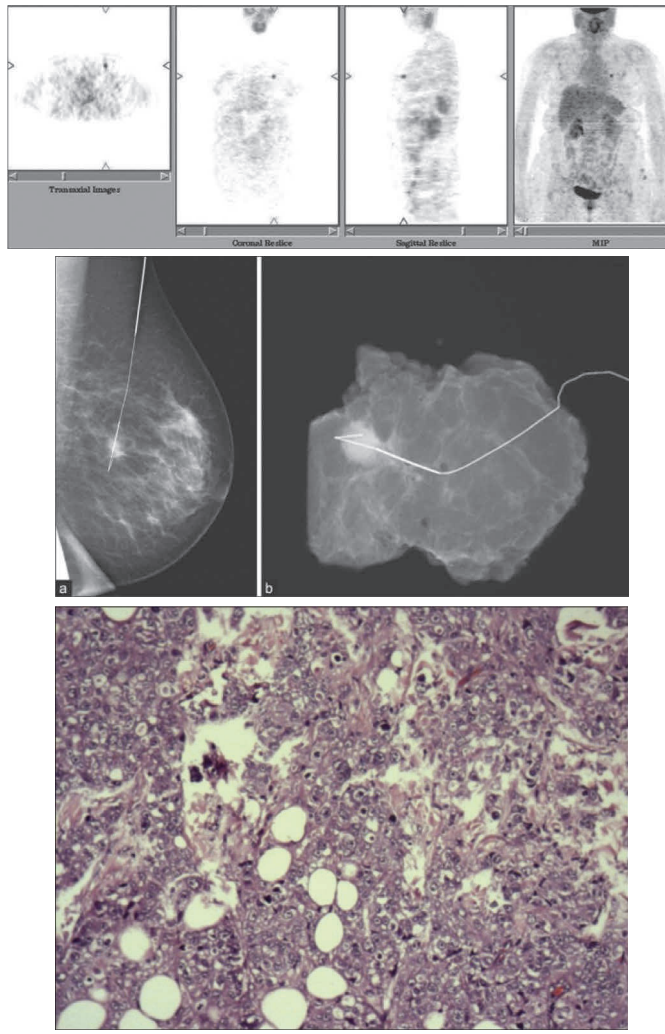

Figure 3.10 (a) Study demonstrating an intense abnormal disease focus ($SUV_{max} = 4.3$) in the left breast (reproduced with permission from Ref. [44]). (b and c) Mammographic hook wire localization was done for the nonpalpable mass corresponding to the FDG-avid left breast focus, and specimen radiography was performed showing the mass with the hook wire needle (reproduced with permission from Ref. [44]). (d) Section shows infiltrating ductal carcinoma (H and E, $\times 40$). The hormone receptor status was triple negative (ER-/PR-/HER2-) on immunohistochemistry (reproduced with permission from Ref. [44]).

assessing angiogenesis, inflammation, the cardiovascular system (e.g., highrisk atherosclerotic plaques) and tumors.

In the field of clinical oncology, the technique can be employed in two ways:

a. By targeting angiogenesis: A lipid-based microbubble contrast agent conjugated to a peptide, which is targeted to $\alpha_v\beta_3$, an integrin highly expressed by activated endothelium in neoangiogenesis. This has opened many new opportunities, including new functional imaging methods, the ability to image capillary flow and the possibility of molecular targeting using labeled microbubbles and the imaging signals that are reflected back to the US transducer. This aids in assessing neoangiogenesis associated with tumor growth at the molecular level. This could have far-reaching implications for angiogenesis-based therapeutic intervention in patients with cancer.

For assessing tumor neovascularity, the application of the reperfusion kinetic method has been a significant development over the power Doppler mode as the former allows capillary imaging.

b. By targeting tumors: Some of the promising applications of microbubble US that have also been examined in the domain of tumor targeting. Certain examples that are highlighted in the peerreviewed literature:

(1) Barbarese et al. studied C6 glioma and L9 glioscarcoma brain tumors by using fluorescence-labeled lipid-coated microbubbles [48].
(2) Targeted breast biopsies for patients with early breast cancer: In one study, microbubble contrast–enhanced US accurately identified the sentinel lymph node (SLN) in 89% of the patients in our study. The results have been promising to be entered into comparison with conventional SLN biopsy method, that is, the blue dye and radioisotope methodology. This has the potential to be combined with less invasive biopsy and thereby preclude the need for a surgical SLN biopsy [49].
(3) Targeted biopsy of prostate carcinoma using transrectal US with perflubutane microbubbles: This was found to enhance detection and provide efficient characterization, especially in the transitional zone area in one study. The investigators

suggested that this procedure might lower the number of biopsies and more accurate diagnosis of prostatic carcinoma [50].

(4) Three-dimensional power Doppler US to monitor response of primary peritoneal papillary serous carcinoma to treatment and to differentiate residual tumor from post-treatment fibrosis [51].

3.6 Functional Molecular Imaging with MRI: The Potential toward Personalization

In the recent literature, the feasibility of obtaining functional information with MRI has been highlighted. This has been particularly possible with hyperpolarized MRI using ^{13}C, ^{3}He, and ^{129}Xe. The advantage is due to the feasibility of achieving a high signal-to-noise ratio through external nuclear polarization. Various methods for hyperpolarization have been employed, which include the following: [a] optical pumping, [b] para-hydrogen-induced polarization, and [c] dynamic nuclear polarization [51–57].

The initial applications of hyperpolarized MRI have been in lung studies using noble gases. However, this technique has now been employed for metabolic studies as well. Among the various molecules, ^{13}C is particularly preferred for metabolic MRI as this can construct many biologically relevant organic compounds (pyruvate, urea, lactate, alanine, etc.). Hence, ^{13}C MRI and MRS have been investigated in tumor metabolic imaging. Among the various labels, hyperpolarized [1-^{13}C] pyruvate has particularly gained popularity in the area of metabolic MRI. The principle of this modality lies in measuring the lactate dehydrogenase (LDH)-catalyzed flux of the ^{13}C label between the carboxyl groups of pyruvate and lactate in the tumor. In an animal experiment, Day et al. observed that flux of the hyperpolarized ^{13}C label between pyruvate and lactate is decreased in mouse lymphoma cells in vitro and in lymphoma tumors in vivo after drug-induced cell death. The authors proposed that the measurements of hyperpolarized ^{13}C label flux between pyruvate and lactate can be employed to assess response to chemotherapy in malignancies in vivo. It is presumable that as pyruvate is

an endogenous substrate, it will be preferable over other MRS techniques due to obvious advantages.

In a recent editorial [57] on ^{13}C-polarized MRI, three areas have been proposed to be of significant promise in the field of hyperpolarized MRI. These are (a) polarization at a higher magnetic field, (b) relaxation time elongation, and (c) addressing of the low aqueous solubility of many interesting compounds. Among these the first issue has already demonstrated impressive results toward increasing the percentage polarization and thereby enhancing the signal achievable with a particular molecule.

In addition to hyperpolarized MRI, another promising approach for studying tumor characteristics is diffusion-weighted imaging (DWI). The principle of DWI is primarily based upon studying the random translational diffusion of water molecules. This modality has been primarily used to study brain tumors, particularly for early monitoring of therapeutic intervention [58]. Changes in tumor diffusion as early as 3 weeks following radiation therapy correlated with structural imaging changes at 10 weeks. It is predicted that this modality can be utilized as an early biomarker for tumor response, time to progression, and overall survival in brain tumors. A similar utility in early assessment of therapeutic response has also been suggested in a few studies in patients with breast cancer [59, 60].

3.6.1 *Molecular Optical Imaging*

The modality of optical imaging is an attractive and promising molecular imaging technique that has the ability to explore cellular and molecular events with high sensitivity [61–63]. The major advantages of the technique are (a) single-cell detection capability, (b) utilization of a large spectrum of contrast and hence probing of a wide range of endogenous and exogenous biomolecules and in vivo processes, and (c) exploration of events in real time that could be translated to assess pathophysiological phenomena and the effect of potential therapeutic interventions and novel agents.

The principle of optical imaging involves designing of biocompatible NIR fluorochromes, development of targeted and activatable "smart" imaging probes, and engineering of activatable fluorescent and bioluminescent proteins. The currently perceived potential

of molecular optical imaging lies primarily in early disease diagnoses, functioning of a number of pathways, and speeding of drug discovery. Multispectral opto-acoustictomography is a new development in the domain of optical imaging that has made possible (a) high-resolution imaging, (b) deep tissue visualization over several millimeters to centimeters of tissue depth, and (c) feasibility to resolve multiple tissue molecules at the same time [62]. The preliminary data validation has been undertaken in animal models. In the clinical area, it is being examined for applications in human intraoperative fluorescence-guided surgery.

The two types of noninvasive optical imaging techniques that are being extensively tested in preclinical, small-animal imaging settings [61] are (a) bioluminescence and (b) fluorescence imaging.

3.6.1.1 Principle of bioluminescence imaging

The underlying principle of this approach is dependent on a gene reporter/probe system, where the firefly luciferase enzyme acts as a gene reporter and *D*-luciferin or coelenterazine as a probe of luciferase gene expression. The enzyme is not normally expressed in mammalian cells, but when introduced as a gene reporter, it can be imaged optically on the administration of *D*-luciferin or coelenterazine as a probe. On oxidation of its substrate, light of 500 to 580 nm is produced and collected via a charge-coupled device (CCD) camera.

3.6.1.2 Principle of fluorescence imaging

NIR fluorescence imaging is based upon directly measuring gene expression without the use of an exogenous probe. There are two important differences of this method from bioluminescence imaging: (a) the optical signal is generated from the expression of an innocuous green or red fluorescent protein as a gene reporter, and (b) an excitation light source is required that illuminates the entire animal. The incident excitation light activates fluorescent proteins, and its subsequent relaxation back to the ground state generates a fluorescent photon that is available for imaging.

3.6.1.3 Translation into molecular imaging

NIR fluorescence can be translated into molecular imaging because of its ability to image signals from targeting compounds conjugated to NIR fluorophores and from those conjugated to chelating moieties for radio-metal sequestration. The increased photon count rate gives this modality superior detection ability compared to other modalities. With optical tomography of biological tissues, it would be possible to quantify agent uptake in terms of percent injection dose per gram (%ID/g) as can be obtained from SPECT and PET imaging. This can aid in the development of personalized medicine further, though presently this is mainly restricted to preclinical animal experiments.

3.7 Conclusion

Current medical imaging data with regard to characterization of an individual's tumor phenotype, especially that obtained from functional radionuclide imaging like PET-CT and SPECT imaging, can answer some of the critical decision-making questions and thus aid in management on an individual basis. The other molecular imaging modalities (e.g., US, MRI, and optical imaging) also hold significant potential and with developments and refinements could be potentially utilized for individualized diagnosis and therapy and further better the clinical management of cancer patients.

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Chapter 4

Boron Neutron Capture Therapy: Active Agents and Lipid Carriers

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In this chapter we place into context the scientific developments that guide the application of boron-rich agents for the neutron capture therapy of brain cancer and also review the evolution of the scientific rationale that underpins current research efforts that are aimed toward the design of liposome-based delivery vehicles that will provide the means of facilitating the transport of boronated agents to their target site.

4.1 Introduction

Many brain diseases (e.g., epilepsy, Parkinson's disease, Alzheimer's disease, schizophrenia, depression, ischemia, oedema) arise from

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local or from peripheral physiological disorders. Others (e.g., encephalitis, meningitis, acquired immunodeficiency syndrome [AIDS], dementia) are caused by brain infections. The blood–brain barrier (BBB) represents the most significant obstacle [1] for drugs that must reach the brain *via* the blood compartment before they can be of therapeutic benefit in the treatment of diseases of the central nervous system (CNS). This cellular and metabolic semipermeable barrier separates the brain and the spinal cord from the blood (circulation) and regulates the entry of molecules into the brain. The surface area of the BBB is 5000 times larger than that of the blood–cerebrospinal fluid barrier located at the choroid plexuses [2].

The BBB is comprised of a complex network of cerebral endothelial cells, which form the capillaries of the brain and the spinal cord and which are connected with astrocytes and pericytes by means of a basal membrane. At their adjacent margins, the endothelial cells form tight junctions (zonula occludens [ZO]) that seal the paracellular pathway consequent to the strong interactions between several transmembrane proteins. These proteins block the diffusion of many blood solutes, inhibiting the access to brain extracellular fluid [3]. Only small (molecular weight <600) lipophilic circulating drug molecules may diffuse through the BBB [4]. The BBB has carrier-mediated transport mechanisms working for influx or efflux of endogenous and exogenous compounds, a receptor-mediated transcytosis mechanism specific to certain peptides (such as transferrin and insulin) and an adsorptive or absorptive-mediated transcytosis mechanism [5–9]. Rationalized by the principle that nutrients and peptides pass through the BBB *via* receptor-mediated or carrier-mediated transport systems (commonly low-density lipoprotein [LDL] receptors, insulin receptors, and transferring receptors), an attempt has been made to deliver actives into the CNS through the deployment of drug-loaded liposomes [10].

While the BBB in patients with high-grade gliomas and brain metastases is typically disrupted, allowing passage of fluid into the extracellular space, the increased permeability of the BBB is primarily owing to opening of the interendothelial tight junctions (and also due to increased endothelial pinocytosis and endothelial fenestrations), demanding that even in these cases the active needs

to be transportable through the intact sections of the BBB if it is to be made available at all sites of tumor nucleation.

Boron neutron capture therapy (BNCT) is a two-step radiotherapeutic technique that involves the selective delivery of ^{10}B-rich agents to tumors and their subsequent irradiation with low-energy neutrons. The excited ^{11}B nuclei that are thus formed undergo fission to yield high-linear-energy transfer particles, essentially highly cytotoxic $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions, which move over short distances—ca.5 μm and ca. 9 μm, respectively—to effect cell death.

4.2 Liposomal Carriers for Delivery to the Brain

Amongst attempts to transport drugs across the BBB, chemical modification of the drug [11, 12] or the opening of this barrier by osmotic methods [13] has received most attention. However, chemical modification invariably alters the pharmacological profile of the drug whereas osmotic methods represent a massive invasive treatment. An alternative strategy to the delivery of drugs to the brain involves the employment of nanostructured formulation [14–17].

Liposomes are vesicular structures in which an aqueous volume is surrounded by a phospholipid membrane. Their size can range between 30 nm and several micrometers. They may consist of one (unilamellar) or more (multilamellar) homocentric bilayers of amphipathic lipids (mainly phospholipids). Liposomes have been initially invented by Alec Bangham [18] to serve as a model for cell membranes in biophysical studies. In the 70's they started to be investigated as promising drug carriers [19, 20]. The suggested use of liposomes in drug delivery has been rationalized in terms of their (i) versatile structure, which can be readily tailored in order to bear the properties needed for each specific application, and (ii) capacity to accommodate any type of drug molecules either in their aqueous compartments (hydrophilic drugs) or in their bilayers (lipophilic drugs) or both (ampliplilic drugs). In addition, there exists a large array of liposomal formulations that are nontoxic, nonimmunogenic, and biodegradable. In this respect, liposomes hold great promise as carriers for drug delivery

to the brain. They offer the promise to maintain the levels of many drugs at a therapeutically desirable range and to increase the half-lives, solubility, stability, and permeability of many drug molecules. However the fast non-specific clearance of liposomes from circulation by the RES cells may be readily addressed by coformulation with polyethylene glycols (PEGs) and/or targeting ligands [21].

4.3 Boron Neutron Capture Therapy

Following the discovery of the neutron by Sir James Chadwick in 1932, a study by H.J. Taylor in 1935 demonstrated the capability of ^{10}B nuclei to capture thermal electrons. This neutron capture is followed by the fission of the resultant ^{11}B nuclei into helium-4, α-particles and lithium-7 particles. It was this combination of scientific findings that allowed G.L. Locher, in 1936, to lay the foundation for neutron capture therapy as an approach toward the treatment of cancer and further led to the development of the basic theory of BNCT being introduced [22, 23].

BNCT is a radiochemotherapeutic technique that provides a way of selectively destroying malignant cells in the presence of normal cells [24]. BNCT involves the nuclear capture and fission reactions that occur when ^{10}B, a nonradioactive, naturally occurring isotope of the element, is irradiated with low-energy (thermal) (LET) neutrons to yield high-linear-energy-transfer (HLET) α-particles and recoiling ^{7}Li (Fig. 4.1).

Within living tissue, HLET α-particles have a specific path length of 5–9 μm, which implies that they offer the potential to selectively destroy cancerous cells. To sustain a lethal dose, BNCT requires the

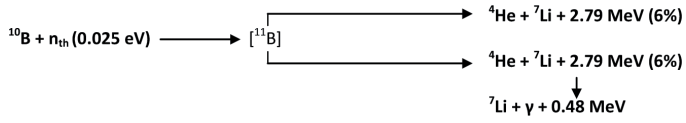

Figure 4.1 The outcome of the interaction between ^{10}B and thermal neutrons.

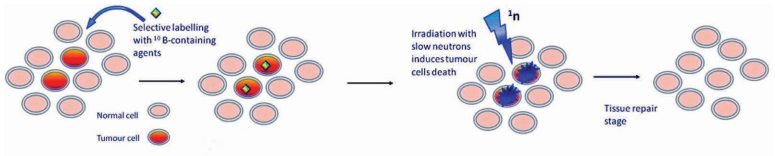

Figure 4.2 Idealized schematic representation of the stages of BNCT.

successful and targeted delivery of therapeutic quantities of ^{10}B (ca. 20 μ of boron/g of tumour [25].

The principle of BNCT is illustrated in Fig. 4.2: the malignant cells take up a formulation/compound that has been designed to selectively deliver ^{10}B to the cancer cells. The tumor cells, which are loaded with ^{10}B, are then irradiated using a source of slow-moving epithermal neutrons. The boron neutron capture process produces HLET particles, which kill the ^{10}B-containing cells, leaving behind healthy cells.

Boric acid and its derivatives represent the first generation of boron compounds to be considered for BNCT applications. Disodium mertacapto-*closo*-dodecaborate (sulfhydryl boron hydride [BSH]) and 1-4-dihydroxyborylphenylalanine (BPA) (so-called second-generation compounds) have both reached the clinical trial stage, owing to low toxicity, longer retention at the tumor site, and favorable (>1) tumor/brain and tumor/blood ratios [26, 27]. In addition to formulation strategies [28, 29], the next advancement in boron-facilitated therapy saw the development of molecules in which a stable boron group or boron clusters (carboranes) are functionalized or coformulated with amphiphilic biomolecules (e.g., porphyrins [30]) that facilitate transport or with tumor-targeting moieties (e.g., monoclonal antibodies [31]).

The carboranes ($C_2B_{10}H_{12}$) are organometallic compounds consisting of carbon, boron, and hydrogen (Fig. 4.3). They are synthesized by the reaction of acetylene or its derivatives with boron hydrides. The polyhedral structure of carborane may exist in one of three isomeric forms: *ortho*, *meta*, or *para*.

BNCT has been the subject of early-stage clinical trials concerned with the treatment of malignant brain tumors, malignant melanoma, hepatoma, and head and neck tumors [32, 33]. Of particular interest