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The third annual BRDS on research and development of nucleic acid-based nanomedicines

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Abstract

The completion of human genome project, decrease in the sequencing cost, and correlation of genome sequencing data with specific diseases led to the exponential rise in the nucleic acid-based therapeutic approaches. In the third annual Biopharmaceutical Research and Development Symposium (BRDS) held at the Center for Drug Discovery and Lozier Center for Pharmacy Sciences and Education at the University of Nebraska Medical Center (UNMC), we highlighted the remarkable features of the nucleic acid-based nanomedicines, their significance, NIH funding opportunities on nanomedicines and gene therapy research, challenges and opportunities in the clinical translation of nucleic acids into therapeutics, and the role of intellectual property (IP) in drug discovery and development.

Keywords

Drug delivery; Gene medicine; Non-coding RNAs; Nanomedicines

Introduction

The annual Biopharmaceutical Research and Development Symposium (BRDS) was introduced by a leading pharmaceutical scientist team in 2014 led by Dr. Ram I. Mahato, currently a professor in the Department of Pharmaceutical Sciences, College of Pharmacy at the University of Nebraska Medical Center (UNMC), Omaha. BRDS was initiated to attract scientists from both academia and industry to discuss the current issues and perspectives for a new drug development from scientific, industrial, and regulatory perspectives. Academic-industry alliance has emerged as a major platform for the development and commercialization of several biomedical and healthcare innovations. The relationship is often centered on mutual benefits and often includes academic-based intellectual property (IP) or cutting edge research in return for financial support or compensation, or access to proprietary technologies and services from the industry. This type of academia-industry collaboration is based on the promise of potential opportunities to develop technologies that benefit the integrated health system and can be brought to the market for the benefit of the healthcare community. Fundamentally, the complementary perspectives and skill sets of

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academia and industry are the major driving forces for this alliance. Industrial partner can provide commercially validated products and services to advance the core mission of any academic institute research, education, patient care, and community service. For collaboration, industry can provide financial and personnel resources in addition to proprietary technologies and services to accelerate the development of academic science and technology. The market and industry insight offered by industry leadership is also instrumental in driving the value of academic translational research. Thus, this relationship involves collective learning from each other.

In the spirit of strengthening the relationships between academic researchers and leading biopharmaceutical industries, research collaboration, and student learning objectives, the first annual BRDS was organized on June 5–6, 2014 at the UNMC. Following the success of the first annual BRDS, the second annual BRDS was organized on April 7–8, 2015 at UNMC. These symposia highlighted the current state-of-the-art and practices in pharmaceutical industries in the discovery and development of new drugs. They featured the invited speaker presentations by leading biopharmaceutical industry scientists and a round-table discussion with the faculty, post-docs, and graduate students of Nebraska University campuses (UNMC, UNO, and UNL), Creighton University and other nearby universities such as South Dakota State University, University of Missouri Kansas City, on topics of mutual interest. For the faculty members and investigators, these meeting provided insights into the functioning and processing of products at the industrial level along with serving as a source of technology transfer and getting recognition and securing financial aid. While for the students and postdocs, these were an opportunity to directly interact with the experts in the field, understand the skill set required to obtain a suitable job in the industry, and thus carve a niche for themselves in today's competitive world. In summary, these symposia became the foundation for exchanging valuable tips to improve techniques, shared new methodologies and discussed how to increase visibility and enable collaborations with industrial partners, guide to successful industry-academia collaboration, scale-up challenges, and considerations for bench-level formulations and bringing academia closer to real industry level pharmaceutical processes.

The third annual BRDS was organized on September 14– 15, 2016 in the new UNMC Center for Drug Discovery and Lozier Center for Pharmacy Sciences and Education which had a grand opening on August 23, 2016 (Fig. 1). This is the (x0024)35 million, 85,000-square-foot new building with state-of-the-art research and educational space to prepare future pharmacists for the changing health care landscape. Student spaces include enclosed one-person work lounges, an open space for larger group study and collaboration, and educational spaces that include two large classrooms, two-midsized classrooms, two auditoriums, and twelve-group meeting rooms. The type of work done in this new building is the embodiment of the medical center's mission to lead the world in transforming lives to create a healthy future for all individuals and communities. Research spaces include laboratory and support spaces for research in three areas: (1) drug discovery and development, (2) drug delivery, and (3) clinical and translational research. The center has a unique focus on infectious diseases where UNMC researchers have made breakthrough in the treatment of tuberculosis, food-borne illnesses, malaria, and HIV/AIDS. Other focus areas include traumatic brain injury, cancer treatment, pharmacogenetics, and nanomedicine.

The theme of the third annual BRDS was the “Research and Development of Nucleic Acid-Based Nanomedicines” to explore the recent advances and challenges of various aspects of drug discovery, delivery, and development. Well-renowned scientists from both academia and pharmaceutical industries and young researchers within the USA gathered to discuss novel findings and to troubleshoot technical problems in the field of regenerative medicines and application of nucleic acid-based nanomedicines (Fig. 2). Dr. David Oupicky (UNMC) initiated the symposium by a welcome speech with a brief introduction about the third annual BRDS. At this symposium, 15 scientists were invited from academia to industries for oral presentations along with podium presentations from four young scientists and 16 poster presentations which helped scientists to promote alliance of diverse interdisciplinary approach, including, but not limited to, drug delivery, pharmacokinetics, discovery, polymer chemistry, cell biology, genomics, bioengineering, informatics, and clinical development. It also facilitated the knowledge sharing and intense discussion among the scientists with broad interest in the field of gene therapy. Attendees presented their case studies, novel approaches and technologies, clinical success and learning from failure, as well as their future directions. In addition, the symposium also promoted a collaboration between scientists and various stockholders of the development of nucleic acid-based therapeutics including academia, pharma-biotech industries, and regulatory bodies.

Nucleic acid-based nanomedicines

Uncovering molecular pathways of human diseases soon after the completion of human genome project have provided hope for the improvement and clinical translation of gene therapy. The opportunity to use nucleic acid molecules such as DNA, RNA, deoxyribonucleic or ribonucleic acid aptamers, ribonucleic acid decoys, ribozymes, small interfering RNAs (siRNAs), complete genes, and noncoding RNAs (ncRNAs) as therapeutics is a novel approach for the treatment of genetic and acquired diseases. Due to their high specificity and low toxicity, many believe that gene medicines will be offered as effective personalized therapies [1]. Unwinding of genes responsible for various pathophysiological conditions and advent of tools to modulate gene expression fueled excitement for gene therapy. However, early excitement has tapered off, and realistic understanding of various issues has been realized over time. Research over the years led to unraveling of the complex barriers to efficient gene medicines. These hurdles include site-specific delivery of nucleic acids to target cells or tissues, stability, and immunogenicity [2]. These potential issues in translation of nucleic acids into therapeutics have diminished the impact of the genomics revolution and led to the decline in innovation and productivity of pharmaceutical research. Scientists from academia and industries have been actively working to overcome these obstacles by designing effective carriers for their in vivo delivery along with the modification of nucleic acid backbone to enhance their stability and reduce immunogenicity. Although these efforts have not yet been fructified, the number of preclinical and clinical trials of gene medicines has grown in the recent years. To further intensify the development of gene medicines, an interdisciplinary approach is required.

Among the invited speakers, Dr. George Calin (Anderson Cancer Center) discussed about the involvement of ncRNAs in the initiation, progression, and metastases of human cancer. Per Dr. Calin, aberrant expression of ncRNAs in multiple diseases strongly suggest its

significance which one or the other is underestimated by scientific and medical communities. Differential expression of ncRNAs in malignant compared to normal cells can be explained by its location, epigenetic mechanisms and alterations in the processing machinery. In addition, ncRNAs profiling from tumors to identify signatures associated with diagnosis, progression, and prognosis responses to treatment and identification of oncogenic genes involved in cancer. Therefore, exploring the correlation between ncRNAs with cancer could potentially be used as biomarkers or novel therapeutics in cancer and other diseases [3]. Similarly, Dr. Anita G. Seto (miRagen Therapeutics) enlightened on the development of a locked nucleic acid (LNA) based on oligonucleotide inhibitor of miR-155 called MRG-106. Treatment of resistant hematological malignancies (HM) remains an area of high unmet need. miR-155 is a well-described oncomiR associated with poor prognosis in multiple malignancies, particularly lymphoma, and leukemia. Cutaneous T-cell lymphoma (CTCL) is a rare HM with limited treatment options and a strong mechanistic link to increased miR-155. Because of the accessibility of cutaneous lesions, CTCL provides a unique opportunity to determine whether inhibition of miR-155 has a therapeutic potential in lymphomas associated. Per Dr. Seto, in vitro studies of MRG-106 on mycosis fungoides (MF) cell lines did not require additional formulation to achieve maximum pharmacodynamic efficacy however, inhibition of miR-155 resulted in transcriptome changes consistent with miR-155 target gene modulation, reduction in cell proliferation, and activation of the programmed cell death. A set of 600 genes was computationally identified to represent the translational pharmacodynamic biomarker signature, both direct and downstream of miR-155 when MF cell lines were treated with MRG-106. Therefore, initiation of a 4-week first-in-human clinical trial in CTCL MF patients was discussed at this symposium. On the other hand, Dr. Jeffrey Waring (AbbVie Inc.) shed light on the development of new classes of nucleic acid-based drugs, with an emphasis on microRNAs (miRNAs) and siRNAs. siRNAs which act through the RNA interference (RNAi) pathway are specific and potent inhibitors of gene expression making them a potential therapeutic agent. Numerous proof-of-concept studies are in clinical trials demonstrating the broad potential and therapeutic value of RNAi therapeutics. The talk of third annual BRDS was not only limited to the discovery of molecular mechanisms of ncRNAs and its therapeutic uses but also to the challenges and opportunities in the clinical translation of nucleic acids into therapeutics. Dr. Paul Burke, who is the principal scientist of Burke Bioventures LLC, a biotechnology consultancy focused his talk on translating research breakthroughs, particularly those based on nanotechnology, targeting, and RNA into products. Per Dr. Burke, new biology ranging from advances in understanding the role of RNA to new approaches to genome editing presents an explosion of an opportunity for therapeutic intervention. Realizing this potential involves in traversing biological barriers at the cellular, tissue, and organismal levels, the commercial hurdles associated with the clinical translation of novel technologies must be overcome. For instance, delivery approaches for a broader range of targets, along with increased safety margins, are important challenges need to overcome. Interdisciplinary approaches to addressing these challenges were discussed among academic and industrial investigators in the symposium.

Other therapeutics approaches

In addition to nucleic acid-based nanomedicines, the third annual BRDS was also framed to cover other important therapeutic approaches. For instance, Dr. Nagendra Chemuturi, from Seattle Genetics highlighted his talk on antibody drug conjugates, a new modality in the treatment of cancer [4]. The purpose of Dr. Chemuturi's talk was to provide an insight into the current knowledge of absorption, distribution, metabolism, and excretion (ADME) properties of antibody drug conjugates and some of the challenges faced, from a drug metabolism and pharmacokinetic (DMPK) perspective. This approach combines with the receptors specificity antibody to selectively deliver potent cytotoxins to tumor cells rather than healthy. Around 30 different kinds of antibody drug conjugates are being investigated in various cancers. On the other hand, Dr. David B. Berkowitz (University of Nebraska Lincoln) spoke about targeting pyridoxal phosphate (PLP) enzymes for mechanism-based inhibition. Dr. Berkowitz is active at the chemistry-biology interface, with interests in reaction development, catalyst screening, using enzymes in organic synthesis, development of tools for chemical biology, mechanism-based enzyme inhibition, and medicinal chemistry. In the latter area, the group is particularly interested in the development of inhibitors targeting PLP-enzymes and in the synthesis of podophyllum cyclolignans as potential antineoplastic agents. He has been dedicated to synthesize new classes of quaternary, alpha-substituted amino acids as "suicide substrates" or mechanism-based inhibitors of PLP-enzymes of the Ca-Cb-lyase family. Therefore, his talk was based on enzyme mechanism, inhibitor design, enzyme kinetic studies, and progress toward demonstrating proof-of-principle for therapeutic agent development.

Dr. Sam Sanderson (University of Nebraska Medical Center and Prommune, Inc.) talked about challenges and opportunities in vaccine-based therapeutics. Over the years, the casual overuse and misuse of antibiotics have imposed mutational pressures on superbugs impelling the development of evasive mechanisms to enhance their survival. The result is the emergence of resistant strains that are not only recalcitrant to the current antibiotic arsenal, but whose rapid worldwide expansion has out-paced the development of new antibiotics to treat them. This alarming rise of resistant bacteria and the dwindling antibiotic treatment options underscore the urgent need for a new therapeutic strategy that extends beyond the scope of antibiotics and their limitations in the face of a rapid bacterial mutation. Thus, an ideal therapy would be one capable of treating normal-resistant infections in both animals and humans. The ability to induce host innate immune responses represents such therapeutic approach. Such "host-directed immune therapy" invokes the body's natural first-line of defense to infection without directly influencing mutable aspects of bacterial replication since this therapy is not imposed directly on the bacteria like antibiotics. Dr. Sanderson's laboratories have been involved in the design and synthesis of immune stimulatory peptides capable of safely and selectively mobilizing the cellular and molecular components involved in induction of innate and acquired immunity. His focus has been on generating libraries of conformationally-constrained pep-tides from the biologically active C-terminal region of human complement component C5a. From this approach, he has generated a lead therapeutic candidate (EP67), which induces innate and acquired immune outcomes via the selective engagement and activation of C5a receptor (C5aR/CD88)-bear-ing antigen

presenting cells, but not C5aR-bearing inflammatory granulocytes [5, 6]. Thus, innate and acquired immune outcomes are generated with little or no inflammatory side effects. The principal objective of Dr. Sanderson's laboratories and collaborative network is to translate EP67 or improved analogs for the invocation of innate and acquired (vaccine) immune outcomes against bacterial, viral, and fungal infections in humans and domestic animals.

Dr. Stelios T. Andreadis (University at Buffalo) talked about directing and monitoring stem cell fate for vascular tissue regeneration. Dr. Andreadis research areas include adult and induced pluripotent stem cells for cardiovascular tissue engineering, gland, skin, tissue engineering and regeneration, molecular design of biomaterials, cell-cell adhesion in stem cell fate decisions, protein and gene delivery, and lentiviral microarrays for high throughput pathway analysis of stem cell differentiation and reprogramming. Cardiovascular disease is the leading cause of mortality worldwide. Regarded as the therapeutic gold standard, treatment with autologous grafts suffers from several technical and patient related risks. Tissue engineered small diameter blood vessels may provide a promising alternative solution as replacement grafts. Therefore, his research group employed adult and induced pluripotent stem cells to engineer fully functional vascular grafts that were implanted into the arterial circulation of a physiologically relevant ovine animal model. Dr. Andreadis also talked about the results on the development of strategies to improve the quality and function of adult mesenchymal stem cells which suffer from the loss of cell function due to poor aging or culture senescence [7, 8].

Dr. Lori A. Henderson from the National Institutes of Health (NIH) talked about the NIH investment in nanotechnology and funding opportunities. Nanomedicine is a specific medical intervention at the molecular scale for curing disease or repairing damaged tissues, such as bone, muscle, or nerve. The NIH's mission is to seek fundamental knowledge about the nature and behavior of living systems and to apply that knowledge to enhance health, lengthen life, and reduce illness and disability. The primary goal of nanotechnology is to characterize the molecular components inside cells at nanolevel which will help to the reengineering components at intracellular level for therapeutic benefits. In support of this mission, the NIH has invested about (x0024)4.2 billion since 2001 in nanotechnology research. This investment is leading to fundamental changes in understanding biological processes in health and disease, as well as enabling novel diagnostics and interventions for treating disease. NIH scientists are developing molecular agents and methods for earlier and more accurate diagnosis, therapies aimed directly and selectively at diseased cells, and are exploring root causes of common and rare diseases at the proper length scales.

Dr. Poonam R. Delvadia from the Food and Drug Administration (FDA) shared her views on the role of biopharmaceutics on orally administered drug development. Biopharmaceutics is the study of interdependence of physiological aspects of the patient (ADME), the physicochemical properties of the drug (e.g., solubility, polymorphism, particle properties, etc.), and the formulation variables (e.g., composition, dosage form, manufacturing process, etc.) that directs the development and helps understand in vivo behavior of the drug. Dr. Delvadia beautifully explained about the tools needed for optimizing the drug performance and study the impact of the changes made during a drug product's lifecycle on clinical

efficacy and safety. She also talked on how biopharmaceutics play a pivotal role in linking quality of product to the patient.

Finally, the talk of the first day was ended by Dr. Michael Dixon from UNeMed Corporation, a company that works with faculty, students, and staff of UNMC to help commercialize innovative, new ideas that have the potential to improve public health for Nebraska residents, and beyond. His talk was focused on the role of IP in drug discovery and development. In his talk, he discussed about the critical role of IP, the valley of death, and how universities and companies work together to achieve the monumental task of developing a new discovery into a lifesaving drug. Similarly, the symposium for the second day was ended by a talk on industry-academia association by Dr. Jasmine Davda (Pfizer). She beautifully presented her experience on how biopharmaceutical companies need academia and vice versa. She also shaded light on the key facts on the biopharmaceutical research and development process which on average takes about 10 years for single drug to be approved by FDA with an average cost of (x0024)2.6 billion including cost of failures. Per Dr. Davda, the relationship between academia and industry is complementary which naturally lends itself to the information of joint research enterprises. She also put light on the important skills the employers are looking while recruiting new employees. For instance, leadership, teamwork, communication, problem solving, relationship building, and decision making are the key transferable skills the employers seek during recruiting graduate students while creativity, entrepreneurial skills, and ability of foreign language skill count the least.

Young scientists' presentations

Besides oral presentations by world leaders, four young scientists were selected to present their works at this third annual BRDS. A graduate student from South Dakota State University shared his research on development of food grade biopolymer based novel core-shell nanoparticles for drug delivery orally. The object of his study was to investigate the feasibility of developing core-shell nanoparticles using food grade biopolymers. The core-shell nanoparticles were developed using corm protein zein as the hydrophobic core and milk proteins (casein, lactoferrin, beta-lactoglobulin, or whey protein isolate) or polyethylene glycol (PEG) as the hydrophilic core. Another, a graduate student from UNMC presented his work related to chloroquine modified starch as a novel approach for multifunctional drug delivery. As hydroxychloroquine is a safe and well-tolerated drug used for 70 years, it may serve as a potential self-assembling moiety with therapeutic benefits in the development of delivery vehicles for cancer treatment. Therefore, the objective of his study was to develop chloroquine-modified starch polymer to encapsulate anticancer drug for treating cancer in a mouse model. Similarly, two postdocs from UNMC were selected to present their work on the biodistribution of micelles containing miRNA and a small molecule drug in pancreatic cancer-bearing mice model along with a combination treatment with nanoorlistat and taxanes for enhanced microtubule stability in taxane-resistant prostate cancer. In addition to these presentations, more than 16 posters were enthusiastically presented by scientists and students from and outside of UNMC, demonstrating their active interest in nucleic acid-based therapeutic research. Finally, the symposium was concluded by Dr. Mahato summarizing the accomplishments of this noteworthy symposium. He acknowledged his colleagues, scientists, and students for their active participation.

Moreover, he shared his experiences, achievements, and the benefits of international collaboration in research. Additionally, awards were handed out to the best podium and poster presenters to encourage research activities among young scientists.

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Fig. 1. UNMC Center for Drug Discovery and the Lozier Center for Pharmacy Sciences and Education

Fig. 2. Participants of the third annual Biopharmaceutical Research and Development Symposium (BRDS)