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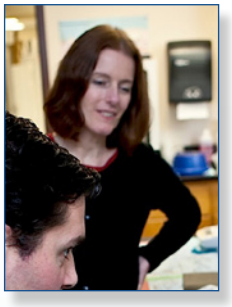

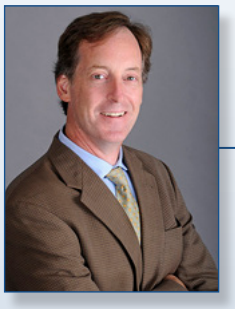

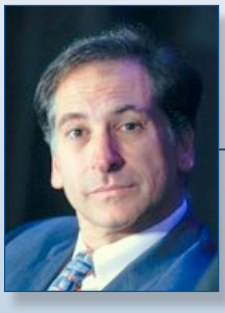

Showcasing Bioscience in Rhode Island

DENICE SPERO, PhD
GUEST EDITOR

Denice Spero, PhD, is a research professor and co-director of the Institute for Immunology and Informatics at the University of Rhode Island (URI) and a founder of Rhode Island BioScience Leaders.

There are a number of well-recognized bioscience companies located in the greater Providence area. They represent a significant and growing source of jobs and future revenue, which promises to play a role in the revitalization and expansion of Rhode Island's economy. In an effort to support these companies and to showcase their research, the *Rhode Island Medical Journal* is highlighting five of these innovative enterprises in this issue. The companies selected are members of the Rhode Island BioScience Leaders organization, and their research spans a wide range of science, from biologics and informatics to innovative coatings for medical devices. They include ProThera Biologics, EpiVax, Tivorsan Pharmaceuticals, BioIntraface, and VeroScience.

COURTESY OF DENICE SPERO

Denice Spero, PhD, in rear of photo, is co-director of the Institute for Immunology and Informatics at the University of Rhode Island.

COMPANIES AT A GLANCE

PROTHERA BIOLOGICS was founded in 2002 by Yow-Pin Lim, MD/PhD, and Douglas Hixson, PhD, to commercialize their research on Inter-alpha Inhibitor Proteins (IAIP) for the treatment of pathological conditions caused by dysregulated inflammatory response such as sepsis/septic shock, Anthrax infection/intoxication, necrotizing enterocolitis and acute ischemic stroke.

EPIVAX, INC., founded by Anne De Groot, MD, William Martin and attorney Fred Stolle, has developed a set of immunoinformatics tools and is leveraging these tools to design better vaccines, de-immunize protein therapeutics and to develop new immune modulatory therapies (T Regulatory Epitopes) to treat a wide variety of diseases.

TIVORSAN PHARMACEUTICALS, formed by Justin Fallon, PhD, professor of neuroscience at Brown University, is a protein therapeutics company pioneering a unique approach to treating serious neuromuscular disorders, including Duchenne Muscular Dystrophy (DMD) and Becker muscular dystrophy (BMD). This method, using recombinant human biglycan (rhBGN), is based on 24 years of basic science work in the Fallon laboratory at Brown University.

BIOINTRAFACE, founded by John Jarrell, PhD, utilizes platform technologies to create economical metal oxide and polymer materials and coatings to control the bioactivity and antimicrobial properties of medical devices and implants.

VEROSCIENCE, founded by Anthony Cincotta, PhD, is developing drugs to treat metabolic and immune system disorders such as type 2 diabetes, obesity, and cancer through its platform technology, Neuroendocrine Resetting Therapy (NRT). This Tiverton-based company has the distinction of being granted FDA marketing approval for its drug Cycloset in May of 2009. Cycloset is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

The Bioscience industry represents a significant part of the future knowledge economy of Rhode Island, and its continued growth and success will lead us into a future with an expanded business base creating new drugs and devices to ultimately improve human health.

ProThera Biologics, Inc.: A Novel Immunomodulator and Biomarker for Life-Threatening Diseases

YOW-PIN LIM, MD, PhD

ABSTRACT

ProThera Biologics is a development stage bio-therapeutics company in East Providence, Rhode Island. The company was founded in 2002 to focus on the critical role and commercial potential of Inter-alpha Inhibitor Proteins (IAIP) for treating acute life-threatening inflammatory diseases. The discovery research originated in the basic research laboratories of the co-founders, Yow-Pin Lim, MD, PhD, and Douglas C. Hixson, PhD, at Rhode Island Hospital, a Lifespan partner. The company is backed by the Slater Technology Fund and has received research grants from the Rhode Island State Science and Technology Council (RI STAC) as well as continuous funding from the National Institutes of Health (NIH), with several Phase I and II Small Business Innovation Research (SBIR) grants over the past 10 years.

ProThera has developed a novel process to purify Inter-alpha Inhibitor Proteins from source material, and has conducted groundbreaking research into the usage of IAIP to fight systemic inflammation.

KEYWORDS: inter-alpha inhibitor proteins, sepsis, septic shock, anthrax

INTER-ALPHA INHIBITOR PROTEINS: ENDOGENOUS PROTECTIVE MOLECULES

Sepsis and Systemic Inflammatory Response Syndrome (SIRS) both refer to a severe physiochemical reaction that may occur following exposure to an infectious agent (e.g. bacterial, viral, fungal) or injury such as burn, or trauma. This systemic hyper-reaction typically includes an excessive production of cytokines, known as a "cytokine storm," and destructive proteases, with disturbances in metabolic, oxygenation, coagulation, and vascular functions leading to multi-organ dysfunction.[1] Consistent findings in the complex pathophysiology of systemic inflammation/sepsis has shown that SIRS activates excess systemic protease activity, neutrophil proteases, proteases of the complement and coagulation systems and matrix metalloproteinases.[2] An array of endogenous protease inhibitors has evolved to prevent excess activation of proteases and to limit the potential injurious actions of protease activation on endothelial and epithelial tissues. Many of these protease inhibitors, such as antithrombin, alpha-1 antitrypsin, C1 esterase inhibitor, tissue inhibitor of metalloproteinases, and secretory leukocyte protease inhibitors are rapidly consumed in acute inflammatory states, often leading to a failure to appropriately regulate protease activity.

Yow-Pin Lim, MD, co-founder of ProThera Biologics, Inc.

One endogenous plasma protease inhibitor family that has recently received increased attention is Inter-alpha Inhibitor Proteins (IAIP). IAIP are a family of trypsin-type protease inhibitors composed of a unique combination of polypeptide subunits (light and heavy chains) covalently linked by a chondroitin sulfate chain.[3] Two major forms of IAIP are found circulating in human plasma:

Inter-alpha-Inhibitor (*IaI*), a larger molecule with 250 kDa composed of two heavy chains (H1 & H2) and a single light chain (L).

Pre-alpha-Inhibitor (*PaI),* a smaller molecule with 125 kDa composed of one heavy (H3) and one light chain (L).

The light chain (also termed 'bikunin'=*bi-kun*itz=inhibitor with two Kunitz domains) is known to have broad inhibitory activity against plasma serine proteases such as trypsin, elastase, plasmin, cathepsin G and furin.[4,5] Upon cleavage, the light chain, or bikunin, is released from the IAIP complex and rapidly excreted in urine. The high level of IAIP normally circulating in plasma, coupled with the fact that persons lacking IAIP have never been reported, suggests that these proteins have an essential physiological role. Relevant in this regard are our previous studies demonstrating that plasma IAIP level in infants are independent of gestational age and that even premature infants born at 24 weeks have similar levels to those found in adults.[6]

Although the full extent of the physiological functions of IAIP remains to be established, numerous studies have implicated that IAIP, or the lack thereof, play a significant role in systemic inflammation. Findings from ProThera and other laboratories have shown that IAIP comprise a crucial component of the body's protective defenses critical to modulating host response to pathological insults.

Decreased IAIP levels in life-threatening diseases

In healthy individuals, the amount of circulating IAIP in blood is relatively high (between 300-600 mg/L). However, IAIP levels rapidly decrease during systemic inflammation and sepsis.[7] In more severe cases, IAIP may be depleted up to 90% of the baseline value found in plasma of septic patients. To date, IAIP levels have been measured in over 2,000 patients serving as study participants in systemic inflammation following bacterial and viral infections.[7-11] The decreased levels of IAIP correlate strongly with the progression of the disease. As the diseases progress to more advanced and life-threatening levels, IAIP levels drop precipitously, suggesting IAIP's clinical utility as a prognostic and theranostic (a diagnostic test that helps the clinician to make the right therapeutic decision for the right patient) marker in assisting clinicians in monitoring disease progression and making informed treatment decisions.

Moreover, we recently studied the receiver operating characteristics of IAIP levels in 573 high-risk infants with suspected sepsis and demonstrated that IAIP level is a reliable biomarker with high sensitivity and specificity (89.5% and 99%) and a high positive and negative predictive value (85% and 98%) for neonatal sepsis.[8] Studies have also demonstrated that the IAIP level is significantly reduced in neonates with necrotizing enterocolitis (NEC), a devastating acute inflammatory condition of the gastrointestinal tract commonly found in premature infants with birth weights of less than 1500 grams. IAIP blood levels in infants with the confirmed NEC (Bell stage 2 and 3) was found significantly lower compared to the matched age control infants with non-specific gastrointestinal disorders.[11]

Thus, IAIP have proven to be a reliable diagnostic marker for neonatal sepsis and systemic inflammatory conditions like NEC. Since these acute conditions present a very serious threat to neonates, there is an urgent need to obtain confirmation as soon as possible. ProThera is currently conducting an NIH-funded project to develop a rapid point-of-care IAIP test that can be used to identify sepsis and necrotizing enterocolitis in high-risk infants with a simple, user-friendly and portable device suitable for use in the NICU setting.

BENEFICIAL EFFECTS OF IAIP THERAPY IN SYSTEMIC INFLAMMATION

As IAIP are found abundantly in human plasma and can be extracted and purified in high yield by a scalable production process similar to other blood derived products such as albumin and immunoglobulin (IVIg), a replacement IAIP therapy to reverse the decrease in systemic levels in pathological conditions is feasible.

Using a rat model of polymicrobial sepsis (Cecal Ligation and Puncture), we demonstrated dramatic improvements following treatment with human plasma derived IAIP administered 1 hour after sepsis induction as evidenced by recovery of hemodynamic stability, decreased organ injury, increased survival (2-3 fold) and arrested progression of sepsis.[12] Similar beneficial effects were achieved even if the IAIP administration was delayed 10 hours after the sepsis challenge, although at this point, the disease had progressed to a severe stage with at least one organ dysfunction.[13]

Similarly, studies in the neonatal model of endotoxin-induced systemic inflammation revealed that administration of IAIP significantly improves survival of the experimental animals.[14] The therapeutic effects of IAIP were also demonstrated in live organism models of sepsis using *E. coli* and Group B hemolytic *Streptococcus* (GBS), two of the most relevant pathogens in sepsis in human infants. Moreover, IAIP was found to attenuate the marked increase in pro-inflammatory cytokine TNF-alpha and to augment anti-inflammatory cytokine IL-10 in the septic animals.[14]

These results suggest that administration of IAIP exerts potent immunomodulatory activity that leads to a significant beneficial effect in adult and neonatal sepsis models. At least three distinct mechanisms in the inflammatory pathways contribute to the protective effects of IAIP: 1) inhibition of the serine proteases (elastase, cathepsin G, and several others)[4]; 2) modulation of pro-inflammatory cytokines (TNF-alpha)[12,14] and 3) blockage of excess complement activation (C5a).[15]

IAIP IN BIODEFENSE APPLICATIONS

Some scientific progress has been made in developing countermeasures for various biothreat pathogens in the post-9/11 era. However, it is becoming clear that the "one bug-one drug" approach such as vaccines or antiserum specific against a single agent is not a practical or sustainable approach.

The life-threatening consequences following exposure to biothreat pathogens do not typically arise directly from the causative agent, but from dysregulated host response leading to lethal systemic inflammation. As potent immunomodulators, IAIP can serve as a "first line of defense" in providing crucial protection against deadly acute systemic inflammation triggered by biothreat pathogens. In addition, as a broad-spectrum serine protease inhibitor, IAIP has a capability to block furin,[5] a cell membrane-associated endogenous serine protease that plays a critical role in the anthrax pathogenesis and several viral diseases.[16] As ProThera's IAIP therapy is independent from the causative agent (bacterial/viral or toxin), it can be applied immediately following exposure without risk of overdosing or misdiagnosis. Subsequently, once the causative agent is identified and confirmed, a more specific and targeted therapy with antibiotics or antiviral drugs to eliminate the pathogens can be initiated.

ProThera's current focus is targeted on Anthrax intoxication and infection, where the company has generated robust and promising data in the experimental animals,[5,17] and is conducting confirmatory studies in large animals including non-human primates (baboons). As the biodefense focus has shifted from the "single agent specific" countermeasures

to more "universal" defenses that address multiple pathogens, IAIP not only can potentially serve as an effective broad-spectrum therapy against biothreat pathogens (CDC Category A, B and C) but also against naturally emerging pathogens. To this end, early investigations have also indicated encouraging protective effects of IAIP in viral diseases (Influenza A and Dengue virus infection).

IAIP IN OTHER ACUTE INFLAMMATORY DISEASES

While ProThera is mainly focusing on the development of IAIP as novel therapeutic proteins in systemic inflammation/sepsis and Anthrax biodefense, recent investigations of IAIP effects in hypoxic and ischemic brain injury have revealed exciting results as well. In collaborations with Barbara Stonestreet, MD, at Women & Infants Hospital and Steve Threlkeld, PhD, at Rhode Island College, ProThera has been able to demonstrate the beneficial effects of IAIP in hypoxic and ischemic brain injury models in fetal sheep and neonatal/adult rats.

IAIP have been detected in neurons, astrocytes, and meningeal cells of the brain and, may function as endogenous neuroprotective molecules.[18] Moreover, studies have observed significant decreases of IAIP in brain tissues of experimental animals following ischemia-reperfusion injury. IAIP might serve as a novel agent to prevent/attenuate brain damage in infants at risk for mental/developmental disorders such as cerebral palsy and in adults following acute ischemic stroke.

SUMMARY

ProThera Biologics develops novel products that are based on its proprietary technology to produce and treat using Inter-alpha Inhibitor Proteins. The key to ProThera's strategy is a single biological product to be developed for the treatment of widespread pathological conditions caused by dysregulated inflammatory response such as sepsis/septic shock, Anthrax infection/intoxication, necrotizing enterocolitis and acute ischemic stroke. ProThera offers a rational targeted solution to treat deadly diseases by combining both the predictive test and the effective replacement therapy of natural occcuring Inter-alpha inhibitors. The successful development of an efficient and scalable manufacturing process combined with the implementation of viral inactivation steps will ensure a safe and effective IAIP product for testing in humans.

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Building Better Biotherapeutics and Vaccines by Design: EpiVax, Inc., an Immunology Company

LEONARD MOISE, PhD; ANTHONY MARCELLO; RYAN TASSONE; LESLIE COUSENS, PhD; WILLIAM MARTIN; ANNE S. DE GROOT, MD

ABSTRACT

EpiVax, Inc., is an early-stage informatics and immunology biotechnology company in Providence, Rhode Island. It applies computational tools to harness immunity in three major areas: immunomodulation, biotherapeutic immunogenicity risk assessment and de-risking, and vaccine development. Immunotherapy, bio-better and vaccine candidates under development at EpiVax promise to improve the health outcomes of millions of people affected by devastating immune-related diseases.

KEYWORDS: vaccines, immunoinformatics, immunotherapy, immunomodulation, autoimmune diseases

A BRIEF HISTORY OF EPIVAX

A talented post-baccalaureate, a statistics major, and a professor who aspired to develop an HIV vaccine are at the root of EpiVax. Gabriel Meister, Bill Jesdale and Anne S. De Groot, MD, were members of the TB/HIV Research Lab team in Brown University's BioMed Center that created two novel computer-driven tools, EpiMer and EpiMatrix, between 1992 and 1996. These 'epitope discovery' algorithms generated the foundation for a whole suite of advanced in-silico tools that now form the core of EpiVax, Inc., a privately held immunoinformatics company in Providence. The late Michael Lysaght, an approachable and optimistic Brown professor of biotechnology, was another instrumental person in the company's establishment; he recognized the promise of EpiVax and connected the founders to the Slater Center for Biotechnology, a source of funding that brought the technology out of the academe into the entrepreneurial world in 1998. With the addition of a programming expert (Bill Martin) and a formidable lawyer (Fred Stolle), a company was born.

Fifteen years later, EpiVax has evolved into a powerhouse of ideas that is changing the way that we think about vaccines and biotherapeutics. EpiVax has also been the source of an unusual spin-out, the Institute for Immunology and Informatics (established in 2008) at the University of Rhode Island, which has exclusive access to the EpiVax technology to research and develop vaccines for neglected tropical diseases and other targets. Team members at EpiVax are now working on a second spin-out devoted to another promising technology that may change the treatment of autoimmune disease.

URI

Dr. Anne S. De Groot, at URI's Institute for Immunology and Informatics at the University of Rhode Island (URI), is the CEO of EpiVax, Inc.

IN-SILICO DESIGN FOR VACCINES AND PROTEINS

Vaccines are among the most important inventions of modern medicine, but the technology for making vaccines was based on empirical rather than hypothesis-driven science until 1996, when molecular biology made bacterial and viral proteins interpretable by computers. EpiVax has harnessed the availability of whole genomes to develop bioinformatics algorithms and apply them to a four-point vaccine design strategy. Immunoinformatics tools are first used to sort through thousands of potential vaccine candidates in a pathogen's genome, comparing those sequences to similar pathogens and identifying sequences that would trigger a human immune response. Protein sequences are then mapped for short, linear, putative T cell epitopes. These epitopes are synthesized as peptides and evaluated in vitro and in vivo for human leukocyte antigen (HLA) binding and antigenicity in survivors of infection or vaccinees. Finally, the optimal composition of immunogenic sequences to drive an effective human immune response is computationally derived (iVAX software suite), and prototype epitope-based vaccines are

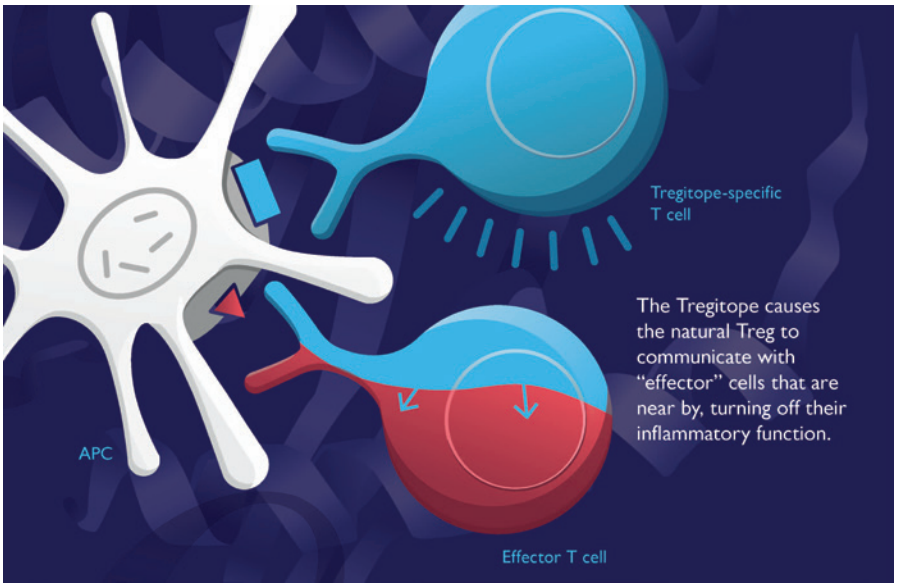
evaluated for immunogenicity and efficacy in humanized transgenic mice. Using this approach, we have demonstrated pre-clinical proof-of-concept for smallpox and tularemia prophylactic vaccination and therapeutic immunization for *H. pylori* infection.[1-4]

The genomes-to-vaccine strategy has two important advantages. First, it strips a pathogen down to the minimal essential antigens, eliciting robust and sustained protective immunity while eliminating non-essential information that could lead to diminished protective immunity and/or immunopathology sometimes associated with whole organism vaccines. This approach may appear to handicap vaccine design because vital elements (i.e., adjuvant, carrier structure) that are normally part of a pathogen are removed, but it creates a valuable opportunity that forms the second advantage. One can combine this novel approach with best-in-class adjuvant and delivery technologies for optimal vaccine construction.

This methodology also forms the core of immunogenicity screening, the process by which protein therapeutics are evaluated for their potential to elicit harmful responses that would impede their effectiveness. Non-vaccine protein therapeutics risk causing harmful immunoreactions, which can render a biologic ineffective and severely compromise patient health. For example, the induction of antibodies known as "inhibitors" against factor VIII in the treatment of hemophilia is a sign of therapeutic protein immunogenicity.[5,6] In 2001, antibodies to a commonly used therapeutic protein drug Erythropoietin, were linked to transfusion dependent anemia.[7] Consequently, unwanted immunoreactions to biologics are a major concern for physicians and drug developers.

EpiVax has thus developed an entire suite of immunoinformatics tools for prospectively identifying and reducing protein therapeutic immunogenicity "in silico," a process that dramatically reduces the time and effort involved, allowing drug developers to accelerate the pre-clinical development of their protein products. The tools are organized in an interactive website called the ISPRI (Interactive Screening and Protein Reengineering Interface) system. Using the ISPRI system, researchers have the ability to screen the protein sequences of product candidates for the presence and immunogenic potential of putative T cell epitopes (EpiMatrix) and epitope clusters (ClustiMer). Protein sequences can be ranked for immunogenic potential in comparison to known proteins on a normalized scale, and an interactive protein reengineering tool (OptiMatrix) allows researchers to modify, or deimmunize, T cell epitope clusters in real time by optimizing the amino acid sequence so that it is no longer able to interact with T cells.

The EpiMatrix toolkit has been extensively validated internally and externally, with several key publications demonstrating the technology and rigorous testing procedures using known protein therapeutic targets.[8,9] In addition, EpiVax incorporates exclusive knowledge of the impact of Tregitopes (T cell regulatory epitopes) on the immunogenicity of protein therapeutics in clinical use, leading to higher accuracy in immunogenicity predictions.



EPIVAX, INC.

TREGITOPES: AN EPIVAX DISCOVERY AND IMMUNOMODULATION POWERHOUSE

The discovery of Tregitopes, or "T Regulatory Epitopes" in one of the most common proteins found in blood (immunoglobulin G, or IgG) can be attributed to keen observation on the part of the scientific team at EpiVax. Tregitopes turned up regularly in the immunogenicity screens that were performed by the scientists at EpiVax as soon as the ISPRI tools were being applied to monoclonal antibody therapeutics, but were only recognized for their regulatory potential by De Groot and Martin in 2008.[10] Tregitopes act as a natural 'off switch' for the immune system. They are naturally part of the arms (Fab) and stem (Fc) of human IgG and are thought to balance the inflammatory triggers that are present in the re-arranged, or hypervariable segments (variable loops) of the antibody arms. Tregitopes are also found in Intravenous IgG (IVIG), a blood-derived product that is used clinically to control autoimmune conditions.[11] Indeed, some of the anti-inflammatory activity of IVIG may be due to the presence of Tregitopes.[12]

The Tregitope discovery has been validated in a range of standard preclinical models and by collaborating laboratories, where Tregitopes have been shown to suppress and treat autoimmune disease and allergies,[13] and to effectively suppress the immunogenicity of co-administered proteins.[14,15] In addition, Tregitopes have been shown to modify immune

responses to biotherapeutics, such as FVIII. In vitro, co-incubation of proteins with Tregitopes leads to suppression of effector cytokine and chemokine secretion, reduced proliferation of effector T cells, and expansion of antigen-specific adaptive Tregs. In vivo, co-administration of Tregitopes with a wide range of proteins (i.e., FVIII, ovalbumin, and autoantigens) leads to antigen-specific suppression of T cell and antibody responses.

Funding for research on Tregitopes has been flowing. For example, EpiVax recently received a Small Business Innovation Research (SBIR) Phase I grant for $600,000 to explore the use of Tregitope in facilitating tolerance to the lifesaving enzyme replacement therapy for Pompe's disease.[16] In 2012 alone, EpiVax scientists were able to obtain $3.4 million in National Institutes of Health (NIH) funding for development of Tregitope therapies; the group has been awarded more than $6 million in grants to develop Tregitopes over the past few years. Once the right formulation of Tregitopes is identified, and they pass the usual regulatory hurdles, their use is expected to have a radical impact on the clinical management of autoimmunity, transplant rejection, and protein replacement therapies.

CONCLUSION

EpiVax will continue to apply the experience gained from these basic research efforts to practical problems in immunotherapy and vaccine design. In the field of protein therapeutics, we are broadly recognized as thought leaders, and we expect to maintain this position through our discovery work on Tregitopes and tolerance. In addition, our work on epitope-driven vaccines – such as the smallpox, Tularemia, and *H. pylori* vaccines in our pipeline – has begun to demonstrate the power of T cell epitopes to generate protective immune responses. We will combine these breakthroughs with advancements in delivery and formulation to bring novel immunomodulatory therapies and vaccines to market.

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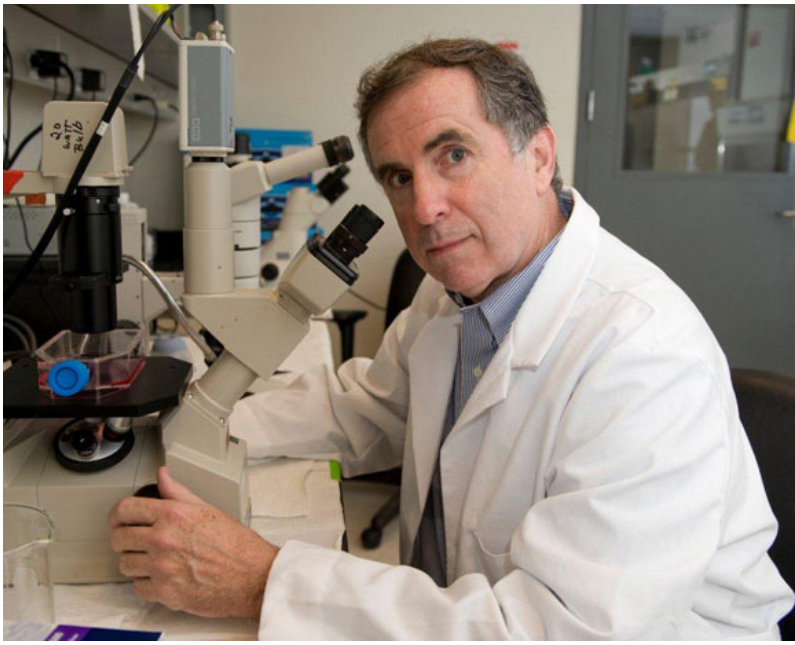
On the Path to a Duchenne Muscular Dystrophy Therapy

JUSTIN R. FALLON, PhD

ABSTRACT

Duchenne Muscular Dystrophy (DMD) is a devastating inherited disease of children with no effective therapies. Here I discuss the landscape for new treatments and the history, current status and prospects for our work developing recombinant biglycan as DMD therapy.

KEYWORDS: Biglycan, Duchenne Muscular Dystrophy, neuromuscular disorders



PETER GOLDBERG, BROWN UNIVERSITY

Justin Fallon in his lab at Brown University.

INTRODUCTION

Duchenne Muscular Dystrophy (DMD) is the most common form of muscular dystrophy. DMD is caused by mutations in dystrophin, a protein that is essential for maintaining the integrity of both cardiac and muscle cells (Emery, 2002; Nowak and Davies, 2004). Starting at about age four, the affected boys exhibit muscle weakness and most are in wheelchairs by their teens. Death is usually caused by failure of the diaphragm and/or cardiomyopathy. Patients rarely survive past their mid-twenties. Effective treatments for this devastating disease are urgently needed.

Current therapies for muscular dystrophies are not disease-modifying and have limited impact on the clinical outcome. The current standard of care is steroids, either prednisone (Mendell et al., 1989) or Deflazacort (a synthetic prednisolone; Biggar et al., 2004). These agents impede inflammatory fibrosis and improve muscle strength. Unfortunately, after an initial increase in strength in the first six months to one year, patients on these medications often exhibit a slow decline after 18 months (Griggs et al., 1991). Both these drugs have significant side effects that can limit their use. Physical rehabilitation, including stretching exercises, can maintain greater flexibility in muscles susceptible to contracture formation. However, most methods of rehabilitation become ineffective once the disease reaches its greatest severity by the second decade of life.

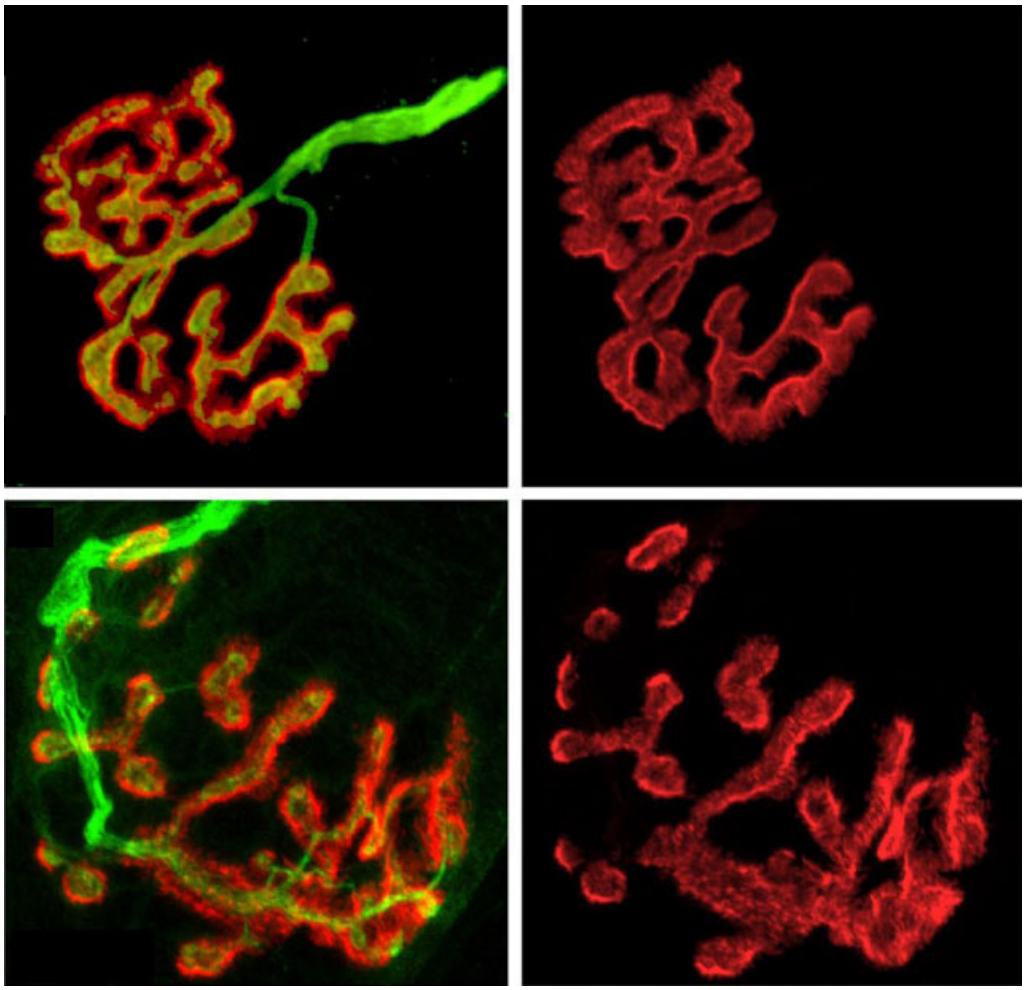
The good news is that a wide range of DMD therapeutic strategies are under investigation, with some promising compounds in late-stage clinical trials. Gene therapies seek to replace, repair or override the mutated dystrophin gene. The most advanced of this class employ a dystrophin minigene delivered by adeno-associated viral vectors (Blankinship et al., 2006). Since muscle is a regenerative tissue, cell-based therapies have drawn much attention. However, it has been difficult to achieve sufficient engraftment of the transplanted cells. Recent approaches using mesangioblasts in mouse models have started to break down this barrier (Sampaolesi et al., 2003), but human studies are still in the planning stages.

Pharmacological interventions include small molecule drugs to induce stop codon read through, which would be effective for ~15% of DMD patients. Ataluran, whose development was inspired by the observation that gentamicin has such activity (Wagner et al., 2001), has been tested in a large clinical trial (PTC Therapeutics). Other pharmacological therapies are also being pursued that either improve muscle performance or mitigate the pathological process in DMD muscle. These include using humanized antibody fusion proteins that neutralize myostatin and related inhibitors of muscle growth. Unfortunately, this class of compounds has not yet proven effective in clinical trials.

Finally, treatments aimed at reducing muscle fibrosis are also in development. Since scarring interferes with the function of remaining normal muscle and degrades the stem cell niches necessary for regeneration, such treatments

could confer significant benefit to the patients.

Exon skipping is an exciting new approach that employs synthetic oligonucleotides to excise selected regions of the mutated dystrophin mRNA by regulating alternative splicing (Cirak et al., 2011). The product is a 'Becker-like' dystrophin protein that, while truncated, harbors activity that would be expected to confer significant benefit to patients. Early small-scale studies in humans targeting exon 51 using two different oligonucleotide chemistries have yielded encouraging results and late-stage trials are currently underway (drisapersen and eteplirsen; GlaxoSmithKline and Sarepta, respectively). Both studies have shown that the expression of (truncated) dystrophin is restored in a subset of muscle fibers. Most importantly, the subjects have maintained ambulation to a remarkable degree when compared to historical controls. These compounds are a leading example of personalized medicine, since the therapy is tailored to specific mutations. However, since mutations can occur virtually anywhere in the very large dystrophin gene, a given compound can only target a subset of patients. For example, exon 51-targeted oligonucleotides could benefit about 15% of patients. With current methodology it is estimated that additional compounds could be developed that would collectively target about 50% of patients. A further limitation of exon skipping is that none of the current oligonucleotide chemistries target the heart.



FALLON LAB/BROWN UNIVERSITY

Segmented synapses

Synaptic structures in mice engineered to lack the protein biglycan (bottom row) appear discontinuous compared to the synaptic structures in normal mice (top).

One of the most long-standing and appealing pharmacological approaches to treat DMD is the upregulation of utrophin, an autosomal homolog of dystrophin (Khurana and Davies, 2003). Utrophin is normally expressed at high levels during fetal development and in early childhood, but in the mature animal it is restricted to the neuromuscular and myotendinous junctions. The high levels of utrophin in young children is likely one of the reasons that the clinical manifestations of DMD only appear after about 4 years of age. Genetic studies have shown that if utrophin is up-regulated it can functionally replace dystrophin in mdx mice (Tinsley et al., 1998). Muscle death is prevented and muscle function is restored to wild-type levels. A utrophin-targeted approach is also appealing since it targets an endogenous, fetal program that could compensate for the loss of dystrophin. Finally, a utrophin-based therapy should target all DMD patients, regardless of mutation.

THE PATH TO BIGLYCAN AS A DMD THERAPEUTIC

Our laboratory is developing a recombinant form of the endogenous extracellular matrix protein biglycan as a DMD therapeutic. Although I did not know it at the time, this idea can be traced back to when I was a postdoctoral fellow with U.J. McMahan at Stanford. The goal of these studies was to identify and characterize the proteins that organize the muscle cell membrane at the synapse. We discovered agrin, an extracellular matrix protein that organizes acetylcholine receptors into discrete domains on the muscle cell surface (Fallon et al., 1985; Nitkin et al., 1987). In my own laboratory

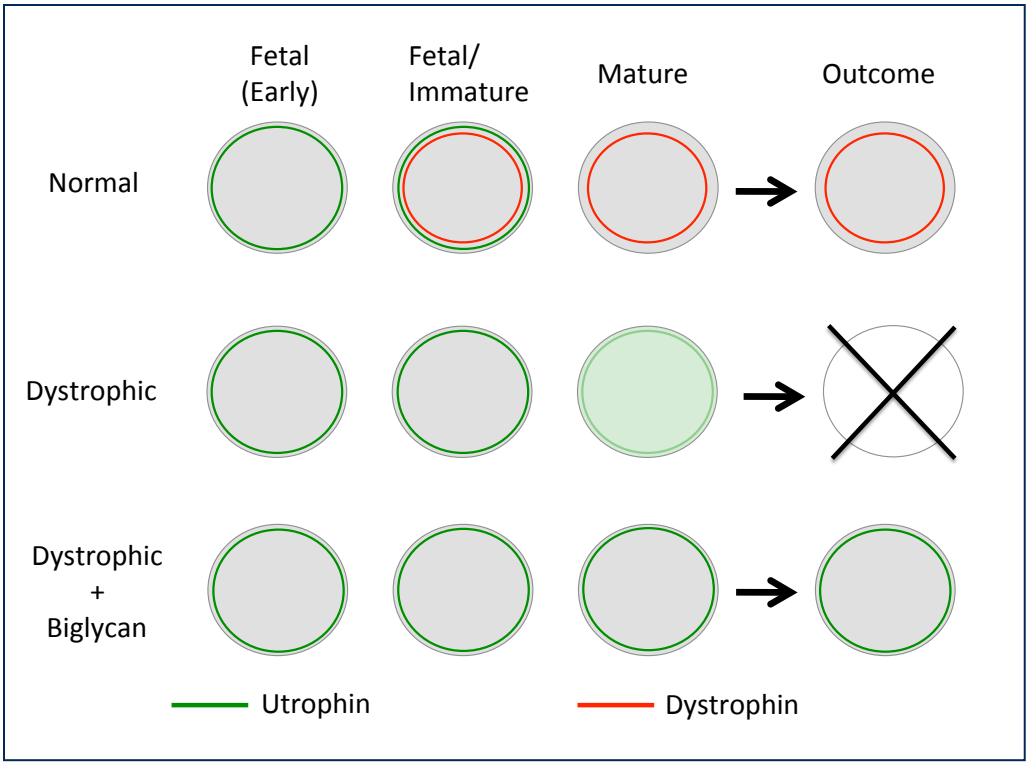


Figure 1. Rationale for Utrophin-directed DMD therapy.
In normal muscle utrophin is highly expressed during development, but then is down-regulated and replaced by dystrophin as the muscle matures. In boys with DMD there is no dystrophin and the levels of utrophin are insufficient to maintain muscle health. Shown are schematic cross-sections of myofibers depicting the distribution of utrophin and dystrophin in normal individuals, DMD patients and proposed therapeutic benefit of delivering recombinant biglycan to upregulate utrophin in dystrophic muscle. Red: dystrophin; Green: utrophin. See text and Amenta et al., 2011 for details.

we went after the cell surface proteins that bind agrin and mediate its activity. This effort led to dystroglycan, which had just been found to be a key member of the complex of proteins that associate with dystrophin (Bowe et al., 1994). Utrophin was known to be at the neuromuscular junction – even in DMD. We began to wonder whether we had tapped into a mechanism that regulates utrophin expression. If so, we realized that it might be possible to harness this pathway to create a new DMD treatment.

We sought to explore this new hypothetical pathway. This work was carried out in my laboratory at Brown University. I have been incredibly fortunate to work with a team of remarkably talented and dedicated scientists. These include Mark Bowe, Katherine Deyst, Mike Rafii, Hiroki Hagiwara, Mary Lynn Mercado, Beatrice Lechner, Sarah Mentzer, Carolyn Schmiedel and Alison Amenta. An especially important member of the team is Beth McKechnie, who has been on this project for over 20 years and has contributed many of the key insights that have brought us to the cusp of clinical trials.

The first clues came from biochemistry; we looked for additional dystroglycan binding proteins and discovered biglycan in this complex (Bowe et al., 2000). Continuing down this biochemical path, we found that biglycan also binds to alpha and gamma sarcoglycans (Rafii et al., 2006). This result was exciting because these sarcoglycans are only found in the two tissues affected by DMD: skeletal muscle and heart. We went on to investigate biglycan function using mutant mice created by Marian Young at the NIH (Young and Fallon, 2012). These studies yielded the critical information that biglycan is important for the proper expression of several dystrophin-associated proteins such as the sarcoglycans and an intracellular signaling complex including nNOS (neuronal nitric oxide synthase; Mercado et al., 2006). However, from a DMD therapy viewpoint, the critical finding was that biglycan also regulates utrophin in early development (Amenta et al., 2011). We now had a link to a potential therapeutic pathway.

The transition from an idea to a viable therapeutic is complex and lengthy. However, the first question is simple – can we produce a candidate compound and show that it can be delivered in a form, route, dose and frequency that is amenable to use as a drug? We therefore produced recombinant biglycan (rhBGN) and asked if it was active in mouse models of DMD. Remarkably, systemically-delivered rhBGN up-regulated utrophin at the muscle membrane and improved the health and function of the dystrophic muscle (Amenta et al., 2011). Equally important, rhBGN was active at doses (2-10mg/kg) and frequencies (once injection every two weeks) that are suitable for use in patients.

With these first results in hand we began a concerted effort to bring rhBGN to clinical trials. These efforts require expertise beyond that of an academic laboratory. Therefore I cofounded Tivorsan Pharmaceuticals, a Rhode Island-based company committed to develop rhBGN as a therapeutic for DMD (www.tivorsan.com). Tivorsan has marshalled the necessary regulatory, manufacturing and clinical expertise that will be needed to complete preclinical work and initiate clinical testing.

FUTURE DIRECTIONS

Biglycan could have therapeutic benefit in amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disease marked by the loss of upper and lower motor neurons (Pasinelli and Brown, 2006). However, the first sign of pathology is destabilization of the nerve-muscle synapse, resulting in deafferentation and muscle paralysis. A therapy that stabilizes this synapse could thus prolong function in ALS patients. As discussed above, our path to biglycan stemmed from an inquiry into the how nerve-muscle synapses are formed. In an exciting recent finding we showed that biglycan binds to the receptor tyrosine kinase MuSK, the central organizer of this synapse. Further, biglycan is important for synapse stability. These basic science findings raise the possibility that rhBGN could stabilize the compromised synapses in ALS patients and delay the progress of the disease. Experiments to test this idea in mouse models of ALS are underway in the laboratory. If these studies in model organisms are favorable, we will be well positioned to initiate testing of rhBGN in ALS patients.

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BioIntraface®: The Next Quantum in Medical Devices

JOHN D. JARRELL, PhD, PE

ABSTRACT

BioIntraface®, Inc., located in Riverside, Rhode Island, was formed in February of 2009 to commercialize its biomaterials surface treatment technologies. The platform technologies involve the creation of economical, multifunctional metal oxide and polymer materials and coatings to control the bioactivity and antimicrobial properties of medical devices and implants. Biointraface® has continued optimizing and validating coatings for promising applications in orthopaedics, dentistry, catheters, wound dressings, topical antimicrobial products, and cosmetics applications. It has also obtained third-party verification of ISO biocompatibility testing for eight coatings with increasing levels of antimicrobial agents, where no cytotoxicity was indicated and similar tests showing long lasting antimicrobial efficacy against multiple strains of bacteria.

KEYWORDS: antimicrobial coatings, medical devices, metal oxide and polymer coatings

UPGRADING THE SURFACE OF MEDICAL DEVICES

Silicones, such as polydimethylsiloxane (PDMS), have a long history of use in medical applications, beginning with a bile duct repair by Lahey in 1946,[1] an artificial urethra in 1948 by DeNicola[2] and a hydrocephalus shunt constructed by Holter for his son in 1956.[3] The wide applicability of PDMS to tissue contact is due to its generally low toxicity and biocompatibility, which was investigated in a publication by Rowe, Spence and Bass in 1948[4] and continues to be extensively studied for general biomedical suitability and specific implant applications.[5,6] From the perspective of chemistry, the strength of the two oxygen and two carbon (methyl group) bonds per silicon atom gives the material thermal stability up to 400°C, allowing autoclave sterilization, and preventing chemical decomposition under most physiological conditions.[7] This inertness has a downside for some applications; PDMS tends to poorly facilitate protein and cell attachment, resulting in poor soft-tissue integration, a lack of skin sealing around percutaneous devices and localized foreign body response with subcutaneous implants.[8]

Titanium has been recognized as the material of choice for many implant applications, especially when contacting bone or to limit contact with nickel. More recently, it has been applied to osseointegrated trans-epithelial prosthetic fixation for dentistry and experimental limb attachment.[9] It is the presence of a spontaneous and self-regenerating passive oxide layer on titanium's surface that is primarily responsible for the corrosion resistance[10] and biointegrative properties of this metal.[11-13] Titanium oxide reduces local inflammatory responses,[14,15] lowers the presence of local reactive oxygen species,[16,17] and dynamically incorporates elements from surrounding tissues after implantation.[18,19] Because of the properties of this (and other) refractory metal oxides, the problem of aseptic osseointegration of medical devices is all but solved.

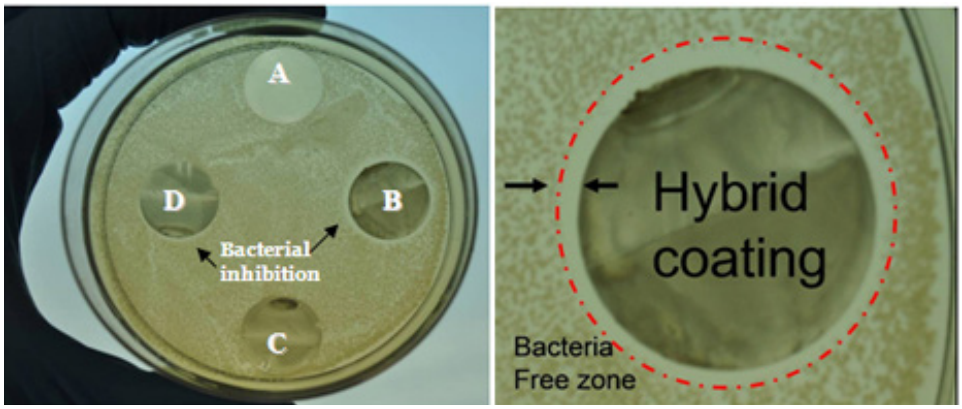
BIOINTRAFACE

John D. Jarrell, PhD

Polyether ether ketone (PEEK) is increasingly finding use in orthopaedic applications like spinal and trauma implants. PEEK has a good combination of formability, mechanical properties, biocompatibility and radio transparency, but lacks some of the bioactive and integrative properties identified with titanium-based implants. Here we explore the use of metal-organic derived, hybrid coatings, as a means of creating antimicrobial treated PEEK biomaterials with a titanium oxide surface interface.

BIOMATERIALS AND BIOFILMS

While biomaterials like titanium, stainless steels, cobalt chrome, polyether ether ketone (PEEK), silicone and polyvinyl chloride (PVC) have been widely used for medical devices, catheters and implants, none of these materials provide active resistance to bacterial infection or prevents biofilm formation. Biofilm formation is a five-step process involving reversible attachment, irreversible attachment, maturation I, maturation II and dispersion.[1,2] Identification of the mechanisms at work in each of these steps has aided investigators in developing targeted approaches.[1-3] These include prevention of bacterial attachment, encouraging release,



BIOINTRAFACE

Figure 1. In vitro results with glass discs with no coating (A), Hybrid #1 coating (B), amorphous Titanium Dioxide only (C), and Hybrid #2 coating (D) incubated in Petri dish with E. Coli-coated agar. Note large zone of bacterial inhibition surrounding disc coated with Hybrid #1 and smaller zone surrounding disc coated with Hybrid #2 (arrows). Right image is a close up view of bacteria free zone around Hybrid #1.

Hybrid coatings

BI's coatings are based on a new hybrid materials technology using metal-organic precursors to create both metal oxide and polymer coatings from liquid solutions. Each component of the coating is already in common use in other medical devices. The prospective coatings are optimized using an innovative rapid screening cell culture platform, inspired by the approaches used by large pharmaceutical companies for new drug discovery.

These promising in vitro results (Figure 1) have been followed by several small animal studies conducted at both Brown University and Rhode Island Hospital. Early applications that have been considered include catheters, fracture fixation devices, and transcutaneous osseointegrative devices (bone-anchored prosthetics for limb replacement).

disrupting quorum sensing and dispersion of the biofilm layer.[1,2] Another approach has been coined as "the race for the interface." That is, there is a competition for implant surfaces between healthy cells/tissues and bacteria. It has also been recently recommended that at least two approaches be employed to prevent bacterial formations on implants and overcome bacterial mechanism of resistance. The Food and Drug Administration (FDA) regulatory requirements and market forces must be taken into consideration in the development of a sensible and commercially feasible approach, which also holds potential for reducing overall healthcare costs. To be clinically relevant, an approach needs to meet at least three criteria: a clinically relevant, 2+ log reduction in bacterial growth, no prevention of tissue healing and no promotion of antibiotic resistance.

FIGHTING INFECTION

Several types of surface treatments have been investigated to reduce bacterial growth and infection, including: the introduction of nano surface topography, manipulation of surface chemistry and the permanent attachment of antibiotic drugs to implants to inhibit bacterial attachment, growth and resulting biofilm formation. Nano surface modifications are well-developed and have been demonstrated to facilitate healing and reduce the growth of bacteria, but not at the log scale level.[1] University of Pennsylvania researchers developed methods for permanently attaching antibiotic drugs to medical devices with good in vitro results against bacteria,[2] but raise the concern of preferentially selecting and encouraging development of drug resistant bacterial infections. Due to the recent increased number of antibiotic resistant bacteria, silver has been studied extensively as an alternative to antibiotics. Silver ion delivery has a history of use on medical devices, does not contribute to drug resistance and provides broad spectrum antimicrobial protection against gram-positive and gram-negative bacteria by at least six different mechanisms.[3-8] Bactericidal effects of silver depend greatly on concentration, particle size and shape in the case of silver particles. Exposure to silver causes changes in bacteria cell membrane morphology, and damage to intracellular proteins and DNA, which affect cell metabolism, cell division, and can result in cell death.

BIOACTIVE AND ANTIMICROBIAL COATINGS

Research conducted at Brown University, Rhode Island Hospital and in collaboration with the U.S. Department of Veterans Affairs shows that metal oxide and polymer hybrid surface treatments have the ability to prevent bacterial attachment and eliminate bacterial growth on the log-scale, while promoting healthy cell growth.[4-7] These coatings are based on a new hybrid-materials technology using metal-organic precursors to create both metal oxide and polymer matrix coatings from liquid solutions. Each component of the coating is already in common use in other medical devices. The prospective coatings are optimized using an innovative rapid screening cell culture platform, inspired by the approaches used by pharmaceutical companies for new drug discovery. Promising in vitro results have been followed by several small and large animal studies conducted at both Brown University and Rhode Island Hospital and third-party in vitro ISO biocompatibility testing and AATCC antimicrobial testing. The coating matrices have novel electro-chemical and controlled release properties to deliver bioactive agents, including metal ions like silver. The main advantages of these treatments over competitive delivery technologies is the ability to control the silver release properties and balance them with the bioactive properties of titanium oxide, the economical methods of application and the formation of a chemical bond between the treatment and the surface of the medical device.

BIOINTRAFACE®, THE COMPANY

BioIntraface®, Inc. (BI), was formed in February of 2009 to commercialize the biomaterials surface treatment technologies. The platform technologies involve the creation of economical, multifunctional metal oxide and polymer materials and coatings to control the bioactivity and antimicrobial properties of medical devices and implants. In 2009, the BI team won the Rhode Island Business Plan Competition, which was followed in 2010 by a Rhode Island Innovation Award in the category of Health Care & Biotechnology Innovations. In 2011, BI was issued two U.S. patents from their original filings and their first international patent claims were allowed in Australia and Mexico, with additional applications pending in the United States, the European Union and six additional industrialized nations. BI has continued optimizing and validating coatings for promising applications in orthopedics, dentistry, catheters, wound dressings, topical antimicrobial products, and cosmetics applications. It has also obtained third-party verification of ISO biocompatibility testing for eight coatings with increasing levels of antimicrobial agents, where no cytotoxicity was indicated and similar tests showing long lasting antimicrobial efficacy against multiple strains of bacteria. On the strength of this technology, BI is raising additional funding to launch multiple companies focused on specific antimicrobial products lines. In 2011, the establishment registered with the FDA as an initial importer of medical devices and plans to begin to obtain FDA approval for upgraded revision of specific device lines for distribution into the United States, Germany, France and the United Kingdom once adequate funding is obtained.

The initial management objective has been to create the infrastructure to warehouse and distribute branded devices into markets ready for a high-value line of antimicrobial devices and implants, while adding in-house manufacturing, quality and R&D capacity. In 2012, BI moved into a pilot manufacturing facility at Quonset to expand processes and research capacity to facilitate rapid growth and a robust new product pipeline.

LEADERSHIP

BI management is led by **John D. Jarrell, PhD, PE**, who is president and founder, with 25 years of experience in failure analysis and product liability of medical devices.

Christopher T. Born, MD, FAAOS, FACS, professor of orthopaedics at Brown University, is head of the BI Clinical Advisory and Chief Technical Officer (CTO).

Brown University Associate Professor of Medical Science and Engineering **Jeffrey Morgan, PhD**, is a co-founder and head of the Scientific Advisory Board. For more information: www.biointraface.com.

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VeroScience: Applying Nature's Genius to Help Improve the Human Condition

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ABSTRACT

VeroScience is a biotechnology company in Tiverton, Rhode Island, focused on the development of therapies and products to improve human health. The company has a strong pipeline of metabolic disease products and therapies for immunological disorders. A major platform technology of the company, Circadian Neuroendocrine Resetting Therapy, is utilized as a generator of multiple therapeutic strategies to treat a variety of disease states. The circadian timed daily (morning) administration of Cycloset®, a quick release formulation of bromocriptine mesylate, a dopamine agonist, was developed for the treatment of type 2 diabetes using this platform technology.

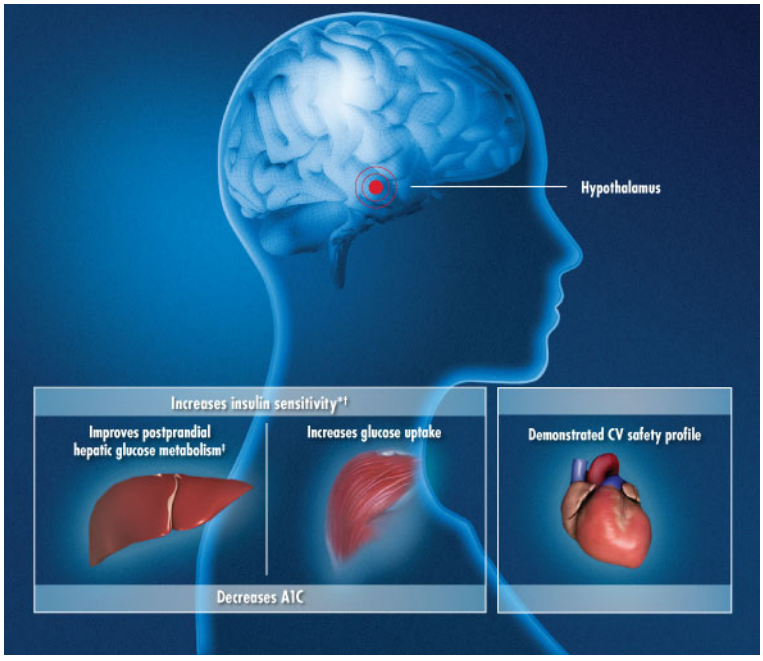
KEYWORDS: Neuroendrocrine Resetting Therapy, type 2 diabetes, cycloset, glycemic control

INTRODUCTION

Since 2001, VeroScience has been in the business of developing drugs to treat metabolic and immune system disorders such as type 2 diabetes, obesity, and cancer through its platform technology, Neuroendocrine Resetting Therapy (NRT). The platform technologies of the company were co-developed by its founder, Anthony Cincotta, PhD, largely in academia during the 1980s, incorporated into a biotechnology company that he founded in 1990 and directed until 2000, ErgoScience, and then ultimately transferred to VeroScience in 2006. NRT is based on research demonstrating that normal physiology is regulated in large part by the temporal interactions of circadian neuroendocrine oscillations within certain centers of the brain. Alterations in the phase relations of these circadian activities alter physiological status of the organism. Resetting aberrations in these activities is an effective means of treating several neuroendocrine disorders.

In 1997, data presented at the 57th Annual Scientific Sessions of the American Diabetes Association (ADA) reported the results of three nationwide, multi-center trials, known as "TRIAD – Time Regulated Intervention in Adult Diabetes" conducted by ErgoScience demonstrating that Ergoset, a timed quick-release formulation of a dopamine agonist (bromocriptine mesylate), used alone or in combination with oral antidiabetic agents, was shown to produce statistically and clinically meaningful reductions in blood sugar levels in obese, type 2 people with diabetes when compared to placebo. In 1998, ErgoScience filed for Food and Drug Administration (FDA) approval of the drug. The FDA initially issued a non-approval letter, owing to concerns about safety of the active agent, bromocriptine. ErgoScience appealed that decision and the FDA issued an approvable letter to ErgoScience for Ergoset in 1999. Thus began efforts for the successful transition of NRT, including Ergoset, to VeroScience and eventual approval of the drug marketed as Cycloset.

After securing private funding in 2003 and 2006, VeroScience was able to initiate the required FDA 3,000-person, one-year trial to evaluate the overall safety and cardiovascular safety of Cycloset. The trial was conducted at 74 sites in the United States, including 22 Veterans Affairs Medical Centers. Briefly, patients with type 2 diabetes were randomized 2:1 to bromocriptine-QR (Cycloset) or placebo in conjunction with the patient's usual diabetes therapy (diet controlled only or up to 2 anti-diabetes medications, including insulin). The all-cause safety endpoint was the occurrence of any serious adverse event (SAE). In a pre-specified analysis, the frequency of cardiovascular (CVD) events defined as a composite of myocardial infarction, stroke, coronary revascularization, hospitalization for angina or congestive heart failure was evaluated.



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RESULTS

Results showed that 176 (8.6%) people in the bromocriptine-QR group reported SAEs compared to 98 (9.6%) in the placebo group, and a lower percentage of people reported a CVD endpoint in the bromocriptine-QR group; 37 (1.8%) versus placebo, 32 (3.2%). In fact, these results demonstrated a 40% relative risk reduction in the CVD endpoint among those taking bromocriptine-QR. Nausea was the most commonly reported adverse event in the bromocriptine-QR group. Mean HbA1c was lower in the bromocriptine-QR group than in the placebo group at one year. [3]

Based on these safety and efficacy results, the FDA granted marketing approval to VeroScience for Cycloset in May of 2009. Cycloset is approved by the FDA, indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus. It has been shown to reduce post-prandial hyperglycemia without increasing insulin levels and while not increasing the risk for hypoglycemia or weight gain.[2] Cycloset was the first diabetes therapy to be approved under the FDA's new cardiovascular guidelines (December 2008) requiring evidence that type 2 diabetes medications do not increase the risk of cardiovascular disease.[4] In additional analyses, five times as many Cycloset-treated patients achieved an A1C goal of ≤7.0% compared to placebo.[5] Cycloset is marketed in the Unites States by Santarus Pharmaceuticals, Inc., in San Diego, California.

VeroScience is headquartered in Tiverton.

ABOUT THE COMPANY

VeroScience is a hybrid of traditional academic inquiry and industrial focus within a small and efficient organization of six full-time employees in the 27,000 sq. ft. building. The facility houses an animal lab, bench laboratory, greenhouse and offices. There are scientists, an animal care professional and a medical/clinical affairs group within the company and several worldwide consultants. The company conducts preclinical and clinical research nationwide, utilizing strong academic and pharmaceutical industry collaborations to advance its development programs.

BACKGROUND – PRECLINICAL STUDIES AND DEVELOPMENT RATIONALE

Decades of work investigating animals in the wild that undergo marked annual cycles of metabolism revealed that seasonal shifts from the obese, insulin-resistant condition to the lean, insulin-sensitive state are driven by shifts in the circadian phase relations of specific hypothalamic neurophysiological events.

By mimicking this neurophysiological shift by pharmacological interventions, it is possible to effectuate the predicted shift in seasonal metabolism in either direction, to or from the insulin-sensitive state. Similar approaches in a variety of genetic and diet-induced animal models of type 2 diabetes have produced similar results.[1]

Animals in the wild under natural conditions express a marked annual cycle of metabolism, shifting between lean insulin-sensitive, and obese insulin-resistant states at specific times of year. Seasonal insulin resistance imparts the ability to withstand long periods of ensuing low food availability and this seasonal mechanism appears to have evolved as a survival strategy to circumvent such an environmental stress. Among the many examples of such seasonal variations in metabolism are bear hibernation, bird migration, and squirrel overwintering. Available evidence indicates that this seasonal mechanism evolved and has persisted over at least 400 million years. Neuroendocrine and neurophysiological studies of seasonal animals among all the major vertebrate classes have implicated an important role for circadian dopaminergic input to the hypothalamus and specifically to the mammalian biological pacemaker (the suprachiasmatic nuclei, SCN) in the regulation of whole-body fuel metabolism.[1]

METABOLIC DISEASE

By employing methods that mimic the neurobiochemical physiology responsible for the seasonal shift from the obese, insulin-resistant condition to the lean, insulin-sensitive state common among vertebrate species in the wild, it is possible to develop new treatment strategies for human metabolic diseases such as type 2 diabetes, obesity, and metabolic syndrome. Changes in the circadian phase relations of distinct neuroendocrine rhythms drive the annual cycle of metabolism among vertebrates in the wild.

Consequently, it is not merely supplying the neuroendocrine factors of the "lean" season that produces leanness but rather supplying the circadian neuroendocrine blueprint that accomplishes this shift. Methods aimed at doing so can function to alleviate and induce the obese, insulin-resistant condition as is the case in the wild. VeroScience is developing different ways of applying this science to provide effective and practical means of treating human metabolic diseases.

METABOLIC AND IMMUNE DISEASE RESEARCH AND THERAPY DEVELOPMENT

VeroScience is committed to developing novel, practical, and effective therapies for chronic debilitating human diseases such as type 2 diabetes, metabolic syndrome, autoimmune disease, and cancer through interdisciplinary basic research.

Our approach to achieving these goals focuses in large part upon readjusting aberrant central nervous system (CNS) modulation of neuroendocrine activities etiologic in or supportive of these disease states.

VeroScience researchers study biological clocks in vertebrates and their organizational influence on neuroendocrine regulation of physiology to develop new treatment strategies for metabolic and immune disorders.

By investigating and mimicking nature's means of regulating biochemical physiology for survival of vertebrates in the wild, VeroScience develops treatment strategies, not products per se, aimed at re-directing pathological biochemistry back towards its 'normal' physiological organization.

IMMUNE DISORDERS

Immuno-suppression and autoimmune diseases are both associated with derangements in the circadian neuroendocrine axis. Once again, it is the critical role of the brain-neuroendocrine axis to regulate and orchestrate the complex immunological interactions that occur at the cellular and tissue levels for the production of an organismal level immunocompetence.

Rather than focusing on specific immunomodulators such chemokines or lymphokines to boost immuno-reactivity, VeroScience focuses on resetting circadian neuroendocrine events that organize overall global immunophysiology to treat immuno-suppressed states. Similarly, autoimmune disorders with genetic components manifest as alterations in the neuroendocrine axis which in turn potentiate the underlying disorder.

Consequently, autoimmune diseases may be improved by appropriately resetting specific aberrations in the circadian neuroendocrine axis. Our interventions are not just pharmaceutical compounds but rather therapeutic treatment regimens employing such compounds in a particular manner to reprogram the master control centers in the brain for the production of whole-body immunological status.

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Important Safety Information

Contraindications

CYCLOSET is contraindicated in:

• Patients with known hypersensitivity to bromocriptine, ergot-related drugs, or any of the excipients in CYCLOSET.

• Patients with syncopal migraine. Bromocriptine increases the likelihood of a hypotensive episode among patients with syncopal migraine. Loss of consciousness during a migraine may reflect dopamine receptor hypersensitivity. CYCLOSET is a dopamine receptor agonist, and may, therefore, potentiate the risk for syncope in these patients.

• Women who are nursing their children. CYCLOSET may inhibit lactation. There are postmarketing reports of stroke in this patient population although causality has not been proven.

Warnings and Precautions

• Hypotension: Can cause orthostatic hypotension and syncope, particularly upon initiation or dose escalation. Use caution in patients taking anti-hypertensive medications. Assess orthostatic vital signs prior to initiation of CYCLOSET and periodically thereafter. Advise patients during early treatment to avoid situations that could lead to injury if syncope was to occur.

• Psychosis: May exacerbate psychotic disorders or reduce the effectiveness of drugs that treat psychosis. Use in patients with severe psychotic disorders is not recommended.

• Somnolence: May cause somnolence. Advise patients not to operate heavy machinery if symptoms of somnolence occur.

• Interaction with dopamine antagonists: Concomitant use with dopamine antagonists such as neuroleptic agents may diminish the effectiveness of both drugs. Concomitant use is not recommended.

• Other dopamine receptor agonists: Effectiveness and safety are unknown in patients already taking dopamine receptor agonists for other indications. Concomitant use is not recommended.

• Macrovascular outcomes: There have been no clinical studies establishing conclusive evidence of macrovascular risk reduction with CYCLOSET or any other antidiabetic drug. CYCLOSET does not increase the risk of macrovascular events.