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MEETING REPORT

BIOTHERAPEUTICS – INNOVATIVE FUTURE MEDICINES

HIGHLIGHTS FROM THE SOCIETY OF MEDICINES RESEARCH SYMPOSIUM, HELD OCTOBER 8, 2015 – NATIONAL HEART & LUNG INSTITUTE, LONDON, UK

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SUMMARY

The Society of Medicine Research (SMR) held a meeting at the beginning of October 2015 entitled "Biotherapeutics – Innovative Future Medicines" at the National Heart and Lung Institute in South Kensington, London. The meeting was well attended by representatives from academia and industry who learnt how biotherapeutics are becoming an integral and valuable part of modern medicine to treat and prevent serious illnesses and disease. Biotherapeutics are an attractive approach to new medicines as many have a good track record with patient safety and in some instances are more efficacious and safer than conventional medicines due the ability to target specific molecules within the human body. Biotherapeutics are a diverse family of medicines ranging from antibodies to bespoke synthetic molecules which regulate cellular functions.

Key words: Romosozumab – Sclerostin – Pertussis antibody – ATX-MS-1467 – IMA-950 – CD19 CAR T cells – AST-VAC2 – MOv18 – Therapeutic oligonucleotides

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SCLEROSTIN: FROM GENE TO DRUG

Dr. Adrian Moore (UCB Celltech, Slough, UK) presented a case study—"Sclerostin: from gene to drug"—on the development of the therapeutic antibody **romosozumab** used for the treatment of osteoporosis. Romosozumab targets the sclerostin protein that plays a key role in the regulation of bone formation. Osteoporosis is a condition characterized by an increased risk of fracture due to a loss of bone strength, often caused by a loss of bone density. It is the principal cause of bone fractures in the elderly and is a key contributor to morbidity and excess mortality to the world's aging population. Normally, about 5-10% of our bone is replaced every year, related in part to the functional characteristics of bone cells (shown in Fig. 1) that are regulated to degrade and rebuild bone in a controlled manner. Sclerosteosis is a condition characterized by exceptionally high bone mass. This condition is caused by the loss of production of the protein sclerostin due to a point mutation. Sclerostin, a 190-residue cysteine-knot glycoprotein, was shown to be a negative regulator of bone formation through the Wnt signaling pathway, and its loss results in increased activity of osteoblasts resulting in increased bone levels of bone density. It was therefore hypothesized that inhibition of the function of this protein could have beneficial effects in individuals suffering from osteoporosis.

Dr. Moore then went on to describe the development of a therapeutic anti-sclerostin antibody. Several animal model studies were conducted, including a study in which rats with postmenopausal osteoporosis treated with this antibody showed a reverse in bone loss (1). Published data from phase I and phase II clinical studies using an

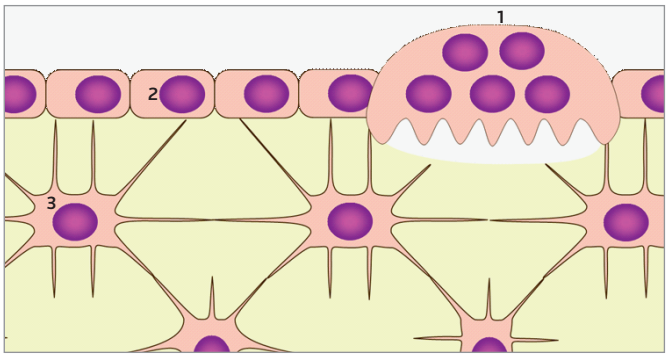

Figure 1. Cellular players of bone remodeling. 1) Osteoclasts: multinucleated giant cells that break down bone; 2) Osteoblasts: cells that lay down bone; 3) Osteocytes: cells within the bone matrix. (Adapted from an image provided Dr. Adrian Moore, UCB Celltech, UK).

anti-sclerostin antibody, romosozumab, formulated for use in humans showed that this biologic stimulated bone formation, decreased bone resorption and increased bone mineral density. In phase II trials these results were obtained in postmenopausal women with low bone mass (2). In addition, Dr. Moore presented data showing that follow-on treatment using a bone-remodeling therapeutic such as denosumab, a human monoclonal antibody that acts as a RANKL inhibitor, can sustain the increased bone mineral density obtained from using romosozumab. Phase III clinical trials of romosozumab with follow-on therapy are ongoing. Dr. Moore concluded his presentation pointing out how this work serves as an excellent example of where the understanding of a human genetic disorder has led to the identification of a novel therapeutic target.

DEVELOPMENT OF AN ANTIPERTUSSIS IMMUNOTHERAPEUTIC ANTIBODY

Professor Jennifer Maynard (The University of Texas at Austin, USA) opened her talk entitled, "Development of an antipertussis immunotherapeutic antibody", by reminding the audience that whooping cough or pertussis, caused by *Bordatella pertussis*, is still a significant public health problem and infection is responsible for the deaths of up to 300,000 children annually. Reduced efficacies of acellular vaccines and a decrease in uptake of vaccination are among the causes cited for rising incidence of pertussis in developed countries. In the U.S., pertussis vaccination usually begins at about 2 months of age and with boosters is usually effective at protecting from infection. However, young infants may be deemed high risk for infection for a number of reasons including premature birth. In such cases, there is a great need for new therapeutics that can be used as prophylactic agents as well as for treating infants who are critically ill with the disease.

To address these needs, Professor Maynard described work characterizing the biological activities and therapeutic efficacies of humanized monoclonal antibodies that are able to neutralize pertussis toxin (PTx). PTx is key virulence factor produced by *B. pertussis*. It impedes the innate immune response and induces severe leukocytosis. Professor Maynard presented results from extensive in vitro analyses of two antibodies, **11E6** and **1B7**. The 11E6 antibody was shown to inhibit the ability of PTx to interact with its receptor, while 1B7 demonstrated an ability to block the enzymatic activity of PTx by interfering with intracellular trafficking. Studies in mice and baboons supported the continued development of these antibodies as therapeutic agents. In particular, data presented showed that weanling baboons infected with 6×10^9 CFU (colony forming units) of *B. pertussis* and treated with both antibodies (20 mg/kg of each i.v.) did not display the severe symptoms of disease. Treated animals showed normal lung pathology at the end of the study, compared to untreated infected animals, which displayed pneumonia and abscess formation. These promising studies suggest that this antibody cocktail will yield improved outcomes to critically ill infants and has potential use as a prophylactic agent, with appropriate formulation, to high-risk infants in both the developed and developing worlds.

SWITCHING OFF IMMUNITY: THE MECHANISM OF ANTIGEN-SPECIFIC IMMUNOTHERAPY

During his talk, "Switching off immunity: the mechanism of antigen-specific immunotherapy", Prof. David Wraith (University of Bristol, UK) eloquently summarized his recent work on peptide immunotherapy.

Current treatments of autoimmune diseases and allergy are focused on treating the symptoms rather than cause of disease or depend on nonspecific immune suppression. Targeting the fundamental cause of disease that is the loss of tolerance to an otherwise innocuous antigen in allergy or self-antigen in autoimmune disease would greatly improve treatment (3). Peptide immunotherapy was first described almost a century ago for the treatment of allergy and today subcutaneous or oral/sublingual administration of allergens is successfully used for the treatment of a wide range of allergies such as bee venom, peanut, birch pollen (3). Progress in allergen-specific immunotherapy has been delayed by the lack of understanding of the underlying immunological mechanisms involved.

Prof. Wraith then summarized his research into the mechanisms of action of antigen-specific immunotherapy, and described the involvement of interleukin-10 (IL-10) producing regulatory T cells (Tregs) specific for potential disease-inducing epitopes as key players. Peptide-induced tolerance induction requires that the peptides mimic naturally processed peptide and are designed to be tolerogenic by binding directly to the MHC (major histocompatibility complex) without the need of processing. $CD4^+$ T cells recognizing these peptide/MHC complexes on antigen presenting cells undergo a negative feedback loop and upregulate IL-10 (4). Furthermore, an in depth analysis of the molecular mechanisms involved (5) and the specific recruitment of molecules to the immunological synapse (IS), comparing Treg cells to effector T cells, were described. Although both cell types form IS to the same extent the duration of molecules within the synapse as well as stability of the IS is shortened in Tregs.

A couple of preclinical examples of the use of peptide therapy were given where a short treatment with **ATX-F8-117** led to suppression of "inhibitor" antibody production in a mouse model of Factor VIII hemophilia while treatment with **ATX-GD-459** prevented antithyroid-stimulating hormone receptor antibody formation in a mouse model of Graves' disease.

Lastly, results from phase I clinical trials of peptide immunotherapy with **ATX-MS-1467** in 49 patients with relapsing multiple sclerosis and progressive multiple sclerosis showed that the treatment indicated no evidence of unexpected safety signals.

These results show that peptide therapy is a valuable new emerging treatment option for prevention and treatment of autoimmune diseases.

ANTIBODY-ENABLED SMALL-MOLECULE DRUG DISCOVERY

"Antibody-enabled small-molecule drug discovery" (Dr. Alastair Lawson, UCB, UK) was a comprehensive summary of UCB's research into combining knowledge obtained from therapeutically successful and function-modifying antibodies and using it in new ways to discover small molecules that are capable of modulating protein–protein interactions (PPI).

Clinical and/or genetic validation of a target with a biological therapy is a helpful way to establish confidence in a target and reduce biological risk and furthermore, the concept of structure-based druggability or ligandability has become an important complementary factor (6). This is especially relevant for PPI which were previously considered to be undruggable and are now thought of as challenging but tractable. Accumulated knowledge from antibodies can then be applied to reduce biological risk during the development of small molecules with similar modes of action. To achieve this, some of the technologies that are readily available are utilized, for example, antibody-enabled crystallography and small molecule screening against antibody-constrained proteins (7). Structural conformations of target proteins are also relevant to inform on active versus inactive states of molecules.

Another useful technique to aid relevant small molecule research is molecular dynamic simulation through definition of predicted conformations, which can be validated using antibody definition. Furthermore, a newly designed NCE fragment library (8) screen against antibody-stabilized targets is used to find initial hits which are then further optimized.

This technology is highly desirable to identify first-in-class small molecules to defined biological targets. It also leads to a portfolio which is well balanced between biological and chemical risks.

Dr. Lawson also described UCB's now fully automated high throughput antibody technology platform which leads to the discovery of highly specific and rare antibodies.

Integration of antibody technology, structural and dynamic information around the target protein and small molecule fragment library design and screening is generating value for patients through the discovery of differentiated and innovative therapies.

DISCOVERING AND DEVELOPING NOVEL ANTICANCER BIOTHERAPEUTICS, THE VIEW FROM CANCER RESEARCH UK

The talk by Dr. Robert J. Williams (Cancer Research UK [CRUK], UK), "Discovering and developing novel anticancer biotherapeutics, the view from Cancer Research UK," sought to give an understanding of the current CRUK organization and its capabilities, partnership approach to drug development and specifically the work it does in developing promising oncology assets shelved by drug companies. CRUK has made a number of investments to achieve these goals, including a GBP 18 million investment in a GMP manufacturing facility for process development and manufacture of biological IMPs, and pursuing several projects in the immune-oncology area. The newly created CRUK Center for Drug Development (CDD) is structured to manage preclinical drug development and sponsor phase I/II clinical studies, proof of concept and proof of principle; currently there are 30 active projects , including, small-molecule therapeutics, gene therapies and imaging agents.

Harnessing the immune system for the treatment of cancer; the idea that tumor regression being associated with infections has existed for quite some time, with the reported use of bacterial extracts to treat cancer (Coleys toxin), and reports of sporadic, but real cures for metastatic cancers receiving the nonspecific immunostimulant IL-2 have been widespread. The failure of therapeutic cancer vaccines in particular in clinical trials contributed to immunotherapy being viewed until recently as being a difficult and controversial approach to cancer treatment. Dr. Williams gave examples of recent failure of the cancer vaccine approach to anticancer therapy, with most failure being due to immunosuppression, suboptimal adjuvants, loss of single antigen expression or antigens not presented in the right context. At CRUK CDD, they have shown that a novel glioblastoma multiforme (GBM)-specific therapeutic vaccine (**IMA-950**) containing 11 tumor-associated peptides (TUMAPs), identified on human leukocyte antigen (HLA) surface receptors in primary human GBM tissue was able to exceed immunogenic success criteria. IMA-950, discovered by Immatics (a German biotech), was designed to activate TUMAP-specific cytotoxic T cells. CRUK-DD licensed in the product and conducted phase I safety and immunogenicity studies of IMA-950 plus standard of care in patients with newly diagnosed GBM. Of the 40 evaluable patients recruited, 36 were single TUMAP responders, and 20 were multi-TUMAP responders, exceeding the study success criteria of more than 30% multi-TUMAP responders and more than 60% single TUMAP responders. Dr. Williams described the difficulties of successfully commercializing therapeutic vaccines, giving the example of the first therapeutic vaccine approved for the treatment of cancer, Provenge® for prostate cancer, which was predicted to be a commercial breakthrough. Its 4-month survival benefit, and the high complexity of the manufacturing process, low uptake and poor sales led to the bankruptcy of its owner.

The first-generation immunotherapeutic **AST-VAC1**, autologous dendritic cells pulsed with telomerase, has been shown to be safe and stimulates anti-telomerase immune responses. Human telomerase reverse transcriptase (hTERT) is an attractive DNA immune therapy target in cancer immunotherapy. High levels of hTERT have been detected in more than 85% of all human cancers, while normal cells showed undetectable levels of telomerase expression. Immunological analysis indicates that the hTERT is a widely applicable target recognized by cells and can be potentially used as a universal cancer vaccine. CRC UK in collaboration with Asterias, are developing a second-generation immunotherapeutic, **AST-VAC2**, which has improvements on AST-VAC1. AST-VAC2, an embryonic stem cell-derived dendritic cell vaccine, is an allogeneic immunotherapy, which has uniform potency and stimulates naive antigen restricted T cells with only a single MHC match, offering broad patient access.

Immunotherapy for cancer could be improved using IgE rather than IgG antibodies. IgE enters tissues more readily and has a much greater affinity for its receptor. In development at CRUK is **MOv18**, a first-in-class IgE antibody against folate receptor (FR)-α, in patients with advanced solid tumors including ovarian, colorectal and mesothelioma. MOv18 IgE is specific for FR, a tumor-specific antigen. Its efficacy is superior to the equivalent IgG_1 antibody in mouse syngeneic WAG tumor model. All therapeutic antibodies developed so far are IgG isotypes. The efficacy of this targeting strategy may be improved using IgE, and therefore this phase I trial being conducted by CRUK could have far-reaching implications if it demonstrates proof-of-concept.

CD19 CAR T CELLS

Dr. Martin Pule (University College London, UK) gave a stimulating talk on "CD19 CAR T cells – a review (2015)," summarizing the recent advances in the field of chimeric antigen receptor (CAR) T cells as an immune-oncology therapy.

Dr. Pule and his group work on engineering mammalian cells for therapeutic applications which involves engineering proteins and vectors for eukaryotic expression, early preclinical testing and then with some promising approaches, moving to clinical studies.

His research focuses on how T cells can be redirected to recognize tumor antigens by genetic modification to express a CAR. These consist of antibody-derived antigen-binding regions linked to T-cell signaling elements. In this context, CD19 is an ideal target because it is expressed on most B-cell malignancies as well as normal B cells but not on other cell types, restricting any 'on target, off tumor' toxicity to B-cell depletion (9).

Dr. Pule further summarized some data from recent clinical studies involving CD19 CAR-directed T cells which have shown unprecedented responses in a range of B-cell malignancies, even in patients with chemorefractory relapse. Durable responses have been achieved, although the persistence of modified T cells may be limited.

Furthermore, the safety aspects of T-cell therapy were described. Cytokine release syndrome and neurotoxicity appear to be frequent but are treatable and reversible.

Dr. Pule leads the UCL Chimeric Antigen Receptor program and his group is now focused around five facets of cellular therapy: i) Synthetic biology approaches for advanced T-cell engineering; ii) preclinical development of promising therapeutic strategies; iii) scale-up, and translation; iv) clinical experimentation; and v) molecular imaging strategies to track engineered T cells in human subjects.

CAR T-cell therapy remains a promising avenue to explore as a specific and tailored treatment in the field of immuno-oncology.

DATA MINING NEW VACCINES

Professor Richard Titball (Department of Biosciences, University of Exeter, UK) presented an excellent talk on "Data mining new vaccines", which was a critical examination of the development processes involved in creating vaccines for use in humans, illustrating pitfalls and successes in the field using examples of vaccines targeted to infectious disease taken from the literature and from work carried out in his laboratory. A key message of his talk was to remind the audience that there are many infectious diseases that lack effective vaccines. He noted that failures in vaccine generation were often associated with the inability of traditional experimental research to identify vaccine candidates that can be formulated to elicit appropriate immune responses capable of conferring protection from exposure to infectious agents. He then introduced the alternative strategy of "reverse vaccinology", and demonstrated how this bioinformatics-driven method has been successfully used to create the recently licensed antimeningitis vaccine Bexsero®. The need for a universal vaccine against serogroup B meningococcus (MenB) and the availability of genomic sequence facilitated the use of data mining techniques to identify surface-associated proteins as vaccine candidates. This use of genomic data for vaccine development resulted in MenB becoming the prototype for this target discovery known as reverse vaccinology (10).

Professor Titball then posed the question, "Can we go beyond predicting potential vaccine components based on cellular location?" He then described two additional approaches that can be used for vaccine discovery: prediction of immunogenicity properties and experimental identification of immunogenic vaccine targets using pathogen-infected patients. He explained how immunogenicity can arise from processing of vaccine antigen epitopes by the MHC Class I or Class II pathways. A wide range of software is now available to predict MHC epitopes and it has been hypothesized that vaccine antigens may be enriched for such peptide sequences. However, he also described data that reported that bacterial vaccine antigens may not be enriched for these epitopes (11), thus questioning the use of MHC prediction software as a tool for bacterial target vaccine discovery. He then described an example of patient-based vaccine discovery research using an example from his own laboratory involving the tropical disease melioidosis, caused by the bacterial pathogen *Burkholderia pseudomallei*. He explained how proteome microarray chips were generated with a wide selection of *B. pseudomallei* recombinant proteins. Chips were then probed with sera from patients infected with the pathogen and naive controls (Fig. 2) to

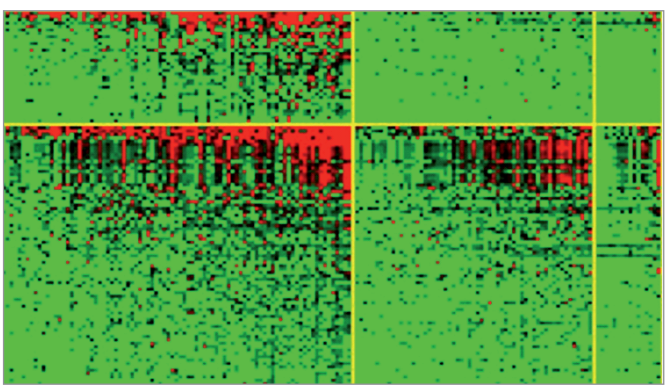

Figure 2. Comparative proteome microarray maps to study antibody responses. Serum antibody responses against *B. pseudomallei* antigens are shown for melioidosis patients (left panel) or control individuals (right panel). (Image provided by Professor Richard Titball, University of Exeter, UK).

identify immunoreactive proteins as potential vaccine candidates (12, 13). He concluded by showing timelines for vaccine development, emphasizing that considerable gains have been made in the vaccine antigen discovery process over the last 30 years, but that more work needs to focus on innovative clinical trials to enable more rapid introduction of new vaccines in the future.

THERAPEUTIC OLIGONUCLEOTIDES

Dr. Stephen A. Hughes gave an excellent summary on recent developments in the field of oligonucleotide research in his talk entitled, "Therapeutic oligonucleotides".

Therapeutic oligonucleotides are short, single or double-stranded DNA or RNA molecules that modulate the expression or pharmacology of selected genes or gene products. They are essentially large, negatively charged "small molecules". Their function includes changes in gene expression via exon skipping, knockdown of gene expression via for example antisense, or they can increase gene expression or alter microRNA expression. Oligonucleotides can also function to stimulate or repress cellular or immunostimulatory responses.

Oligonucleotides include a wide range of modalities including RNA interference (RNAi), antisense, aptamers, miRNA antagomirs and mimics as well as immunomodulators. While the idea of using oligonucleotides to specifically target single genes dates back to the 1970s, early attempts to translate this approach into the clinic were unsuccessful. The field has, however, made incremental progress with the regulatory approval of Vitravene® (fomivirsen) for cytomegalovirus retinitis (1998), Macugen® (pegaptanib) for wet macular degeneration (2004) and Kynamro® (mipomersen sodium) for homozygous familial hypercholesterolemia (2013).

Development activity has continued to grow with over 80 oligonucleotides now in active clinical development. Of these, several assets have delivered positive clinical efficacy data that supports continued progression. Key challenges and developments in the field include improving delivery and chemistries which should lead to an improved therapeutic index.

The example of antisense oligonucleotides (ASOs) including all stages from initial ASO design to pre-candidate selection using advanced third-generation chemistry was discussed. The great advances in nucleic acid chemistry allowed it to be made not only in large quantities but also in a large variety of molecules with distinctly unique and desirable properties.

Now with our recent insight in how nature uses oligonucleotides for the regulation of cellular functions and defense mechanisms, one of the most fascinating insights of recent years has been that nature itself uses endogenous oligonucleotides for the regulation of gene function and control of cellular homeostasis on a large scale. Thus, like antibodies, oligonucleotides can be regarded as natural compounds that act through natural mechanisms.

To combat the decline in new drug development productivity, alternative therapeutic modalities need to be pursued. Oligonucleotide therapeutics offer a promising option as they have the potential to treat disease targets previously "undruggable" using small molecules or monoclonal antibodies.

DISCLOSURES

J.C. Holder is an employee of Roslin Cell Sciences. F. Fallah-Arani is an employee of UCB. M. Konneh is an employee of BioTrial. The other author states no conflicts of interest.

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