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MEETING REPORT

NEW DEVELOPMENTS IN TRANSLATIONAL TECHNOLOGIES. HIGHLIGHTS FROM THE SOCIETY FOR MEDICINES RESEARCH SYMPOSIUM, HELD ON MARCH 17, 2016 – ROYAL VETERINARY COLLEGE, LONDON, UK

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SUMMARY

An area of drug discovery and development that has seen a great increase in focus in recent years has been the need for more translational approaches in many if not all therapeutic areas. This has stemmed from the increasing appreciation that many of the cellular and animal models, traditionally used for the assessment of efficacy, have limited predictive validity to the human diseases we are trying to treat. This 1-day Society for Medicines Research Symposium hosted by Transpharmation Ltd at the Royal Veterinary College, London, U.K. and sponsored by Pharmidex Ltd was organized by Lee Dawson, Wendy Alderton, Mo Alavijeh and Peter Weber. The meeting brought together experts from a variety of imaging and preclinical model backgrounds across a range of diseases to discuss how the drug discovery community can better target disease processes, more optimally test novel therapeutics in appropriate populations and ultimately enhance clinical success in producing efficacious therapeutics for patients.

Key words: Alzheimer's disease – Markers – Cognitive dysfunction – Neuropharmacokinetics – Translational imaging – Pain – Oncology

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SCIENCE, BIOMARKERS FOR ALZHEIMER'S DISEASE: WHAT FOR?

Professor Simon Lovestone, Translational Neuroscience, University of Oxford, gave an overview of his research over the last 10 years on the discovery of blood-based biomarkers for selection and stratification approaches for clinical trials in Alzheimer's disease (AD). The majority of clinical trials in AD, to date, have tested therapies in patients following diagnosis of dementia and have failed to demonstrate efficacy. A key reason for this failure is likely the advanced progression of neuronal loss by the time patients are diagnosed. Therefore, it is of most utility to find biomarkers to identify asymptomatic individuals in the preclinical phase of the disease as earlier diagnosis will enable potential disease-modifying agents to be tested more effectively in the clinic.

Professor Lovestone described his early 2D gel electrophoresis case-control blood-based biomarker studies that identified complement factor H as significantly separating AD samples from controls. Interestingly, complement H had been previously identified in another amyloid-based disease: macular degeneration. In order to search for biomarkers in preclinical disease, studies used an endophenotype design such that biomarkers could be correlated with hippocampal atrophy, cognition (as measured using mini-mental state examinations) and progression and speed of decline. Such studies identified clusterin as a candidate biomarker, which was found to correlate with amyloid PET imaging. Concomitantly the clusterin gene was also identified in genome-wide association studies as a risk factor for late-onset AD. Clusterin has subsequently

been shown to possibly be involved in β-amyloid-mediated toxicity via the Dickkopf-1/wnt-PCP-JNK pathway (1). Further biomarkers identified were α_2-macroglobulin and fibrinogen gamma chain, which are associated with neocortical amyloid burden. A number of blood-based biomarkers have now been and continue to be identified, all of which now need replication. These may ultimately form a signature panel that can identify patients early and predict disease progression. To this end, Professor Lovestone described a validation study using 1,100 plasma samples and a design based on imaging measures as surrogate for disease severity, which found a panel of 10 plasma proteins that predict conversion to dementia from prodromal disease with an accuracy of 87% (2). Other biomarker studies aiming to find subjects in early disease have used PET imaging amyloid endophenotype and proteomics in plasma samples as well as measurement of tau and $A\beta_{42}$ in cerebrospinal fluid. Proteomics discovery of plasma protein biomarkers of AD pathology was undertaken in cognitively healthy elderly using three timepoints across 12 years with PET imaging at the last time point. Seven proteins (α_2-macroglobulin, apolipoprotein A-1, complement C3, complement C4-B, haptoglobin, Ig κ chain C region and fibrinogen γ chain) were found as candidate biomarkers of cerebral amyloid burden consistently across all three time points.

Replication and cross validation of these biomarker discoveries requires many cross-center studies in large cohorts, which has been the premise behind three large public–private partnerships with the aim of facilitating translational research in dementia. Professor Lovestone went on to describe the structure of the Dementias Platform UK which is building a discovery cohort of 2 million subjects and has access to samples from extensively phenotyped volunteers from the UK Biobank, which includes whole body MRI in 8,000 subjects to date; the Innovative Medicines Initiative (IMI) European Medical Information Framework is aiming to access data from 48 million Europeans and more than 50,000 samples from volunteers from multiple cohorts; and finally the IMI European Prevention of Alzheimer's Disease initiative has a registry of 24,000 volunteers who are willing to take part in clinical studies and is building a longitudinal cohort in 6,000 volunteers as well as initiating a first proof-of-concept trial to test multiple compounds from industry and academia. These U.K./E.U. initiatives may be paralleled by similar efforts in Canada and the U.S., all of which will hopefully lead to the identification of diagnosis and prognostic biomarkers and ultimately successful clinical studies to identify treatments for AD.

ELECTROPHYSIOLOGICAL MARKERS OF COGNITIVE DYSFUNCTION

Dr. Paul Dockree, School of Psychology, Trinity College, Dublin, Ireland, presented his research in the measurement of human scalp electroencephalogram (EEG) to identify electrophysiological signatures of sustained attention and decision making which can ultimately be used as a translational methodology from animals to humans. Sustained attention refers to the ability to maintain alertness in circumstances in which there is little change or novelty in the environment. It is a fundamental process underlying the more complex forms of cognition, such as awareness and executive function. Increased frequency of lapsing attention is apparent in aging and a range of clinical syndromes including attention deficit hyperactivity disorder (ADHD), stroke, traumatic brain injury and bipolar disorder. Dr. Dockree described high temporal resolution of the surface event-related potentials (ERPs), which are characteristic time varying scalp fields that result from the summation of electrocortical activity, generated in sustained attention to response tests and continuous temporal monitoring tasks. In the continuous temporal monitoring task, stimuli are presented at a 25-Hz flicker, thus eliciting a steady-state visual-evoked potential. Data are analyzed at three timescales: long-term pre-target processing (–30 seconds), short-term pre-target processing (–4 seconds) and post-target processing (+1 second). This revealed failures of sustained attention involving maladaptive neural patterns operating at the different timescales and the results showed that the specific neural signatures of attentional lapses are registered in the EEG up to 20 seconds before an error (3). The continuous temporal monitoring task offers a paradigm which can be used to better understand the mechanism of action of a drug. Dr. Dockree gave the ADHD therapy Ritalin or methylphenidate (MPH) as an example (4). MPH was administered within a placebo-controlled, double-blinded, within-subject design to 40 neurological healthy male adults. MPH reduced both attentional lapses and response variability to target detections compared to placebo without significant change across conditions in mean response time. These results suggested that MPH improves indices of sustained attention during the task but does not alter processing speed to the same extent. MPH also modulated attention-relevant signals in surface EEG including α-band suppression and P3 amplitude increases, which predicted target detection. By contrast, there were no changes to early visual processes (visual P1 and 25 Hz steady-state response) indicating that MPH exerts its influence primarily on higher-order endogenous mechanisms instead of facilitating bottom-up sensory processing. These data show the physiological basis by which MPH improves attention, offering candidate markers for remediation in ADHD or other attention disorders.

Decision-making deficits are seen in a range of psychiatric and neurological disorders and manifest in simple tasks in common ways. These basic task-derived measures may then serve as markers for more profound changes to thinking and reasoning. Dr. Dockree described a novel task where participants were required to detect brief changes in a single feature of a continuously presented stimulus and using a simple button-push response were able to track preparatory activity of premotor areas during decision formation (5). These results isolated freely evolving neural signatures of sensory evidence encoding, decision formation and motor preparation in the human brain that mirror the dynamics observed in single-neuron counterparts as can be measured in animals. The homologous methodology in human and animal studies offers greater scope for application and translational purposes in novel drug development.

NEUROPHARMACOKINETICS OF CNS ACTIVE MOLECULES

Dr. Mo S. Alavijeh, Pharmidex, London, gave an overview of the importance of monitoring pharmacokinetics of a CNS active drug in brain compartments termed "neuropharmacokinetics" (6). CNS active molecules, in addition to having the usual drug-like characteristics (i.e., solubility, permeability, metabolic stability and appropriate protein binding), need to have the correct balance of physicochemical properties to facilitate transit across the blood–brain barrier (BBB), the structural and dynamic barrier that separates the CNS from the blood. As opposed to fenestrated peripheral

capillaries, brain capillaries are tightly sealed by cell adhesion molecules which forming tight junctions and this together with a number of influx and efflux transporter systems vigilantly monitors and limits the types of substances that can pass through the BBB and into the CNS. Typical physicochemical properties of a CNS drug are MW < 400, log D 1-3, H-bond acceptors < 6, H-bond donors < 2, neutral or basic pKa 7.5-10.5, polar surface area < 90 Å) and low substrate affinity transports such as P-glycoprotein (P-gp). Once a molecule enters the CNS there is an independent brain drug metabolism (7); brain P450 enzymes are 1/10 to 1/15 of the activity of their liver counterparts but metabolism rates can vary significantly and can produce differential metabolism versus the peripheral system. One example given was the anxiolytic drug alprazolam (8): the brain metabolism produces the pharmacologically active metabolite α-hydroxy-alprazolam, whereas the inactive metabolite 4-hydroxy-alprazolam is the major liver metabolite. Dr. Alavijeh did highlight that a small number of structures within the brain, such as midline of the ventricular system referred to as circumventricular organs (CVOs), lacks a BBB and could thus be a potential target for drug delivery; a challenging task, however, as CVOs' surface area is 1/1000 that of the BBB.

Dr. Alavijeh went to discuss the pros and cons of the current methodologies utilized. In vitro models provide a useful and rapid means to get an early indication of BBB permeability. One of the most common in vitro models used by industry is Madin–Darby Canine Kidney (MDCK) cell lines as they form tight junctions and lend themselves to higher throughput screening. Perhaps more informative, but low throughput systems are available such as the co-cultured mixed cell models (e.g., bovine, porcine and human brain endothelial cells cultured with astrocytes). Ex vivo models provide an intermediary step between in vitro and in vivo assessments. One of most common methods used is in-situ brain perfusion, which measures the rate of brain uptake and is mostly suitable for both slow and fast brain-penetrating compounds. In vivo the most common method is measuring drug concentration in the brain and blood compartments following systemic administration and using these numbers to calculate the so-called brain-to-blood ration ($B/B = C_{brain}/C_{blood}$). However, one has to be careful on the interpretation of these data as many studies have indicated a 250- to 400-fold difference in B/B ratios with some marketed drugs and differences can occur between species. One alternative means of assessment is the use of in vivo microdialysis as a method for measuring the free drug levels within tissue. With this method, drug levels can also be correlated to the pharmacodynamic consequence of a compound on tissue biochemistry and/or neurotransmitter changes. It was noted, however, that free drug concentrations can vary across different brain regions and not all compounds are amenable to the technique.

PATIENT-DERIVED TUMOR XENOGRAFT MODELS FOR TARGETED ANTICANCER DRUG DEVELOPMENT

Professor Heinz Herbert Fiebig, CEO of 4HF GmbH and former CEO of Oncotest GmbH, gave an overview of patient-derived in vivo and in vitro cancer models developed by Oncotest. The company was founded in 1993 as a spin-out of the University of Freiburg, where Prof. Fiebig pioneered the development of novel xenograft models derived from primary tumor tissue directly taken from patients (PDX). The approach is based on the initial propagation of tumor tissue in nude mice, followed by phenotypic and molecular characterization. The tumors obtained are frozen in liquid nitrogen and can be utilized in colony formation assays, monolayer cultures and in vivo subcutaneous xenografts. The platform established by Oncotest currently consists of approximately 360 different validated xenografts from a large number of different solid tumors. Further models, including a variety of liquid tumor models, are currently under development. Established xenografts are characterized on a molecular level, including Affymetrix-based assessment of mRNA profiles, genetic mutation profiles, SNP analysis and gene copy number variation. Whole genome sequencing has been performed in ca. 90% of the PDX models and 70 proprietary cell lines. In addition, selected genes/proteins have been characterized by FISH/PCR, Western blot and IHC analysis. As an outcome of this analysis it was found that common oncogenic mutations in human cancers, e.g., KRAS mutation, ErbB2 amplification or PTEN loss, are well represented in the panel. PDX models also compare well to human primary tumors at a phenotypic level: For example, histology studies show that the structural architecture of PDX tumors observed by IHC shows strong similarity to that found in human cancers. This is in strong contrast to conventional, cell line-based xenograft models, which display limited differentiation and a lack of stromal elements. Another advantage of PDX models is the good reflection of drug response profiles seen in patients: A comparison between remission after therapy in cancer patients versus response of PDX models derived from these patients showed a > 90% match in outcome. Oncotest has established a number of in vitro assays with patient-derived material, similar to PDX in vivo models, that have shown utility in drug profiling (9). Clonogenic assays have been used to characterize targeted therapies, identify biomarkers and predict responder populations. For example, analysis of the BRAF inhibitor vemurafenib in a large cell panel derived from 17 solid tumors revealed a strong response in melanoma-derived cells compared to other lines. Similarly, in a screen with 67 different tumor models, the BRAF inhibitor PLX-4720 gave a strong response on BRAF V600E mutants derived from melanoma compared to other tumors. In the next part of his presentation, Prof. Fiebig described how the PDX platform can serve the development of predictive gene signatures for the individualization of cancer chemotherapy. This process is based on initial drug profiling in colony formation assays, followed by confirmation of activity in vivo. Activity data are then correlated with gene expression profiles to generate a gene signature that could serve as a response biomarker. This signature is then further validated in additional test sets. Using the profiling of trabectedin (Yondelis, Johnson & Johnson) as an example, Prof. Fiebig showed how a set of 19 genes can predict response in vitro and in vivo with great accuracy.

In summary, well-characterized PDX models can serve the selection of appropriate model systems, the testing of treatment hypotheses and identification of biomarkers, which can ultimately lead to marker-guided selection of patients for clinical trials.

MECHANISTIC STUDIES OF NEURODEGENERATION IN HUMAN STEM CELL SYSTEMS

Professor Rick Livesey's team at the Gurdon Institute, University of Cambridge, U.K., have developed novel stem cell methodologies to assess the neuropathy associated with both Down syndrome (DS) and familial Alzheimer's disease (fAD). Using their methods they differentiate human embryonic and inducible pluripotent stem cells

(iPSCs) into human cortical neurons, which over a long period of growth (up to 90 days) can form complex excitatory neuronal networks. These can then be used to track disease progression, changes in neuronal network conductivity and changes in protein processing and subsequent disease pathology. Patient-derived cells can be used to increase our understanding of some of the genetic changes that have been associated with diseases such as fAD and DS. Thus, it provides a valuable tool to help understand the process of neurodegeneration associated with disease and facilitates the identification of potential new therapeutics for these disorders. Dr. Livesey provided examples of how human stem cells systems are used in his laboratory to study the molecular mechanisms of neurodegeneration.

DS individuals have an extra copy of chromosome 21, which carries the gene for amyloid precursor protein (APP), and due to this DS individuals have a much higher incidence of dementia (six times) than the rest of the population. Interestingly, this is not 100% penetrant suggesting that mechanisms of resistance may also be isolated from these systems. By generating cortical neurons from skin cells of people with DS, Dr. Livesey's team has observed the disease process over a much shorter period (weeks versus months). Compared to cells derived from healthy subjects, the cortical neurons from DS patients showed more than twice the amount of $A\beta_{42}$ within 28 days which went to form amyloid plaques within 2 months. Tau protein (the microtubule binding protein also thought to be involved in AD) became abnormally altered and distributed in the cells, one of the common later-stage characteristics of the disease (10). What is promising about this stem cell technique is that functioning human cortex cells are created in a dish, allowing closer modeling of what is happening in our brains. The new model has added benefit of showing many of the characteristic features of human AD and will allow testing of new treatments more easily. In this regard, Dr. Livesey also touched on the joint work with Professor John Hardy at the Institute of Neurology, University College London, focusing on answering a number of questions on the APP metabolism regulation and the consequences of the familial genetic risk factors. While distinct genetic forms of AD all increase $A\beta_{42}$ generation, neurons from different genetic forms of AD differ in APP processing and ultimately tau pathology; interestingly the PSEN1 (presenilin 1 protein coding gene) mutations do not. This suggests that APP processing is involved in AD pathogenesis in multiple different ways and the cell autonomous metabolism can produce a variety of APP-derived peptides that can have very different synaptotoxicities in different genetic forms of AD. He also showed how some of the pharmacological tools that have been generated produce very different outcomes in both amyloid and tau pathology in these systems, potentially informing about mechanisms of actions and possible liabilities of these compounds.

TRANSLATIONAL IMAGING AT GLAXOSMITHKLINE

Dr. Christine Parker, from the GlaxoSmithKline (GSK) translational team based at GSK's Stevenage research site, provided insight into how their innovative imaging technologies are progressively being applied to aid translational research by allowing drug–target interactions to be monitored in clinical trials.

Experimental medicine imaging (EMI) at GSK has a broad role with a cross-therapeutic area focus. Research projects are collaborative in nature with extensive external links to help with method selection for bioavailability in tissue exposure, confirmation that target is on the pathological path, demonstration of target binding, pharmacokinetic/pharmacodynamic correlations to inform dose selection, proof of mechanism/pharmacology and determination of efficacy in patient populations. Dr. Parker gave two working examples across the two therapeutic focuses.

The CNS-focused example described the development of PET ligands for I_2-imidazoline binding sites (I_2BS) in collaboration with Imperial College, London, and funded by an MRC academic industry grant (11-13). Monitoring I_2BS could aid the understanding of psychiatric and neurological conditions and potentially act as a biomarker for early disease diagnosis. The collaborative project successfully synthesized and pharmacologically profiled (in vitro and ex vivo in rat) a potential PET radioligand, BU-99008. BU-99008 demonstrated excellent affinity and selectivity for the I_2BS (K_i of 1.4 nM; K_d = 1.3 nM), good selectivity compared with the α_2-adrenoceptor (909-fold) and achieved uptake into the rat brain following systemic administration. Studies in pig and nonhuman primates demonstrated a distribution consistent with expected I_2BS localization. This, together with the amenability of BU-99008 to radiolabeling with a positron-emitting radioisotope, indicated its potential utility as a PET radioligand for imaging the I_2BS in vivo.

Dr. Parker went on to discuss the development and usage of 3D multi-turnover spheroids (MTS), in vivo animal xenograft models and imaging in humans as a translational path for development, using the AKT pathway and specifically AKT inhibitor GSK-2141795 as the example (14). GSK-2141795 treatment in vitro and in vivo resulted in ~50-90% decrease in phospho-PRAS40 and 20-80% decrease in fluoro-deoxyglucose (FDG) uptake. Proteomic analysis of GSK-2141795 in vitro and in vivo identified a signature of pathway inhibition including changes in AKT and p38 phosphorylation. In patient biopsies, prior to treatment with GSK-2141795 in a phase I clinical trial, this signature was predictive of post-treatment changes in the response markers. Development of this signature represents an opportunity to demonstrate the clinical importance of AKT inhibition and also the use of 3D spheroid methodologies as a bridging technology into xenograft models and beyond. Currently, a number of clinical studies are underway with AKT inhibitor GSK-2141795 either alone or in combination to treat patients.

ASSESSMENT OF NATURALLY OCCURRING NEUROPATHIC PAIN IN COMPANION ANIMALS

Prof. Holger Volk, Royal Veterinary College (RVC), Hatfield, U.K., described the characteristics of the canine syringomyelia (SM) in Cavalier King Charles Spaniels. SM is a disorder clinically defined as non-cerebrospinal fluid filled cavities within the spinal cord often seen in dogs bred to have characteristic shortened noses resulting in cranial overcrowding and cerebellar herniation (15). However, this disorder is increasingly becoming common in humans. SM can be also seen in human patients as a consequence of spinal trauma. Both diseases produce a range of symptoms/behavioral phenotypes, including marked clinical neuropathic pain brought about by altered somatosensory processing of aberrant sensory information producing the perception of pain. The perception of pain per se is somewhat difficult to measure in dogs, although

obvious indicators such as vocalization, a reluctance to exercise or movement/postural changes that result in alterations in CSF pressure are common, and along with coughing and defecation are characteristic phenotypes. Phantom scratching or "air guitaring" probably due to abnormal sensory sensations are behaviors often described. These behaviors are often akin to the burning, pins and needles and other strange sensations experienced by human SM sufferers. In order to more quantifiably assess these behaviors, a sensory threshold examination test (STEP) has been developed by the RVC group; this includes tactile sensitivity (von Frey filaments test), mechanical threshold or weight bearing and sensitivity to heat/cold stimuli. The group has shown this to be feasible, safe and well tolerated with good robustness on re-test and hence it appears to be a good tool for assessing this debilitating and complex disorder.

Pharmacological assessment shows that, as in people, the dogs glean no efficacy from anti-inflammatory drugs. However, agents such as gabapentin, pregabalin and topiramate (the only drugs currently of any utility in human neuropathic pain) do show some clinical efficacy in some animals, interestingly with less side effects than seen in human subjects. SM is clearly an unmet medical need in the veterinary arena and better therapeutics are thus needed. But additionally, the similarities between canine and human neuropathic pain may also offer the potential for improved translatability to human disease.

ETHOLOGICALLY RELEVANT NON-EVOKED PAIN ENDPOINTS AND MULTICENTER APPROACHES IN PRECLINICAL STUDIES

Dr. Rachel Wodarski, Imperial College London, presented her work and the work of a coordinated network of researchers across Europe to evaluate burrowing behavior as an ethological endpoint for the assessment and translation of pain (www.imieuropain.org). Dr. Wodarski presented the shortcomings of the traditionally used reflex measures of sensory pain models and their lack of construct and predicative validity. Pain perception is a multifactorial experience which goes beyond sensory perception alone and thus a more global measure of pain may be more relevant. Burrowing in rodents is a social behavior used to build underground homes and protect from predation and is highly conserved in laboratory animals (16). Deficits in this behavior have been shown in the context of inflammatory and neuropathic pain and can be reversed by known analgesics.

One major limitation of these types of preclinical models is the reproducible and cross-laboratory robustness. Thus, a multicenter coordinated effort was established to assess the replication and inter-laboratory variation in this model and determine the issues and factors associated with any variability observed. The rodent complete Freund's adjuvant (CFA)-induced inflammatory pain model was used in 11 studies across 8 centers and data were collected centrally before analysis. The studies demonstrated that following CFA injection, significant deficits in burrowing were replicated across the various centers with duration and recovery following similar trajectories, although some cross-site variability was observed. These data suggested that the model may be a useful tool with good reproducibility for global assessment of pain in rodents.

DISCLOSURES

L.A. Dawson, W. Alderton, M.S. Alavijeh and P. Weber are in paid employment of their respective organizations. All authors are SMR Committee members for which no remuneration is paid.

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