

## BIOMARKERS FOR DISEASE MODIFICATION TRIALS – THE INNOVATIVE MEDICINES INITIATIVE AND ADDNEUROMED

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In a previous issue of the Journal we reviewed the pressing and urgent need for biomarkers in Alzheimer's Disease (AD) (1). This need has become no less urgent or pressing and indeed arguably becomes more so as increasing numbers of compounds pass the phase II barrier and move to phase III trials. To recap briefly, AD presents difficulties with diagnosis, with prediction and with monitoring progress that might, if not be solved then might be assisted by biomarkers. In the context of clinical trials all three potential uses of biomarkers might be important. Diagnostic biomarkers would potentially increase specificity of diagnosis and thus help to recruit a homogenous subject group. Predictive biomarkers might be used in trials with conversion to dementia as an end point – increasing the power of Mild Cognitive Impairment (MCI) trials for example. However the most important potential use of biomarkers is as markers of progression in disease modifying trials.

The developing field of biomarkers in AD has been extensively reviewed recently (2-5). Most advanced are imaging markers which are increasingly being used as secondary end-points in clinical trials in an attempt to demonstrate disease modification. Significant advances are being made in the search for biochemical markers in AD with the most promising being various measures of tau and Ab in CSF. To date most studies have been of the candidate approach, assaying proteins, lipids or other metabolites implicated in AD pathology. Increasing numbers of studies have also now reported the outcomes of more systematic proteomic or other unbiased technologies (6-9). These studies, both candidate and systematic, have found changes in both CSF and in blood and strongly suggest that biomarkers for disease modifying trials are a realisable goal although currently no marker has test characteristics enabling it to be used as an outcome measure in therapeutic trials.

Currently the primary end-point for trials are clinical measures. However clinical measures of cognition and function are problematical as markers of progression. As an instance of this, one study of change in cognition over time as measured by the mini mental state examination showed some patients to improve by more than the average effect size of the cholinesterase inhibitors, even though this study was conducted prior to these drugs being developed (10). If a biomarker could be developed that mirrored disease progression then this would have huge potential in clinical trials. This potential has been widely recognised by the pharmaceutical industry and in this

paper we describe a process where an initiative driven by the industry has resulted in a large European biomarkers in Alzheimer's disease initiative and has had an important influence on EU funding decisions.

### The Innovative Medicines Initiative (IMI)

In 2004 the European Federation of Pharmaceutical Industries and Associations (EFPIA) and the European Union (EU) published a strategic research agenda for biomedicine in Europe (second version published 2006; <http://www.imi-europe.org/>). This process resulted from the work of over 350 senior representatives of patient organisations, academic and health organisations together with small and large biopharmaceutical companies. The document sets out recommendations for predicting safety and efficacy of medicines as well as plans to bridge gaps in knowledge management, training and education. It is noted that "these are the principal causes of delays in the complex process of developing new medicines". A key element of the recommendations of the IMI strategic research agenda concerns biomarkers in the context of drug development. Amongst the benefits biomarkers might bring to drug development are increased probability of programme success and reduced cycle time, matching patients with therapy (individualised medicine), faster optimisation of therapy, improved compliance and reduced complications and improved efficacy. All of these might potentially be goals for AD biomarkers in disease modification trials. In addition the strategic research agenda highlights the potential for earlier intervention with the use of biomarkers – something that is of particular importance in AD. Validating biomarkers is "a lengthy and expensive" exercise albeit an absolutely essential one, for which there is no real consensus. However in developing the technologies – especially 'omics and imaging – and in establishing and strengthening existing networks in order to deliver the patients, the European Union has an opportunity to deliver on this core translational medicine goal.

### AddNeuroMed

Following from the work leading to the strategic research agenda, EFPIA proposed to EU two projects utilising biomarker approaches – Predictive Toxicology (PredTox) and

## NEUROIMAGING OUTCOMES FOR CLINICAL TRIALS

AddNeuroMed. Following the normal peer review led funding process these projects were awarded and over 40 Pharma, SMEs and academic centres came together under a single management structure as Innovative Medicines (InnoMed). The goals of the AddNeuroMed component of InnoMed are to use pre-clinical and clinical studies to discover biomarkers for AD with a specific focus on biomarkers for progression.

### Identifying biomarkers from improved models of Alzheimer's disease

Animal models have two potential roles in the discovery of biomarkers for AD – discovery and biological validation. A model that recapitulated the disease process, or some element of that process, might be used in discovery – assaying biological fluids (blood, CSF) in much the same ways as in human studies. However animal models have three distinct advantages over clinical studies – the effects of individual and environmental variation are minimised, fluids can be obtained at defined time points relative to disease and to perturbations and biomarker outcomes can be correlated directly with neuropathology. AddNeuroMed aims to develop improved animal and cellular models of AD processes for biomarker discovery studies. In addition biological validation is an important part of biomarker programmes. When using unbiased technologies ('omics), potential biomarkers will be discovered that have no known role in disease process. In order for a biomarker to become widely accepted it may be necessary to demonstrate a direct influence on disease process and models of disease may be the only way to achieve this convincingly.

AddNeuroMed is using four inter-linked models - organotypic slice cultures, intracerebral injections of Ab in rodents, transgenic *Drosophila* and transgenic mice. The first model system uses organotypic slice culture of rat hippocampus (11) exposed to AD-related insults as a source of markers in-vitro. Organotypic cultures represent one the best in-vitro systems available because of the integrity of neuronal circuits and the presence of all CNS cell types. *Drosophila* is a useful model system for examining the effect of mis-expression of genes linked to AD on aetiology of pathology (12). We aim to develop novel *Drosophila* models of AD by exploring the interaction of known AD genes with environmental risk factors for AD. The effects of these interactions on gene and protein expression in the fly nervous system, together with data from the organotypic cultures, will be used to inform the development and refinement of mouse models.

Only partial reproduction of AD pathology has been achieved to date in transgenic mouse lines, so there is a pressing need for models with greater face and construct validity. Therefore, we aim to develop new transgenic mice models by adding additional genes identified from the *Drosophila* studies and combining novel and established genetic perturbation with additional stressors implicated in AD to precipitate overt neurodegeneration and synapse loss. Sample

fluids from these animals will be used in biomarker discovery using a range of unbiased technologies.

In addition to developing cellular and animal models, AddNeuroMed is using both systems biology and bioinformatics to drive biomarker discovery.

### Identifying progression biomarkers for use in disease modifying trials

The largest biomarker study reported to date in the AD field is the Alzheimer's Disease Neuroimaging Initiative (ADNI; www.adni-info.org) (13). This large, North American, multi-centre trial has as its primary aims to "define the rate of progress of mild cognitive impairment and Alzheimer's disease, to develop improved methods for clinical trials in this area, and to provide a large database which will improve design of treatment trials". These aims include the assessment of imaging and CSF based biomarkers. These aims overlap with and are complementary to the aims of AddNeuroMed. With this in mind the clinical component of AddNeuroMed was designed to be at least partially compatible to ADNI although the aims are somewhat different.

Currently phase II/III trials for potential disease modification in AD are frequently 12-24 months in duration, although longer trials may be necessary. Measuring deterioration over such a relatively short time span is problematical using clinical endpoints in a slowly deteriorating condition such as AD and a biomarker is sought that would show reliable change over this time-frame. The clinical component of AddNeuroMed is, in effect, a mock clinical trial designed to collect data and samples for progression biomarkers in this context. Six clinical sites in Europe (London, Perugia, Thessaloniki, Toulouse, Lodz, Kuopio) are in the process of recruiting a cohort of subjects with AD, with MCI and unaffected elderly controls. Neuroimaging at baseline, at 3 months and at 12 months is structural MRI using ADNI protocols. Clinical measures include scales routinely used in clinical trials for cognition, function, behaviour and global deterioration and a comprehensive set of samples at 3 monthly intervals in cases and at baseline and at 12 months are being archived for biomarker analyses. Samples include plasma, serum, DNA, samples for RNA and cellular protein and urine.

### Identifying biomarkers for disease modifying trials – a European perspective

AddNeuroMed represents the largest European effort to identify biomarkers for use in trials in AD. Using animal and cellular models together with samples from a cohort assessed as if in a clinical trial, the collaborative group is focussed on identifying markers that might be used as alternative end points to monitor progression of disease. The efforts of more than 30 Pharma, SMEs and academic centres are bringing to bear on this critical goal in AD research, a wide range of technologies,

a considerable research effort and a large number of patients and samples. Although early signs are promising and the first fruits of this effort are appearing (14), the scale of the task should not be underestimated. Discovering potential biomarkers is taxing but validating such biomarkers and implementing their use in clinical or trials contexts is a far greater task. The scale of the task, of validating a biomarker, has been placed in the same order of magnitude as obtaining sufficient data for the licensing of a new compound. It is likely that the introduction of biomarkers into the world of disease modifying trials, and even more importantly, of obtaining the support of the regulatory authorities, will only become possible with the concerted action of many similarly large studies acting in concert.

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