ERA-NET NEURON Cofund

Symposium

‘Biomakers in neurology and psychiatry’

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This symposium is part of the Work Package

“Outreach and interaction activities”

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Welcome

Dr. Marlies Dorlöchter (PT-DLR, Neuron Coordinator, Bonn, Germany)

Marlies Dorlöchter introduced the scientific symposium on "Biomarkers in Neurology and Psychiatry" by addressing a few welcoming words to the speakers and the NEURON Scientific Advisory board. She emphasized the value of such a meeting for funding organizations to understand what is important in the field of biomarkers in neurology and psychiatry.
The ERA-NET NEURON is a European network of 27 funding organizations in the area of disease-related neurosciences, from 14 countries in the EU, as well as Switzerland, Norway, Israel, Canada, and Turkey. Its purpose, as a network, is to improve interactions between the research community, policy makers, funding organizations and the general public, by discussing the main areas of interest in research and preparing joint activities, such as calls for research proposals. NEURON aims to overcome issues such as bottlenecks in biomarker development and biobanks, to improve the transfer of technology from bench to bedside, and to help promote interactions between scientists, clinicians, and the society as a whole. Moreover, one of NEURON's specific aims is to promote early career researchers by developing support measures. NEURON's priorities cover neurological, psychiatric and sensory organs' diseases, ranging from understanding disease mechanisms to disease progression, as well as preventive and interventional treatments. As a funding network for translational neurosciences, it seeks to have multidisciplinary projects ranging from basic research to clinical research.

Dr Dorlöcher also described regular symposia organised by ERA-NET, for instance on how to reinforce the relations between scientists, clinicians and the society in the field of brain research. The results of this symposium were published in Lancet Neurology. Moreover, open science is of great interest to NEURON, and how it can be improved. On another occasion, Lancet Psychiatry featured last year's "Emerging Fields in Mental Health" scientific symposium in its editorial. New hot topics are being discussed during such scientific symposia in order to prepare joint calls. One purpose of this symposium is therefore to characterise how best to shape the next call topics, to make them precise enough so that there isn't an excess of applications, and at the same time open enough for original novel approaches to be considered.

ERA-NET NEURON has been funding projects for the last ten years, and spent more than 110 million Euros. NEURON funding scheme aims to be complementary to the European Commission calls, by focusing mainly on small to middle sized consortia of five or six, which are still flexible and without management issues.

Introduction

Dr. Etienne Hirsch (Paris, France) and Dr. Bernard Poulain (Paris, France)

Etienne Hirsch and Bernard Poulain presented the general objectives of this symposium. Its purpose is to shape the next call which will focus on biomarkers in neurology and psychiatry. The topic of biomarkers was chosen because there is a great demand both from basic researchers and clinicians for biomarkers to be very sensitive and very specific. Presently, the scientific community faces a bottleneck situation, where due to a lack of biomarkers, there is a lack of investment from big pharmaceutic in developing new therapeutic agents. Indeed, biomarkers represent a major issue in the field of brain related disorders. The drugs are working perfectly in preclinical models, but very few are tested at clinical levels, and even fewer yield positive results in such clinical settings. It is all the more an issue as even the best clinicians are doing wrong diagnoses. For instance, in the field of Parkinson's disease (not covered by ERA-NET NEURON), 25% of the patients are misdiagnosed. Likewise, in the field of psychiatric disorders, bipolar patients for instance suffer from a huge delay between the onset of the disease and the correct diagnosis.

Therefore, the hope of this symposium is to establish a call to fund the development of biomarkers that meet four criteria:

1) biomarkers to have an accurate diagnosis;
2) biomarkers to follow the disease progression;
3) biomarkers to verify the target engagement of drug, its downstream effects on the pathophysiological pathway, to make sure the target of interest is engaged when the drug is tested;
4) biomarkers that will predict which clusters of patients are more or less prone toside effects – a last category that is often overlooked; indeed there are many drugs that are known to be efficient, but are stopped because they lead to side effects in some patients; so the ability to predict which patient groups are vulnerable to side effects, would allow for the use of drugs that are safe and efficient in the vast majority of patients.

The other approach of biomarkers that will be covered in this symposium is the one distinguishing them by type: fluid, imaging and behavioural biomarkers. Moreover, this symposium will consider the question of biomarker quality, as it needs be reproducible and to be shared. Finally, one needs also to discuss the appropriate infrastructure and coordination of research in this field.

So the questions to be answered by the speakers today will be about the bottlenecks to overcome and how to shape the next call for proposals, in the very wide field of biomarker research.

What are biomarkers? What is their use?

Kaj Blennow, Göteborg, Sweden

Prof. Kaj Blennow works in Sweden in clinical neurochemistry, and leads a combined research lab that creates antibodies, does assay development and validation in clinical studies but also provides tests for patients as well as pharmaceutic companies to do their trials. As first speaker in this symposium, Prof. Blennow opened the discussions by talking about the definition of a biomarker, which he characterised as an objective measure of a biological or pathogenic process that can be used to evaluate disease risk or prognosis, guide clinical diagnosis, monitor therapeutic interventions, including side effects. He focused his talk on the example of biomarker development in Alzheimer's disease (AD).

Since Dr Alzheimer's 1901 index case of a 51 year old woman with memory disturbances and dementia, and her autopsy which showed plaques and tangles, AD was considered to be a disease of middle age people (50-65 yo). Older groups with poor memory and dementia were classified as senile dementia (SD), until the 1970s, when neuropathologists found that brains from elderly people with SD also presented plaques and tangles. This led it to be called senile dementia of the Alzheimer type. Now it all merged into one disease, Alzheimer's disease, characterised by memory problems, dementia, plaques and tangles. A rare familial form (1%) has also been described, which today is known to present with mutations in the amyloid precursor protein (APP) and presenilin genes. In the very large group of patients ranging from 75 to 90 yo, AD clearly appears to be age related and is called sporadic AD.

Following the discovery of beta amyloid and mutations in familial AD, but not sporadic AD, it was hypothesized that all neurons normally produce soluble beta-amyloid monomers, that for unknown reasons in AD patients change their conformation to beta-shaped, which start to agregate in Abeta oligomers (small soluble agregates) then Abeta fibrils and then Abeta plaques, that lead to neuronal degeneration, with atrophy of the brain. On the other side, the tangles are today known to be composed of tau in a phosphorylated form (PHF-tau), which lead to memory problems and cognitive symptoms. This hypothesis has been the lead for drug development for at least fifteen years. Thus, the treatment options today are: beta secretase (BASE) inhibitors that would reduce production of beta amyloid; gamma secretase modulators that will shift the clivage to shorter Abeta peptides (gamma secretase inhibitors have also been tried but stopped because of side effects); there are also molecules that aim at inhibiting agregation; and finally there is immunotherapy – either active immunization or passive administration of antibodies against either soluble Abeta or agregated Abeta.

<u>Why do we need biomarkers ?</u>

Prof. Blennow raised many issues with the current understanding of the physiopathology and treatment of AD, issues being faced in most brain disorders. First, clinical criteria for AD have poor diagnostic accuracy, with sensitivity and specificity around 70 %, meaning that 30 % of patients included in clinical trials have other diseases. So biomarkers are needed for diagnosis, to select true AD cases for inclusion in clinical trials and to make a correct diagnosis for initiation of treatment. Conversely, in the general population, should one want to use a drug like the aducanumab antibody, which reduces Abeta plaques in AD, there will be a need for cheap and first-in-line simple blood test screening which does not need to be disease specific, as long as it has a high negative predictive value (> 90-95%).

Furthermore, neuropathological examination of late-onset clinical AD (75 to 85 yo people who died with a clinical diagnosis of AD) showed that although 80% had a pathological diagnosis of AD with plaques and tangles, it was associated with multiple other pathologies in different combinations: TDP-43 pathology (65%), arteriolosclerosis (32%), microscopic infarcts (29%), Lewy bodies (25%), hippocampal sclerosis (11%). Therefore, Blennow explained that there will be a lower chance of identifying the effect of a drug aiming at reducing plaques and tangles when many other pathologies are intertwined. This is why biomarkers are needed to study disease pathogenesis directly in patients to understand the relative contribution from multiple pathologies, and the temporal evolution of the different pathologies. This would lead in clinical trials to a stratification of patients, according to the relative amount of different pathologies, following a "personalised medicine" principle.

From the first transgenic mouse model for AD (1990), with APP and presenilin mutations leading to a rapid increase of plaques in the brain, more than 200 molecules have been identified to reduce amyloid pathology or cognitive deficits in AD transgenic mice. Amongst them, there are 25 approved drugs, such as antibiotics, hypertension, diabetes, NSAIDs, estrogen, antidepressives, but also foods, drinks and supplements: garlic, blueberries, curry, green tea, coffee, red wine, omega-3, ginseng, Q10, vit3, Cu2+… Nevertheless, many of these have been tested in trials and failed. Therefore Blennow underlines the very poor translatability of these types of animal models to human sporadic AD, and stresses the need of biomarkers to verify target engagement in patients, and to evaluate its clinical effect on different types of scales (MMSE or ADAScog for instance). However, because of a minimal change in the preclinical stage as well as a very large variability in cognitive decline over time, very long trials would be needed to identify a clinical benefit. So theragnostic biomarkers are needed to identify downstream effects on neurodegeneration by anti-Abeta and tau drugs. Biomarkers could thus be used as primary endpoints (similarly to low cholesterol levels in cardiovascular prevention).

To summarise, biomarkers can be used for:

1. Diagnostic enrichment, as clinical diagnosis for AD, for instance, is difficult, especially in the MCI stage. Biomarkers are essential to select true AD cases for inclusion.
2. Target engagement, to validate in man the effects shown in cell cultures or mouse models. This pharmacodynamic data is essential to launch large clinical trials.
3. The assessment of downstream effects: the proof of disease-modifying effects by a drug.

Moreover, from a methodological perspective, biomarkers need to be validated using different cohorts from different research centres in different countries, using different research methods on different stages of disease to show that it really holds up.

<u>What is a good biomarker ?</u>

Prof. Blennow addressed the question of quality of a good biomarker both from a clinical chemistry perspective and a physiopathological one. To show the usefulness of biomarkers, he mentionned a study in which he collaborated, which is the first with a long follow-up (4 to 7 years) of 134 patients with mild cognitive impairment amongst whom 57 developed AD dementia (Hansson et al, Lancet Neurology 2006), where the combination of the three core biomarkers measured in the CSF

– total tau (associated to neuronal and axonal degeneration), phospho tau (phosphorylation state of tau associated to the development of tangles) and Abeta 42 (beta amyloid metabolism associated with the development of senile plaques) – led to identifying 95 % of patients with a 87% specificity. This combination of markers at the MCI stage was associated with a probability of developing AD 25.5 times higher than in the patients lacking it, thus leading to a biological definition of Alzheimer's disease: it is the combination of amyloid, tau and neurodegeneration that defines the disease, while the presence or not of symptoms is only linked to the stage.

To do such a study, from a chemistry perspective, it then becomes very important to have a valid and standardised biomarker. This requires a reference measurement procedure (RMP); mass spectrometry is the gold standard method, with absolute quantification and exact levels. And one also requires a certified reference material (CRM); in the case of CSF biomarkers, the gold standard is a CSF pool, all aliquoted and tested in many pools and with exact levels that have been set using the RMP. Prof. Blennow works with an international federation of clinical chemistry on this type of projects. For instance, for Abeta42, he established a CRM method that has been approved as a reference measurement procedure by the Joint Committe for Traceability and Laboratory Medicine. He is now working with the Joint Research centre in Gheel, Belgium on these CRMs which today are available. This is important to harmonise between assays and laboratories and produce exact table results throughout the world. Furthermore, there is a need for good analytical techniques. Blennow's laboratory is therefore using fully automated immunoassays that have very high performance (high lot-to-lot comparability: r=0.99; high precision: repeatability of coefficients of variation <2%). He also takes part in a quality control program to check the methods used for diagnostics throughout the world. Interestingly, between lab variability of measurements for ELISA is 15%, while it is only 3% for immunotests, meaning the immunotests' results can be trusted. Prof Blennow thus insists on the importance of insuring high quality results and introducing global cut-off levels, which are absolutely need in order to introduce biomarkers in clinics and for trials.

Also, it is important to understand how biomarkers relate to the brain pathophysiology. In AD, Abeta comes first and then Tau, then brain atrophy, then memory impairment then clinical function loss. Total-TAU and Phospho-Tau in the CSF are more related to the intensity of the disease, not the stage; they are high early and they won't change. On the other side, there is a number of biomarkers for AD that reflect the stages of disease: MRI atrophy, amyloid PET, Tau PET; the more abnormal they are, the more advanced the disease is). Blennow suggests that a good way to associate a biomarker to pathophysiology is, for instance, to take a biomarker known to be associated with brain pathology, and another which is a fluid biomarker, then look at their distribution, do a cut-off and then do a validation cohort to check. Low Abeta42 matches positive amyloidPET. (amyloid pet and csf abeta for instance:(Palmquist S et al JAMA neurology 2014).

A few examples of biomarkers' use in Alzheimer's disease

CSF Total Tau (T-Tau) seems to be a marker for the intensity of neurodegeneration in AD; there is indeed a moderate to marked increased in AD, while it is normal in depression and normal to minimally increased in Parkinson's. It is very increased in Creutzfeldt-Jakob's disease. There is no clear change in some disorders with tau pathology, e.g. FTD, PSP. Higher CSF T-Tau predicts future cognitive impairment in cognitively normal elderly (CDR= 0), time to conversion to dementia in MCI patients, cognitive decline in amyloid positive MCI patients, and cognitive decline and mortality in AD dementia patients.

CSF Phospho-Tau (P-Tau) is more poorly understood: it's not a marker for neurodegeneration but it seems to be a marker for tau pathology, although high levels are only found in AD, despite high levels of Total-Tau in other disorders. Also, CSF P-tau correlates poorly to static measurements of tau pathology (tangle pathology post-mortem or tau PET in AD dementia). This is why there is a need for clinical studies comparing static CSF P-tau with longitudinal tau PET scans to learn more, to see whether higher CSF P-tau predict the rate of future accumulation of tau pathology.

CSF neurogranin seems to be a possibility for an AD specific marker of synaptic degeneration. It is a synaptic protein, abundant in cortex, hippocampus and amygdala, the same regions that are affected in AD. It is concentrated in dendritic spines, and is important for memory consolidation and LTP induction. Different immunoassays and mass spectrometry showed a marked increase in CSF neurogranin in AD and prodromal AD, and high CSF neurogranin predicts future rate of cognitive decline, like T-Tau. Interestingly, it was not increased in other diseases (FTD, LWB, PD, PSP, MSA), only in AD and familial AD, hence its specificity.

A good example of *target engagement* studies is the one by Kennery et al (2016), who tested the BACE1 inhibitor verubecestat in different species, inclulding man, using different doses. After 14 days of treatment, with 40mg, 80% of reduction in CSF Abeta was found with this type of drug, which confirms the target engagement. However, trials look for a clinical effect have been much more disappoing: the EPOCH trial in mild to moderate AD was stopped for lack of efficacy; a phase 3 trial on prodromal cases was also stopped. So Dr Blennow explains that despite evidence of target engagement for amyloid drugs in AD, this may not directly translate to disease-modifying effect or clinical benefit.

Downstream effects of a drug need to be shown, to prove it is disease-modifying. For instance, the bapineuzumab trial (Salloway et al, NEJM 2014) showed a small reduction of CSF T-Tau and CSF P-Tau by anti soluble Abeta antibodies, which might indicate downstream effects on tau pathology, suggesting effects on neurodegeneration, but there is no hard data however. Other trials like the aducanumab trial (Sevigny et al) showed reduction in Abeta plaques in AD, while reduction in amyloid PET indicates target engagement. But Dr Blennow highlights the fact that biomarkers need to be found for neurodegeneration specifically, to support disease-modification.

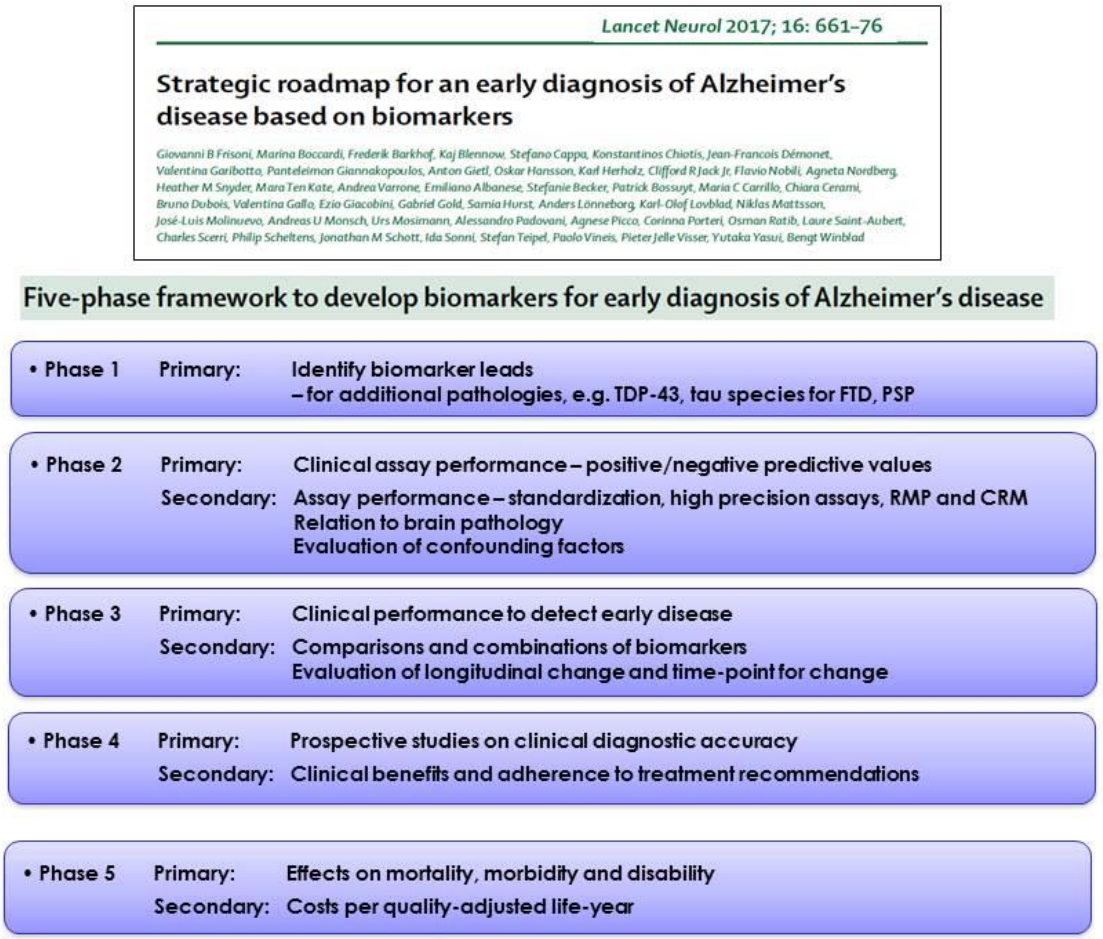
Finally, *blood biomarkers* are one of the most important objectives of biomarker research as they may be useful in first screening of patients to select for 2nd grade diagnostic evaluation (CSF, PET, MRI), and to monitor drug effects on amyloid/tau pathology and neurodegeneration in trials. They would require a diagnostic performance suitable for clinical use: a high enough percent change as compared with cognitively unimpaired elderly, a high negative predictive value (>90-95%), although it does not need to be disease specific (positive predictive value > 40-50 %). However, the challenges to develop protein blood biomarkers for brain pathophysiology include the fact that there are very low levels of brain-derived proteins in plasma, a high amount of plasma proteins (albumin) (50 g/L), with a risk for matrix effects, the noise made by the expression in peripheral tisses, reducing the performance, and the rapid peripheral metabolism, by proteases degrading the potential biomarkers. To this purpose, the speaker presented the Single Molecule Array (SIMOA) technique his team used to detect neurofilament in blood, a protein which has been shown to be highly correlated to CSF neurofilament, and to correlate with neurodegeneration in patients with AD (Mattsson et al, JAMA Neurology), although it is not disease specific, as its levels have been high in several neurodegenerative disorders. The SIMOA technique uses antibodies attached to the surface of paramagnetic beads that link to single molecules of the target protein, which is sufficient to make a detectable signal. This leads to a lower limit of quantification, of 0.3 pg/ml instead of 70 pg/ml as in the usual ELISA tests.

Conversely, plasma Tau does not reflect the levels of the protein in the CSF, as there is a large overlap in Tau levels in blood between AD, MCI and controls. However, SIMOA assays showed that other AD markers, such as Abeta42 and Abeta40 were significantly correlated between plasma and CSF, and between plasma and amyloid PET, although the correlations themselves were poor. Nevertheless, according to Prof. Blennow, plasma Abeta shows potential as a future screening test for brain amyloidosis.

Conclusion

Prof. Blennow suggested the following strategic roadmap for biomarkers development:

- Phase 1 studies: there is a need to identify more biomarker leads, as it has already been done in AD, but for other pathologies – for instance TDP-43 and Tau species for FTD and PSP.
- Phase 2 studies: first, one needs to do a clinical assay performance for evaluating the positive and negative predictive values; secondly, to improve the assay's performance by standardization, high precision assays, RMP and CRM, to understand its relation to brain pathology, and evaluate the confounding factors.
- Phase 3 studies: first, to test the biomarker's clinical performance to detect early disease; secondly to compare and combine biomarkers, and look for longitudinal change, to see whether they are stable over time, increase or decrease, and to find time-points associated to these changes.
- Phase 4 studies: first, to do prospective studies on clinical diagnostic accuracy; and secondly to assess the clinical benefits and adherence to treatment recommendations
- Phase 5 studies: first, to assess the effects on mortality, morbidity and disability, as well as the costs per quality-adjusted life-year.



Fluid biomarkers

Christoph W Turck

Prof. Christoph Turck works at the Max Planck Institute of Psychiatry in Munich, which is organised as a basic research institute associated to a clinic. His talk focused on fluid biomarkers. He began by highlighting the need for biosignatures, particularly in psychiatry where there is no underlying lesion to focus on. Such biomarkers would be essential for diagnosis, disease follow-up, pre-symptomatic detection and monitoring therapy response.

Furthermore, taking into account biomarkers information might improve GWAS dataset queries, by allowing to select a limited list of candidate genes associated to these biomarkers. Also, drug development in psychiatry would benefit from patient stratification by a biosignature. Finally, biomarkers would help monitor clinical response to treatment, improve the understanding of disease processes, and identify dysfunctional pathways. Prof. Turck also stressed the need to differentiate between dynamic and static biomarkers. An example for the latter are genomic biomarkers, characterising one individual against another, while the former are represented by proteomic, metabolomic or microbiota biomarkers, distinguishing between different states of the same individual.

There are more than 100 FDA approved protein-based assays in plasma or serum (immunoassays, enzyme assays, functional coagulation assays), but none are directly relevant for brain disorders diagnosis and treatment.

A strategy to identiy biomarker candidates

Prof. Turck discussed the two strategies for biomarker research: bottom-up, starting with animal models, or top-down, starting with patients. The advantage of animal models is that inbred species make for good homogenous phenotypes. In addition, they can be housed under a controlled environment, and brain tissues can be obtained. On the other hand, patient groups represent an outbred population of heterogenous phenotypes that are exposed to variable environments. Only peripheral body fluids can be obtained from cohorts of limited numbers. According to Prof. Turck, it is not possible to mimic complex phenotypes, like depression, in a mouse. However, animal models can mimic selected endophenotypes and can be used to test the efficacy and toxicity of pharmacological compounds. They can also be used to study similarities in cellular and molecular processes, such as mitochondrial pathways or energy metabolism, which are highly conserved between man and mouse. Starting from a heterogenous multidimensional phenotype like depression, behavioural biologists model certain aspects of these very complex phenotypes in the mouse. Then, the affected molecular pathways are disentangled by looking at protein and metabolite level alterations. Translating the identified candidate biosignature into applications of clinical day to day life is a major leap.

As an example for such a bottom-up strategy, Prof. Turck presented a project from his laboratory that deals with the unpredictibility of the antidepressant treatment response. The goal was to delineate molecular pathways relating to response to drug treatment in an inbred mouse strain treated with the SSRI paroxetine. With the help of the forced swim test, depressive-like behaviour in mice was assessed. Responder and non-responder mice had similar amounts of paroxetine in their brains after one month of treatment. Proteomics and targeted metabolomics analyses of hippocampi and blood, followed by computational analysis, resulted in two pathways that distinguished responders versus non-responders: the purine and pyrimidine metabolic pathways were identified where a number of proteins and metabolites had altered levels between the two groups. These pathways were not only found to be affected in the hippocampus, but were also reflected in the periphery, suggesting that biosignatures in the CNS can also be monitored in the periphery. Prof. Turck's team then went one step further by interrogating blood cells from patients that were part of the 'Munich Antidepressant Response Signature' study, where patients were rated at baseline and then after a month of treatment with an antidepressant. Levels of two enzymes from the purine and pyrimidine pathways identified in the mouse model were shown to correlate with the antidepressant response. Although this result needs to be validated in larger cohorts, it is a good example of a bottom-up strategy, from mice to men.

What are the knowledge gaps in science and medicine ?

The pathophysiology of psychiatric disorders is poorly understood due to multidimensional phenotypes leading to heterogenous diagnostic categories. These phenotypes are the product of interactions between multiple environmental and developmental events, stressors and traumas, and complex gene expressions including genetic mosaicism (not every cell in the brain has the same

genome sequence), differential spatial expression, and different functions at different stages of development. Consequently, there is a continuum of symptoms with a large overlap between classical phenotypes. Stratification with biomarkers would make for a more personalised medicine. For instance, biomarkers could help predict the response to antidepressant treatment. At the moment, psychiatrists need 2 to 4 weeks to realise whether or not treatment is efficient, resulting in prolonged suffering, suicide risk and high healthcare costs.

There are several difficulties impeding translation from preclinical models to clinical endpoints: some symptoms are uniquely human; there are differences in drug metabolism, in drug target affinities, and in genotypes that affect drug effects and placebo response. All these factors complicate clinical trials and are responsible for the so called "valley of death".

Moreover, we must take into account the two-fold challenge associated with peripheral biomarkers. Are peripheral biomarkers indeed a reflection of what is happening in the brain?. The other issue is that blood proteome analysis is complicated by the large dynamic range of proteins making the detection of low abundant proteins difficult.

The characterisation of the blood metabolome is challenging because of its diversity and heterogeneity. There are two ways of doing metabolomics, targeted and non-targeted in an unbiased fashion. However, a typical non-targeted metabolomic experiment usually results in thousands of "features", mass to charge ratios of metabolites, with only a handful that can be annotated (meaning "one knows what the identity of this particular feature is").

Prof. Turck then presented results of a ketamine treatment response case study using a blood cell biomarker. Ketamine is a fast acting antidepressant, which acts as a non-competitive antagonist of the NMDA receptors, leading downstream to the phosphorylation of mTOR. Patients treated with ketamine and showing a fast response on the MADRS and BDI scales gave blood samples at different time points. A simple Western Blot analysis for the phosphorylation of mTOR at serine residue Ser2448 showed a correlation with the MADRS and BDI ratings. This demonstrated that the effect of ketamine could be monitored in periphery.

Likewise, biomarkers in the CSF can be used for disease stratification. Prof. Turck enquired whether it was possible to distinguish schizophrenic, bipolar, and depressed patients, using CSF protein biomarkers. A nano-array platform and 24 different antibodies were used. It was shown that there was a disease group stratification using these 24 protein expression levels. Whereas schizophrenic patients could be well distinguished, the bipolar and depressed patients showed an overlap, which, according to Prof. Turck, is not too surprising because samples from bipolar disorder patients were collected while in a depressed stage. It appeared that the diagnostic continuum was also reflected by the biomarkers in the blood. This is why one needs more specific biomarkers for a better stratification.

Recommendations

First, Prof. Turck elaborated about new proteomic methods, with the goal to increase the number of identified peptides and to improve quantification to validate a biomarker candidate. Mass spectrometry methods using Data Independent Acquisition have been a major step forward in this regard, making it possible to detect low abundant proteins that may represent biomarkers, even in very complex matrices like blood. Target enrichment can be used when one already has a particular biomarker candidate. The protein is enriched with an antibody which makes quantitative mass spectrometry much more sensitive. Nakamura et al. (Nature, 2018) have used this method to assess Amyloid-beta peptide ratios in the blood with very good specificity and sensitivity. They were also able to show that their data was in good agreement with the PET and CSF data.

Prof. Turck also highlighted the fact that one needs to calibrate plasma protein concentrations against genetic and temporal factors. Blood protein level variations are not only caused by differences in expression levels based on genetics, but also depend on the environment, the diet, the lifestyle, and

the age. There are some proteins that are less affected by the environment and should be considered for an assay in a clinical laboratory.

Prof. Turck's recommendations in terms of topics for future research included the field of immuno-inflammation for psychiatric disorders, now being considered by the Wellcome Trust for neurodegenerative disorders, AD, but also psychiatric disorders. Furthermore microglia, which has been poorly studied so far, may be worth focusing on.

Microbiota biology with respect to inflammatory processes, also needs to be studied more. There is 1 kg of microbiota in our bodies, with close to 40 trillion microbial cells, adding to the 20000 genes from the human genome another 20 million genes that might have a significant impact on health and disease. This is why Prof. Turck suggests to investigate the gut-brain axis and mediating metabolites. He compared fecal pellet extracts of susceptible and resilient animals from the social stress mouse model. Mass spectrometry was used to compare the metabolite profiles and it appeared that some lipids are involved in stratifying resilience or susceptibility toward social stress in mice.

Conclusion

Prof. Turck concluded that longitudinal studies are needed, with periodic sampling, to follow-up patients and treatment over time. However, because they are very clinical in nature, these might be too costly for an ERANET project of 3 years.

Imaging biomarkers

Giovanni Frisoni

The use of imaging biomarkers in the clinic requires throughout Europe large numbers of specialized memory clinics, which can provide well phenotyped patients and consistency in the use of biomarkers. This is what Prof. Frison will illustrate, by taking for example the use of imaging biomarkers in Alzheimer's disease and other neurodegenerative disorders. He began by presenting the two major corner stones in the pathophysiology of Alzheimer's and other neurodegenerative disorders.

- First, the field is moving towards a molecular biology classification taxonomy of neurodegenerative disease; the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) Alzheimer's Criteria are now a syndrome and not a disease. The disease is defined histopathologically, by β-amyloid plaques, tau pathology, and it is also the case of Lewy body's and Parkinson's disorders (alpha-synuclein), fronto-temporal dementia and amyotrophic lateral sclerosis (tau, TDP43, FUS) and other non Alzheimer diseases.
- Secondly, these diseases develop over a long period of time and the symptomatic phase is just a fraction of the whole disease. This is why focusing on the clinical window (MCI and dementia) to understand these diseases is not appropriated to explore the pathophysiology, since the causal triggers might have taken place long before. But biomarkers, whether they are imaging or not, could track this sequence of events over its 15 years of evolution.

The use of imaging biomarkers in clinic

In the clinic, patients are classified in three groups according to their symptoms and complaints, and for each of them the clinician's challenge is different.

1) **Patients with dementia, who have a progressive acquired cognitive impairment with impact on self-sufficiency and autonomy.** Once secondary reversible causes of dementia are excluded, the challenge is to make a differential diagnosis among the different etiologies impacting on cognitive impairment (AD, DLB, FTP, nonamyloid pathologies, PSP, etc.).

For instance, Boccardi et al (JAMA Neurology, 2016) used amyloid-PET imaging to assess patients diagnosed with AD by specialists, based on the current work-up. It appeared that in one case out of three, patients did not have brain amyloidosis, highlighting that even in memory clinics in developed countries, AD diagnosis is failing. Prof. Frisoni finds that this is consistent with trial failures such as with solumezemab. The situation is even worse in the groups of patients diagnosed as non-Alzheimer dementia due to depression, Parkinson or fronto-temporal symptoms. Half of the patients had brain amyloidosis, suggesting there is likely a group of AD there. This is why imaging biomarkers are critical to do an accurate imaging diagnosis in the clinical practise. Moreover, such biomarkers are useful even when there is an accurate diagnosis, to assess prognosis. Indeed, in patients with a solid DAT-scan diagnosis of Lewy body dementia, a positive amyloid-PET seems to predict a decline of cognitive performance, whereas a negative amyloid-PET is associated to a stable cognitive performance, despite the other LBD features such as psychiatric disturbances and parkinsonism.

2) **Patients with mild cognitive impairment (MCI), without impact on self-sufficiency.** In this case the challenge is early diagnosis and prognosis – which comes with diagnosis. Indeed, at this stage, 2/5 cases of MCI are due to AD, but there are also all the other non-Alzheimer neurodegenerative conditions going through an MCI stage (vascular dementia), but most interestingly, normal aging can lead to MCI in a same proportion as Alzheimer. In this last situation, with no positive marker of neurodegenerative disease, patients will stay stable and the prognosis is good for the foreseeable future.

For instance, Prestia et al (Neurology 2012) did a multicentre study of more than 70 patients with MCI and he measured the combination of three different biomarkers: CSF Aβ42, FDG PET and MRI for hippocampal volume. It appeared that patients negative in all three almost never converted, while patients positive in all three invariably converted over the following 3 to 7 years, a result that has since been confirmed in a much larger group of patients by Vos et al (Brain 2015). Prof. Frisoni noted that although CSF was used at the time to measure amyloid proteins, now amyloid-PET could replace it and give the same results.

3) **Patients with subjective cognitive decline** are seen increasingly frequently in clinics. This group encompasses people with subjective cognitive decline, but also the "worried well" and people with psychiatric symptoms (anxiety, depression), making any diagnostic prediction very challenging. Furthermore, in the pre-Alzheimer patients, even if there is amyloidosis, or Tau, it is not known if or when the symptoms will appear, and biomarkers could help stratify this risk.

For instance, Vos et al (Lancet Neurology, 2013) stratified cognitively unimpaired patients with subjective complaints according to their CSF Aβ42 and Tau levels, as well as their scores in cognitive tests (those in the low end of normal suggesting neurodegeneration). It appeared that 50% of those who were positive for Aβ42 and tau had developed cognitive deterioration after 14 years of follow-up, while 80% of those who in addition scored low on the cognitive tests developed cognitive deterioration after 14 years. So biomarkers are good tools for risk stratification and profiling, however Prof. Frison noted that one should take into account that not all risks have the same weight. Indeed lifestyle risk factors are not significant in comparison with amyloid, amyloid+tau or even the genetic factors (APOE).

<u>Recommendations</u>

1) **Imaging biomarkers need further standardization and validation.**

Prof Frisoni took part in a work group that interrogated the scientific community to understand why there wasn't a stable, consistent and valid use of biomarkers, and it appeared it was due to the fact there were big discrepancies amongst EU countries in the reimbursement of these tests. This was due to the lack of a single strategy to validate biomarkers, like the frameworks used for drug validation. So the work group borrowed the development framework used by oncologists (more advanced in the

development of biomarkers) to compare it to what is presently available in the AD biomarker field. The framework encompasses: a phase 1, for preclinical, exploratory studies; a phase 2, for case-control; a phase 3, for early diagnosis with retrospective studies (using longitudinal data available in repositories); a phase 4, for prospective diagnostic accuracy studies; and a phase 5, for disease burden reduction studies and health outcome studies. It quickly appeared that even the most acknowledged imaging biomarker in the AD field, medial temporal atrophy on MRI, did not comply with all of these phases of validation. So a strategic roadmap was put in place to collect the evidence needed for various biomarkers at various phases, and Prof. Frisoni participated to the working group on harmonizing the imaging biomarkers for Alzheimer's disease in order to promote their use in the clinic and the research.

2) **There is a need to develop tracking markers that mirror the multi-dimensionality of the disease.** AD for instance can be seen in a multi-dimensional way, as the combination of many biomarkers over time: amyloid build up, Tau build up, synaptic loss, atrophy, cognitive impairment. And although studying and bringing one biomarker at a time to the best accuracy is important, there is a dire need to combine them in a wider approach, like a unique mathematic model of the disease which links all the different biomarkers together over time. This is what the European Progression of Neurological Disease initiative (EUROPOND) is doing. Villemagne et al. (Lancet Neurology, 2013) outlined the build up of amyloid over 40 years, the whole history of the disease, not by observing one group of patients, but by integrating piece-wise observations of different patients studied longitudinally in the different stages of the disease from no symptoms to mild to severe dementia. He obtained one sigmoid equation recapitulating the course of amyloid accumulation in the brain. Going further, Lorenzi et al (Neuroimage, 2017) computed the trajectory of a number of biomarkers based on piece-wise informations at different times in the disease. It only assumed that each biomarker had a monotonic sigmoid curve, only getting worse, and that the sigmoid curves were linked. It did not assume anything else, and was completely data-driven. From synthetic data, the model was then tested on the ADNI cohort data and was shown to hold. Therefore, Prof. Frisoni explained that once you have a mathematical model which can model whole brain amyloidosis, it is possible to imagine to model amyloidosis on a voxel by voxel basis, linking the evolution of brain amyloidosis in 3D with the evolution of hypometabolism in 3D, with atrophy in 3D, with cognition, with Aβ42 in the CSF and with neurogranin, etc.

3) **There is a need for imaging biomarkers of neuroinflammation.**
Activated microglia has always been found around plaques, but never has it really been studied whether inflammation follows the deposition of plaques or it is the other way around. Prof. Frisoni presented early results on the association between pro-inflammatory and anti-inflammatory bacteria in the gut and brain amyloidosis. He showed that pro-inflammatory bacteria (Escherichia Coli and Shigella) were present in greater abundance in amyloid positive, ie AD patients, compared to controls and to the amyloid negative ones. Vice versa, the anti-inflammatory bacteria were less represented in amyloid positive patients (cognitively impaired and AD). Furthermore, in the periphery, patients with AD had a greater concentration of pro inflammatory cytokines and a lower concentration of anti inflammatory cytokines, while there also is good association between pro-inflammatory cytokines in the gut and in the blood. To take it one step further, transgenic mice raised germ-free did not develop amyloid (or far less at least) and it was possible to restore the ability to produce amyloid by doing gavage with stools from transgenic mice raised conventionally. So there appears to be a deterministic relation between inflammation and amyloidosis, which needs to be further explored. However, in the majority of patients in clinics, when attempts are made to measure activated microglia, it leads to very low, heterogenous signals, that cannot be used. Prof. Frisoni suggests this might be due either to unknown features of our ligands, or to our poor categorization of AD. This is why it would be useful to couple an inflammation biomarker with all the new metagenomic techniques to study bacteria.

In conclusion, there is a need for further standardization and validation of imaging biomarkers, for developing tracking biomarkers that mirror the multi-dimensionality of the disease over time, and to research imaging biomarkers for neuroinflammation.

Digital biomarkers

Heleen Riper, Amsterdam,The Netherlands

Heleen Riper is a professor at the Vrije Universiteit Amsterdam, specializing in e-Mental Health. She was therefore speaking from an applied science perspective, not a fundamental one, but she believed the results could be of a lot of interest as the collaboration has been more and more increasing between applied and fundamental sciences. Prof. Riper developed in her presentation the concept of behavioural biomarkers, she described the setting of the European concerted research and she illustrated it by an example of depression treatment through e-CBT, as well as key preliminary results on internet interventions.

In terms of prevalence, one out of four patients will experience a major psychiatric disorder, and when looking specifically at depression from a European perspective, yearly prevalence in Europe is around 7%. On WHO projections, depression has been perceived as the most burdensome of all diseases both from a somatic and psychological nature in disability adjusted life years (DALY's 7%). The cost of treating depression but also indirect costs like economic losses are extremely high, around 90 billion in one year. And only one out of three patients who could benefit from it actually do receive the treatment. There is a huge delay between onset and treatment.

Another important aspect to take into account to explain the increasing importance of digital mental health is that when looking specifically at mental health resources (institutions and health professionals) available over Europe, and at the availability of cognitive behavioural therapy, they are very heterogeneous. For instance, in France, per 100 000 inhabitants, there are 160 GPs, and around 5 clinical psychologists; in the Netherlands, around 50 GPs, and 28 clinical psychologists for this same number of inhabitants. So there are big differences in how we treat depression, not only in the treatment itself, but also in who provides it.

However, despite differences in health policies across countries, all countries move towards community and public mental health approaches.

The example of e-CBT for the treatment of depression

There is ample room for improvement at many different levels: in terms of reach (to aim at more than just one in three depressed patients treated), of access, of effectiveness (improvement to be done in the biopsychosocial evidence-based perspective), of stigma, of prevention (with early on prevention, with CBT, in patients with sub-clinical complaints, the incidence of depression can be decreased with 25%), of treatment and care. Mobile phones and internet access being now available everywhere, Prof. Riper wondered whether these could be used to improve some of these aspects like increase effectiveness or access or reach, by making smart use of technology.

When looking at the E-Mental-Health Care domain, one can see a variety of interventions available - although not to a similar degree in all countries. There are interventions available throughout the whole mental health care continuum, from even before the disease, like improving the mental fitness, to early detection and prevention, treatment, relapse prevention, and chronic conditions, with support for daily functioning. And the type of interventions that are available range from completely unguided interventions (people go on the web, find an intervention and they can follow it without any guidance) to guided interventions: treatment can be provided from a distance for a patient, by email or phone. The more guided the treatment was, the more the costs increased. But many combinations are possible like blended therapies, associating new technology and face to face

therapies. Whereas internet treatments appear clearly to be cheap, and mobile applications on smartphones seem to be also available, although they never have been evaluated in terms of cost-effectiveness, other already known technologies become increasingly cheaper, such as virtual reality for anxiety disorders.

When studies compare the different types of internet based treatments for depression for example, it appears that unguided treatments are better than doing nothing (compared to a control group on a waiting list) and in terms of clinical effect, they have a small effect size. If some guidance is provided to it, then a small to moderate effect size is found. Compared to routine face to face care, evidence shows on a number of meta-analysis that the internet or smartphone based ones are equivalent. But the number of studies is still very much limited. Oddly enough, these studies did not show any cost-effectiveness.

However, not only is it important to know that patients profit from these types of treatment, it is also crucial that they do not deteriorate. For instance, one often hears that when people engage with this type of treatment, they may delay seeking the routine ones, hence deteriorating their health status. Prof. Riper did not find so far on this type of studies any proof of that. She worked in a collaborative project between Germany and the Netherlands, which showed that a guided internet intervention for depression led to 25% of patients with sub-clinical depression not to develop depression.

EU collaborative projects on e-Mental Health

Despite a lot of evidence in favour of the efficacy of such e-therapies, it has been mainly done on self-referred patients who react to an invitation to partake in clinical trial, and there are still many bottlenecks between the efficacy studies and interventions of such types with patients in real clinical practice settings. Blended CBT treatment in routine care still needs scoping studies, pilot RCT studies, assessment studies, protocol studies, routine care use. There are however certain EU collaborative projects leading the way, which Prof. Riper presented.

The projects started with “ICT 4 Depression” in 2009: clinical psychologists worked with computational scientists to develop technology platforms allowing to treat patients by making use of the internet, also combined with wearables – it was the first time in the field one started to measure physical signs such as heart variability and provided some support of monitoring progress on the smartphone. But at that time it was too early to use wearables to obtain data in an unobtrusive manner because the technology was not advanced enough; one needed to use gloves, which were found cumbersome by depressed people.

This led, however, to the “Mastermind” project, which looked if it was possible to implement this type of treatment in real practice, and to the “E-COMPARED” project (Kleiboer, Riper et al, 2016, Trials). The latter is a clinically effectiveness driven project consisting of nine multi-site centres but also of some countries that conduct the technological development (the Netherlands, the UK and Sweden are front-runners, while France and Poland are at the back). It also engaged with a number of patients, therapists and stakeholders.

The aim of this specifically randomized control trial was to see whether blender treatments integrating standardized CBT-treatment protocol with face-to-face and digital components, in routine care for unipolar depression, would be clinically non-inferior to face to face treatment and less cost-effective, as the number of face to face sessions was decreased as patients could be more self-active with the internet based system. The study included Sweden, Spain, Germany, Poland, United Kingdom, Switzerland, France, the Netherlands and Denmark. It was a study in routine care which meant that one could not control the setting where this type of intervention was provided so all patients had similar characteristics but sometimes the care was provided in specialist care settings and in other countries it was provided in primary care. The primary outcome was a MDD diagnostic free at 12 months follow-up and the PHQ9 score. Were included patients above 18 years old with a MDD diagnosis and a PHQ9 score of at least 5. Were excluded patients with a high suicidal risk, co-morbid severe psychiatric disorders, or psychological depression treatment.

The digital platform allowed the researchers to look at everything the patient was doing on the platform, their self-management capacities as well as what their therapist did. Patients also started to rate their mood and sleep patterns on a daily basis, when prompted, which is a progress because normally psychiatrists can only assess the mood retrospectively. It is to be noted, though, that such recordings were still obtrusive since people were prompted to answer through their smartphones.

Pr Riper first highlighted the good sample characteristics, more representative of the general population than in the usual efficacy trials: in this study there was a high co-morbid population (co-morbid anxiety in 52% of patients), 50 % of patients had already a mood medication at baseline, and the education level showed a high degree of variability than classic trials. The first results showed that blended-CBT (bCBT) was non-inferior to treatment as usual (TAU) at 12 months (on the PHQ-9 scale), but surprisingly, it did not appear to be less expensive than TAU. From a societal perspective, this might be due to the fact that patients were made more aware of their disease. However, from a healthcare provider's perspective, bCBT was more effective, with no differences in costs. Patients were as satisfied with bCBT as with TAU (CSQ-8). Three quarters of bCBT patients rated the usability of the digital platforms above average.

Overall, this was the first large scale bCBT trial in routine care. And EU and national stakeholders opted for increase in e-Mental Health. The lessons learned were that bCBT treatment and technical intervention sharing among EU countries was possible and accelerated uptake.

Recommendations

This is why Prof. Riper recommended:

- to personalize treatment protocols for the division of face-to-face and online sessions, tailored to the patient's needs
- to embed bCBT as treatment option in routine care, target lower educated and male populations
- to increase depression awareness among all stakeholders and at the workplace
- to evaluate the active therapeutic components of bCBT to increase cost-effectiveness
- to foster EU and national eMH policies and professional guideline development and curricula
- to increase involvement of patient in research and development

Overall, since 2014, the E-COMPARED project was a success and led to 14 PhD trajectories, and already 26 papers published.

Now, another domain left to be explored is unobtrusive ecological monitoring, by retrieving data without prompting (with the patients' permission), such as the GPS location, the amounts of steps one makes, the type of social media one uses, to see whether one could use these changes in those type of patterns to predict changes in mood patterns. Prof Riper's team followed many such variables, markers of social interactions (call duration, call frequency, sms frequency, social apps, social app duration, image frequency), of activity (distance (GPS), activity (accelerometer)), of smartphone usage (CPU, data traffic, screen on frequency, screen time, app frequency, app duration), on the principle that when depressed, people are less active and more isolated. Very individual patterns were found, leading the team to believe that with this type of proxy measures one could indeed spot people being at the phase of developing depression or monitor when they are in treatment to see if it is effective. Moreover, in the prevention field, changes in the shopping behaviour, such as buying more alcohol or sugar could be proxy markers at early stages to predict entry into psychiatric behaviours.

Prof Riper suggests ideally to combine proxy behavioural markers with biomarkers.

Conclusion

Unobtrusive measures can make small but significant contributions to predictive models of mood, especially when model selection procedures are free to develop personalized models for individual users.

Unobtrusive ecological momentary assessment provides a feasible new method to study dynamic relations between behaviour, the social environment and emotions, and may be the key element of adaptive treatment pathways.

The E-COMPARED project contributed to show that long-term EMA is feasible, clinically relevant and provides feedback; the log-files provided detailed accounts of usage and exposure to therapeutic interventions; blended CBT enables rich data collection on the individual level, all this taking the field one step closer to personalized modeling and real-time momentary interventions.

Genetic biomarkers

Alexandra Durr, Paris, France

Alexandra Durr is a professor at the Genetic department of the Pitié-Salpêtrière's hospital and also a researcher at Institut du Cerveau et de la Moelle, with experience in neurodegenerative and rare disorders. She began her talk by discussing the present burning issues in the field of genetic biomarkers in neurodegenerative disorders, before giving examples of how one could develop more solid genetic biomarkers, and how such research in the field of rare diseases can have an important bearing on frequent disorders.

The challenges of genetic biomarkers for neurodegenerative disorders

The main problem in a Mendelian neurodegenerative disorder like Huntington's disease, is that although the major genetic susceptibility is present from the start, the symptoms appear however only after a certain amount of time. Despite the presence of the incriminating genes and their toxic proteins, nothing happens until 40 – 60 years of age, when the disease becomes symptomatic and is diagnosed, but with a significant variability in time and presentation. This begs the question whether genes, which are always there and do not change over time, can indeed be markers of prognosis. Conversely, the pathological onset and the subclinical signs could be good markers to approach this kind of diseases.

Furthermore, it is very challenging to run therapeutic trials with patients with neurodegenerative disorders, as the evolution is very slow, and although changes can be seen on clinical scales, they are too small, have a small effect size and therefore would require at least one or two hundred patients in each arm to see an effect, which is impossible – in best case scenarios, one can nowadays recruit 50 patients in the setting of a multiple centre study. This is why biomarkers need to be very sensitive, have a longitudinal involvement to assess evolution over time, and help stratify and recruit patients whom we know we'll develop the disease early.

Finally, one also needs to re-think the measured outcome itself. It can not be survival, since it is too variable; for instance, in amyotrophic lateral sclerosis, where many genes have been found, different genetic entities have different survival probabilities, with a mean age of onset around 40 and death around 80, making any survival study unpractical. For instance, Tabrizi et al (Neurology 2012) showed that in Huntington's disease, where the use of biomarkers is most developed through initiatives likes TrackHD or Track On, if one expects a 40% improvement with a drug, one would still need at least 400 patients in each arm. This is a difficult problem one needs to overcome, as there are today many treatments, especially antisense therapies, which need to be compared to each other.

How to improve genetic biomarkers ?

In 2006, genomic markers were introduced by regulatory agencies and defined as "DNA or RNA indicators of normal biologic processes, pathogenic processes, and/or response to therapeutic or other intervention". Most significantly, it was added that genomic biomarkers specifically associated with the phenotype of interest will have more clinical utility than the ones associated with a range of phenotypes. It is therefore important, when trying to develop a biomarker, to select a specific phenotype of interest to look at. Prof. Durr discussed about the "age of onset" phenotype in diseases

linked to polyglutamine-coding (CAG)n repeat expansions, causing Huntington's disease and spinocerebellar ataxias. Indeed, it could be interesting to see whether when starting treatment in people known as carriers, it is possible to delay age at onset. She showed that age of onset not only depended on the length of the CAG repeats in the causative gene, but also on a single nucleotide polymorphism (SNP) affecting the DNA repair pathway, and thirdly on the length of CAG repeats in causative sister genes.

In CAG repeats' disorders, like Huntington and the ataxias, the mutation marker gives a real threshold for developping the disease: until 35 repeats, everything is fine, but after 36, the diseases will develop. These repeats are translated in proteins and lead to polyglutaminic expansions in these proteins that will accumulate, and can be measured. However when one looks at the age of onset of Huntington and other ataxias, it appears that age of onset is very variable: with a 40 CAG repeat, the disease can start at 45 or 80. There is a correlation between the length of the CAG repeats and the age of onset, since the longer they are, the sooner it starts. But there still is a significant probability for a patient with many repeats to develop the disease only very late in life. So even in a very homogeneous group of patients with a Mendelian disorder, defined by a genetic marker, there will be a very heterogeneous expression of the disease anyway; making genetic markers very difficult to handle by themselves. Prof. Durr illustrated this with the example of the SCA2 gene, leading to spinocerebellar ataxia. Even for 55 repeats, the disease's onset could go from as long as birth to 45 years old. So studies wouldn't be possible just with the CAG repeats genetic marker and the age of onset as the outcome. Moreover, the fact that there is in the brain a somatic mosaicism not reflected in the blood means that one doesn't even precisely know what is actually expressed in the organ of interest.

To answer this issue of unpredictability of age of onset, the GEM-HD consortium did a genome-wide association analysis on 4000 patients to identify loci of genetic variations that alter the age at neurological onset of Huntington's disease (Cell 2015). One SNP was found on chromosome 15 that could accelerate onset by 6.1 years or delay it by 1.4 years. It appeared that this SNP was in a gene coding for a DNA repair pathway, and that it had the same major modifying properties of age at onset in other SCAs (Bettencourt et al, Ann Neurol, 2016). Prof. Durr suggested this could be an interesting target in a trial looking for other strategies than treating the gene.

Furthermore, in a study to find other factors involved in the "age of onset" variability, Tezenas du Montcel et al. (Brain 2014) did a regression analysis in 1255 affected individuals with identified expansions to see whether age at onset is also influenced by the size of normal allele in eight causal (CAG)n-containing genes. And indeed it appeared that other loci in the genome, which are not causally linked to the disease, but have polyglutamine expansions, will influence the phenotype. However, the variability in age of onset is still not completely explained by the combination of these causal and "sister" genes, and other genetic or environmental factors are still to be found.

From rare to frequent disorders

The polymorphism of CAG repeats in the normal population is quite strictly controlled. There are 22/22 repeats on both chromosomes, and CAG repeats are interrupted by CAA, which both give glutamine. In patients with spinocerebellar ataxia, there are more than 34 repeats in the SCA2 gene that will be purely CAG repeats. However, Charles et al (Neurology, 2007) shown that alleles of SCA2 with large CAG repeats, interrupted by CAA, so still within the norm, are the most frequent genetic risk factor of autosomal dominant parkinsonism. Also, the same alleles, but without interruption, can lead to amyotrophic lateral sclerosis. So whether in the same gene there is an interruption or not, there are completely different phenotypes, some of rare diseases, other of frequent ones.

This is why Prof. Durr highlights the importance of overlapping regulatory elements between frequent and rare diseases. Whilst 2% of the genome is coding, 98% is made of introns, regulatory, interspersed, non coding RNA; amongst these miRNA are regulatory elements which are found both in frequent and rare diseases. For instance, miRNA-34 is found in normal ageing, in Alzheimer's disease, Huntington's disease, Parkinson's disease, autophagy, Amyotrophic Lateral Sclerosis and Prion disease

(Basak et al, 2016). These miRNAs will change over time. miRNA-34 is reversely correlated to the anti-ageing protein SIRT 1. It could be a good ageing indicator one should take into account when people are included in cohorts, since some people will age differently than others. Prof. Durr emphasizes however the fact there is a lack of proper longitudinal studies that follow the evolution of such transnosographic markers. This is all the more important as more and more studies are measuring miRNAs in the blood, finding some that are differently expressed in prodromal HD and claiming these are correlated with the disease, despite the fact there is a major overlap with normal patients. Should there be however a longitudinal difference, these markers could indeed be used.

Furthermore, Prof Durr described another possible frequent application stemming from rare diseases' research. Swedish registries showed there is less cancer in polyglutamine diseases carriers and patients as well as in Parkinson's disease or other neurodegenerative diseases. This has been found not to be linked to the mutation itself, as there was no correlation to the CAG number, but rather to probable cofactors upregulated in the system. It led however Murmann et al. (Embor reports, 2018), to develop a cancer therapy by CAG repeats, which was efficient in mice.

Using a combination of markers as a more efficient predictor of outcome

Prof Durr gave the example of the use of neurofilament (NfL), a completely non-specific marker of neural loss. There is indeed a clear association between baseline NfL concentration in plasma and progression to manifest Huntington's disease in HTT mutation carriers who were premanifest at baseline (Wild et al, Neurology, 2017). If the probability for a pre-manifest person to remain pre-manifest or to get the disease is clearly correlated to the amount of neurofibrilin, one could use neurofilament to stratify groups. Based on CAG repeats, age and neurofilament, one would recruit patients having the disease fast enough to run a trial with them and use the onset of disease as a read out. But this means including even less people, which will increase the risk of not seeing an effect in therapeutic trials if the effect size is small.

To improve this, it is possible to further associate, to neurofilament and genetic markers, MRI markers of caudate volume atrophy – since premanifest HD are losing caudate volume much faster than controls - or MRI markers of changes in white matter, which is changing longitudinally much faster than the caudate atrophy (the same is true for imaging studies in SCAs, where there is atrophy of the cerebellar volume or the brain stem). The sample size required with this combination of markers to see an effect size becomes much smaller (Hobbs et al, J Neurol Neurosurg Psychiatry, 2015). For instance, using the caudate atrophy imaging, which has an efficacy of 50%, one would need 100 subjects in one arm, which is a much more achievable number.

Conclusion

Prof Durr concluded that:
- one can use genetics to calculate the burden of disease onset but one needs two players, the mutation and the age;
- the presence of the mutation leads to more homogeneous groups, but only to some extent;
- genetics gives access to premanifest cohorts, which is an interesting and useful way to start treatment in people;
- the genetic modifiers could represent a target;
- non coding RNAs can be used as indicators of pathology;
- there is an important need to think about the connection between genetics in rare and frequent diseases

The key needs she sees in biomarker research are:
- longitudinal follow-ups of well defined patient cohorts with multimodal assessment for biomarker development and validation;

- research modulation of genetic modifiers;
- identification of the « good timing » to administer potential treatment;
- increasing precision of individual trajectories to run trials in small numbers

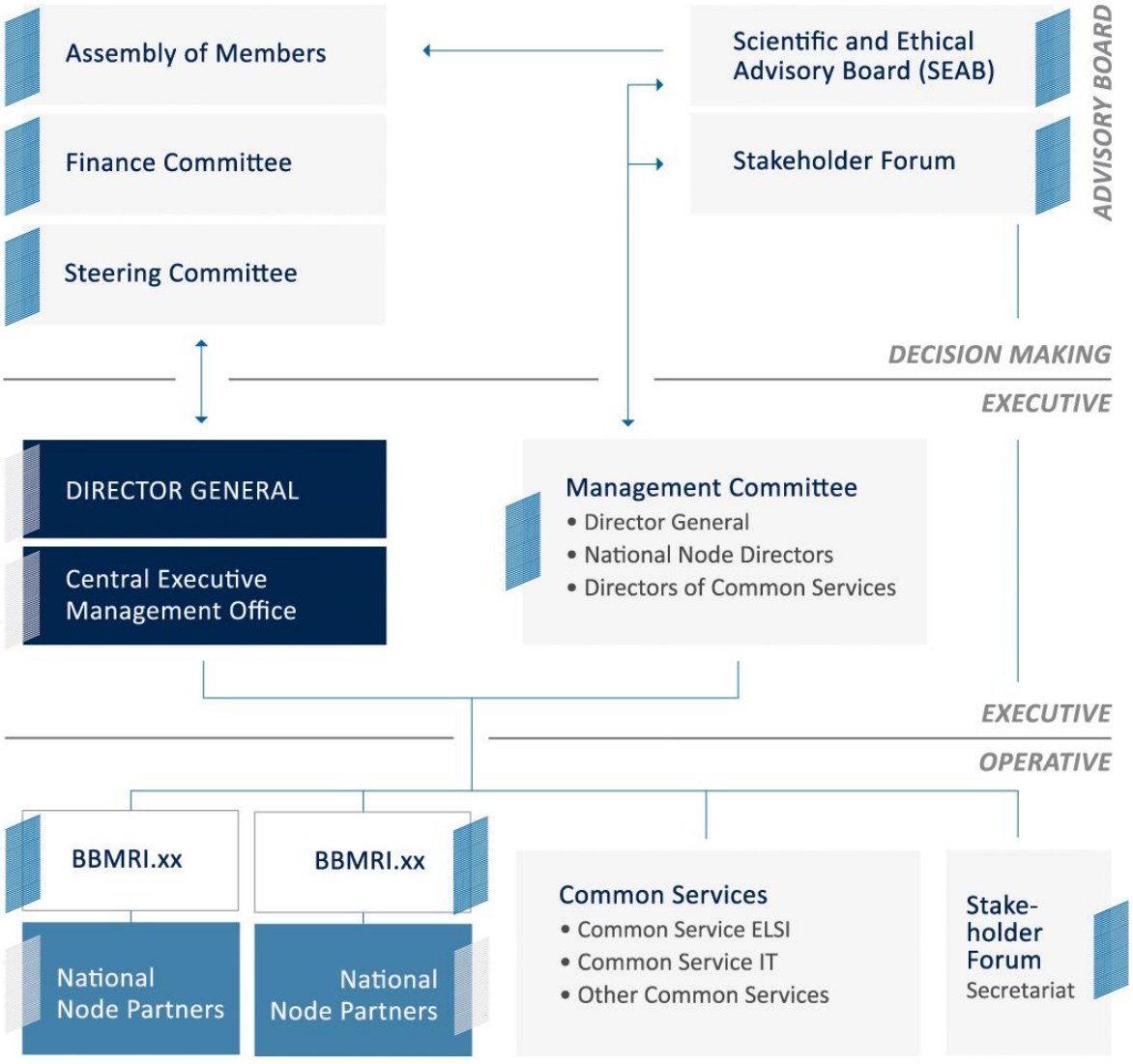
Indeed, if the natural history before the treatment starts and how it is affect afterwards is known, then one can do the correlation afterwards and before and less patients will be needed.

Biobanking

Andreas Wutte, Graz, Austria

BBMRI-ERIC is the Biobanking and BioMolecular resources Research Infrastructure – European Research Infrastructure Consortium. It provides expertise and services in order to facilitate the use of European sample collections and data for the benefit of human health.

Originally, BBMRI emerged from the first European Strategy Forum on Research Infrastructures in 2006, then went through a preparatory phase, with an application from 2008 to 2013 and the BBMRI-ERIC status was obtained in December 2013. **The governance structure can be seen Fig 1.**



The members of this consortium are: Austria, Belgium, Czech Republic, Estonia, Finland, France, Germany, Greece, Italy, Latvia, Malta, Netherlands, Norway, Poland, Sweden, and the United Kingdom. Observer countries are Switzerland, Cyprus, Turkey and the International Agency for Research on Cancer.

The BBMRI-ERIC Members can be seen Fig 2

The BBMRI-ERIC serves researchers, biobankers, patients, clinicians, politicians, partners and the industry. It offers: 1) support with ethical, legal and societal issues; 2) tools and expertise; and 3) quality management services.

1) Support with ethical, legal and societal issues. It is provided by the service of Ethical Legal and Social Issues (ELSI). All BBMRI-ERIC members and observers countries nominate participating experts. This service offers practical interpretation on new legislation, a custom-based helpdesk, and it also monitors relevant ethical and legal frameworks in development. Importantly, it develops ELSI guidelines for researchers (e.g. how-to-engage with patient organizations).

2) The IT service contributes to help main partners develop and operate services and helps as well smaller partners to focus on piloting and testing services. The core IT services are a directory, a locator and negotiator, a BIBBOX, and a helpdesk.

3) Quality management services Andrea Wutte firmly believes that if we want researchers to be able to produce reliable findings, one needs to make sure that they have access to samples and data of appropriate defined quality. As a European research infrastructure, BBMRI's ultimate goal is to make samples comparable across different countries and different biobanking systems.

The BBMRI-ERIC quality management services, for basic and applied research are comprised of:

The BBMRI-ERIC QM Service can be seen Fig 3

- Knowledge hub:

- following international standards (new ISO 20187 for general requirements for biobanking publication approx. Q4/2018, ISO 9001, ISO 15189, CEN Technical Specifications, etc.)
- quality management in EU funded projects
- quality management in national and international research projects
- webinars, counseling sessions

- Training and support for

- achieving international biobanking standards
- general quality management systems
- integrated management systems
- interface management systems
- inhouse training, workshops
- summerschools, master courses

- Auditing

- there is indeed a BBMRI-ERIC self-assessment survey for biobanks and researchers and also a BBMRI-ERIC audit programme developed and can be offered to all Members and Partners.

The BBMRI-ERIC quality management network is made of 109 participants all over the member and observer countries.

The partner charter encompasses that all partners should commit themselves to implement quality management procedures compliant with applicable European and International standards, following OECD best practice guidelines for global biological resource centres networks, WHO/IARC guidelines common minimum technical standards and protocols for biobanks dedicated to cancer research, with the establishment of standards of practice made publicly available for all processes related to sample collection, processing, storage, retrieval and dispatch.

The International standards recommended to be followed are:
New ISO/FDIS 20387 Biobanking – General requirements for Biobanking (soon published Q4/2018)
- ISO 9001:2015 Quality management systems – Requirements
- ISO 15189:2012 Medical laboratories – Requirements for quality and competence
- ISO 17025:2005 General requirements for the competence of testing and calibration laboratories
- ISO Guide 34:2009 General requirements for the competence of reference material producers
- ISO 17043:2010 Conformity assessment – General requirements for proficiency testing
- ISO 19011:2011 Guidelines for auditing management systems
All these, amongst many others as deemed applicable to research organisations and biobanks

Andrea Wutte highlighted the importance of insuring a proper quality assessment, in the setting of many published complaints regarding the lack of reproducible data (Lippi et al. Preanalytical quality improvement: from dream to reality. Clinc Chem Lab Med. 2011; Stephen A Bustin. The reproducibility of biomedical research: sleepers awake! Biomolecular Detection and Quantification 2014; Freedman LP et al. The Economics of Reproducibility in Preclinical Research. Plos Biol. 2015)
This is why BBMRI-ERIC recommend implementing CEN Technical Specifications (CEN/TS) – CEN standing for "Comité Européen de Normalisation" or "European Standardization Committee" in daily sample handling procedures for intended purposes.

These encompass:
- CEN/TS 16826-1, snap frozen tissue – Part 1: Isolated RNA
- CEN/TS 16826-2, snap frozen tissue – Part 2: Isolated proteins
- CEN/TS 16827-1, FFPE tissue – Part 1: Isolated RNA
- CEN/TS 16827-2, FFPE tissue – Part 2: Isolated proteins
- CEN/TS 16827-3, FFPE tissue – Part 3: Isolated DNA
- CEN/TS 16835-1, venous whole blood – Part 1: Isolated cellular RNA
- CEN/TS 16835-2, venous whole blood – Part 2: Isolated genomic DNA
- CEN/TS 16835-3, venous whole blood – Part 3: Isolated circ. cell-free DNA from plasma
- CEN/TS 16945 metabolomics in urine, serum and plasma

These recommendations cover methods outside the laboratory, such as primary specimen collection manual, sample donor, sample processing and transport; as well as methods inside the laboratory, such as sample reception, fixation, evaluation of the pathology, post fixation, processing of embedding, aliquoting, storage, isolation processes (RNA, DNA, Proteins..), using commercial kits, laboratories' own protocols, quantity and quality assessment, storage of isolated RNA, DNA, ccfDNA, proteins, etc; and finally they also cover quality control of RNA, DNA, proteins, and the impact of preanalytical workflow steps on specimen quality, time dependencies of analyte integrity

Furthermore, BBMRI-ERIC is partner of the Standardisation of generic Pre-analytical procedures for Invitro DIAgnostics for Personalized Medicine, SPIDIA4P, a European Union's Horizon 2020 research and innovation programme under grant agreement No 733112 with the aim to develop new standards for biomedical research:

- 4 CEN/TS for venous whole blood circulating Tumor and Organ Cells (DNA, RNA, Proteins, staining procedures)
- 1 CEN/TS for Venous Whole Blood Exosomes / cell-free circulating RNA
- 1 CEN/TS for Saliva (DNA)
- 1 CEN/TS for Frozen Tissues (DNA)
- 1 CEN/TS for Urine and other body fluids (cell-free DNA)
- 3 CEN/TS for Fine Needle Aspirates (RNA, DNA, Proteins)

- 1 CEN/TS for Saliva and Stool Microbiomes (DNA)
- 1 CEN/TS for FFPE Tissues (in-situ staining procedures)

In terms of visibility of Biobanks complying to highest quality levels, the strategy of the BBMRI-ERIC Quality Service is to encourage biobanks to comply with the highest quality requirements available, were put in place BBMRI-ERIC Quality Expert Working Groups currently involving 106 experts and researchers from 20 Member and Observer States and the WHO/IARC. They provide solutions to better meet sample quality requirements, and they developed Self-Assessment Surveys (BBMRI-ERIC SAS) based on the pre- examination processes published by CEN/Technical Specifications (CEN/TS by cen.eu). The BBMRI-ERIC SAS provides a complimentary tool to biobankers to 1) implement quality requirements and 2) assess their performance. If biobanks fulfill the criteria of the BBMRI-ERIC SAS, they will receive recognition by being flagged in the BBMRI-ERIC Directory. Hence, BBMRI-ERIC promotes those biobanks that are able and willing to give access to high quality samples/data. An added value and service for both biobanks and their customers.

Andrea Wutte gave two examples of BBMRI's quality management involvement in EU funded projects: in the first, the above mentioned SPIDIA4P, in the second, EurOPDX Distributed Infrastructure for Research on patient-derived cancer Xenografts, EDIReX, BBMRI-ERIC is providing support to implement standards to biobanks and reference centres, education and training programmes, and industry-academia stakeholder workshops.

Last but not least, BBMRI-ERIC is a Liaison Observer to International Standardization Organization (ISO). Andrea Wutte is Liaison officer to ISO/TC 276 Biotechnology, ISO/TC 212 Clinical laboratory testing and in vitro diagnostic test systems and CEN/TC 140 In vitro diagnostic medical devices. Within these ISO Technical Committees' relevant International standards relevant for biobanking and research based on human specimens are currently under development.

Futur needs for translational research Futur needs for translational research

Toni Andreu, EATRIS,

Dr Toni Andreu presented the European Advanced Translational Research Infrastructure in Medicine, where he is scientific director. He first set the background by showing how biomarkers are supporting therapeutic development in neurodegeneration, then discussed the current bottlenecks and ways to accelerate translational research, before presenting new perspectives.

How do biomarkers support the therapeutic development in neurodegeneration ?

When looking at the biomarkers being moved from phase 2 to phase 3 clinical trials in the field of Alzheimer's disease, for instance, it appears that there is a positive move, but that not all biomarkers pass the phases at the same speed (Cummings et al, 2017). CSF amyloid and Tau have shown a modest increase moving from phase 2 to phase 3, nothing particularly exciting or relevant; there was a modest decrease with FDG PET biomarkers. Studies using as clinical endpoint volumetric MRI showed a sharp increase between both phases. This is due to the fact that MRI measures are a cost-effective biomarker since they were already used as a safety read for trials about amyloid mechanism. Most interestingly, amyloid-PET has shown the sharpest increase among all biomarkers. From 8% of the total to 30% of the total, doubling the number from phase 2 to phase 3, it is indicating that it's becoming a very useful tool for identifying clinical endpoints. So despite positive advancements from phase 2 to 3, there's an imbalance depending on the type of biomarker. Plasma biomarkers, for

instance, are still in an early phase; and there's even a longer way to go in terms of the biology of the biomarker itself.

In addition to the research in the biology and quality of biomarkers, Dr Andreu highlighted however a very serious concern regarding the strategic element of biomarker development, by the pharmaceutical industry. Indeed, pharmaceutical industries have in the last few years shut down 50 % of the portfolio in their CNS programmes, which is not their top priority anymore. The reason for that is their need to get fast results, and research in CNS disorders appears risky from this point of view. This is leading the big Pharma to move towards research in inflammation in oncology; and even more dangerously, it is moving to phase 3 and marketing, absolutely forgetting phase 2. This is creating a gap, "a valley of death" for the field of clinical trials for drugs in the CNS.

Another element of complexity is the issue of quality and reproducibility of research. Freedman et al. analysed the money that has been spent in the US for preclinical development and what happened with that investment (PLOS Biology, 2015). From 56 billion dollars spent in the US in 2014, in biomedical research, 50% of the money led to irreproducible research. In most cases, it was due to an inappropriate use of biological reagents and reference materials, but also problems with the study design, which was often not appropriated for the development, inappropriate data analysis and data reporting, as well as inappropriate use of laboratory protocols.

This led in the last few years to a continuous decline in the production of novel drugs by the pharmaceutical industry, which needs every year more and more money to produce less and less drugs. According to Dr Andreu, there might be a near collapse in CNS drug development in the next few years. There is therefore a dire need to improve this situation.

Current bottlenecks and how to accelerate translational research

It appears that 65% of clinical trials fail at phase 2, which is the critical moment when molecules face for the first time the real landscape, the real patient with whom the molecule has to deal. If a clinical trial is able to pass this barrier of phase 2, the situation gets a little better. This failure has to do with the quality issue.

Dr Andreu underlined several bottlenecks in translational research, not only from a scientific point of view, but also from a managerial one: there is an issue with the reproducibility of bio-medical research (both in academia and industry); there is a "valley of death" between phase 2 and 3, that very few are incentivized to cross. Furthermore, the way one is incentivized to produce science is based by policy-makers on publications, making it difficult to identify real and fair indicators of development in bio-medical research. The validation of tools and models is a very long and expensive process, and public money is used for development, but rarely for validation. On the other side, the private sector is subjected to completely different external and internal elements of regulation, making a public-private interface for research very challenging. All this is leading to high costs, long time-lines, hence an unsustainable path.

This is why the research infrastructure programme was created: to make sure that there will be a tool at the European level that would be able to provide, to help, to support the academic community and the industry with these quality elements that will help science to evolve in a positive and productive way. The programme of which EATRIS, BBMRI and other infrastructures are part of started in 2002 as a decision of the European Commission who created the European strategic forum on research infrastructures and the idea was to identify research infrastructures that were able to create stable translational networks of research capacities with the idea of providing to the community facility resources, services to conduct research and foster innovation. And from the beginning, the commission and the member states insisted that the concept of infrastructure did not relate exclusively to scientific equipment, but also to knowledge-based translational resources (social research infrastructures for instance, collections, infrastructures, large amount of scientific data, etc). Regardless whether it is biobanking, translational processors, data management or clinical trials, the purpose is to work on key systemic issues like reproducibility, standardization, harmonization with the final objective of

improving the conditions for development and generation of knowledge and innovation across the European landscape (in the same way as BBMRI, from a different perspective).

This programme encompasses three medical research infrastructures ("medical" because they have the patients as the target of their activity): BBMRI-ERIC, EATRIS and ECRIN (the European clinical research infrastructure); for all of them, "Elixir", the life sciences data management research infrastructure is going to play a central role in the next few years, in terms of managing the big data policy.

In EATRIS' case, the composition is different due to different research infrastructures depending on the different member states and their internal strategic decisions. There are 12 participating countries, plus other non official members of the infrastructure who have academic teams that have a particular strong relationship with EATRIS. It supports 90 academic and non profit research institutions of excellence in translational medicine (universities, hospitals), amongst which 45 centres have a more direct presence in the field of translational medicine, and 14 have a specific expertise in the field of neurology and psychiatry. These institutions are organized in product platforms so when an academic group of these institutions belongs to EATRIS, according to its expertise and interest, it is assigned to one of these platforms: biomarkers platform, vaccines platform, tracer and imaging platform, advance therapy medicinal products' platform, small molecules platform. It is to be noted that 36 institutions throughout Europe are working together on the biomarker platform. Also, Dr Andreu stresses the fact that the denomination of these platforms has a lot to do with regulatory pathways of drug development, which is one of the focus of their activity.

What does the future look like?

The aim is to create a stable "co-creation" strategy to improve Europe's ability to bring therapies to patients. This is why multi-sector collaboration is essential: to jointly develop tools and models, to understand mechanisms, to validate biology, to increase regulatory transparency, to develop standards. And it not only involves the academic community, but also the policy-makers, the scientific associations and all the actors that have a particular voice in that process.

One example is a project that started not long ago, a COST action that is developed in collaboration with BBMRI but also with Elixir and other organizations for increasing the quality standard in biomarker validation, to develop standards for biomarker validation.

For the last couple of years, Dr Andreu and his colleagues have been analyzing the landscape and they believe now is the moment to make a strategic agenda for the development and implementation of personalized medicine with a lead in the field of biomarkers, for stratifying patients' subgroups within particular pathologies. This should be made by the international community, under the umbrella of the international consortium of personalized medicine, in which many European institutions, public founders, member states, and the European commission participate.

This is why they are in the process of building a project called "EATRIS plus" that wants to identify the key elements of the process of biomarker validation in the biomarker validation pipeline, specifically focused, from the beginning, on personalized medicine, and on a particular stratified group of patients showing a particular phenotype, rather than focusing on a disease. One of the elements of this project is the precise fine characterization of the biological phenotype cascade; to this purpose, they are recruiting a cohort of normal individuals, stratifying them by age, gender, trying to identify their omic profile, not only with the typical approach (DNA, met-DNA, RNA, Protein, metabolites) but through the whole biological cascade. In this context, they have started an ambitious project, the Human Omic Reference Atlas, developed in 6 centers of the networks that will perform the omic readouts at the DNA level, methylation DNA level, metabolome, proteome analysis, transcriptome and microRNA sequencing in normal individuals with the idea of developing a multiomic signature that should be a reference value for future validation of biomarkers in stratified individuals, but also, through a digital research environment to identify not only the multiomic signature but the cross-omic readout of that particular phenotype.

Conclusion

To accelerate translation, one should improve:
- reproducibility, by insuring high quality biomarker validation in the preclinical phase, towards a more predictive translation, with the idea of using that robust data in the identification of biomarkers for further development to identify new drugs;
- stratification, by maximizing the predictive use of biomarkers in well-stratified patient cohorts – ie to give the right drug, at the right patient, at the right time
- the use of molecular imaging to support drug development;
- collaboration in phase 2 of CNS research (experimental medicine), as a de-risking strategy, using existing consortia (IMI, EATRIS, ERANET NEURON, etc) to work on a gap that is getting bigger and bigger due to the fact that the industry is going towards marketing as their main priority, specifically in areas where the level of risk is minimum to ensure their investments.

It is very important to convince academia, public founders, and large consortia that one really needs to foster their capacity to develop promising phase 2 clinical trials.

Dr Andreu suggested five key criteria for selecting missions:
- bold, inspirational with wide societal relevance
- a clear direction: targeted, measurable and time-bound
- ambitious but realistic research and innovative actions
- cross-disciplinary, cross-sectoral and cross-actor innovation
- multiple, bottom-up solutions

Annex I

List of participants

Speakers at the scientific workshop "Biomarkers in neurology and psychiatry"

1. **Toni Andreu**, EATRIS,
2. **Kaj Blennow,** University Göteborg, Göteborg, Sweden
3. **Alexandra Durr,** ICM, Paris, France
4. **Giovanni Frisoni,** Brescia, Italy - University of Geneva/Geneva University Hospital, Switzerland
5. **Heleen Riper,** VU University Amsterdam, Amsterdam, The Netherlands
6. **Christoph W Turck,** Max Planck Institute of Psychiatry**,** Munich, Germany
7. **Andrea Wutte,** BBMRI-ERIC**,** Graz, Austria

Neuron SAB members and other Pannelists

1. Carlos Belmonte, Spain
2. Chapman Joab, Tel Aviv, Israel
3. Jean-Antoine Girault, Paris, France
4. Luc Mallet, Paris, France
5. Fabrizio Tagliavini, Milan, Italy
6. Ana-Maria Zagrean, Romania / UEFISCDI

7. Frauke Zipp, Mainz, Germany

Guests

1. Anton Iftimovici, Psychiatry Resident, Paris Descartes University, France
2. Silvia Villanueva, Scientific officer, European Commission

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