INDUSTRY

No biomarkers → No drug development

No targets

Negative outcome of Phase III studies:
loss of 800 – 1500 million Euro
Biomarkers

- Diagnostic biomarkers: Differential diagnosis
- Prognostic biomarkers: Prognosis/Chance for healing
- Predictive biomarkers: Response to therapy/disease probability

- Surrogate biomarkers: Intervention influences the endpoint of interest
- Traitmarkers: Invariable characteristics (gene-mutation)
- Statemarkers: Observation of disease progression (enzymes, ions,...)
Criteria for biological markers (1)

• Features of an ideal biomarker


– linked to fundamental features of the neuropathology
– validated in neuropathologically confirmed cases
– able to detect the disease early in its course and distinguish it from other dementias
– non-invasive, simple to use and inexpensive
– not influenced by symptomatic drug treatment

Gerlach, Riederer et al.
Criteria for biological markers (2)

- Criteria that must be evaluated before acceptance as a biomarker

(Shaw et al. 2007, Biomarkers of neurodegeneration for diagnosis and monitoring therapeutics. Nature Reviews 6: 295-303)

- **Sensitivity** (>85%; 100% indicates that all patients are identified with the disease)
- **Specificity** (>85%; 100% a test identifies all individuals free of the disease)
- Prior probability (the background prevalence of the disease in the population tested)
- **Positive predictive value** (>80%; refers to % of people who are positive for the biomarker and have definite the disease at autopsy)
- **Negative predictive value** (The % of people with a negative test, no disease at autopsy)
To meet endophenotype criteria, candidate markers have to be:

- Heritable
- Relatively state-independent and stable over time
- Associated with the illness
- To be found in affected as well as unaffected family members at a higher rate than in the general population
Relevance of BIOMARKERS

- PRESYMPTOMATIC DIAGNOSIS
- EVIDENCE FOR PROTECTIVE THERAPY
- DIFFERENTIATING „DEMENTIAS“
Biomarkers can identify a large population of individuals that may benefit from prevention strategies.
Biomarker key functions for AD clinical trials

1. assess drug safety
2. patient selection, enrichment & stratification
3. monitor effects of treatment (mechanisms & outcomes)

Biomarkers should support/replace clinical endpoints

1. Earlier/easier/quicker to measure
2. Reduce trial duration, size & cost
3. More mechanistic, accurate & reproducible
4. Change dynamic in proportion to what they represent

Hampel et al. (2010) Nature Reviews Drug Discovery
Aisen, Vellas, Hampel et al. (2013) Nature Reviews Drug Discovery
The four categories of biomarkers: target, mechanism, pathophysiological and diagnostic
RECOMMENDATIONS FOR BIOMARKERS DURING ALL STAGES OF THE DRUG DEVELOPMENT PROCESS

Blennow, Zetterberg, Hampel (2014) Neuropsychopharmacology
Sporadic Alzheimers Disease

- more than 15 million people worldwide
- multiple subtypes
- multiple phenotypes
- mutation-related stimulus is lacking
- causation is enigmatic
Dementias

- Alzheimer Dementia (AD)

- Neurodegeneration + / - vascular pathology
  - Amyloid
  - τau - Pathology
  - Synapse

- Multiple Pathologies in most of aged people
  - AD + SVD (small vessel disease)
  - AD + CAA or SVD
Dementias

- FTLD (Pick): CA1, CA 4 round inclusions; early (50 – 70 yrs.)
- PSP (Progressive Supranuclear Palsy): astrocytes; (60 – 100 yrs.)
- CBD (Cortico Basal Disease): 60 – 90 yrs.
- AGD (Argyrophilic Grain Disease): τau-pathol. in spines; (60 – 100 yrs.)
- FTLD-TDP: nuclear inclusions in nerve-cells subtypes
- LBD (Lewy Body Disease): Lewy neurites, LB

Braak, Thal 2011; Thal 2012
Montine 2012
Vascular Disorders

- multi infarct dementia
- strategic infarct dementia
- subcortical vascular encephalopathy
- small vessel disease (cerebral microangiopathy, lipohyalinosis, no calcification, no arteriosclerosis; basal ganglia!)

Grinberg, Thal 2010

- cerebral amyloid angiopathy → correlates with AD stages

Thal 2003
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Entry criteria (early diagnosis)*</th>
<th>Stratification (prognosis)*</th>
<th>Monitoring effect (individualization of treatment)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE genotype</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Aβ_{42}∗ Aβ_{40} (plasma)*</td>
<td>−</td>
<td>−</td>
<td>−/+</td>
</tr>
<tr>
<td>Aβ_{42}∗ Aβ_{40} (CSF)</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Tau (CSF)</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>P-tau (CSF)</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>BACE1 (CSF)</td>
<td>+</td>
<td>−/+</td>
<td>−/+</td>
</tr>
<tr>
<td>MR volumetrics</td>
<td>++</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>MR functional</td>
<td>−/+</td>
<td>−</td>
<td>−/+</td>
</tr>
<tr>
<td>MR spectroscopy</td>
<td>−</td>
<td>−</td>
<td>−/+</td>
</tr>
<tr>
<td>FDG-PET</td>
<td>++</td>
<td>−/+</td>
<td>−</td>
</tr>
<tr>
<td>Amyloid-PET</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

− not useful; −/+ useful in limited circumstances, for example plasma amyloid-β (Aβ) for passive immunization; + generally useful; ++ always useful, as discerned from recent meetings of the Alzheimer's Association Research Roundtable. APOE, apolipoprotein E; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; CSF, cerebrospinal fluid; FDG, 18F-2-fluoro-2-deoxy-D-glucose; MR, magnetic resonance; P-tau, phosphorylated tau; PET, positron emission tomography. *Text in parentheses indicates potential application in clinical practice.

*Measurements in plasma may be confounded due to other proteins present in plasma, and so may not accurately reflect the pathology.
Problems with GWAS - studies

- significance levels too high; loss of information
- subtyping spectrum disorder
- high N means many clinical subtypes included!
- interaction clinicians / basic researchers is missing

- regional gene expression different?
- social support
- life events

- epigenetics
- copy number variation
- Splicing
- de novo mutations
- gene interactions
1. Markers of Amyloidogenic Pathway
   - Aβ Peptides
   - Autoantibodies against Aβ
   - APP isoforms in platelets membranes
   - BACE 1 activity

2. Markers of Cholesterol Metabolism
   - Cholesterol
   - Oxysterols/24S-Hydroxycholesterol
   - Apolipoprotein E (Apo E)
   - Apo E Genotype

3. Markers of Oxidation
   - Antioxidants
   - Isoprostanes/8,12-iso-iPF2α-VI

4. Markers of Immunologic Mechanisms & Inflammation
   - α1-Antichymotrypsin
   - Interleukin-6 (IL-6)
   - Soluble IL-6 receptor complex (sIL-6RC)
   - TNF alpha receptor complex & TACE

5. Markers of Microvascular Changes
   - CT-proET-1
   - MR-proADM
   - MR-proANP
   - MR-proANP / CT-proET-1 Ratio

6. Integrity of the mTOR Pathway
   - GenoTor (gene-based assay)
   - PhenoTor (cell-based assay)
Conclusion

• there is no evidence to enrol one specific and selective biomarker

• combined compound / gene measures may be suitable as „biomarker“

• selectivities and specificities of combined biomarkers to be evaluated
Chronic Model and phenotypes for PD

Pre-Clinical Phase
- Smell / taste deficits

Clinical Phase
- Begin of PD Symptoms
- Depression
- Insomnia
- Tremor
- Rigidity
- Bradykinesia / Akinesia
- Postural instability
- Micrographia
- Short-term memory loss
- Apathy
- Apraxia
- Anxiety
- Incontinence
- Exhaustion
- Diagnosis
- Death
RESEARCH SYNOPSIS AND META-ANALYSES IN SPORADIC PD GENETICS

PD GENE DATA BANK

27,000 articles → 828 eligible articles
7 million polymorphisms were screened
meta-analyses on 147 SNPs from unpublished GWAS

Result: 11 loci with GWAS significant (p < 5x 10^{-8}) association

BST1, CCDc62, HIP1R, DGKQ/GAK, GBA, LRRK2, MAPT, MCCC1 / LAMP3, PARK 16, SNCA, STK 39, SYT11 / RAB 25, ITGA 8

Lill et al. 2012
### Combined Genes as „Biomarker“

#### VARIABLES IN THE PREDICTED PROBABILITY EQUATION

<table>
<thead>
<tr>
<th>Gene</th>
<th>B</th>
<th>P value</th>
<th>OR</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>L_ALDH1A1</td>
<td>-0.22</td>
<td>0.011</td>
<td>0.80</td>
<td>0.67</td>
<td>0.95</td>
</tr>
<tr>
<td>L_HSPA8</td>
<td>0.43</td>
<td>0.002</td>
<td>1.54</td>
<td>1.17</td>
<td>2.03</td>
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<tr>
<td>L_PSMC4</td>
<td>-0.30</td>
<td>0.009</td>
<td>0.74</td>
<td>0.58</td>
<td>0.93</td>
</tr>
<tr>
<td>L_SKP1</td>
<td>-0.26</td>
<td>0.026</td>
<td>0.77</td>
<td>0.61</td>
<td>0.24</td>
</tr>
<tr>
<td>L_UBE2K</td>
<td>0.24</td>
<td>0.030</td>
<td>1.27</td>
<td>1.02</td>
<td>1.59</td>
</tr>
<tr>
<td>L_EGLN1</td>
<td>-0.19</td>
<td>0.035</td>
<td>0.83</td>
<td>0.69</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Molochnikov et al. 2012
Combined Genes as "Biomarkers"

Molochnikov et al. 2012
<table>
<thead>
<tr>
<th>Application</th>
<th>Method</th>
<th>Biomarkers</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient enrichment</td>
<td>CSF biomarkers analyzed for diagnostic purposes before enrollment into a clinical trial</td>
<td>High T-tau, high P-tau and low Aβ42 are indicative of AD</td>
<td>Improved diagnostic accuracy in mild AD and enrichment of MCI trials with prodromal AD cases may improve the possibility to identify a clinical effect of the drug candidate</td>
</tr>
<tr>
<td>Patient stratification</td>
<td>CSF samples taken before trial initiation, and analyses performed after the end of the clinical trial</td>
<td>Post hoc analysis</td>
<td>AD cases with CSF biomarker evidence (low Aβ42) of a disturbance in Aβ metabolism might be more responsive to anti-Aβ drugs than patients without clear evidence of disturbed Aβ metabolism</td>
</tr>
<tr>
<td>Safety monitoring</td>
<td>CSF samples taken before trial initiation for comparison and new samples taken if an adverse event occurs</td>
<td>CSF cell count, CSF/serum albumin ratio, IgG/IgM index, and isoelectric focusing to identify IgG/IgM oligodendral bands to identify inflammatory processes and disturbances in the blood–brain barrier</td>
<td>Aβ immunotherapy might elicit adverse effects, such as meningoencephalitis or ARIA-E/vasogenic edema</td>
</tr>
<tr>
<td>Pharmacokinetics</td>
<td>Analysis of plasma and CSF samples after a single dose or multiple dosing</td>
<td>The therapeutic antibody</td>
<td>Antibody ratio between CSF and plasma will indicate whether the therapeutic antibody passes the blood–brain/CSF barrier to the same degree as endogenous IgG</td>
</tr>
<tr>
<td>Theragnostics</td>
<td>CSF biomarkers analyzed before study initiation and at time points during the trial including last week of the study</td>
<td>Aβ1–42 as the main biomarker for Aβ metabolism and deposition; other Aβ isoforms (e.g., AβX-42, Aβ1–40, Aβ1–16, Aβ5-X, and total Aβ) for complementary information on the Aβ metabolism, and APP isoforms (sAPPα and sAPPβ) and BACE1 activity for information on APP processing</td>
<td>Pharmacodynamical information on whether, and how, the drug candidate affects Aβ metabolism and deposition and APP processing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downstream biomarkers (e.g., T-tau, P-tau, HFABP, and VLP-1)</td>
<td>Biomarker information on whether the drug candidate has downstream effects on the intensity of neuronal degeneration and tau phosphorylation state/tangle formation</td>
</tr>
</tbody>
</table>

Blennow, Zetterberg, Hampel (2014) Neuropsychopharmacology