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PO.20.11: Application of toxicologic approaches to develop a test for diagnosis of Alzheimer's disease before it strikes.

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ABSTRACT Decades of animal-based research aiming at the elucidation of the pathophysiological mechanisms driving **Alzheimer's disease (AD)** have not yet translated into effective therapeutic treatments for AD patients. One possible reason behind this translational failure may be the disproportionately little attention that has been given to external risk factors believed to contribute to the fast majority of the known Alzheimer's cases.
The Interreg. project ''Herinneringen'' applies human-based toxicological approaches to identify genetic biomarkers representing pathological processes triggered by external factors that seem associated with Alzheimer's pathology. The selected genetic biomarkers are evaluated directly on human clinical samples, and age-matched patients and controls, to validate human pathophysiological relevance and to demonstrate accurate early diagnosis. The envisaged deliverable of the project is an inexpensive and minimally invasive test method diagnosing Alzheimer's during initiation and early development of the disease, and facilitating novel drug development.
The anticipated impact of the test method is expected to be beneficial for patients, caregivers, the community and companies who want to develop Alzheimer's diagnostics and therapies. These benefits include: (1) better quality of life for patients and their family, (2) faster certainty about treatment (as soon as available), (3) lower costs for health care, and (4) new possibilities for the development of active drugs.

1. MODIFIABLE RISK FACTORS FOR DEMENTIA have been identified, but there remains a substantial proportion of unexplained risk. There is evidence that several classes of environmental chemicals and life style may explain some of this risk.

1. Heavy metals

Heavy metal exposure	Human/ Animal/ In vitro Study	Toxicity Outcome
Aluminum	Human	Aggregation of *Aβ42* forming the amyloid plaques.
Arsenic	Human	Hyper phosphorylation of protein tau.
Cadmium	Human	Increased aggregation of tau protein.
Lead	Human	Affects cognitive function.
Mercury	In vitro	Affect the tau protein function.
Copper	*Human*	*Influence the Aβ 40, 42 homeostasis.*
Cobalt	In vitro	Oxidative stress reason to induce AD.
Iron	Human	Aggregation of *Aβ42* to form plaques.
Zinc	Human	Affects the Cu, Fe levels in AD.
Selenium	Rat	Damage the acetylcholine neurons in hippocampus region of brain.

2. Pesticides

Pesticides exposure	Human/ Animal/ In vitro Study	Toxicity Outcome
Organochlorine pesticides (OCPs) [lindane, HCH, Aldrin, Dieldrin, Endosulfan, DDT, DDE]	Children	Cognitive dysfunction.
Organophosphate insecticides (OPIs) [Methyl parathion, Dimethyl parathion, Trichlorfon, Chlorpyrifos]	Children	Cognitive dysfunction.
Carbamates (carbofuran)	Rat	Neurodegeneration in the hippocampus of brain
Fipronil and its metabolites	*In vitro*	*Increased the toxic Aβ42, 43 expression.*

3. Industrial Chemicals

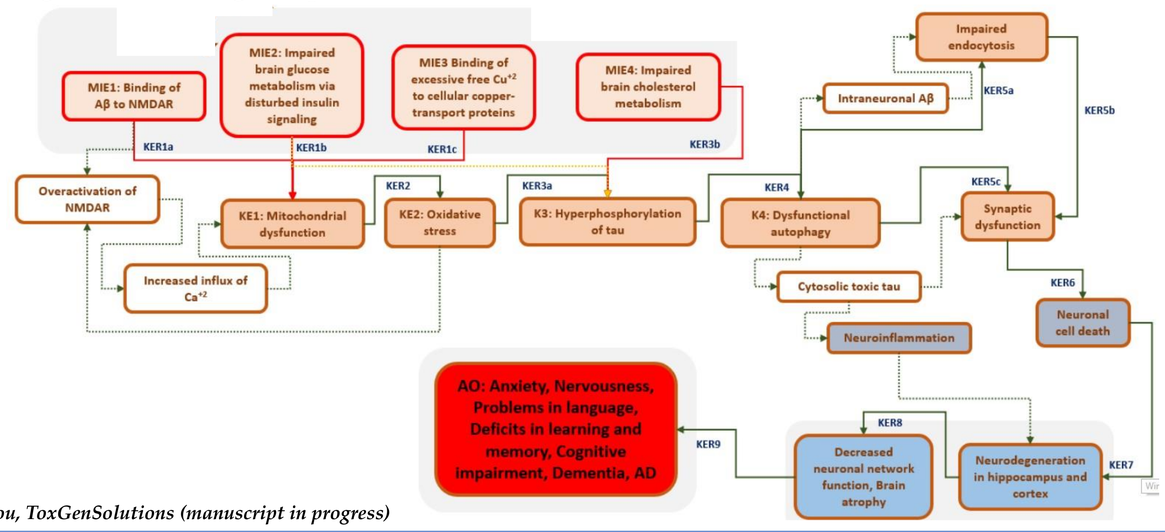
Industrial chemical exposure	Human/ Animal/ In vitro Study	Toxicity Outcome
Brominated flame retardants	Rat	Cognitive dysfunction.
Dioxins	Rat	Dementia.
Parabens	Rat	Neurodevelopmental disorder.
Bisphenol A (BPA)	Rat	Inhibit synapsis formation in hippocampus.
Phthalates	Human	Impaired neurodevelopment.

4. Air Pollutants

Air pollutants	Human/ Animal/ In vitro Study	Toxicity Outcome
Particulate matter (PM)	Mice	Amyloid-β40 and 42 levels double in mice brains.
Volatile Organic Compounds	Human	Memory impairment.

Venkatanaidu Karri, JJacco J Briedé et al., University of Maastricht; Killin et al (2016), BMCGeratrics 16; 175

2. BUILDING AN ADVERSE OUTCOME PATHWAY (AOP) FOR AD by collecting and structuring the knowledge acquired from *in vivo* (human and animal) and *in vitro* studies.



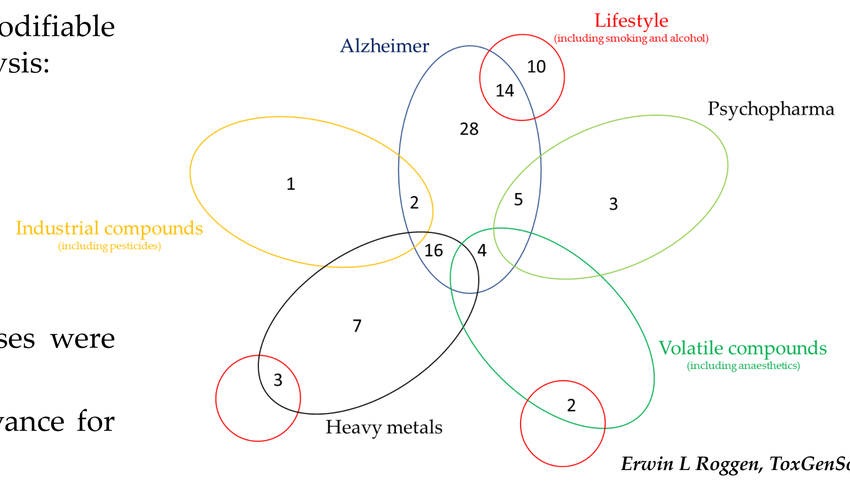
Maria Tsamou, ToxGenSolutions (manuscript in progress)

3. IDENTIFICATION OF MECHANISTIC OVERLAPS between Alzheimer's and neurotoxicity induced by modifiable risk factors. The pathophysiological processes of interest address the key events (KE) identified by the AOP analysis:

- Glucose and cholesterol metabolism;
- ROS production and Ca2+ homeostasis during oxidative stress;
- Neuroinflammation;
- Pre- and postsynaptic receptor signalling, and synaptic function;
- APP and Tau processing, and function;
- Cell death.

Based on the available mechanistic data (45 peer reviewed publications and 5 reviews) 95 relevant processes were identified, of which 41 were shared by KE's with relevance for both Alzheimer's and modifiable risk factors.

An additional 20-25 processes triggered by environmental factors affected selected KE's, but a potential relevance for Alzheimer's initiation and development needs to be substantiated.



Erwin L Roggen, ToxGenSolutions

4. SETTING UP THE *IN VITRO* TOXICOLOGY TOOLS FOR FILLING OUT THE IDENTIFIED GAPS using copper, fipronil and inflammatory cytokine mix.

Step 1: From human iPSC to cortical neurons

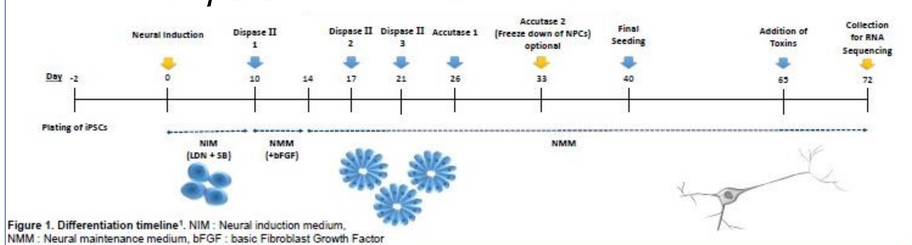


Figure 1. Differentiation timeline[1]. NIM : Neural induction medium, NMM : Neural maintenance medium, bFGF : basic Fibroblast Growth Factor

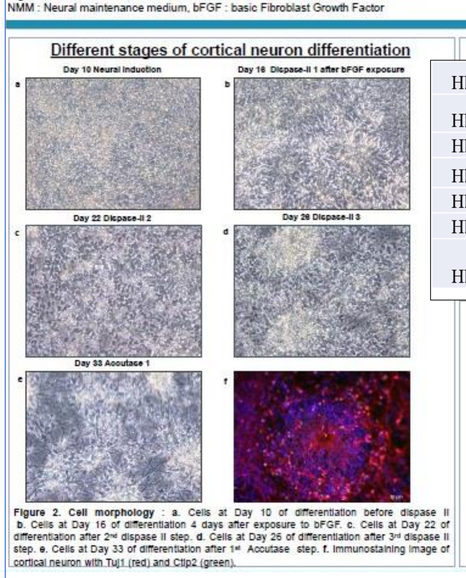
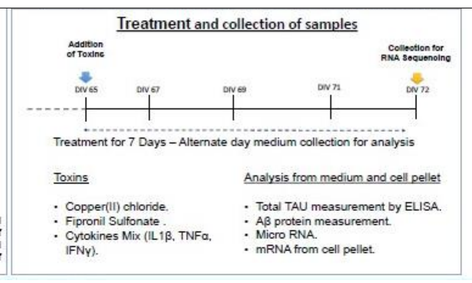


Figure 2. Cell morphology : a. Cells at Day 10 of differentiation before dispase II b. Cells at Day 16 of differentiation 4 days after exposure to bFGF. c. Cells at Day 22 of differentiation after 2nd dispase II step. d. Cells at Day 26 of differentiation after 3rd dispase II step. e. Cells at Day 33 of differentiation after 1st Accutase step. f. Immunostaining image of cortical neuron with Tuj1 (red) and Ctip2 (green).

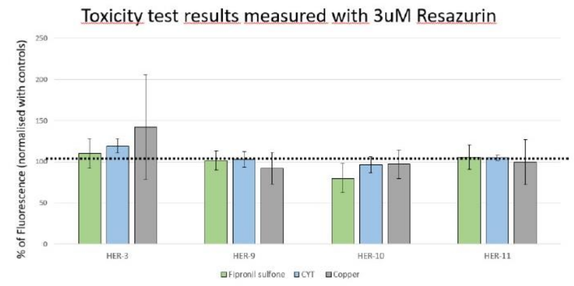
IPSCs lines used in the project

HER-02	male, 75-79 years old	Controls
HER-03	male, 86 years old	Controls
HER-06	male, 83 years old	sporadic AD
HER-08	male, 82 years old	sporadic AD
HER-09	male, 75 years old	APOE4/4, familial AD
HER-10	male, 24 years old (not really AD, but causes 4RTau)	familial AD
HER-11	male, 24 years old,iPSC used to create the HER-0010 TAU 3xmutant line	familial AD

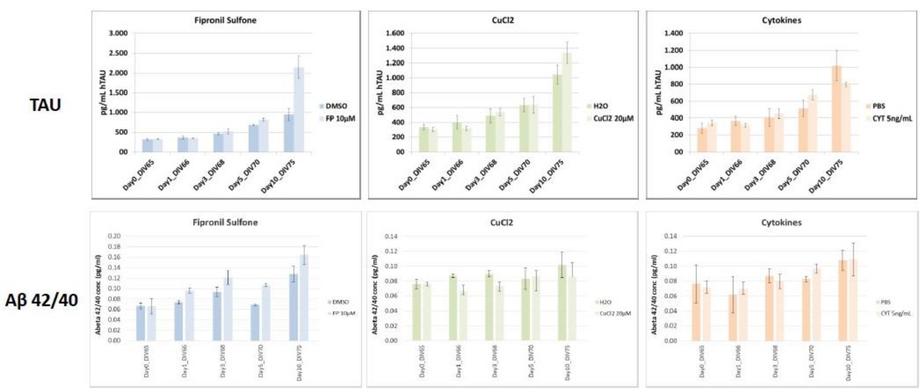


Kristy Vogels and Elisa Malki, Katholieke Universiteit Leuven

Step 2: Optimizing exposure regime: Finding dose and timing avoiding cytotoxicity



Step 3: Quality control: Checking responsiveness of differentiated cells to selected risk factors using widely accepted Alzheimer's specific protein markers



Katrien Princen, reMYND

STATUS AND PERSPECTIVES

From the existing literature it seems plausible that modifiable risk factors (e.g. drugs, chemicals, metals) are able to push an aging brain further towards degeneration. However, several gaps exist which the project ''Herinneringen'' intends to fill out. The differentiated iPSC cell lines were exposed to noncytotoxic concentrations of copper, fipronil and inflammatory cytokine mix, respectively, according to an optimised exposure protocol. Cells and cell medium are being analysed for differential expression of mRNA and microRNA, extracted as described in pre-established protocols. The obtained information is to fill out the gaps in the mechanistic understanding that emerged from the AOP analysis and the KE comparison between established Alzheimer's and emerging neurotoxicity pathways. The acquired (epi-)genetic information is evaluated against mRNA and microRNA data from age-matched human clinical samples (brain slices, CSF and blood) to demonstrate relevance. Finally, the capacity of a selection of peripheral microRNA's to identify humans prone to develop Alzheimer's (before clinical symptoms occur) is pre-validated using human cohorts of patients and age- and gender matched controls (N=150).