Mechanistic early phase clinical pharmacology studies with disease-modifying drugs for neurodegenerative disorders

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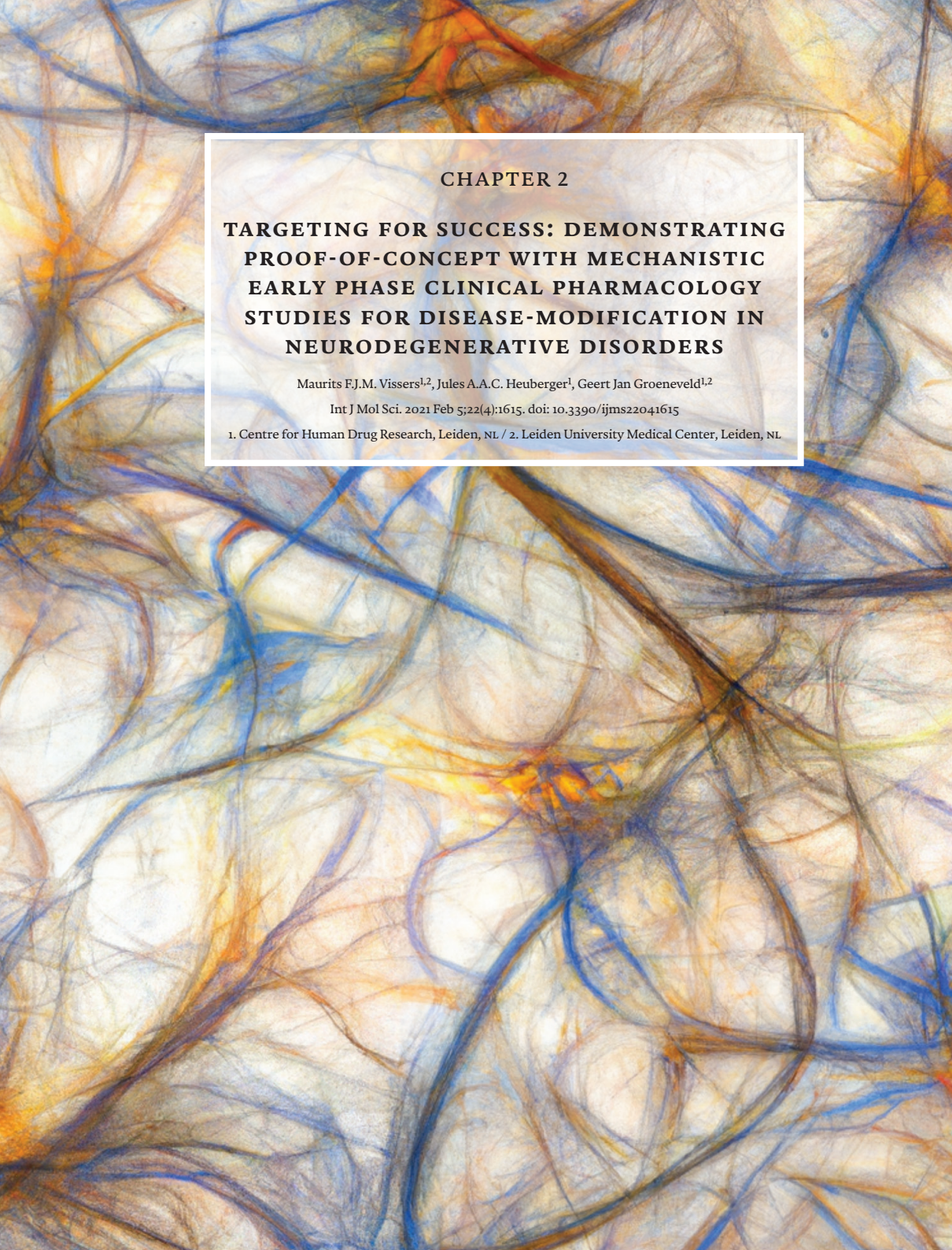

CHAPTER 2

TARGETING FOR SUCCESS: DEMONSTRATING PROOF-OF-CONCEPT WITH MECHANISTIC EARLY PHASE CLINICAL PHARMACOLOGY STUDIES FOR DISEASE-MODIFICATION IN NEURODEGENERATIVE DISORDERS

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ABSTRACT

The clinical failure rate for disease-modifying treatments (DMTs) that slow or stop disease progression has been nearly 100% for the major neurodegenerative disorders (NDDs), with many compounds failing in expensive and time-consuming phase 2 and 3 trials for lack of efficacy. Here, we critically review the use of pharmacological and mechanistic biomarkers in early phase clinical trials of DMTs in NDDs, and propose a roadmap for providing early proof-of-concept to increase R&D productivity in this field of high unmet medical need. A literature search was performed on published early phase clinical trials aimed at the evaluation of NDD DMT compounds using MESH terms in PUBMED. Publications were selected that reported an early phase clinical trial with NDD DMT compounds between 2010 and November 2020. Attention was given to the reported use of pharmacodynamic (mechanistic and physiological response) biomarkers. A total of 121 early phase clinical trials were identified, of which 89 trials (74%) incorporated one or multiple pharmacodynamic biomarkers. However, only 65 trials (54%) used mechanistic (target occupancy or activation) biomarkers to demonstrate target engagement in humans. The most important categories of early phase mechanistic and response biomarkers are discussed and a roadmap for incorporation of a robust biomarker strategy for early phase NDD DMT clinical trials is proposed. As our understanding of NDDs is improving, there is a rise in potentially disease-modifying treatments being brought to the clinic. Further increasing the rational use of mechanistic biomarkers in early phase trials for these (targeted) therapies can increase R&D productivity with a quick win/fast fail approach in an area that has seen a nearly 100% failure rate to date.

INTRODUCTION

While there have been successes in neuropharmacology, most central nervous system (CNS) pharmaceutical approaches treat symptoms rather than disease cause. Such symptomatic treatments can be very successful at suppressing disease symptoms at first, however, the effects eventually diminish over time and do not stop disease progression. Therefore, there is an urgent need for better treatments that can slow or stop disease progression of neurodegenerative disorders (NDDs), especially since the burden of these debilitating diseases on patients and society is on the rise as populations age.[1] Alarmingly, the clinical failure rate for such disease-modifying treatments (DMTs) for NDDs has been nearly 100% to date.[2–5] Exceptions include the approval of riluzole and edaravone as treatments for amyotrophic lateral sclerosis (ALS), however both arguably show only marginal effects.[6,7] With the recent approval of nusinersen for the treatment of spinal muscular atrophy (SMA)[8] new hope may be on the horizon.

In fact, our understanding of underlying NDD pathophysiological mechanisms is rapidly expanding,[9–13] and this has sparked a new interest in the development of (targeted) disease-modifying treatments. This is reflected for example, by the >100 compounds currently in clinical development for Alzheimer's disease[4] and close to 150 compounds in clinical development for Parkinson's disease,[14] many of which can be categorized as DMTs.

Compared to most other fields, the clinical development path of NDD DMTs faces some important additional challenges that contribute to the high failure rate experienced to date. First, preclinical and animal models have historically shown poor translatability to predict drug efficacy in human NDDs because of the complexity of the pathophysiology of neurodegenerative disorders and our incomplete understanding of these processes.[2,15,16] Secondly, in NDDs it may take a long time from disease onset to the manifestation of clinical symptoms to objectifiable disease progression and clinical trials have struggled to separate out symptomatic effects from disease-modifying effects.[2,16,17] Moreover, by the time of diagnosis significant (irreversible) damage to the CNS has often already occurred, and it has been challenging to identify robust diagnostic biomarkers to initiate treatment in earlier disease stages.[18] Thirdly, unlike diseases of most other organ systems, CNS disorders are localized to a body compartment that is not easily accessible for obtaining tissue samples in clinical studies to verify molecular pathophysiologic mechanisms and drug effects. And finally, there has been a lack of validated biomarkers as outcome measures for disease progression in disease-modification trials.[16]

However, considerable progress is being made in the development of biomarkers for NDDs,[19,20] that cannot only help diagnose or track progression of NDDs, but can also be used as tools during clinical development to demonstrate central exposure, (peripheral) target engagement and functional responses to guide dosing-decisions or facilitate patient enrichment in later stage clinical trials.[21] In particular, peripheral biomarkers for their relatively easy clinical accessibility hold a promise to help overcome some of the fundamental challenges in CNS drug development and allow for more efficient screening of drug candidates in early-phase clinical trials.[22] In a field where nearly 100% of investigational drugs fail to make it to market, the use of such biomarkers can offer an indirect yet relatively quick strategy

to confirm (peripheral) target and pathway-engagement and provide early proof-of-concept in short-duration mechanistic early-phase trials in both healthy volunteers and patients.[23,24] This quick win / fast fail approach can increase research and development (R&D) productivity and help guide dosing-decisions for maximizing success rates in later stage trials.[25]

Here we present a review and a roadmap for the use of pharmacodynamic biomarkers in early phase clinical trials of DMTs in NDDs. First, we present an introduction on NDD mechanisms, considerations for drug development of innovative disease-modifying compounds and the role of biomarkers in clinical drug development for context. Then we categorize the pharmacodynamic biomarkers that were reported in early phase clinical pharmacology studies identified from a literature review of the past decade, including an overview of bodily sources that can be used for biomarker analysis, and present considerations for biomarker selection in early clinical development. Finally, we summarize and conclude this overview with a proposal for a roadmap for designing mechanistic, data-rich early phase clinical pharmacology studies for disease-modifying therapies in neurodegenerative disorders.

NEURODEGENERATIVE DISEASE MECHANISMS

Neurodegenerative disorders, including Alzheimer's disease (AD), frontotemporal- (FTD) and Lewy body dementia (LBD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD), and spinocerebellar ataxias (SCAs), are characterized by a progressive degeneration of neurons in various regions of the brain and result in losses in cognitive and/or motor function.[26,27] As it appears, these NDDs share multiple overlapping pathological mechanisms including misfolding, aggregation, and accumulation of proteins, dysfunctional mitochondrial homeostasis, formation of stress granules, and maladaptive innate immune responses eventually leading to cellular dysfunction, loss of synaptic connections, and brain damage.[28,29] In AD amyloid-β protein fragments that cluster together and form amyloid plaques, as well as TAU proteins forming neurofibrillary tangles, disrupt neurological functioning and contribute to neurotoxicity leading to inflammation and neuronal cell death. In PD clumping of α-synuclein into so-called Lewy bodies in dopaminergic neurons is believed to play an important role in neuroinflammation and eventually neurodegeneration, while in ALS the aggregation of TAR DNA-binding protein 43 (TDP-43) in cell stress granules may contribute to disease pathology, neuroinflammation and motor neuron death. Because of an overlap in the underlying pathological mechanisms, as well as involvement of the same cell types, it is not surprising that many DMT mechanisms under development often target multiple NDDs. For example, inhibition of receptor-interacting serine/threonine-protein kinase 1 (RIPK1), a regulator of inflammation, cytokine release, and necroptotic cell death, is being investigated as treatment for AD, ALS and multiple sclerosis (MS),[30] while TAU protein is being targeted with antibodies for both progressive supranuclear palsy (PSP) and AD.[31] In addition to the more general mechanisms of neurodegeneration, genetic studies have begun identifying risk-associated alleles and disease-causing rare mutations in NDDs.[13,32] These genetic studies may pave the way for targeted therapies in selected subpopulations, such as an antisense oligonucleotide targeting the mutated superoxide dismutase (SOD1) enzyme in ALS,[33] or glucocerebrosidase (GBA)-activators or leucine-rich repeat kinase 2 (LRRK2)-inhibitors targeting disease-causing mutations in GBA or LRRK2 respectively in Parkinson's disease.[34]

INNOVATIVE DRUG DEVELOPMENT OF DISEASE-MODIFYING TREATMENTS

The development of innovative disease-modifying treatments for these NDDs with novel mechanisms of action is radically different from the development of a generic version of an existing effective drug from a well-established class.[25] For innovative compounds, the uncertainty about the different aspects of the drug is far greater, which is also reflected in the high clinical failure rate in the field of DMTs for NDDs. This uncertainty requires a high level of flexibility in the drug development program, the use of innovative methods and a high level of integration of information rather than the purely operational requirements of a generic development program.[25] Innovative drug development in essence starts with the preclinical development of assays to identify and validate a novel pharmacological target and subsequently demonstrating safety and efficacy in a (relatively standardized) battery of laboratory and animal studies. Hereafter the clinical development trajectory starts in humans and revolves around answering a set of 6 basic scientific questions in a series of what are traditionally called phase 1-3 clinical trials:

1 What is the safety and pharmacokinetic behavior of the drug?
2 Does the drug occupy the intended pharmacological target?
3 Is the drug capable of activating the target?
4 Does this target activation lead to the intended physiological response?
5 And subsequently to the intended pathophysiological response?
6 And does the drug result in a sufficient clinical response?[25]

Traditionally these questions are addressed in a chronological order, starting with small-scale phase 1 clinical studies focusing on safety and pharmacokinetics in healthy volunteers or patients and ending with large-scale, often global and multi-center, phase 3 studies to demonstrate safety and efficacy versus placebo or an active comparator in the intended drug label target population. However, as stated above, drug development does not need to take this linear approach. Especially if one considers that development becomes more and more expensive the further a compound progresses into later stage trails. In fact, for truly innovative compounds such as the development of DMTs in NDDs, there is a strong scientific and financial argument to be made to demonstrate proof-of-concept for a new compound in humans as early as possible.[35] From a scientific perspective, an early demonstration of proof-of-concept helps focus future efforts to the most promising leads. From a financial perspective early proof-of-concept contributes to a quick win / fast fail development approach thereby increasing R&D productivity and preventing investments in compounds only to fail in the most expensive later stages of drug development.

Demonstrating proof-of-concept of DMTs in early-stage trials is challenging, however. Considering the definition of a neurodegenerative DMT: 'an intervention that produces an enduring change in the clinical progression of the NDD by interfering in the underlying pathophysiological mechanisms of the disease process leading to cell death',[36] proof-of-concept for the first part of this definition is difficult to demonstrate because of the short-duration of early phase clinical trials. Moreover, traditional clinical outcomes – such as disease progression scales or patient-reported outcomes (PROs) – are not suitable for demonstrating effects of DMTs in NDDs in healthy subjects for a lack of disease, nor in patients because of the general short duration and small group sizes in phase 1 trials and large placebo-effects in PROs often seen in these patient populations. The ability of an investigational compound to 'interfere in the underlying pathophysiological mechanisms leading to cell death' on the other hand, is something that could be demonstrated with the use of pharmacodynamic biomarkers in short-duration early phase trials, even in healthy subjects.

BIOMARKERS

A biomarker (biological marker) is defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention'.[37] When the level of a biomarker changes in response to exposure to a medical product, it can be called a response or pharmacodynamic biomarker.[38] Other types of biomarkers can include diagnostic biomarkers (detecting or confirming the presence of a disease), predictive biomarkers (presence or change in the biomarker predicts an individual or group to experience a favorable or unfavorable effect from the exposure to a medical product), prognostic biomarkers (identify the likelihood of a clinical event, disease recurrence, or disease progression in untreated patients) and safety biomarkers (indicates the likelihood, presence, or extent of a toxicity as an adverse event)[38,39] – *Table 1*. In some cases a biomarker can be used as surrogate to substitute for a clinical endpoint, but to qualify as a surrogate, a biomarker must correlate with the clinical outcome and the change in the biomarker must also explain the change in the clinical outcome;[38] evidence that is currently lacking for the majority of biomarkers.

Recent reviews have described the current status of biomarkers in ALS,[40] Alzheimer's disease,[41] Parkinson's disease,[42] Huntington's disease,[43] and spinocerebellar ataxias,[44] although for most of these indications reliable indicators of disease severity, progression, and phenotype are still lacking.

EARLY PHASE PROOF-OF-CONCEPT WITH MECHANISTIC BIOMARKERS

Even without a proven correlation with clinical outcome, biomarkers are useful in early phase trials of DMTs for NDDs. At this stage of development, it is more important and feasible to demonstrate that the investigational drug engages its molecular pathway in humans as envisioned (mechanistic proof-of-concept). This can be accomplished with mechanistic biomarkers, by demonstrating pharmacologic activity of the compound both in healthy subjects as well as patients, allow for the application of mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) modelling,[46] and help define the optimal dose for phase 2/3 efficacy trials. This maximizes the eventual chance of clinical development success, or can save valuable resources by supporting an early 'no-go' decision in case the compound fails to reach or appropriately modulate its target.[21,47] In fact, disease specific regulatory guidance for drug development in NDDs also recommends the use of biomarkers in the early phases of the clinical development to:

1 Establish the pharmacological mechanism(s) on which the drug may be thought to have therapeutic activity.
2 Demonstrate target engagement and proof-of-concept.
3 Determine the PK/PD relationship and the dose-response curve.[48–50]

Additionally, by including a pharmacological effect or target engagement biomarker in a first-in-human (FIH) study, the dose-response curve in humans can be linked to the non-clinical experience, thereby supporting more informed dose escalation decisions. This is especially true for innovative drugs with a novel mode of action, where the relationship between the minimally pharmacologically active dose and a safe therapeutic dose in humans is not yet known.[51] Inclusion of a pharmacodynamic measure in FIH trials is now also recommended by the regulatory bodies for safety reasons.[52]

REPORTED USE AND CLASSIFICATION OF EARLY CLINICAL PHASE BIOMARKERS

As indicated above, biomarkers can play an important role in early phase drug development. To investigate the current use of pharmacodynamic response biomarkers for the development of DMTs for NDDs, a literature search was performed for published early phase clinical trials using medical subject headings (MESH) terms in PUBMED (*Supplement 1, available online via chapter reference*). Publications between 2010 and November 2020 were selected that reported an early phase clinical trial with NDD DMT compounds. Publications of early phase trials identified from references in the reviewed literature that were not identified by the MESH search strategy were also included. Only the first and original reports of early phase clinical trials were selected to avoid duplication (*Supplemental Figure S2*). An overview of all included trials and the reported peripheral and central pharmacodynamic biomarkers is presented in *Table 2*.

The early clinical phase pharmacodynamic response biomarkers retrieved from this search can be subdivided into proximal mechanistic biomarkers that are primarily used to demonstrate target occupancy and target activation (target engagement), and physiological and pathophysiological response (distal) biomarkers (*Table 1*).[25,46]

Overall, 89 out of 121 (74%) NDD DMT early phase trials that were published over the past decade reported the use of one or more pharmacodynamic response biomarkers (*Figure 1*). Given the significant added value of using pharmacodynamic response biomarkers in early phase trials this might not be surprising. Less than half of all trials (46%) reported the use of central pharmacodynamic biomarkers. The use of peripheral pharmacodynamic biomarkers was slightly higher at 50%. Only 65 trials (54%) reported the use of proximal mechanistic biomarkers (*Figure 1*) and there are clear differences in the use of biomarkers between different disorders and different types of drugs (*Table 2*).

Clinical outcome data was collected even more frequently in early clinical phase NDD trials (74% of all trials involving patients, or 60% of all trials) than mechanistic biomarker read-outs (54% of all trials) (*Figure 1*). This despite the fact that early phase trials are often of too short a duration and have a too limited sample size to expect a significant effect on any clinical or surrogate response biomarkers.

In the next sections we will break down the different types of identified biomarkers. For each stage of drug development, these different types of biomarkers can help answer different relevant clinical development questions, see also *Figure 2*.

Target occupancy

Only 26% of early clinical phase NDD DMT trials reported target occupancy biomarkers (*Figure 1* and *Table 2*). Target occupancy in first-in-human studies is used to demonstrate that the same target binding observed in the preclinical animal models holds true in humans.[171] The importance of this from a safety perspective is exemplified by the clinical study with the CD28 targeting immunomodulating agent, TGN1412. Because of differences in TGN1412 pharmacology between nonhuman primates and humans, the starting dose of the FIH trial directly resulted in 90% receptor occupancy, leading to life-threatening cytokine release syndrome in healthy volunteers.[172,173]

Demonstrating target engagement is also critical from the drug-development perspective. When a novel compound fails to demonstrate disease-modifying properties and no target engagement data is available, it will be difficult if not impossible to conclude whether the mechanism of action does not produce NDD disease-modification per se, or if this specific compound was just not successful in sufficiently engaging the intended target in humans.[174,175]

Ideally target occupancy is demonstrated by biomarker evidence of:

1 The compound reaching its site of action;
2 The compound binding to the intended molecular target;
3 Occupancy of the target increases with increasing dose.

Demonstrating that a compound reaches its site of action is one of the major challenges in CNS drug development, and in fact often not even possible to demonstrate directly (except post-mortem). As an alternative, often the presence of the compound at pharmacologically active concentrations in the cerebrospinal fluid (CSF) is used as a surrogate for CNS exposure.[2,23,30,54] While this is not an absolute guarantee that the compound reaches its site of action in the brain, it does provide a relatively uncomplicated method (it

can even safely be used in pediatrics)[176] to demonstrate that the compound does cross the blood-brain barrier in sufficient concentrations to expect an effect based on preclinical cellular dose-response assays. In addition, further translational approaches can be used to predict human brain distribution and target site kinetics.[177]

Besides measuring compound concentration in CSF, positron emission tomography (PET) can be used to demonstrate compound distribution into specific brain compartments and can in some cases also be used as a direct occupancy assay for receptor, transporter or enzyme targets.[178,179] However, PET imaging cannot always be applied for the lack of an appropriate radioligand or unfavorable radioligand characteristics, e.g. high non-specific binding.[159] Actual binding of the compound to the molecular target could in some cases be demonstrated in the CSF, for example for monoclonal antibodies binding to a circulating extracellular target protein such as amyloid β[54–56,60] or α-synuclein[146] (*Table 2*). However, this may not always be possible because assays are either not sensitive enough to detect the low abundance pathological target (e.g. aggregated αSYN concentrations in CSF) or drug concentrations in the CSF are not sufficient to demonstrate an effect on a more abundant surrogate biomarker (e.g. total αSYN in CSF).[145]

For (intra)cellular targets in CNS tissue it may be even more difficult to demonstrate that the compound binds the intended molecular target, mainly because of the fact that these cellular molecules are likely not present in biofluids in detectable amounts and the target neuronal cells cannot be sampled from living human beings for cell lysis and subsequent target engagement assays. In these cases, an alternative indirect strategy could be to demonstrate target engagement in peripheral cells, on the condition that the molecular target is expressed in these cells. For example, peripheral receptor occupancy on cell surfaces can be measured with the use of flow cytometry on fresh blood.[180] In a similar fashion, intracellular target occupancy can be demonstrated peripherally in blood cells such as done for LRRK2-inhibitor binding measured via the dephosphorylation of SER935 on the LRRK2 protein in lymphoblastoid cells,[181] or the reduction of phosphorylated S166 RIPK1 in peripheral blood mononuclear cells (PBMCs) after dosing of an RIPK1-inhibitor.[30] When combined with the plasma-to-CSF drug concentration ratio, such peripheral target occupancy can give an indirect indication of expected target occupancy in the CNS.

Target activation

After confirming that a novel compound occupies its molecular target, the next step in innovative clinical development is to demonstrate that upon target occupation the investigational compound activates the intended molecular pathway to a sufficient extent for possible disease modification (*Figure 2*). Such mechanistic proof-of-concept can often be demonstrated by evaluating a substrate biomarker that is downstream in the pathway of the compound's direct molecular target. When quantitatively measured, changes in such a so-called 'pathway activation biomarker' at different dose-levels can help generate a dose-response curve of the investigational compound's agonistic (stimulatory or inhibitory) molecular effects. This dose-response curve can be linked to the preclinical *in vitro* and animal model studies to determine a human dose level at which maximum disease modification can be expected in patients. Target activation biomarkers have been used more frequently than target occupation biomarkers, but still only 40% of early clinical phase NDD DMT trials reports the use of target activation biomarkers (*Figure 1*).

An example of a molecular pathway activation biomarker is the quantification of amyloid β1-42 (Aβ) concentrations in the CSF in response to BACE1-inhibitors (*Table 2*).[84–90] BACE1 (β secretase) is a protease that cleaves the amyloid precursor protein at the β-site, which eventually leads to the production and release of Aβ peptide in the brain. A decrease in Aβ brain concentrations may help prevent the progression of Alzheimer's disease.[182] However, as indicated before, such an apparently obvious relationship between the molecular pathway activation biomarker to the neurodegenerative disease that the compound is being develop is not a necessity. It is more important that the biomarker has a direct relationship to the true molecular target that the investigational compound activates or inhibits, and that the biomarker can reliably be measured with a robust and validated assay. An example is the quantification of phosphorylation of RAB10 (pRAB10), a bonafide substrate of LRRK2 kinase activity, in response to the administration of LRRK2-inhibitors under development for Parkinson's disease.[183] The fact that at the time of discovery it was not entirely clear how the activity of RAB GTPases contributes to degeneration of the nervous system[184] does not impact the usability of pRAB10 as target activation biomarker to quantify the inhibitory effects of LRRK2-inhibitors.

Similar to target occupancy, it may not always be possible to demonstrate target activation in the CNS, especially for intracellular molecular pathways, in which case an alternative strategy can also be to demonstrate target activation peripherally in blood or tissues expressing the same molecular target (*Figure 2*).[120,126,127,130]

Demonstrating target activation can be complicated by the fact that the targeted molecular pathway activation status may only be present in diseased tissue. For example, RIPK1 regulates inflammation, cytokine release, and necroptotic cell death and inhibition of RIPK1 activity protects against inflammation and cell death in multiple animal models. RIPK1 is also expressed in circulating PBMCs offering a peripheral opportunity to demonstrated target activation of RIPK1-inhibitors. However, in these non-diseased PBMCs RIPK1 activity levels will not be similar to that in the CNS of ALS and AD patients. To overcome this problem and quantify the effects of different dose levels of a RIPK1-inhibitor peripherally, PBMCs can be collected from study subjects after dosing and then be stimulated *in vitro* with e.g. the pan-caspase inhibitor ZVAD-FMK (TSZ) to stimulate these cells to increase phosphorylated RIPK1.[30] In a similar fashion, lipopolysaccharide (LPS) has been used in an early phase study in MS patients to stimulate 6-SULPHO LACNAC+dendritic cells *in vitro*, to demonstrate that laquinimod therapy is capable of reducing CD83 expression and TNF-α production.[138] The possibility to demonstrate target activation *in vitro* in human cells is supported by regulatory guidance,[50] and could be used to demonstrate target activation in first-in-human studies with healthy volunteers.[30] Some molecular targets are really only present in patients with the target disease, such as mutated huntingtin protein in patients with Huntington's disease. In such a case the best strategy may therefore be to directly include patients in the earliest clinical trials, to be able to demonstrate target activation as early as possible in the clinical development trajectory.[131]

Other types of target activation biomarkers may be used for different classes of investigational drugs (*Table 2*). For example, in the case of immunotherapy target activation could be demonstrated by the formation of antibody titers in plasma,[156] and in the case of an antisense oligonucleotide target activation may be demonstrated by a reduction in target protein levels.[33,167] For other types of drugs such as monoclonal antibodies against amyloid β[53,54,63,55–62] it may not be possible to demonstrate target activation, as the goal of these treatments is to clear the molecular target either by macrophage phagocytosis and complement activation or by altering the equilibrium of amyloid across the blood–brain barrier in favor of efflux from the brain to the blood.[185]

Physiological response

Physiological response biomarkers are reported in 23% of early phase NDD DMT clinical trials (*Figure 1*). These provide insight into more general or systemic (distal) responses to the investigational compound that are expected to contribute to, or be indicative of, possible disease modification. Examples of physiological response markers that have been used in early phase NDD DMT clinical trials include the evaluation of brain glucose metabolism after administration of nerve growth factor gene therapy[68] or deep brain stimulation[76,78] for Alzheimer's disease, and CSF cytokine production after transfusion of stem cells[101] or administration of granulocyte colony-stimulating factor (G-CSF)[115] in ALS patients (*Table 2*). However, it is important to realize that while such biomarkers can indicate that a compound exerts a physiological response, they often do not provide direct information about the actual clinical effects of the compound,[25] nor that the intervention can produce an enduring change in the clinical progression of the NDD. Nevertheless, when combined with target occupancy and activation biomarkers, physiological response biomarkers can contribute to the total amount of evidence for proof-of-concept (*Figure 2*). Additionally, physiological response markers can offer an opportunity to get a better understanding of an intervention's potential effects when no direct molecular target is involved or when the exact mechanism of action is not yet fully understood, e.g. in the case of stem cell trials in ALS patients (*Table 2*).[101,104]

Pathophysiological response

Pathophysiological response biomarkers are also distal biomarkers, and contrary to the physiological response biomarkers, should have a clear and direct link to the disease pathophysiological mechanisms. For early phase trials these biomarkers do not necessarily need to be validated surrogate substitutes for clinical endpoints, however, when available, a validated surrogate would of course provide stronger evidence for possible disease modification. It should be considered though that most early phase trials are only of a short duration and for most NDDs the disease progresses too slow to measure a significant change over a short period of time. Moreover, early phase trials usually only recruit small sample sizes and there can be significant interindividual variation in disease phenotype and progression. Therefore, chances are that it may not be possible to demonstrate a significant effect of the investigational compound on pathophysiological response biomarkers in early phase trials, which would not necessarily equal a lack of effect of the investigational compound. It is therefore not surprising that pathophysiological response biomarkers are only reported in 33% of early phase clinical trials involving patients (*Figure 1*). In healthy volunteer studies pathophysiological response biomarkers obviously cannot be included for a lack of disease presence.

Examples of pathophysiological response wet biomarkers that have been used in early phase NDD DMT trials include quantification of CSF TAU phosphorylated at threonine 181 (p-TAU181)[54,60] and evaluation of amyloid β by PET[75] for Alzheimer's disease pathology, phosphorylated neurofilament heavy chains (and post-hoc neurofilament light chain) concentrations as general axonal damage biomarker in ALS,[33] FTD,[129] and Huntington's disease,[131] and CSF mitochondrial dysfunction markers (GDF15, lactate) in MS (*Table 2*).[139] Other types of more physical pathophysiological response biomarkers include the evaluation of retinal nerve fiber layer thinning in MS[139] and electromyogram (EMG) study of the tibialis anterior muscles in ALS patients receiving stem cell treatment.[109] Also neuroimaging techniques can be used as pathophysiological response biomarkers, such as the evaluation of disease progression via dopaminergic function with the use of 18F-DOPA PET,[153] or reduction of whole brain or hippocampal atrophy (MRI) or reduction of cerebral metabolism on fluordeoxyglucose (FDG) PET,[36] although it is unlikely that an effect on these markers can be observed in short-duration trials.

Clinical response

It appears that clinical outcomes are most frequently included (74%) as exploratory endpoints in early phase trials with NDD patients (*Figure 1*). These clinical outcome measures included disease rating scales [e.g. Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-COG),[53,70,73,78] Mini-Mental State Examination (MMSE),[58,61] Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R),[33,106,119] Neuronal Ceroid Lipofuscinosis Type 2 Clinical Rating Scale (CLN2 score),[143] Unified Huntington's Disease Rating Scale (UHDRS),[132] Hammersmith Functional Motor Scale Expanded (HFMSE),[167] and Movement Disorders Society Unified Parkinson Disease Rating Scale (MDS-UPDRS)[153,154,161]], pulmonary functioning evaluation,[100,128] muscle power assessments,[99,103,113] and quality of life questionnaires.[68,120,152] We would argue, however, that due to small samples sizes in early phase trials, potentially significant placebo effects or sometimes lack of a placebo control, and the relatively low sensitivity of these disease rating scales such instruments may at best be useful as safety biomarkers but not as outcome markers at this stage of clinical development. Even in longer-duration open label extensions of early phase trials clinical outcomes are not expected to yield reliable results because of the small sample sizes and lack of a placebo control.[186] However, the high percentage of early phase trials reporting clinical outcomes may result from regulatory guidance that recommends to explore clinical outcomes in early phase trials to investigate how these can be further used in subsequent pivotal trials.[49] A more sensitive future tool for assessing exploratory clinical outcomes on disease progression could be the use of continuous digital biomarkers, such as smartphone-based assessments.[187]

BIOMARKER SOURCES

Cerebrospinal fluid (31% of trials) and blood (45% of trials) are the most frequently used biofluids for biomarker analysis in NDD research. These biofluids are relatively easily accessible in the clinical setting and well-established bioanalytical methods for these matrices are available. CSF could arguably be the most proximal source for physiological and pathological response biomarkers related to the intended CNS target. Moreover, concentrations of CNS biomarkers outside of CSF are often extremely low making them difficult to detect using standard assays, and in blood endogenous antibodies and proteases may be present that interfere with assays or shorten the lifespan of peripheral protein biomarkers.[18] However as discussed previously, mechanistic proof-of-concept of target engagement by DMT compounds can often be demonstrated very well peripherally without being hampered much by such challenges. Moreover, NDDs are found to also be influencing some peripheral tissues outside the CNS.[188] Therefore, in early stage drug development pharmacodynamic biomarkers can be used from a large variety of bodily sources (*Table 2*). Besides whole blood, plasma or serum, leukocytes and in particular the subset of PBMCs can be an easily accessible source for evaluating intracellular pathways *ex vivo*, which also offers the possibility to simulate disease states (also in heathy volunteer studies). When working with PBMCs though, it is important to realize that these cells represent a heterogeneous group that includes lymphocytes, monocytes and macrophages and the molecular target of interest may not be expressed to similar levels in all of these cells. For example, LRRK2 kinase and its direct substrate RAB10 are only abundantly expressed in monocytes and are virtually undetectable in B and T lymphocytes as well as natural killer and dendritic cells that constitute most of the PBMCs.[189] Moreover, both these proteins are expressed to an even higher degree in neutrophils, making neutrophils potentially the best source for demonstrating mechanistic proof-of-concept of LRRK2-inhibitors.[189] Another easily accessible biofluid that can be a source for biomarker analysis is urine,[190] but also more challenging matrices, such as stool samples, ocular fluids, and mucosal secretions can be considered for biomarker analyses.[191] The challenge of accurate analysis, however, is much higher in such matrices

and therefore feasibility of sampling as well as analyte extraction should be considered and demonstrated prior to implementation in clinical trials.[191] Also tissue biopsies, such as from muscle[99] or nasal olfactory neural tissue,[192] and surgical byproducts[191] can be considered as sources for biomarker analysis. And even the body surface has proven to be an easily accessible source for biomarker analysis in NDD drug development via the use of skin fibroblasts[193] and hair follicle RNA.[194]

As there may be relatively large intra- and interindividual variability in some of the biomarkers in these matrices, it could be necessary to normalize the biomarker readouts to a quantifiable reference value to draw more robust conclusions between different sampling times and individuals. This is especially important given the small numbers of subjects usually included in early phase trials. Examples of normalization factors used in biomarker analysis include normalization to total protein or creatinine to correct for the number or concentration of cells in a specific sample or matrix for gene expression analysis,[191] relating analysis of SOD1 activity in erythrocytes to the content of hemoglobin in erythrocyte lysates,[120] relating phosphorylated glycogen synthase (GS) to the total levels of GS,[129] and using the survival of motor neuron 2 full length (SMN2FL)/SMN2Δ7 mRNA ratio to reduce the confounding effects of SMN2FL and SMN2Δ7 mRNA level fluctuations for monitoring the inclusion of SMN2 exon 7 and the effect of risdiplam.[169] Also using patients as their own controls with crossover designs in early phase clinical trials helps limit the potential effects of often large intersubject variability in studies with small numbers of subjects.[81] Finally it can be worth considering using patient enrichment strategies for early phase trials,[195] to optimize the chance of success in demonstrating proof-of-concept by including the most suitable patient population (e.g. with a specific genetic mutations, disease onset state, or a slow or fast disease progression prognosis). The scientific benefit of targeting a specific subpopulation, however, should be balanced to the recruitability of the trial and potentially the targeted mode of action.

BIOMARKER SELECTION, DEVELOPMENT, AND VALIDATION

The decision to evaluate biomarkers in early phase clinical trials should be taken well in advance in order to select appropriate biomarkers to address the key scientific early phase clinical development questions and develop robust bioanalytical methods.[25,191] In fact, the biomarker strategy planning for first-in-human studies should ideally start during the preclinical development phase (*Figure 2*). Steps to consider when selecting biomarkers for use in early phase clinical trials include defining the scientific questions that the biomarker should help answer, performing a thorough literature review to select fit-for-purpose biomarker, bioanalytical method development or assay and laboratory selection, analytical model validation testing, and defining the clinical sampling, data reduction and analysis strategy.[191,196] Preferably the selected biomarkers are validated in the preclinical models used during drug development as well as in patients or patient biofluid repositories.[197] Characteristics to select a useful biomarker include that the biomarker should give a consistent response across studies and drugs with the same mode of action, must respond clearly to therapeutic doses, must have a clear dose-response relationship and ideally there should be a plausible relationship between the biomarker, pharmacology of the drug class, and disease pathophysiology (although for mechanistic biomarkers this not an absolute necessity as discussed previously).[25]

Biomarkers used in early phase clinical development do not fall under standardized regulatory requirements and therefore the clinical development team has to decide on the level of method characterization and documentation that is needed by weighing how the biomarker may provide the most value to the clinical development program goals.[191] For an early go/no-go decision a qualified assay may fit the purpose, whereas for proof-of-concept of clinical responses a fully validated method may be required.[191] Some biomarkers used in early phase trials may evolve over time to become diagnostics or surrogate endpoints, but this requires the biomarkers to become accepted for use through submission of biomarker data during the drug approval process or via the biomarker qualification program developed by the Center for Drug Evaluation and Research.[39]

LIMITATIONS

It is clear that the use of pharmacodynamic biomarkers in early phase clinical trials can help optimize clinical development in an area that has seen a near 100% failure rate to date, and that the frequency of rational use of these pharmacodynamic biomarkers should be improved (*Figure 1*). However, the use of pharmacodynamic biomarkers in itself is obviously not a guarantee for clinical development success. There are still some major challenges that the development of DMTs for NDDs faces that the use of biomarkers will not be able to solve.

DMT development has been struggling with a poor translatability of preclinical and animal models to human disease,[15] though in the past decade great advances have been with neurons derived from induced pluripotent stem cells (IPSCs) and 3D cell cultures technologies as preclinical models for neurodegenerative diseases.[198] While the use of biomarkers will not directly impact the quality of the animal models, biomarkers may help identify subsets of patients or early versus late stage disease states to better align the preclinical work with the target population for human proof-of-concept studies. Moreover, when preclinical and early stage clinical biomarker programs are well aligned, they can help demonstrate early proof-of-concept and translatability of target engagement in humans. Especially when combined with upcoming preclinical or translational PK/PD modeling and simulation (M&S) techniques,[199] mechanistic biomarkers can in this way contribute to early 'go/no-go' development decisions and thereby help improve R&D productivity in the development of NDD DMTs.

Another challenge for the development of DMTs for NDDs is that our current disease understanding or hypotheses may be wrong, and that even when biomarkers demonstrate target engagement in humans there may be no clinical disease-modifying effects of the compound.[2] However, in this case it is essential that target engagement was demonstrated in the early phase trials, as this would point towards limited clinical relevance of the targeted pathway as a whole, rather than possibly just a lack of effect of the specific compound itself.

The usefulness of biomarkers must also not be overestimated. Early phase clinical trials may be of too short a duration to demonstrate an effect on disease progression biomarkers and therefore a lack of effect on a pathophysiological response marker in early phase trials does not necessarily mean that there can be no long-term clinical effect. Another caveat to be aware of is that treating a biomarker may not treat the disease, as has become clear in the development of anti-amyloid therapies. While anti-amyloid antibodies, BACE inhibitors, and γ-secretase inhibitors all demonstrated target engagement in early phase trials, they all subsequently failed to demonstrate clinical effect in later stage trials.[200] This could potentially indicate that targeting amyloid β may after all not contribute to disease modification in Alzheimer's disease, or that amyloid β-targeting therapies need to be administered in a much earlier disease state for which we currently still lack robust diagnostic biomarkers.

Moreover, as no single one biomarker to date has been demonstrated to be indicative of NDD disease progression, it is recommended to use multiple response biomarkers when available to establish a pattern or fingerprint of treatment effects,[201,202] contributing to the overall persuasiveness of proof-of-concept for a disease-modifying effect.

Finally, it should be kept in mind that developing a robust biomarker strategy can be a very lengthy and time-consuming process, and this process should therefore already be initiated well in advance of the first-in-human studies. This requires a strong collaborative effort between the preclinical scientists and the clinical development team to ensure a seamless integration of the preclinical and early-stage clinical biomarker strategies,[25] which in the end might prove to be the most critical parameter for success in early stage NDD DMT development.

ROADMAP FOR MECHANISTIC, DATA-RICH EARLY PHASE CLINICAL PHARMACOLOGY STUDIES

Over the past decade the toolbox for early phase clinical development for NDDs has expanded significantly, which will hopefully help bring the first DMTs to patients in the decade to come. In AD (79%) and PD (71%) pharmacodynamic biomarkers by now have a well-established role in early clinical development, but in for example ALS (52%) and PSP (25%) there is still room for significant improvement (*Table 2*). In *Figure 2* we therefore propose a best-practice roadmap for mechanistic, data-rich early phase clinical pharmacology studies for disease-modifying therapies in neurodegenerative disorders. Even if modifying the course of NDDs could ultimately prove to require a multi-drug approach, it will remain essential to clearly demonstrate pathway engagement of each individual drug component to get to rational multi-drug treatment regimens.

CONCLUSION

As our understanding of NDDs is improving, there is a rise in potentially disease-modifying treatments being brought to the clinic. Further increasing the rational use of mechanistic biomarkers in early phase trials for these (targeted) therapies can increase R&D productivity with a quick win / fast fail approach in an area that has seen a nearly 100% failure rate to date.

Table 1 Biomarker categories and examples of use in NND DMT drug development (adapted from Cummings and Amur et al).[39,45]

Biomarker category	Use in drug development	Examples from NND DMT drug development
Response	Pharmacodynamic biomarker as indicator of intended drug activity • *Proximal* (molecular target occupancy and activation) • *Distal* ([patho]physiological response) Efficacy response marker as a surrogate for a clinical endpoint	CSF total amyloid-β and fragments in response to amyloid-β antibody treatments Braak staging with TAU PET as a surrogate biomarker for clinical AD (though no validated surrogate biomarkers are available yet for NDDs)
Diagnostic	Patient selection	GBA1 gene mutation in PD patients SOD1 gene mutation in ALS patients
Predictive	Patient stratification Trial enrichment via inclusion criteria	TAU PET to identify AD patients more likely to respond to anti-TAU therapies
Prognostic	Patient stratification Trial enrichment with patients likely to have disease	Percentage of weight loss at baseline for life expectancy and disease progression in ALS patients
Safety	Detect AEs and off-target drug responses	MRI for structural changes (including tumor or syrinx formation) within the brain after stem cell transplantation for ALS

Figure 1 Percentage of early clinical phase reporting the use of different categories of pharmacodynamic biomarkers and clinical outcomes. Thirty-one trials (26%) reported the use of target occupancy biomarkers and forty-eight trials (40%) reported the use of a target activation biomarkers. Sixty-five trials included at least 1 proximal (mechanistic) biomarker (target occupancy and/or activation). Twenty-eight trials (23%) reported the use of physiological response biomarkers. Thirty-two trials used patho-physiological response biomarkers, which comes down to 33% of all early phase NDD DMT trials (98) that were performed in patients. Forty-seven trials (39%) reported the use of at least 1 distal biomarker. In total 89 of 121 trials reported at least one pharmaco-dynamic biomarker and seventy-three trials reported clinical outcomes, which comes down to 74% of all early phase NDD DMT trials (98) that were performed in patients.

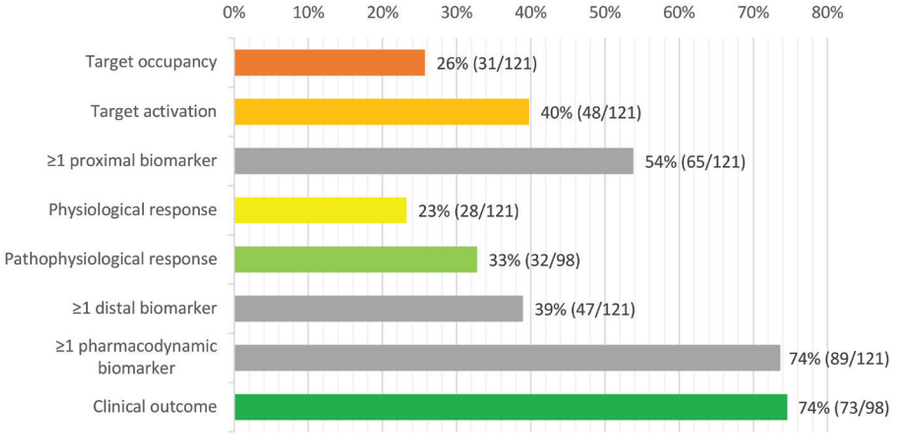


Table 2 Overview of published early phase clinical trials for disease-modifying compounds in neurodegenerative disorders between 2010 and November 2020 and reported peripheral and central pharmacodynamic biomarker outcomes.

Indication	Drug category	Drug target	Trials reporting mechanistic biomarker	Peripheral biomarkers	Central biomarkers	Types of biomarkers	Study population	References
AD	Antibody	Amyloid β	10/11 (91%)	Plasma total Aβ and Aβ fragments (Aβ1-x, Aβ1-40, Aβ1-42, Aβ3-42, Aβ1-38, Aβ18-35)	CSF Aβ species (Aβ1-x, Aβ1-40, Aβ1-42), t-TAU, and p-TAU181	Target occupancy and pathophysiological response	HVs and patients	53-63
		TAU protein	1/1 (100%)	-	CSF N-terminal TAU, mid-domain TAU, Aβ40, and Aβ42	Target occupancy and physiological response	HVs	64
	Cell therapy	Cytotropic factors, anti-inflammatory, neurogenesis	1/1 (100%)	-	CSF Aβ, t-TAU and p-TAU; PIB-PET changes in parenchymal amyloid deposition; FDG-PET metabolic changes	(patho)physiological response	Patients	65
		Nerve growth factor	0/1 (0%)	-	-	-	Patients	66
	Dietary	Xanthophyll Carotenoids, Omega-3 Fatty Acids	0/1 (0%)	-	-	-	Patients	67
	Gene therapy	Nerve growth factor	1/1 (100%)	-	PET brain glucose metabolism (post-mortem brain autopsy gene-mediated NGF expression and bioactivity)	Physiological response (and post-mortem target occupancy and activation)	Patients	68
	Growth factor	Nerve growth factor	1/1 (100%)	-	MRI for implant position; CSF Aβ1-42, t-TAU, p-TAU181, NFL, glial fibrillary acidic protein (GFAP), AChE and choline acetyltransferase (ChAT) activity and protein levels	Target occupancy, activation and (patho) physiological response	Patients	69
	Immunotherapy	Amyloid β	3/3 (100%)	Plasma anti-Aβ40 antibodies, Aβ peptides (Aβ40, Aβ42) and cytokines (IL-6, TNF-α, IL-1β, MCP-1, IL-2, sIL-2R); Serum antibody titres (Aβ IGM, Aβ IGG), Aβ1-40, Aβx-40, Aβ1-42; *In vitro* lymphocyte proliferation and cytokine production; PBMC β-specific and Qβ-specific responses of T-cells	CSF antibody titres, Aβx-40, Aβx-42, Aβ1-42, Aβn-42, t-TAU, p-TAU181; MRI brain volumetric assessment	Target activation and (patho)physiological response	Patients	70-72
		TAU protein	1/1 (100%)	IGG and IGM titre anti-vaccin peptide, anti-KLH antibody titre, anti-pathological-TAU antibody titre; Lymphocyte immunoprofiling	-	Target activation and physiological response	Patients	73,92
	Peptide	Amyloid β	0/1 (0%)	-	-	-	HVs	74
	Focused ultrasound with injected micro-bubbles	BBB-opening to amyloid β and TAU	1/1 (100%)	-	PET BBB opening and amyloid β deposition	Target occupancy and pathophysiological response	Patients	75
	DBS	Cerebral glucose metabolism	3/4 (75%)	-	PET cerebral glucose metabolism	Physiological response	Patients	76-79
	Small molecule	5-HT2A receptor	0/1 (0%)	-	-	-	HVs	80
		Amyloid precursor protein (APP) synthesis	1/1 (100%)	-	CSF sAPPα, sAPPβ, t-TAU, p-TAU, Aβ42 and inflammatory markers (complement 3, factor H, MCP-1, YKL-40, sCD14)	Target activation and (patho)physiological response	Patients	81
		Amyloid production and associated inflammatory response	0/1 (0%)	-	-	-	HVs	82
		BACE1	7/8 (89%)	Plasma total Aβ and Aβ fragments (Aβ1-37, Aβ1-38, Aβ1-40, Aβ1-42, Aβx-40), total sAPP and fragments (sAPPα, sAPPβ)	CSF total Aβ and fragments (Aβx-38, Aβx-40, Aβx-42, Aβ1-37, Aβ1-38, Aβ1-40, Aβ1-42), total sAPP and fragments (sAPPα, sAPPβ), BACE1, t-TAU, p-TAU181	Target occupancy, activation and pathophysiological response	HVs and patients	83-90
		ET(B) receptor	0/1 (0%)	-	-	-	HVs	91
		Glutaminyl cyclase (QC)	1/1 (100%)	Serum QC activity	CSF QC activity	Target occupancy and activation	HVs	92
		Glycogen synthase kinase-3β (GSK3β)	1/1 (100%)	Lymphocyte GS phosphorylation	-	Target occupancy	HVs	93
		Sigma-2 receptor complex	0/1 (0%)	-	-	-	HVs	94
		γ-secretase	2/2 (100%)	Plasma Aβx-42	CSF total Aβ and Aβ fragments (Aβ42, Aβ40, Aβ37, Aβ38)	Target activation	HVs	95, 96
		RIPK1 inhibitor*	1/1 (100%)	PBMCs reduction of ps166 RIPK1	-	Target occupancy and activation	HVs	30
		Microtubule stabilization	1/1 (100%)	-	CSF NFL, t-TAU, p-TAU, Aβ42, YKL-40	Pathophysiological response	Patients	97
	Cell therapy	Neuroprotective effects	1/1 (100%)	-	CSF t-TAU, p-TAU, Aβ42	Pathophysiological response	Patients	98
Overall use of mechanistic biomarkers in early phase AD trials			37/47 (79%)					
ALS	Antibody	Neurite outgrowth inhibitor NOGO-A	1/1 (100%)	Muscle biopsy NOGO-A RNA and protein expression; Plasma NOGO-A protein gamma sarcoglycan; EMG (MUNE)	-	Target occupancy and activation	Patients	99
	Antisense Oligonu-cleotide	SOD1	2/2 (100%)	Plasma p-NFH, NFL	CSF SOD1, p-NFH, NFL	Target activation and pathophysiological response	Patients	33, 40, 60, 100
	Cell therapy	Neurotrophic growth factors and cytokines secretion, immunomodulation and cell proliferation or replacement	5/13 (38%)	MRI muscle volume CD4+CD25+FOXP3+TREGS, proliferation of autologous responder T lymphocytes; EMG of TA muscles (CMAP, FD, SMUP, MUNE, MUNIX, MUSIX); EIM	CSF cytokines (TGF-B1, TGF-B2, TGF-B3, IL-6, IL-10, MCP-1)	(patho)physiological response	Patients	101, 102, 111-113, 103-110
	Gene therapy	Hepatocyte growth factor	1/1 (100%)	Serum HGF; Muscle circumference	-	Target activation and pathophysiological response	Patients	114
	Growth factor	Granulocyte colony-stimulating factor	1/1 (100%)	Blood cell counts, CD34+cells serum cytokines/chemokines (IL-1B, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, EOTAXIN, bFGF, FGF-2, TGF-A, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1A, MIP-1B, PDGF-BB, RANTES, TNF-α, VEGF)	CSF BMC presence, cytokines/chemokines (IL-1B, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, EOTAXIN, bFGF, FGF-2, TGF-A, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1A, MIP-1B, PDGF-BB, RANTES, TNF-α, VEGF)	Target activation and (patho)physiological response	Patients	115
		Hepatocyte growth factor	0/1 (0%)	-	-	-	Patients	116
	Small molecule	EAAT2	0/1 (0%)	-	-	-	Patients	117
		Putative mitochondrial modulation	0/1 (0%)	-	-	-	HVs	118
		Inflammatory macrophages and monocytes regulation	1/1 (100%)	Blood monocyte immune activation markers CD16, HLA-DR	-	Target activation	Patients	119
		SOD1	2/2 (100%)	Erythrocyte SOD1 enzymatic activity; Leukocyte actin-normalized SOD1	CSF SOD1 protein and enzymic activity	Target activation	Patients	120, 121
	Supplement	Lysosomal Cathepsins B and L	0/1 (0%)	-	-	-	Patients	122
		Stabilize the mitochondrial transition pore, buffer intracellular energy stores, stimulate synaptic glutamate uptake, and scavenge reactive oxygen species	1/1 (100%)	-	MRS brain glutamate and glutamine (GLX)	Physiological response	Patients	123
Overall use of mechanistic biomarkers in early phase ALS trials			14/27 (52%)*					

[*Continuation Table 2*]

Indication	Drug category	Drug target	Trials reporting mechanistic biomarker	Peripheral biomarkers	Central biomarkers	Types of biomarkers	Study population	References
ATTR amyloidosis	Antisense oligonucleotide	Transthyretin (TTR)	1/1 (100%)	Plasma TTR	-	Target activation	HVs	124
	RNA interference	Transthyretin amyloid	1/1 (100%)	Serum transthyretin, retinol-binding protein and vitamin A	-	Target occupancy and activation	HVs and patients	125
Overall use of mechanistic biomarkers in early phase ATTR trials			2/2 (100%)					
FRDA	Small molecule	FXN gene expression	1/1 (100%)	Whole blood FXN mRNA, frataxin protein; PBMC chromatin modification via H3 lysine 9 acetylation	-	Target occupancy and activation	Patients	126
	Supplement	FXN gene expression	1/1 (100%)	PBMC FXN mRNA and frataxin protein); Blood heterochromatin modifications at the FXN locus	-	Target occupancy and activation	Patients	127
	Polyunsaturated fatty acid	Lipid peroxidation	1/1 (100%)	RBC compartment D2-LA	-	Target occupancy	Patients	128
Overall use of mechanistic biomarkers in early phase FRDA trials			3/3 (100%)					
FTD	Small molecule	Progranulin protein (PGRN)	1/1 (100%)	Plasma PGRN, PGRN-related inflammatory markers (CRP, ESR), blood cytokines (IL-10, IL-2, IL-6, IL-8, TNF-α)	CSF PGRN, NFL, Aβ42, TAU, cytokines (IL-10, IL-2, IL-6, IL-8, TNF-α); MRI volumetric assessment	Target activation and (patho)physiological response	Patients	129
Overall use of mechanistic biomarkers in early phase FTD trials			1/1 (100%)					
GM2 gangliosidosis	Small molecule	β-hexosaminidase (HEX)	1/1 (100%)	Leucocyte and plasma HEX A, β-galactosidase and glucocerebrosidase activity, β-glucuronidase and acid phosphatase	-	Target activation	Patients	130
Overall use of mechanistic biomarkers in early phase GM2 gangliosidosis trials			1/1 (100%)					
HD	Antisense oligonucleotide	HTT mRNA	1/1 (100%)	-	CSF mutant HTT, NFL; MRI ventricular volume	Target activation and pathophysiological response	Patients	131
	Peptide	Cardiolipin	1/1 (100%)	MRI skeletal muscle dynamic 31P-MRS; PBMC mitochondrial membrane potential ($\Delta\Psi m$)	MRI brain 31P-MRS; CNS functional domain test battery (NeuroCart®)	Target activation and (patho)physiological response	Patients	132
Overall use of mechanistic biomarkers in early phase HD trials			2/2 (100%)					
Leber Hereditary Optic Neuropathy	Gene therapy	Mitochondrial gene encoding NADH: ubiquinone oxidoreductase subunit 4 (ND4)	1/2 (50%)	-	OCT average retinal nerve fiber layer (RNFL) thickness; Pattern electroretinogram amplitudes	Physiological response	Patients	133, 134
Overall use of mechanistic biomarkers in early phase Leber Hereditary Optic Neuropathy trials			1/2 (50%)					
MS	Antibody	Semaphorin 4D	1/1 (100%)	T-CELL CSEMA4D expression and saturation; Serum SSEMA4D	-	Target occupancy and activation	Patients	135
	Cell therapy	Neurotrophic and immunomodulatory effects, neurogenesis	2/2 (100%)	Lymphocyte subsets (CD4+, CD25+ and CD40+lymphocytes and CD83+, CD86+, and HLA-DR+myeloid dendritic cells); PBMC cytokine production	MRI labeled cell localization and volumetric assessment; OCT average retinal nerve fiber layer (RNFL); Vision (HCVA, LCLA)	Target occupancy and (patho)physiological response	Patients	136, 137
	Small molecule	Anti-inflammatory	1/1 (100%)	PBMC monocyte and 6-sulpho LACNAC+dendritic cell (slanDC) frequency, properties, and activation status	-	Target activation and physiological response	Patients	138
		Mitochondrial ATP production (coenzyme Q10)	1/1 (100%)	-	CSF mitochondrial dysfunction markers (GDF15, lactate), NFL, sCD14; BBB leakage (albumin quotient); OCT retinal nerve fiber layer thinning; MRI brain ventricular volume	(patho)physiological response	Patients	139
Overall use of mechanistic biomarkers in early phase MS trials			5/5 (100%)					

Indication	Drug category	Drug target	Trials reporting mechanistic biomarker	Peripheral biomarkers	Central biomarkers	Types of biomarkers	Study population	References
MSA	Cell therapy	Neurotrophic factors secretion	1/1 (100%)	-	CSF neurotrophic factors (NGF, GDNF, BDNF)	Physiological response	Patients	140
	Immunotherapy	α-synuclein	1/1 (100%)	Serum immunopeptide titers, α-synuclein native epitope titers	-	Target activation	Patients	141
Overall use of mechanistic biomarkers in early phase MSA trials			2/2 (100%)					
NCLS	Cell therapy	Palmitoyl-protein thioesterase 1 (PPT-1) and tripeptidyl peptidase 1 (TPP1) enzymes production	0/1 (0%)	-	-	-	Patients	142
CLN2 disease	Enzyme replacement	Lysosomal enzyme TPP1	0/1 (0%)	-	-	-	Patients	143
Overall use of mechanistic biomarkers in early phase NCLS trials			0/2 (0%)					
NPC1	Cyclodextrin	Neuronal cholesterol homoeostasis	1/1 (100%)	Serum 24(S)-hydroxycholesterol (24(S)-HC)	CSF 24(S)-hydroxycholesterol (24(S)-HC), fatty acid binding protein 3 (FABP3) and calbindin D19	Target activation and (patho)physiological response	Patients	144
Overall use of mechanistic biomarkers in early phase NPC1 trials			1/1 (100%)					
PD	Antibody	α-synuclein	3/3 (100%)	Plasma antibody/α-synuclein complexes; Serum total and free α-synuclein	CSF total and free α-synuclein, total Aβ, Aβ42, DJ-1, DAT scan	Target occupancy, activation, and pathophysiological response	HVs and patients	145-147
	Cell therapy	Neurotrophic factors to restore dopaminergic cell function	0/1 (0%)	-	-	-	Patients	148
	Gene therapy	Aromatic L-amino acid decarboxylase (AADC)	3/3 (100%)	-	PET FMT brain AADC expression and activity	Target occupancy and activation	Patients	149-151
		Tyrosine hydroxylase, AADC, cyclohydrolase 1	1/1 (100%)	-	PET cortical excitability and reflex recordings	Physiological response	Patients	152
	Growth factor	Granulocyte colony-stimulating factor (G-CSF)	0/1 (0%)	-	PET 18 F-DOPA for disease progression	Pathophysiological response	Patients	153
		Granulocyte-macrophage colony-stimulating factor (GM-CSF)	1/1 (100%)	Expression of Treg phenotype and function (CD4+Teffs (CD4+CD127hiCD25hi), CD4+Tregs (CD4+CD127loCD25hi), FOXP3+CD4+ Tregs, ICTLA4+CD4+Tregs, CD39+CD4+ Tregs, and fFAS+CD4+Tregs), T cell proliferation mRNA (GATA4, IL2, HOXA10, and KIF2C), anti-inflammatory gene expression (PPARG, LRRC32, FOSL1, IL1R2, IL13RA1, NR4A3, GFI1), tryptophan pathway targeted metabolomics	-	Target activation and physiological response	Patients	154
		rhPDGF-BB (proliferation of SOX-2/OLIG-1– POSITIVE periventricular progenitor cells)	1/1 (100%)	-	[11C]PE2I DAT binding	Pathophysiological response	Patients	155
	Immunotherapy	α-synuclein	1/1 (100%)	Serum antibody titres	CSF antibody titres, total α-synuclein, Aβ1–42, p-TAU	Target activation and pathophysiological response	Patients	156
	Deep brain stimulation (DBS)	Unknown	0/1 (0%)	-	-	-	Patients	157
	Small molecule	Glucosylceramide synthase (GCS)	1/1 (100%)	Plasma glucosylceramide (GL-1), globostriaosylceramide (GL-3), and GM3 ganglioside (GM3)	-	Target activation	HVs	158
		Myeloperoxidase	1/1 (100%)	-	PET distribution volume of 11C-PBR28 binding to microglia marker TSPO	Target occupancy	Patients	159
		Flavonoid (regulating dopaminergic system function, anti-oxidative damage and anti-inflammatory effects)	0/1 (0%)	-	-	-	HVs	160
	Supplement	Antioxidant	0/1 (0%)	-	-	-	Patients	161
Overall use of mechanistic biomarkers in early phase PD trials			12/17 (71%)					

[*Continuation Table 2*]

Indication	Drug category	Drug target	Trials reporting mechanistic biomarker	Peripheral biomarkers	Central biomarkers	Types of biomarkers	Study population	References
PSP	Antibody	TAU protein	0/2 (0%)	-	-	-	Patients	31, 162
	Cell therapy	Trophic, anti-apoptotic and regenerative effects	0/1 (0%)	-	MRI, SPECT and PET with tropanic tracers (FP-CIT and BETA-CIT) longitudinal neuroimaging	Pathophysiological response	Patients	163
	Small molecule / Blood product	Acetylation of TAU /unknown	1/1 (100%)	Plasma NFL concentrations	CSF amyloid beta Aβ, t-TAU, p-TAU181; MRI brain volumetric assessment	(patho)physiological response	Patients	164
Overall use of mechanistic biomarkers in early phase PSP trials			1/4 (25%)					
SCA	Cell therapy	Trophic factor secretion, immunomodulation	1/1 (100%)	-	PET brain glucose metabolism	Physiological response	Patients	165
	Growth factor	Antiapoptotic, antioxidative, anti-inflammatory, neurotrophic and angio- genic properties	0/1 (0%)	-	-	-	HVS	166
Overall use of mechanistic biomarkers in early phase SCA trials			1/2 (50%)					
SMA	Antisense oligonucleotide	SMN2 mRNA splicing	1/1 (100%)	-	CSF SMN protein	Target activation	Patients	167
	Small molecule	SMN2 splicing	2/2 (100%)	Blood mRNA (full-length SMN2, SMN1, SMNΔ7), SMN protein	-	Target activation	HVS and patients	168,169
	Gene therapy	SMN	0/1 (0%)	-	-	-	Patients	170
Overall use of mechanistic biomarkers in early phase SMA trials			3/4 (25%)					

*AADC = aromatic L-amino acid decarboxylase / Aβ = amyloid β / AChE = Acetylcholinesterase / AD = Alzheimer's disease / ALS = amyotrophic lateral sclerosis / AAP = amyloid precursor protein / ATP = adenosine triphosphate / ATTR = amyloid transthyretin / BACE1 = beta-secretase 1 / BBB = blood-brain barrier / BDNF = brain-derived neurotrophic factor / BETA-CIT = 18F-Fluoro-2-deoxyglucose labeled tropanic SPECT tracer / BMC = bone marrow concentrated cells / CD# = cluster of differentiation # / ChAT = choline acetyltransferase / CLN2 = classic late infantile neuronal ceroid lipofuscinosis / CMAP = compound muscle action potential / CRP = C-reactive protein / cSEMA4D = T-cell semaphorin 4D / CSF = cerebrospinal fluid / CTLA4 = cytotoxic T-lymphocyte-associated protein 4 / DAT = dopamine active transporter / DBS = deep brain stimulation / DJ-1 = protein deglycase DJ-1 (PARK7) / D2-LA = di-deutero isotopologue of linoleic acid ethyl ester / EAAT2 = excitatory amino acid transporter 2 / EIM = electrical impedance myography / EMG = electromyogram / ESR = erythrocyte sedimentation rate / ET(B). = endothelin receptor type B / FABP3 = fatty acid binding protein 3 / FD = fiber density / FDG = fluorine-18-deoxyglucose / FGF-# = fibroblast growth factor # / FMT = [18F]fluorometatyrosine / FOSL1 = FOS like 1, AP-1 transcription factor subunit / FOXP3 = forkhead box P3 / FRDA = Friedreich ataxia / FTD = frontotemporal dementia / FP-CIT = [123I] labeled tropanic SPECT tracer / FXN = frataxin / GATA4 = transcription factor GATA-4 / GCS = glucosylceramide synthase / GDF15 = growth/differentiation factor 15 / GDNF = glial cell-derived neurotrophic factor / GFAP = glial fibrillary acidic protein / GFI1 = growth factor independent 1 transcriptional repressor / GLCR = β-glucuronidase / GL-1 = glucosylceramide / GL-3 = globotriasylceramide / GLX = glutamate and glutamine / GM3 = mono-sialodihexosylganglioside / GM-CSF = granulocyte-macrophage colony-stimulating factor / GS = glycogen synthase / GSK3β = glycogen synthase kinase-3β / G-CSF = granulocyte colony-stimulating factor / HCVA = high-contrast visual acuity / HD = Huntington's disease / HEX = β-hexosaminidase / HGF = Hepatocyte growth factor / HLA-DR = human leukocyte antigen DR / HOXA10 = homeobox A10 / HTT = huntingtin / HVS = healthy volunteers / IFN-γ = interferon gamma / IGG = immunoglobulin G / IGM = immunoglobulin M / IL-# = Interleukin # / IP-10 = interferon gamma-induced protein 10 / KIF2C = kinesin Family Member 2C / KLH = keyhole limpet hemocyanin / LACNAC = N-acetyllactosamine / LCLA = low-contrast letter acuity / LRRC32 = leucine rich repeat containing 32 / MCP-1 = monocyte chemoattractant protein 1 / MIP-# = macrophage inflammatory protein # / MRI = magnetic resonance imaging / mRNA = messenger RNA / MRS = magnetic resonance spectroscopy / MS = multiple sclerosis / MSA = multiple system atrophy / MUNE = motor unit number estimation / MUNIX = motor unit number / MUSIX = motor unit size / NADH = nicotinamide adenine dinucleotide / NCLS = neuronal ceroid lipofuscinoses / ND4 = NADH-ubiquinone oxidoreductase chain 4 / NGF = nerve growth factor / NFL = neurofilament light chain / NPC1 = Niemann-Pick disease type C1 / NR4A3 = nuclear receptor subfamily 4 group A member 3 / OCT = optical coherence tomography / PBMCs = peripheral blood mononuclear cells / PD = Parkinson's disease / PDGF-BB = platelet-derived growth factor BB / PET = positron emission tomography / PGRN = progranulin protein / PIB = Pittsburgh compound B / PPARG = peroxisome proliferator-activated receptor gamma / PPT-1 = palmitoyl-protein thioesterase 1 / PSP = progressive supranuclear palsy / pS166 = phosphorylation of serine 166 / P-NFH = phosphorylated neurofilament heavy chain / p-TAU181 = TAU phosphorylated at threonine 181 / QC = glutaminyl cyclase / RANTES = regulated on activation, normal T cell expressed and secreted / RBC = red blood cells / rhPDGF-BB = recombinant human platelet-derived growth factor-BB / RIPK1 = receptor-interacting serine/threonine-protein kinase 1 / RNA = ribonucleic acid / RNFL = retinal nerve fiber layer / SAPP = soluble amyloid precursor protein / SCA = spinocerebellar ataxia / SCD14 = soluble CD14 / SIL-2R = soluble IL-2 receptor / slanDCS = 6-sulfo / LACNAC dendritic cells / SMA = spinal muscular atrophy / SMN# = survival of motor neuron # / SMNΔ7 = exon 7-deleted SMN protein / SMUP = single motor unit potential / SOD1 = superoxide dismutase 1 / SOX-2 / OLIG-1 = SRY-box transcription factor 2 / oligodendrocyte transcription factor 1 / SPECT = single photon emission computed tomography / SSEMA4D = soluble semaphorin 4D / TA = tibialis anterior / Teffs = effector T cells / TGF-# = transforming growth factor # / TNF-α = tumor necrosis factor / TPP1 = tripeptidyl peptidase 1 / Tregs = regulatory T cells / TSPO = translocator protein / TTR = transthyretin / t-TAU = total TAU / VEGF = vascular endothelial growth factor / YKL-40 = chitinase-3-like-1 protein / 24[S]-HC = α24(S)-hydroxycholesterol / 31P-MRS = 31P-magnetic resonance spectroscopy / 5-HT2A = 5-hydroxy-tryptamine 2A / ΔΨM = mitochondrial membrane potential / [11C]PE21 = selective dopamine active transporter (DAT) radiotracer / [11C]-PBR28 = 18pkD translocator protein (TSPO) radiotracer. *RIPK1 was under development for multiple indications (AD and ALS) in healthy subjects and has been added to the totals for both indications. It is only listed once AD in the Table to avoid duplication.*

Figure 2 Roadmap for early phase clinical development of disease-modification therapies in neurodegenerative disorders, focusing on demonstrating proof-of-concept with mechanistic early phase clinical pharmacology studies. Innovative clinical drug development revolves around confirming the pharmacokinetic behavior of the drug, occupation and activation of the intended pharmacological target in humans, quantifying the subsequent physiological and pathophysiological response before moving into large late-stage trials to demonstrate a clinical response (long-term disease modification). Safety evaluation is not specifically mentioned but is obviously an essential component at each stage of clinical drug development. For each stage of drug development different biomarker techniques can be used to come to an early mechanistic proof-of-concept, define the optimum dose, and facilitate a validated 'go/no-go' decision before moving into expensive late stage trials.

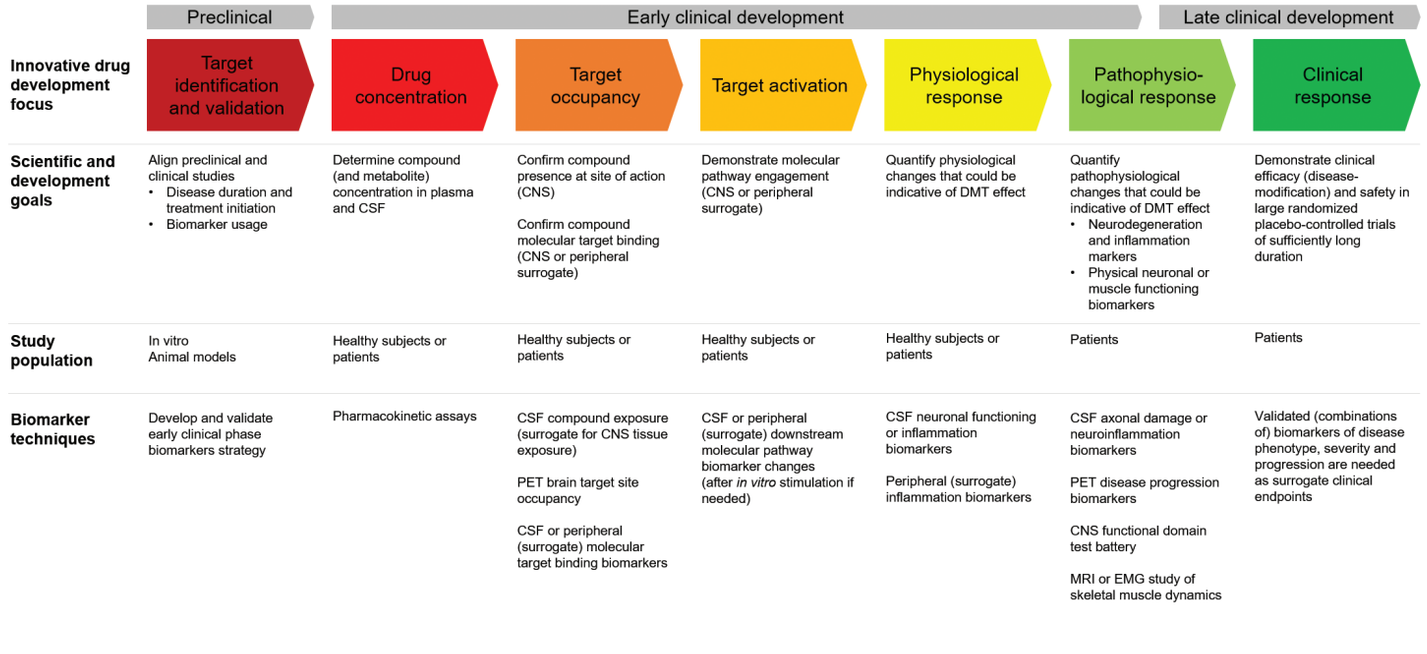
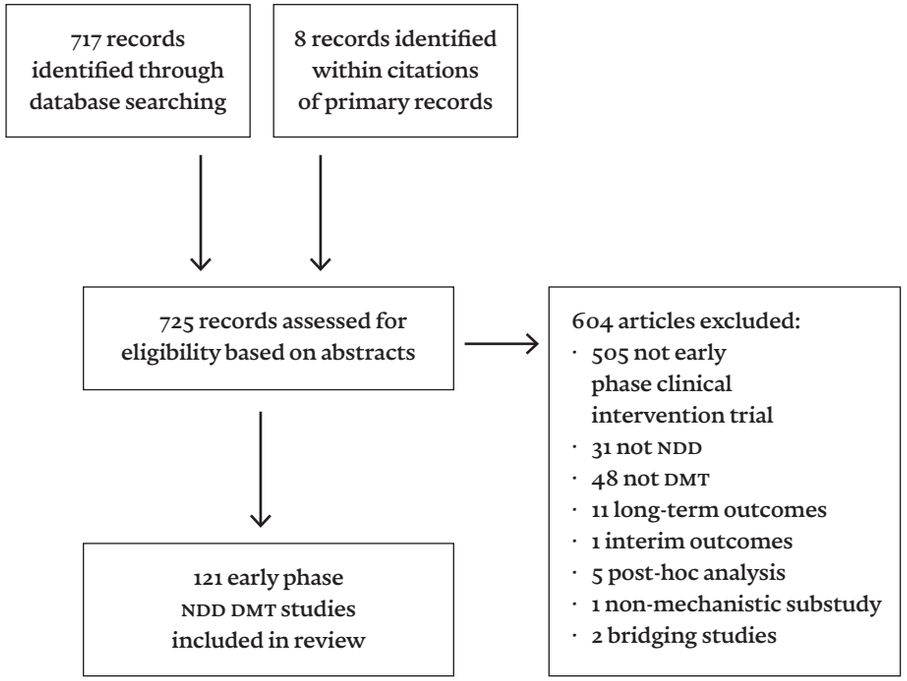


Figure S2 Study selection overview. Flow diagram of studies' screening and selection for this review.



REFERENCES

1 Feigin VL, Nichols E, Alam T, et al. Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019;18(5):459-480. Doi:10.1016/S1474-4422(18)30499-X

2 Gribkoff VK, Kaczmarek LK. The need for new approaches in CNS drug discovery: Why drugs have failed, and what can be done to improve outcomes. Neuropharmacology. 2017;120:11-19. Doi:10.1016/j.neuropharm.2016.03.021

3 Cummings J. Disease modification and Neuroprotection in neurodegenerative disorders. Transl Neurodegener. 2017;6(1). Doi:10.1186/s40035-017-0096-2

4 Plascencia-Villa G, Perry G. Status and future directions of clinical trials in Alzheimer's disease. In: International Review of Neurobiology. Vol 154. Academic Press Inc.; 2020:3-50. Doi:10.1016/bs.irn.2020.03.022

5 Travessa AM, Rodrigues FB, Mestre TA, Ferreira JJ. Fifteen years of clinical trials in Huntington's disease: A very low clinical drug development success rate. J Huntingtons Dis. 2017;6(2):157-163. Doi:10.3233/JHD-170245

6 Miller R, Mitchell J, Lyon M, Moore D. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). In: Cochrane Database of Systematic Reviews. John Wiley & Sons, Ltd; 2002. Doi:10.1002/14651858.cd001447

7 Hardiman O, van den Berg LH. Edaravone: a new treatment for ALS on the horizon? Lancet Neurol. 2017;16(7):490-491. Doi:10.1016/S1474-4422(17)30163-1

8 Maharshi V, Hasan S. Nusinersen: The First Option Beyond Supportive Care for Spinal Muscular Atrophy. Clin Drug Investig. 2017;37(9):807-817. Doi:10.1007/s40261-017-0557-5

9 Hodges JR, Piguet O. Progress and Challenges in Frontotemporal Dementia Research: A 20-Year Review. J Alzheimer's Dis. 2018;62(3):1467-1480. Doi:10.3233/JAD-171087

10 Farrar MA, Park SB, Vucic S, et al. Emerging therapies and challenges in spinal muscular atrophy. Ann Neurol. 2017;81(3):355-368. Doi:10.1002/ana.24864

11 Ashizawa T, Öz G, Paulson HL. Spinocerebellar ataxias: prospects and challenges for therapy development. Nat Rev Neurol. 2018;14(10):590-605. Doi:10.1038/s41582-018-0051-6

12 Zeuner KE, Schäffer E, Hopfner F, Brüggemann N, Berg D. Progress of Pharmacological Approaches in Parkinson's Disease. Clin Pharmacol Ther. 2019;105(5):1106-1120. Doi:10.1002/cpt.1374

13 Mejzini R, Flynn LL, Pitout IL, Fletcher S, Wilton SD, Akkari PA. ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now? Front Neurosci. 2019;13. Doi:10.3389/fnins.2019.01310

14 McFarthing K, Buff S, Rafaloff G, Dominey T, Wyse RK, Stott SRW. Parkinson's Disease Drug Therapies in the Clinical Trial Pipeline: 2020. J Parkinsons Dis. 2020;10(3):757-774. Doi:10.3233/JPD-202128

15 Dawson TM, Golde TE, Lagier-Tourenne C. Animal models of neurodegenerative diseases. Nat Neurosci. 2018;21(10):1370-1379. Doi:10.1038/s41593-018-0236-8

16 McGhee DJM, Ritchie CW, Zajicek JP, Counsell CE. A review of clinical trial designs used to detect a disease-modifying effect of drug therapy in Alzheimer's disease and Parkinson's disease. BMC Neurol. 2016;16(1):92. Doi:10.1186/s12883-016-0606-3

17 Henchcliffe C, Severt WL. Disease modification in Parkinson's disease. Drugs and Aging. 2011;28(8):605-615. Doi:10.2165/11591320-000000000-00000

18 Obrocki P, Khatun A, Ness D, et al. Perspectives in fluid biomarkers in neurodegeneration from the 2019 biomarkers in neurodegenerative diseases course – A joint PhD student course at University College London and University of Gothenburg. Alzheimer's Res Ther. 2020;12(1):16. Doi:10.1186/s13195-020-00586-6

19 Bakkar N, Boehringer A, Bowser R. Use of biomarkers in ALS drug development and clinical trials. Brain Res. 2015;1607:94-107. Doi:10.1016/j.brainres.2014.10.031

20 Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline: 2020. Alzheimer's Dement Transl Res Clin Interv. 2020;6(1). Doi:10.1002/trc2.12050

21 Degroot A. Biomarker-Guided Drug Development for Better Defined Early Patient Studies and Clinical Trial Efficiency. In: Handbook of Behavioral Neuroscience. Vol 29. Elsevier B.V.; 2019:17-23. Doi:10.1016/B978-0-12-803161-2.00002-3

22 Beach TG. A Review of Biomarkers for Neurodegenerative Disease: Will They Swing Us Across the Valley? Neurol Ther. 2017;6(Suppl 1):5-13. Doi:10.1007/s40120-017-0072-x

23 West AB. Achieving neuroprotection with LRRK2 kinase inhibitors in Parkinson disease.

Exp Neurol. 2017;298:236-245. Doi:10.1016/j.expneurol.2017.07.019
24 Macaluso M, Krams M, Savitz J, Drevets WC, Preskorn SH. New Approaches in Translational Medicine for Phase I Clinical Trials of CNS Drugs. In: Handbook of Behavioral Neuroscience. Vol 29. Elsevier B.V.; 2019:81-91. Doi:10.1016/B978-0-12-803161-2.00006-0
25 Cohen AF, Burggraaf J, Van Gerven JMA, Moerland M, Groeneveld GJ. The use of biomarkers in human pharmacology (Phase I) studies. Annu Rev Pharmacol Toxicol. 2015;55(1):55-74. Doi:10.1146/annurev-pharmtox-011613-135918
26 Nagai Y, Eiko M. Drug development for neurodegenerative diseases. In: Neurodegenerative Disorders as Systemic Diseases. Springer Japan; 2015:183-216. Doi:10.1007/978-4-431-54541-5_9
27 Erkkinen MG, Kim MO, Geschwind MD. Clinical neurology and epidemiology of the major neurodegenerative diseases. Cold Spring Harb Perspect Biol. 2018;10(4). Doi:10.1101/cshperspect.a033118
28 Gan L, Cookson MR, Petrucelli L, La Spada AR. Converging pathways in neurodegeneration, from genetics to mechanisms. Nat Neurosci. 2018;21(10):1300-1309. Doi:10.1038/s41593-018-0237-7
29 Soto C, Pritzkow S. Protein misfolding, aggregation, and conformational strains in neurodegenerative diseases. Nat Neurosci. 2018;21(10):1332-1340. Doi:10.1038/s41593-018-0235-9
30 Grievink HW, Heuberger JAAC, Huang F, et al. DNL104, a Centrally Penetrant RIPK1 Inhibitor, Inhibits RIP1 Kinase Phosphorylation in a Randomized Phase I Ascending Dose Study in Healthy Volunteers. Clin Pharmacol Ther. 2020;107(2):406-414. Doi:10.1002/cpt.1615
31 West T, Hu Y, Verghese PB, et al. Preclinical and Clinical Development of ABBV-8E12, a Humanized Anti-Tau Antibody, for Treatment of Alzheimer's Disease and Other Tauopathies. J Prev Alzheimer's Dis. 2017;4(4):236-241. Doi:10.14283/jpad.2017.36
32 Hernandez DG, Reed X, Singleton AB. Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. J Neurochem. 2016;139(Suppl 1):59-74. Doi:10.1111/jnc.13593
33 Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. N Engl J Med. 2020;383(2):109-119. Doi:10.1056/NEJMoa2003715
34 Sardi SP, Cedarbaum JM, Brundin P. Targeted Therapies for Parkinson's Disease: From Genetics to the Clinic. Mov Disord. 2018;33(5):684-696. Doi:10.1002/mds.27414
35 de Visser SJ, Cohen AF, Kenter MJH. Integrating scientific considerations into R&D project valuation. Nat Biotechnol. 2020;38(1):14-18. Doi:10.1038/s41587-019-0358-x
36 Cummings J, Fox N. Defining Disease Modifying Therapy for Alzheimer's Disease. J Prev Alzheimer's Dis. 2017;4(2):109-115. Doi:10.14283/jpad.2017.12
37 Atkinson AJ, Colburn WA, DeGruttola VG, et al. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89-95. Doi:10.1067/mcp.2001.113989
38 Califf RM. Biomarker definitions and their applications. Exp Biol Med. 2018;243(3):213-221. Doi:10.1177/1535370217750088
39 Amur S, Lavange L, Zineh I, Buckman-Garner S, Woodcock J. Biomarker qualification: Toward a multiple stakeholder framework for biomarker development, regulatory acceptance, and utilization. Clin Pharmacol Ther. 2015;98(1):34-46. Doi:10.1002/cpt.136
40 Verber NS, Shepheard SR, Sassani M, et al. Biomarkers in motor neuron disease: A state of the art review. Front Neurol. 2019;10(APR). doi:10.3389/fneur.2019.00291
41 Blennow K, Zetterberg H. The Past and the Future of Alzheimer's Disease Fluid Biomarkers. J Alzheimer's Dis. 2018;62(3):1125-1140. Doi:10.3233/JAD-170773
42 Parnetti L, Gaetani L, Eusebi P, et al. CSF and blood biomarkers for Parkinson's disease. Lancet Neurol. 2019;18(6):573-586. Doi:10.1016/S1474-4422(19)30024-9
43 Silajdzic E, Bjorkqvist M. A critical evaluation of wet biomarkers for huntington's disease: Current status and ways forward. J Huntingtons Dis. 2018;7(2):109-135. Doi:10.3233/JHD-170273
44 Coarelli G, Brice A, Durr A. Recent advances in understanding dominant spinocerebellar ataxias from clinical and genetic points of view [version 1; referees: 3 approved]. F1000Research. 2018;7. Doi:10.12688/f1000research.15788.1
45 Cummings J. The Role of Biomarkers in Alzheimer's Disease Drug Development. In: Advances in Experimental Medicine and Biology. Vol 1118. Springer New York LLC; 2019:29-61. Doi:10.1007/978-3-030-05542-4_2
46 Danhof M, Alvan G, Dahl SG, Kuhlmann J, Paintaud G. Mechanism-based pharmacokinetic-pharmacodynamic modeling – A new classification of biomarkers. Pharm Res. 2005;22(9):1432-1437. Doi:10.1007/s11095-005-5882-3
47 Paul SM, Mytelka DS, Dunwiddie CT, et al. How to improve RD productivity: The pharmaceutical industry's grand challenge. Nat Rev Drug Discov. 2010;9(3):203-214. Doi:10.1038/nrd3078
48 US Food and Drug Administration. Amyotrophic Lateral Sclerosis: Developing Drugs for Treatment Guidance for Industry.; 2019.
49 Committee for Medicinal Products for Human Use (CHMP). Guideline on the Clinical Investigation of Medicines for the Treatment of Alzheimer's Disease.; 2018.
50 Committee for Medicinal Product for Human Use (CHMP). Guideline on Clinical Investigation of Medicinal Products for the Treatment of Amyotrophic Lateral Sclerosis (ALS).; 2013.
51 Cohen AF. Developing drug prototypes: Pharmacology replaces safety and tolerability? Nat Rev Drug Discov. 2010;9(11):856-865. Doi:10.1038/nrd3227
52 Committee for Medicinal Products for Human Use (CHMP). Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products. 20 Jul. Published online 2017.
53 Ferrero J, Williams L, Stella H, et al. First-in-human, double-blind, placebo-controlled, single-dose escalation study of aducanumab (BIIBo37) in mild-to-moderate Alzheimer's disease. Alzheimer's Dement Transl Res Clin Interv. 2016;2(3):169-176. Doi:10.1016/j.trci.2016.06.002
54 Logovinsky V, Satlin A, Lai R, et al. Safety and tolerability of BAN2401 – a clinical study in Alzheimer's disease with a protofibril selective Aβ antibody. Alzheimers Res Ther. 2016;8(1):14. Doi:10.1186/s13195-016-0181-2
55 Miyoshi I, Fujimoto Y, Yamada M, et al. Safety and pharmacokinetics of PF-04360365 following a single-dose intravenous infusion in Japanese subjects with mild-to-moderate Alzheimer's disease: A multicenter, randomized, double-blind, placebo-controlled, dose-escalation study. Int J Clin Pharmacol Ther. 2013;51(12):911-923. Doi:10.5414/CP201816
56 Landen JW, Zhao Q, Cohen S, et al. Safety and pharmacology of a single intravenous dose of ponezumab in subjects with mild-to-moderate hosphor disease: A phase I, randomized, placebo-controlled, double-blind, dose-escalation study. Clin Neuropharmacol. 2013;36(1):14-23. Doi:10.1097/WNF.0b013e31827db49b
57 Li L, Zhen EY, Decker RL, et al. Pharmacokinetics and Pharmacodynamics of LY2599666, a PEG-Linked Antigen Binding Fragment that Targets Soluble Monomer Amyloid-β. J Alzheimer's Dis. 2019;68(1):137-144. Doi:10.3233/JAD-180925
58 Lu M, Brashear HR. Pharmacokinetics, Pharmacodynamics, and Safety of Subcutaneous Bapineuzumab: A Single-Ascending-Dose Study in Patients With Mild to Moderate Alzheimer Disease. Clin Pharmacol Drug Dev. 2019;8(3):326-335. Doi:10.1002/cpdd.584
59 Arai H, Umemura K, Ichimiya Y, et al. Safety and pharmacokinetics of bapineuzumab in a single ascending-dose study in Japanese patients with mild to moderate Alzheimer's disease. Geriatr Gerontol Int. 2016;16(5):644-650. Doi:10.1111/ggi.12516
60 Leyhe T, Andreasen N, Simeoni M, et al. Modulation of β-amyloid by a single dose of GSK933776 in patients with mild Alzheimer's disease: A phase © study. Alzheimer's Res Ther. 2014;6(2):19. Doi:10.1186/alzrt249
61 Black RS, Sperling RA, Safirstein B, et al. A single ascending dose study of bapineuzumab in patients with hosphor disease. Alzheimer Dis Assoc Disord. 2010;24(2):198-203. Doi:10.1097/WAD.0b013e3181c53b00
62 Adolfsson O, Pihlgren M, Toni N, et al. An effector-reduced anti-β-amyloid (Aβ) antibody with unique Aβ binding properties promotes neuroprotection and glial engulfment of Aβ. J Neurosci. 2012;32(28):9677-9689. Doi:10.1523/JNeurosci.4742-11.2012
63 Arai H, Ichimiya Y, Shibata N, et al. Safety and tolerability of immune globulin intravenous (human), 10% solution in Japanese subjects with mild to moderate Alzheimer's disease. Psychogeriatrics. 2014;14(3):165-174. Doi:10.1111/psyg.12055
64 Qureshi IA, Tirucherai G, Ahlijanian MK, Kolaitis G, Bechtold C, Grundman M. A randomized, single ascending dose study of intravenous BIIB092 in healthy participants. Alzheimer's Dement Transl Res Clin Interv. 2018;4:746-755. Doi:10.1016/j.trci.2018.10.007
65 Kim HJ, Seo SW, Chang JW, et al. Stereotactic brain injection of human umbilical cord blood mesenchymal stem cells in patients with

Alzheimer's disease dementia: A phase 1 clinical trial. Alzheimer's Dement Transl Res Clin Interv. 2015;1(2):95-102. Doi:10.1016/j.trci.2015.06.007
66 Wahlberg LU, Lind G, Almqvist PM, et al. Targeted delivery of nerve growth factor via encapsulated cell biodelivery in Alzheimer disease: A technology platform for restorative neurosurgery – Clinical article. J Neurosurg. 2012;117(2):340-347. Doi:10.3171/2012.2.JNS11714
67 Nolan JM, Mulcahy R, Power R, Moran R, Howard AN. Nutritional Intervention to Prevent Alzheimer's Disease: Potential Benefits of Xanthophyll Carotenoids and Omega-3 Fatty Acids Combined. J Alzheimer's Dis. 2018;64(2):367-378. Doi:10.3233/JAD-180160
68 Rafii MS, Baumann TL, Bakay RAE, et al. A phase1 study of stereotactic gene delivery of AAV2-NGF for Alzheimer's disease. Alzheimers Dement. 2014;10(5):571-581. Doi:10.1016/j.jalz.2013.09.004
69 Eyjolfsdottir H, Eriksdotter M, Linderoth B, et al. Targeted delivery of nerve growth factor to the cholinergic basal forebrain of Alzheimer's disease patients: Application of a second-generation encapsulated cell biodelivery device. Alzheimer's Res Ther. 2016;8(1). Doi:10.1186/s13195-016-0195-9
70 Wang CY, Wang PN, Chiu MJ, et al. UB-311, a novel UBITh® amyloid β peptide vaccine for mild Alzheimer's disease. Alzheimer's Dement Transl Res Clin Interv. 2017;3(2):262-272. Doi:10.1016/j.trci.2017.03.005
71 Winblad B, Andreasen N, Minthon L, et al. Safety, tolerability, and antibody response of active Aβ immunotherapy with CAD106 in patients with Alzheimer's disease: Randomised, double-blind, placebo-controlled, first-in-human study. Lancet Neurol. 2012;11(7):597-604. Doi:10.1016/S1474-4422(12)70140-0
72 Lacosta AM, Pascual-Lucas M, Pesini P, et al. Safety, tolerability and immunogenicity of an active anti-Aβ 40 vaccine (Abvac40) in patients with Alzheimer's disease: A hosphord, double-blind, placebo-controlled, phase © trial. Alzheimer's Res Ther. 2018;10(1). Doi:10.1186/s13195-018-0340-8
73 Novak P, Schmidt R, Kontsekova E, et al. Safety and immunogenicity of the tau vaccine AADvac1 in patients with Alzheimer's disease: a hosphord, double-blind, placebo-controlled, phase 1 trial. Lancet Neurol. 2017;16(2):123-134. Doi:10.1016/S1474-4422(16)30331-3
74 Kutzsche J, Jürgens D, Willuweit A, et al. Safety and pharmacokinetics of the orally available antiprionic compound PRI-002: A single and multiple ascending dose phase I study. Alzheimer's Dement Transl Res Clin Interv. 2020;6(1). Doi:10.1002/trc2.12001
75 Lipsman N, Meng Y, Bethune AJ, et al. Blood–brain barrier opening in Alzheimer's disease using MR-guided focused ultrasound. Nat Commun. 2018;9(1):1-8. Doi:10.1038/s41467-018-04529-6
76 Smith GS, Laxton AW, Tang-Wai DF, et al. Increased cerebral metabolism after 1 year of deep brain stimulation in Alzheimer disease. Arch Neurol. 2012;69(9):1141-1148. Doi:10.1001/archneurol.2012.590
77 Noreik M, Kuhn J, Hardenacke K, et al. Changes in nutritional status after deep brain stimulation of the nucleus basalis of Meynert in Alzheimer's disease — Results of a phase I study. J Nutr Heal Aging. 2015;19(8):812-818. Doi:10.1007/s12603-015-0595-8
78 Laxton AW, Tang-Wai DF, McAndrews MP, et al. A phase © trial of deep brain stimulation of memory circuits in Alzheimer's disease. Ann Neurol. 2010;68(4):521-534. Doi:10.1002/ana.22089
79 Scharre DW, Weichart E, Nielson D, et al. Deep Brain Stimulation of Frontal Lobe Networks to Treat Alzheimer's Disease. J Alzheimer's Dis. 2018;62(2):621-633. Doi:10.3233/JAD-170082
80 Family N, Maillet EL, Williams LTJ, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of low dose lysergic acid diethylamide (LSD) in healthy older volunteers. Psychopharmacology (Berl). 2020;237(3):841-853. Doi:10.1007/s00213-019-05417-7
81 Maccecchini ML, Chang MY, Pan C, John V, Zetterberg H, Greig NH. Posiphen as a candidate drug to lower CSF amyloid precursor protein, amyloid-β peptide and τ levels: Target engagement, tolerabilityand pharmacokinetics in humans. J Neurol Neurosurg Psychiatry. 2012;83(9):894-902. Doi:10.1136/jnnp-2012-302589
82 Brazier D, Perry R, Keane J, Barrett K, Elmaleh DR. Pharmacokinetics of Cromolyn and Ibuprofen in Healthy Elderly Volunteers. Clin Drug Investig. 2017;37(11):1025-1034. Doi:10.1007/s40261-017-0549-5
83 Forman M, Palcza J, Tseng J, et al. Safety, Tolerability, and Pharmacokinetics of the β-Site Amyloid Precursor Protein-Cleaving Enzyme 1 Inhibitor Verubecestat (MK-8931) in Healthy Elderly Male and Female Subjects. Clin Transl Sci. 2019;12(5):545-555. Doi:10.1111/cts.12645
84 Chris Min K, Dockendorf MF, Palcza J, et al. Pharmacokinetics and Pharmacodynamics of the BACE1 Inhibitor Verubecestat (MK-8931) in Healthy Japanese Adults: A Randomized, Placebo-Controlled Study. Clin Pharmacol Ther. 2019;105(5):1234-1243. Doi:10.1002/cpt.1258
85 Timmers M, Streffer JR, Russu A, et al. Pharmacodynamics of atabecestat (JNJ-54861911), an oral BACE1 inhibitor in patients with early Alzheimer's disease: Randomized, double-blind, placebo-controlled study. Alzheimer's Res Ther. 2018;10(1):85. Doi:10.1186/s13195-018-0415-6
86 Sakamoto K, Matsuki S, Matsuguma K, et al. BACE1 Inhibitor Lanabecestat (AZD3293) in a Phase 1 Study of Healthy Japanese Subjects: Pharmacokinetics and Effects on Plasma and Cerebrospinal Fluid Aβ Peptides. J Clin Pharmacol. 2017;57(11):1460-1471. Doi:10.1002/jcph.950
87 Cebers G, Alexander RC, Haeberlein SB, et al. AZD3293: Pharmacokinetic and pharmacodynamic effects in healthy subjects and patients with Alzheimer's disease. J Alzheimer's Dis. 2017;55(3):1039-1053. Doi:10.3233/JAD-160701
88 Kennedy ME, Stamford AW, Chen X, et al. The BACE1 inhibitor verubecestat (MK-8931) reduces CNS b-Amyloid in animal models and in Alzheimer's disease patients. Sci Transl Med. 2016;8(363):363ra150-363ra150. Doi:10.1126/scitranslmed.aad9704
89 Timmers M, Van Broeck B, Ramael S, et al. Profiling the dynamics of CSF and plasma Aβ reduction after treatment with JNJ-54861911, a potent oral BACE inhibitor. Alzheimer's Dement Transl Res Clin Interv. 2016;2(3):202-212. Doi:10.1016/j.trci.2016.08.001
90 Qiu R, Ahn JE, Alexander R, et al. Safety, Tolerability, Pharmacokinetics, and Pharmacodynamic Effects of PF-06751979, a Potent and Selective Oral BACE1 Inhibitor: Results from Phase © Studies in Healthy Adults and Healthy Older Subjects. J Alzheimer's Dis. 2019;71(2):581-595. Doi:10.3233/JAD-190228
91 Gulati A, Hornick MG, Briyal S, Lavhale MS. A Novel Neuroregenerative Approach Using ET B Receptor Agonist, IRL-1620, to Treat CNS Disorders. Physiol Res. 2018;67:95-113. Doi:10.33549/physiolres.933859
92 Lues I, Weber F, Meyer A, et al. A phase 1 study to evaluate the safety and pharmacokinetics of PQ912, a glutaminyl cyclase inhibitor, in healthy subjects This work was previously published as abstracts presented at AAIC 2013 Conference Boston. Alzheimer's Dement Transl Res Clin Interv. 2015;1(3):182-195. Doi:10.1016/j.trci.2015.08.002
93 Georgievska B, Sandin J, Doherty J, et al. AZD1080, a novel GSK3 inhibitor, rescues synaptic plasticity deficits in rodent brain and exhibits peripheral target engagement in humans. J Neurochem. 2013;125(3):446-456. Doi:10.1111/jnc.12203
94 Grundman M, Morgan R, Lickliter JD, et al. A phase 1 clinical trial of the sigma-2 receptor complex allosteric antagonist CT1812, a novel therapeutic candidate for Alzheimer's disease. Alzheimer's Dement Transl Res Clin Interv. 2019;5:20-26. Doi:10.1016/j.trci.2018.11.001
95 Ahn JE, Carrieri C, Dela Cruz F, et al. Pharmacokinetic and Pharmacodynamic Effects of a γ-Secretase Modulator, PF-06648671, on CSF Amyloid-β Peptides in Randomized Phase I Studies. Clin Pharmacol Ther. 2020;107(1):211-220. Doi:10.1002/cpt.1570
96 Yu Y, Logovinsky V, Schuck E, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of the novel γ-secretase modulator, E2212, in healthy human subjects. J Clin Pharmacol. 2014;54(5):528-536. Doi:10.1002/jcph.249
97 Tsai RM, Miller Z, Koestler M, et al. Reactions to Multiple Ascending Doses of the Microtubule Stabilizer TPI-287 in Patients with Alzheimer Disease, Progressive Supranuclear Palsy, and Corticobasal Syndrome: A Randomized Clinical Trial. JAMA Neurol. 2020;77(2):215-224. Doi:10.1001/jamaneurol.2019.3812
98 Duma C, Kopyov O, Kopyov A, et al. Human intracerebroventricular (ICV) injection of autologous, non-engineered, adipose-derived stromal vascular fraction (ADSVF) for neurodegenerative disorders: results of a 3-year phase 1 study of 113 injections in 31 patients. Mol Biol Rep. 2019;46(5):5257-5272. Doi:10.1007/s11033-019-04983-5
99 Meininger V, Pradat PF, Corse A, et al. Safety, pharmacokinetic, and functional effects of the Nogo-A monoclonal antibody in amyotrophic lateral sclerosis: A randomized, first-in-human clinical trial. PloS One. 2014;9(5). Doi:10.1371/journal.pone.0097803
100 Miller TM, Pestronk A, David W, et al. An antisense oligonucleotide against SOD1 delivered intrathecally for patients with SOD1 familial amyotrophic lateral sclerosis: A phase 1, hosphord, first-in-man study. Lancet Neurol. 2013;12(5):435-442. Doi:10.1016/S1474-4422(13)70061-9

101 Oh K-W, Moon C, Kim HY, et al. Phase I Trial of Repeated Intrathecal Autologous Bone Marrow-Derived Mesenchymal Stromal Cells in Amyotrophic Lateral Sclerosis. Stem Cells Transl Med. 2015;4(6):590-597. Doi:10.5966/sctm.2014-0212

102 Mazzini L, Ferrero I, Luparello V, et al. Mesenchymal stem cell transplantation in amyotrophic lateral sclerosis: A Phase I clinical trial. Exp Neurol. 2010;223(1):229-237. Doi:10.1016/j.expneurol.2009.08.007

103 Blanquer M, Moraleda JM, Iniesta F, et al. Neurotrophic bone marrow cellular nests prevent spinal motoneuron degeneration in amyotrophic lateral sclerosis patients: A pilot safety study. Stem Cells. 2012;30(6):1277-1285. Doi:10.1002/stem.1080

104 Petrou P, Gothelf Y, Argov Z, et al. Safety and clinical effects of mesenchymal stem cells secreting neurotrophic factor transplantation in patients with amyotrophic lateral sclerosis. JAMA Neurol. 2016;73(3):337-344. Doi:10.1001/jamaneurol.2015.4321

105 Staff NP, Madigan NN, Morris J, et al. Safety of intrathecal autologous adipose-derived mesenchymal stromal cells in patients with ALS. Neurology. 2016;87(21):2230-2234. Doi:10.1212/WNL.0000000000003359

106 Syková E, Rychmach P, Drahorádová I, et al. Transplantation of mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: Results of phase I/IIa clinical trial. Cell Transplant. 2017;26(4):647-658. Doi:10.3727/096368916X693716

107 Mazzini L, Gelati M, Profico DC, et al. Human neural stem cell transplantation in ALS: Initial results from a phase I trial. J Transl Med. 2015;13(1). Doi:10.1186/s12967-014-0371-2

108 Feldman EL, Boulis NM, Hur J, et al. Intraspinal neural stem cell transplantation in amyotrophic lateral sclerosis: Phase 1 trial outcomes. Ann Neurol. 2014;75(3):363-373. Doi:10.1002/ana.24113

109 Geijo-Barrientos E, Pastore-Olmedo C, De Mingo P, et al. Intramuscular Injection of Bone Marrow Stem Cells in Amyotrophic Lateral Sclerosis Patients: A Randomized Clinical Trial. Front Neurosci. 2020;14. Doi:10.3389/fnins.2020.00195

110 Thonhoff JR, Beers DR, Zhao W, et al. Expanded autologous regulatory T-lymphocyte infusions in ALS A phase I, first-in-human study. Neurol Neuroimmunol NeuroInflammation. 2018;5(4):465. Doi:10.1212/NXI.0000000000000465

111 Nabavi SM, Arab L, Jarooghi N, et al. Safety, feasibility of intravenous and intrathecal injection of autologous bone marrow derived mesenchymal stromal cells in patients with amyotrophic lateral sclerosis: An open label phase I clinical trial. Cell J. 2019;20(4):592-598. Doi:10.22074/cellj.2019.5370

112 Riley J, Glass J, Feldman EL, et al. Intraspinal stem cell transplantation in amyotrophic lateral sclerosis: A phase I trial, cervical microinjection, and final surgical safety outcomes. Neurosurgery. 2014;74(1):77-87. Doi:10.1227/NEU.0000000000000156

113 Glass JD, Boulis NM, Johe K, et al. Lumbar intraspinal injection of neural stem cells in patients with amyotrophic lateral sclerosis: Results of a phase I trial in 12 patients. Stem Cells. 2012;30(6):1144-1151. Doi:10.1002/stem.1079

114 Sufit RL, Ajroud-Driss S, Casey P, Kessler JA. Open label study to assess the safety of VM202 in subjects with amyotrophic lateral sclerosis. Amyotroph Lateral Scler Front Degener. 2017;18(3-4):269-278. Doi:10.1080/21678421.2016.1259334

115 Chiò A, Mora G, La Bella V, et al. Repeated courses of granulocyte colony-stimulating factor in amyotrophic lateral sclerosis: Clinical and biological results from a prospective multicenter study. Muscle and Nerve. 2011;43(2):189-195. Doi:10.1002/mus.21851

116 Warita H, Kato M, Asada R, et al. Safety, Tolerability, and Pharmacodynamics of Intrathecal Injection of Recombinant Human HGF (KP-100) in Subjects With Amyotrophic Lateral Sclerosis: A Phase I Trial. J Clin Pharmacol. 2019;59(5):677-687. Doi:10.1002/jcph.1355

117 Berry JD, Shefner JM, Conwit R, et al. Design and Initial Results of a Multi-Phase Randomized Trial of Ceftriaxone in Amyotrophic Lateral Sclerosis. PloS One. 2013;8(4). Doi:10.1371/journal.pone.0061177

118 Bozik ME, Mather JL, Kramer WG, Gribkoff VK, Ingersoll EW. Safety, tolerability, and pharmacokinetics of KNS-760704 (dexpramipexole) in healthy adult subjects. J Clin Pharmacol. 2011;51(8):1177-1185. Doi:10.1177/0091270010379412

119 Miller RG, Zhang R, Block G, et al. NP001 regulation of macrophage activation markers in ALS: A phase I clinical and biomarker study. Amyotroph Lateral Scler Front Degener. 2014;15(7-8):601-609. Doi:10.3109/21678421.2014.951940

120 Lange DJ, Shahbazi M, Silani V, et al. Pyrimethamine significantly lowers cerebrospinal fluid Cu/Zn superoxide dismutase in amyotrophic lateral sclerosis patients with SOD1 mutations. Ann Neurol. 2017;81(6):837-848. Doi:10.1002/ana.24950

121 Lange DJ, Andersen PM, Remanan R, Marklund S, Benjamin D. Pyrimethamine decreases levels of SOD1 in leukocytes and cerebrospinal fluid of ALS patients: A phase © pilot study. Amyotroph Lateral Scler Front Degener. 2013;14(3):199-204. Doi:10.3109/17482968.2012.724074

122 Levine TD, Miller RG, Bradley WG, et al. Phase I clinical trial of safety of L-serine for ALS patients. Amyotroph Lateral Scler Front Degener. 2017;18(1-2):107-111. Doi:10.1080/21678421.2016.1221971

123 Atassi N, Ratai EM, Greenblatt DJ, et al. A phase I, pharmacokinetic, dosage escalation study of creatine monohydrate in subjects with amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 2010;11(6):508-513. Doi:10.3109/17482961003797130

124 Ackermann EJ, Guo S, Benson MD, et al. Suppressing transthyretin production in mice, monkeys and humans using 2nd-Generation antisense oligonucleotides. Amyloid. 2016;23(3):148-157. Doi:10.1080/13506129.2016.1191458

125 Coelho T, Adams D, Silva A, et al. Safety and Efficacy of RNAi Therapy for Transthyretin Amyloidosis. N Engl J Med. 2013;369(9):819-829. Doi:10.1056/nejmoa1208760

126 Soragni E, Miao W, Iudicello M, et al. Epigenetic Therapy for Friedreich ataxia. Ann Neurol. 2014;76(4):489-508. Doi:10.1002/ana.24260

127 Libri V, Yandim C, Athanasopoulos S, et al. Epigenetic and neurological effects and safety of high-dose nicotinamide in patients with Friedreich's ataxia: An exploratory, open-label, dose-escalation study. Lancet. 2014;384(9942):504-513. Doi:10.1016/S0140-6736(14)60382-2

128 Zesiewicz T, Heerinckx F, De Jager R, et al. Randomized, clinical trial of RT001: Early signals of efficacy in Friedreich's ataxia. Mov Disord. 2018;33(6):1000-1005. Doi:10.1002/mds.27353

129 Sha SJ, Miller ZA, Min S won, et al. An 8-week, open-label, dose-finding study of nimodipine for the treatment of progranulin insufficiency from GRN gene mutations. Alzheimer's Dement Transl Res Clin Interv. 2017;3(4):507-512. Doi:10.1016/j.trci.2017.08.002

130 Clarke JTR, Mahuran DJ, Sathe S, et al. An open-label Phase I/II clinical trial of pyrimethamine for the treatment of patients affected with chronic GM2 gangliosidosis (Tay-Sachs or Sandhoff variants). Mol Genet Metab. 2011;102(1):6-12. Doi:10.1016/j.ymgme.2010.09.004

131 Tabrizi SJ, Leavitt BR, Landwehrmeyer GB, et al. Targeting Huntingtin Expression in Patients with Huntington's Disease. N Engl J Med. 2019;380(24):2307-2316. Doi:10.1056/nejmoa1900907

132 Diemen MPJ, Hart EP, Abbruscato A, et al. Safety, Pharmacokinetics and Pharmacodynamics of SBT-020 in Patients with Early Stage Huntington's Disease, a two-part study. Br J Clin Pharmacol. Published online November 16, 2020:bcp.14656. doi:10.1111/bcp.14656

133 Guy J, Feuer WJ, Davis JL, et al. Gene Therapy for Leber Hereditary Optic Neuropathy: Low- and Medium-Dose Visual Results. Ophthalmology. 2017;124(11):1621-1634. Doi:10.1016/j.ophtha.2017.05.016

134 Feuer WJ, Schiffman JC, Davis JL, et al. Gene therapy for leber hereditary optic neuropathy initial results. Ophthalmology. 2016;123(3):558-570. Doi:10.1016/j.ophtha.2015.10.025

135 LaGanke C, Samkoff L, Edwards K, et al. Safety/tolerability of the anti-semaphorin 4D antibody VX15/2503 in a randomized phase 1 trial. Neurol Neuroimmunol NeuroInflammation. 2017;4(4):367. Doi:10.1212/NXI.0000000000000367

136 Cohen JA, Imrey PB, Planchon SM, et al. Pilot trial of intravenous autologous culture-expanded mesenchymal stem cell transplantation in multiple sclerosis. Mult Scler J. 2018;24(4):501-511. Doi:10.1177/1352458517703802

137 Karussis D, Karageorgiou C, Vaknin-Dembinsky A, et al. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol. 2010;67(10):1187-1194. Doi:10.1001/archneurol.2010.248

138 Ziemssen T, Tumani H, Sehr T, et al. Safety and in vivo immune assessment of escalating doses of oral laquinimod in patients with RRMS. J Neuroinflammation. 2017;14(1):172. Doi:10.1186/s12974-017-0945-z

139 Kosa P, Wu T, Phillips J, et al. Idebenone does not inhibit disability progression in primary progressive MS. Mult Scler Relat Disord. 2020;45:102434. Doi:10.1016/j.msard.2020.102434

140 Singer W, Dietz AB, Zeller AD, et al. Intrathecal administration of autologous mesenchymal stem cells in multiple system atrophy. Neurology. 2019;93(1):E77-E87. Doi:10.1212/WNL.0000000000007720

141 Meissner WG, Traon AP Le, Foubert-Samier A, et al. A Phase 1 Randomized Trial of Specific Active α-Synuclein Immunotherapies PD01A and PD03A in Multiple System Atrophy. Mov Disord. 2020;35(11):1957-1965. Doi:10.1002/mds.28218
142 Selden NR, Al-Uzri A, Huhn SL, et al. Central nervous system stem cell transplantation for children with neuronal ceroid lipofuscinosis. J Neurosurg Pediatr. 2013;11(6):643-652. Doi:10.3171/2013.3.PEDS12397
143 Kim A, Grover A, Hammon K, et al. Clinical Pharmacokinetics and Pharmacodynamics of Cerliponase Alfa, Enzyme Replacement Therapy for CLN2 Disease by Intracerebroventricular Administration. Clin Transl Sci. Published online November 30, 2020:cts.12925. doi:10.1111/cts.12925
144 Ory DS, Ottinger EA, Farhat NY, et al. Intrathecal 2-hydroxypropyl-β-cyclodextrin decreases neurological disease progression in Niemann-Pick disease, type C1: a non-randomised, open-label, phase 1–2 trial. Lancet. 2017;390(10104):1758-1768. Doi:10.1016/S0140-6736(17)31465-4
145 Brys M, Fanning L, Hung S, et al. Randomized phase I clinical trial of anti–α-synuclein antibody BIIB054. Mov Disord. 2019;34(8):1154-1163. Doi:10.1002/mds.27738
146 Jankovic J, Goodman I, Safirstein B, et al. Safety and Tolerability of Multiple Ascending Doses of PRX002/RG7935, an Anti–Synuclein Monoclonal Antibody, in Patients with Parkinson Disease: A Randomized Clinical Trial. JAMA Neurol. 2018;75(10):1206-1214. Doi:10.1001/jamaneurol.2018.1487
147 Schenk DB, Koller M, Ness DK, et al. First-in-human assessment of PRX002, an anti–α-synuclein monoclonal antibody, in healthy volunteers. Mov Disord. 2017;32(2):211-218. Doi:10.1002/mds.26878
148 Horne CG Van, Quintero JE, Gurwell JA, Wagner RP, Slevin JT, Gerhardt GA. Implantation of autologous peripheral nerve grafts into the substantia nigra of subjects with idiopathic Parkinson's disease treated with bilateral STN DBS: a report of safety and feasibility. J Neurosurg. 2016;126(April):1-8. Doi:10.3171/2016.2.JNS151988.1140
149 Christine CW, Bankiewicz KS, Van Laar AD, et al. Magnetic resonance imaging–guided phase 1 trial of putaminal AADC gene therapy for Parkinson's disease. Ann Neurol. 2019;85(5):704-714. Doi:10.1002/ana.25450
150 Mittermeyer G, Christine CW, Rosenbluth KH, et al. Long-term evaluation of a phase 1 study of AADC gene therapy for hosphor's disease. Hum Gene Ther. 2011;23(4):377-381. Doi:10.1089/hum.2011.220
151 Muramatsu SI, Fujimoto KI, Kato S, et al. A phase © study of aromatic l-amino acid decarboxylase gene therapy for hosphor's disease. Mol Ther. 2010;18(9):1731-1735. Doi:10.1038/mt.2010.135
152 Palfi S, Gurruchaga JM, Scott Ralph G, et al. Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: A dose escalation, open-label, phase 1/2 trial. Lancet. 2014;383(9923):1138-1146. Doi:10.1016/S0140-6736(13)61939-X
153 Tsai ST, Chu SC, Liu SH, et al. Neuroprotection of granulocyte colony-stimulating factor for early stage Parkinson's disease. Cell Transplant. 2017;26(3):409-416. Doi:10.3727/096368916X694247
154 Gendelman HE, Zhang Y, Santamaria P, et al. Evaluation of the safety and immunomodulatory effects of sargramostim in a randomized, double-blind phase 1 clinical Parkinson's disease trial. Npj Park Dis. 2017;3(1):1-11. Doi:10.1038/s41531-017-0013-5
155 Paul G, Zachrisson O, Varrone A, et al. Safety and tolerability of intracerebroventricular PDGF-BB in Parkinson's disease patients. J Clin Invest. 2015;125(3):1339-1346. Doi:10.1172/JCI79635
156 Volc D, Poewe W, Kutzelnigg A, et al. Safety and immunogenicity of the α-synuclein active immunotherapeutic PD01A in patients with Parkinson's disease: a hosphord, single-blinded, phase 1 trial. Lancet Neurol. 2020;19(7):591-600. Doi:10.1016/S1474-4422(20)30136-8
157 Charles D, Tolleson C, Davis TL, et al. Pilot study assessing the feasibility of applying bilateral subthalamic nucleus deep brain stimulation in very early stage Parkinson's disease: Study design and rationale. J Parkinsons Dis. 2012;2(3):215-223. Doi:10.3233/JPD-2012-012095
158 Peterschmitt MJ, Crawford NPS, Gaemers SJM, Ji AJ, Sharma J, Pham TT. Pharmacokinetics, Pharmacodynamics, Safety, and Tolerability of Oral Venglustat in Healthy Volunteers. Clin Pharmacol Drug Dev. Published online August 26, 2020:cpdd.865. doi:10.1002/cpdd.865
159 Jucaite A, Svenningsson P, Rinne JO, et al. Effect of the myeloperoxidase inhibitor AZD3241 on microglia: A PET study in Parkinson's disease. Brain. 2015;138(9):2687-2700. Doi:10.1093/brain/awv184
160 Li M, Shi A, Pang H, et al. Safety, tolerability, and pharmacokinetics of a single ascending dose of baicalein chewable tablets in healthy subjects. J Ethnopharmacol. 2014;156:210-215. Doi:10.1016/j.jep.2014.08.031
161 Mischley LK, Leverenz JB, Lau RC, et al. A randomized, double-blind phase I/Iia study of intranasal glutathione in Parkinson's disease. Mov Disord. 2015;30(12):1696-1701. Doi:10.1002/mds.26351
162 Boxer AL, Qureshi I, Ahlijanian M, et al. Safety of the tau-directed monoclonal antibody BIIB092 in progressive supranuclear palsy: a hosphord, placebo-controlled, multiple ascending dose phase 1b trial. Lancet Neurol. 2019;18(6):549-558. Doi:10.1016/S1474-4422(19)30139-5
163 Canesi M, Giordano R, Lazzari L, et al. Finding a new therapeutic approach for no-option Parkinsonisms: Mesenchymal stromal cells for progressive supranuclear palsy. J Transl Med. 2016;14(1):127. Doi:10.1186/s12967-016-0880-2
164 VandeVrede L, Dale ML, Fields S, et al. Open-Label Phase 1 Futility Studies of Salsalate and Young Plasma in Progressive Supranuclear Palsy. Mov Disord Clin Pract. 2020;7(4):440-447. Doi:10.1002/mdc3.12940
165 Tsai YA, Liu RS, Lirng JF, et al. Treatment of spinocerebellar ataxia with mesenchymal stem cells: A phase I/Iia clinical study. Cell Transplant. 2017;26(3):503-512. Doi:10.3727/096368916X694373
166 Santos-Morales O, Díaz-Machado A, Jiménez-Rodríguez D, et al. Nasal administration of the neuroprotective candidate NeuroEPO to healthy volunteers: A randomized, parallel, open-label safety study. BMC Neurol. 2017;17(1). Doi:10.1186/s12883-017-0908-0
167 Chiriboga CA, Swoboda KJ, Darras BT, et al. Results from a phase 1 study of nusinersen (ISIS-SMN Rx) in children with spinal muscular atrophy. Neurology. 2016;86(10):890-897. Doi:10.1212/WNL.0000000000002445
168 Kletzl H, Marquet A, Günther A, et al. The oral splicing modifier RG7800 increases full length survival of motor neuron 2 mRNA and survival of motor neuron protein: Results from trials in healthy adults and patients with spinal muscular atrophy. Neuromuscul Disord. 2019;29(1):21-29. Doi:10.1016/j.nmd.2018.10.001
169 Sturm S, Günther A, Jaber B, et al. A phase 1 healthy male volunteer single escalating dose study of the pharmacokinetics and pharmacodynamics of risdiplam (RG7916, RO7034067), a SMN2 splicing modifier. Br J Clin Pharmacol. 2019;85(1):181-193. Doi:10.1111/bcp.13786
170 Mendell JR, Al-Zaidy S, Shell R, et al. Single-Dose Gene-Replacement Therapy for Spinal Muscular Atrophy. N Engl J Med. 2017;377(18):1713-1722. Doi:10.1056/nejmoa1706198
171 Stewart JJ, Green CL, Jones N, et al. Role of receptor occupancy assays by flow cytometry in drug development. Cytometry B Clin Cytom. 2016;90(2):110-116. Doi:10.1002/cyto.b.21355
172 Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. N Engl J Med. 2006;355(10):1018-1028. Doi:10.1056/NEJMoa063842
173 Muller PY, Brennan FR. Safety assessment and dose selection for first-in-human clinical trials with immunomodulatory monoclonal antibodies. Clin Pharmacol Ther. 2009;85(3):247-258. Doi:10.1038/clpt.2008.273
174 Cummings J, Ritter A, Zhong K. Clinical Trials for Disease-Modifying Therapies in Alzheimer's Disease: A Primer, Lessons Learned, and a Blueprint for the Future. J Alzheimer's Dis. 2018;64(s1):S3-S22. Doi:10.3233/JAD-179901
175 Cudkowicz ME, Titus S, Kearney M, et al. Safety and efficacy of ceftriaxone for amyotrophic lateral sclerosis: A multi-stage, hosphord, double-blind, placebo-controlled trial. Lancet Neurol. 2014;13(11):1083-1091. Doi:10.1016/S1474-4422(14)70222-4
176 Haché M, Swoboda KJ, Sethna N, et al. Intrathecal Injections in Children with Spinal Muscular Atrophy: Nusinersen Clinical Trial Experience. J Child Neurol. 2016;31(7):899-906. Doi:10.1177/0883073815627882
177 de Lange EC. The mastermind approach to CNS drug therapy: translational prediction of human brain distribution, target site kinetics, and therapeutic effects. Fluids Barriers CNS. 2013;10(1):12. Doi:10.1186/2045-8118-10-12
178 Takano A, Varrone A, Gulyás B, et al. Guidelines to PET measurements of the target occupancy in the brain for drug development. Eur J Nucl Med Mol Imaging. 2016;43(12):2255-2262. Doi:10.1007/s00259-016-3476-4
179 Johnström P, Bergman L, Varnäs K, Malmquist J, Halldin C, Farde L. Development of rapid multistep carbon-11 radiosynthesis of the myeloperoxidase inhibitor AZD3241 to assess brain exposure by PET microdosing. Nucl Med Biol. 2015;42(6):555-560. Doi:10.1016/j.nucmedbio.2015.02.001

180 Liang M, Schwickart M, Schneider AK, et al. Receptor occupancy assessment by flow cytometry as a pharmacodynamic biomarker in biopharmaceutical development. Cytom Part B – Clin Cytom. 2016;90(2):117-127. Doi:10.1002/cyto.b.21259
181 Dzamko N, Deak M, Hentati F, et al. Inhibition of LRRK2 kinase activity leads to dephosphorylation of Ser(910)/Ser(935), disruption of 14-3-3 binding and altered cytoplasmic localization. Biochem J. 2010;430(3):405-413. Doi:10.1042/BJ20100784
182 Coimbra JRM, Marques DFF, Baptista SJ, et al. Highlights in BACE1 inhibitors for Alzheimer's disease treatment. Front Chem. 2018;6(MAY):178. Doi:10.3389/fchem.2018.00178
183 West AB, Cookson MR. Identification of Bona Fide LRRK2 Kinase Substrates. Mov Disord. 2016;31(8):1140-1141. Doi:10.1002/mds.26647
184 Steger M, Tonelli F, Ito G, et al. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. Elife. 2016;5:e12813. Doi:10.7554/eLife.12813.001
185 Prins ND, Scheltens P. Treating Alzheimer's disease with monoclonal antibodies: Current status and outlook for the future. Alzheimer's Res Ther. 2013;5(6):56. Doi:10.1186/alzrt220
186 Collins R, Bowman L, Landray M, Peto R. The Magic of Randomization versus the Myth of Real-World Evidence. N Engl J Med. 2020;382(7):674-678. Doi:10.1056/nejmsb1901642
187 Lipsmeier F, Taylor KI, Kilchenmann T, et al. Evaluation of smartphone-based testing to generate exploratory outcome measures in a phase 1 Parkinson's disease clinical trial. Mov Disord. 2018;33(8):1287-1297. Doi:10.1002/mds.27376
188 Htike TT, Mishra S, Kumar S, Padmanabhan P, Gulyás B. Peripheral Biomarkers for Early Detection of Alzheimer's and Parkinson's Diseases. Mol Neurobiol. 2019;56(3):2256-2277. Doi:10.1007/s12035-018-1151-4
189 Fan Y, Howden AJM, Sarhan AR, et al. Interrogating Parkinson's disease LRRK2 kinase pathway activity by assessing Rab10 phosphorylation in human neutrophils. Biochem J. 2018;475(1):23-44. Doi:10.1042/BCJ20170803
190 Seol W, Kim H, Son I. Urinary Biomarkers for Neurodegenerative Diseases. Exp Neurobiol. 2020;29(5):325-333. Doi:10.5607/en20042
191 Shen J, Swift B, Mamelok R, Pine S, Sinclair J, Attar M. Design and Conduct Considerations for First-in-Human Trials. Clin Transl Sci. 2019;12(1):6-19. Doi:10.1111/cts.12582
192 Sattler R, Ayukawa Y, Coddington L, et al. Human nasal olfactory epithelium as a dynamic marker for CNS therapy development. Exp Neurol. 2011;232(2):203-211. Doi:10.1016/j.expneurol.2011.09.002
193 Teves JMY, Bhargava V, Kirwan KR, et al. Parkinson's Disease Skin Fibroblasts Display Signature Alterations in Growth, Redox Homeostasis, Mitochondrial Function, and Autophagy. Front Neurosci. 2018;11(JAN):737. Doi:10.3389/fnins.2017.00737
194 Tanis KQ, Podtelezhnikov AA, Blackman SC, et al. An Accessible Pharmacodynamic Transcriptional Biomarker for Notch Target Engagement. Clin Pharmacol Ther. 2016;99(4):370-380. Doi:10.1002/cpt.335
195 Sevigny J, Suhy J, Chiao P, et al. Amyloid PET screening for enrichment of early-stage Alzheimer disease clinical trials: Experience in a phase 1b clinical trial. Alzheimer Dis Assoc Disord. 2016;30(1):1-7. Doi:10.1097/WAD.0000000000000144
196 Yee LM, Lively TG, McShane LM. Biomarkers in early-phase trials: fundamental issues. Bioanalysis. 2018;10(12):933-944. Doi:10.4155/bio-2018-0006
197 Huang F, Zhu Y, Hsiao-Nakamoto J, et al. Longitudinal biomarkers in amyotrophic lateral sclerosis. Ann Clin Transl Neurol. Published online 2020. Doi:10.1002/©3.51078
198 Bordoni M, Rey F, Fantini V, et al. From neuronal differentiation of iPSCs to 3D neuro-organoids: Modelling and therapy of neurodegenerative diseases. Int J Mol Sci. 2018;19(12). Doi:10.3390/ijms19123972
199 Houghton R, Chamberlain J. Conference Report: Analytical challenges in the qualification and validation of pharmacodynamic biomarkers. In: Bioanalysis. Vol 3. Future Science Ltd London, UK ; 2011:945-948. Doi:10.4155/bio.11.90
200 Mullard A. News in Brief: Innovative antidepressants arrive; Anti-amyloid failures stack up as Alzheimer antibody flops. Nat Rev Drug Discov. 2019;18(may):2019.
201 van den Brink WJ, van den Berg DJ, Bonsel FEM, et al. Fingerprints of CNS drug effects: a plasma neuroendocrine reflection of D2 receptor activation using multi-biomarker pharmacokinetic/pharmacodynamic modelling. Br J Pharmacol. 2018;175(19):3832-3843. Doi:10.1111/bph.14452
202 Fda, Cder, Mccrayk. Early Alzheimer's Disease: Developing Drugs for Treatment Guidance for Industry Draft Guidance.