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A Review of NeuroAiD™ II (MLC901) Development in Alzheimer's Disease Treatment: Promises of A Multimodal Pathway

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ABSTRACT

Background: Alzheimer's disease (AD) is a clinical and economic burden on society. Without new treatment, the impact of AD on society could triple by 2050.

Aim: After a brief overview of treatments and challenges of new drug developments for AD, we reviewed the preclinical and clinical development program of NeuroAiD (MLC901, MLC601).

Method: A literature search was conducted by using different web sources. The initial screening was based on keywords contained in the subtitles of each corresponding paragraphs of this article. We sorted the reviewed publications by relevance and publication date selecting 74 references out of the 319 initially shortlisted for review.

Review: Since 1998, only symptomatic drugs were marketed. Intensive research has continued, aimed at delaying the onset of the disease and/or slowing its progression. However, the predictive value of delaying the onset of AD remains debated. Since 2003, aducanumab is the first new treatment approved and registered by US-FDA as an amyloid beta-directed antibody indicated for the treatment of Alzheimer's disease under post-marketing conditions. Traditional medicines (TM) have shown interesting results, but many of TM clinical studies leave much to be desired from a methodological point of view. Among TM, NeuroAiD (MLC901/601), a botanical-derived combination, acts in a multimodal pathway combining neuroprotective and neuro-regenerative properties. It has demonstrated sustained symptomatic benefits, slowing the disease progression in AD with a good safety profile.

Discussion/Conclusions: The discovery of treatments preventing or slowing down the disease progression, are necessary to get reliable diagnostic tools to confirm AD diagnosis, and follow its evolution and long-term therapy. A growing consensus is emerging on the need for a multi-factorial approach to the treatment and the development of suitable AD drug combinations. Such an approach has been that of TM for a long time. This is the case for NeuroAiD, that it may be integrated safely either after symptomatic treatments have failed or on top of symptomatic treatments.

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List of Abbreviations

Aβ: β-amyloid

AChEIs: Acetylcholinesterase Inhibitors

AD: Alzheimer's Disease

ADAS-Cog: Alzheimer's Disease Assessment Scale - Cognitive subscale

AE(s): Adverse Event(s)

APP: Amyloid Precursor Protein
Bax: Bcl-2-associated X-protein
BrdU: 5'-Bromodeoxyuridine
BDNF: Brain-Derived Neurotrophic Factor
CA1: Cornu Ammonis
CDR: Clinical Dementia Rating
C-EXIT25: Chinese version of the executive interview
CI: Confidence Interval
DG: Dental Gyrus
DLPFC: Dorsolateral Prefrontal Cortex
DMT: Disease Modifier (or Modifying) Treatment
DCX: Doublecortin
I^2: I Square Index to Quantify the Dispersion of Effect Sizes in A Meta-Analysis.
ITT: Intention-To-Treat
IV: Intravenous
KATP: ATP-sensitive potassium
MMSE: Mini-Mental State Examination
NFT: Neurofibrillary Tangles
NMDA: N-methyl-d-aspartate receptor antagonist
NMDAR: NMDA Receptor
NSE: neuron-specific enolase
PP: Per-Protocol
S100b: S100 beta
SAE(s): Serious Adverse Event(s)
sAPPα: soluble APPα
TBI: Traumatic Brain Injury
TCM: Traditional Chinese Medicine
TM(s): Traditional Medicine(s)
VaD: Vascular Dementia
VCIND: Vascular Cognitive Impairment No Dementia
VE: Vascular Events
WM: Western Medicine

Background: Alzheimer's Disease Overview

Method

We searched PubMed, Clintrials.gov, Z-library and Google for reviews and original articles published in English using the following keywords (singular or in a phrase): 'Alzheimer's', 'new drugs', 'pharmacology', 'clinical development', 'neuroaid', 'MLC601/901'. We also furthered our search by scanning the bibliographies of systematic review articles. For this review, we have selected the most relevant and most recent articles for Alzheimer's Disease, brain lesions and NeuroAiD using the keywords contained in the subtitles of each corresponding paragraphs of this article. Therefore, after screening 319 articles, 82 references were selected for this review.

Epidemiology and Costs

Since Alois Alzheimer first described and identified this type of progressive dementia in 1906, there has been significant development on the knowledge and treatment of the condition [1]. However, the number of people living with Alzheimer's disease (AD) and other types of dementia continues to rise. This is mainly due to population growth and ageing, with other dementia risk factors such as high BMI, high fasting blood sugar, smoking and diet high in sugary drinks, and the lack of treatment able to decelerate or prevent the progression of the disease [2].

Among more than 100 diseases that can lead to dementia, the most common types are strokes and AD – with AD by far the most common type of dementia in later life - accounting for up to 80 % of all cases worldwide [3]. In the World Alzheimer's Report 2018, it was estimated that 50 million people worldwide were living with dementia and that this number will more than triple to 152 million by 2050. If new treatments are not found, one new case of dementia will be diagnosed every 3 seconds worldwide [4]. The total worldwide cost of dementia was estimated at US$1tn in 2018 and it could double to US$2tn by 2030.

Conventional Therapies: From Symptomatic to Disease-Modifying Treatments

Since 1998, only four out of 100 drugs tested have been marketed as symptomatic treatments with either acetylcholinesterase inhibitors (AChEIs: donepezil, rivastigmine, galantamine) in the mild to moderate stages of the disease, or with an N-methyl-d-aspartate receptor antagonist (NMDA: memantine) in moderate to severe stages [5, 6]. In view of their effects on cognitive functions in everyday life, their benefit: risk ratio is questionable if they lead to side effects in frail patients and are quickly discontinued [3].

In December 2014, the G8 designated finding a cure or an approved disease-modifying therapy (DMT) to dementia as a priority by 2025 [7, 8]. A review of the AD drug development pipeline, published in 2021 by Cummings et al., identified 126 agents with 152 trials evaluating new therapies for AD. In Table 1, the numbers and rates of agent categories in 2021 are given, with the main changes from the 2020 pipeline [5, 6]. Clearly, research is increasingly focusing on agents targeting a disease-modifying therapeutic effect with the authors concluding that the diversity of biological targets and modes of action of developing agents has widened. Since 2003, only one new pharmaceutical treatment (aducanumab, 1 IV infusion every 4 week) has been approved in the United States as an amyloid beta-directed antibody indicated for the treatment of Alzheimer's disease. This indication was obtained through a debated and disputed accelerated approval pathway, based on a surrogate endpoint and the reduction of amyloid beta plaque [9]. US-FDA has since limited the offerings to people with mild cognitive impairment or mild dementia [10]. A recent safety review of the two phase 3 studies of aducanumab (EMERGE and ENGAGE) testing 3285 participants indicates that most frequent AEs are amyloid-related imaging abnormalities (ARIA), with about a quarter of patients experiencing symptoms such as headaches, confusion, dizziness, and nausea [11]. Despite the ups and downs around this registration, it is hoped that it will stimulate the need for new clinical trials with innovative and novel treatments for AD. And, why not looking at other pathways than those of hallmarks such as that of protein kinase C mainly involved in the progression of age-related neurodegeneration, or that of the sigma-1 receptors acting as amplifiers of neurodegeneration and neuroprotection [12, 13]. Finally, let's not forget that alongside the well-established links between stroke and AD, those linking traumatic brain injury (TBI) and AD are no less important, with the aim of implementing effective treatments to correct trauma-related early biochemical changes and prevent later protein aggregation [14].

Table 1: Drug development pipeline 2021 in Alzheimer's disease (AD): number (n) and rate (%) of agents, trials, and changes from 2020 to 2021 pipelines [5, 6]

Categories	Development Phases							
	3		2		1		Total	
	n	%	n	%	n	%	n	%
Agents								
- all indications[1]	28	22%	74	59%	24	19%	126	100%
- repurposed agents[2]	10	36%	30	41%	10	42%	50	40%
- putative DMTs[2]	17	61%	64	86%	23	96%	104	83%
- cognitive enhancers[2]	6	21%	6	8%	1	4%	13	10%
- neuropsychiatric symptoms[2]	5	18%	4	5%	0	0%	9	7%
Trials								
- all indications[1]	41	27%	87	57%	24	16%	152	100%
- total number of subjects needed[1]	25 373	65%	12 414	32%	1 039	3%	38 826	100%
- actual and estimated duration in months: mean (SD) / min-max	52.7 (20.9)	25-115.1	43.1 (27.1)	4-146.2	40.8 (54.4)	5-273.4	N/A	N/A
- prevention[2]	5	18%	2	3%	0	0%	7	6%
Changes 2021 vs. 2020 pipelines								
Agents 2020 total[1]	29	24%	65	54%	27	22%	121	100%
- stopped and new agents[3]	-1	-4%	9	12%	-3	-13%	5	4%
Trials 2020 total[1]	36	26%	73	54%	27	20%	136	100%
- completed, terminated, suspended or unknown[3]	7	25%	18	24%	9	38%	34	27%

1- % vs. raw Total; 2- % vs all indications; 3- % vs. 2020 total
From Cummings et al. Alzheimer's Dement. 2021;7:e12179 / Alzheimers Dement (N Y) 2020;6(1):e12050.

A Remaining Challenge: Slowing Down the Symptomatic Disease Progression

One important challenge is to slow down the progression of AD to severe dementia in millions of symptomatic patients with a probable diagnosis of AD, who may or may not receive one of the marketed standard treatments [3]. Whatever their stage of AD, the challenge of discovering and developing new treatments is mainly related to the complexity and multiplicity of mechanisms and pathophysiological disorders involved in its evolution. The main molecular and cellular mechanisms underlying the pathogenesis of AD include Amyloid Precursor Protein (APP) processing with β-amyloid (Aβ) protein - the main component of AD-associated amyloid plaques and tau-associated mechanisms. Multiple molecular and cellular mechanisms underlie the formation of AD with neurofibrillary tangles (NFTs), neuroinflammation, microglia activation and gliosis, and neuronal loss, neurodegeneration combining with cerebrovascular amyloidosis, and huge synaptic changes [15]. Requiring a multimodal intervention acting on several targets at both molecular and cellular levels, this might be achieved by combining drugs with various modes of action. This would increase the risk of interactions and adverse events (AEs) in an ever aging and frail population [16].

For millennia, this multimodal approach has been the basis of using traditional medicines (TM). These are based on using multi-ingredient formulations acting on multiple targets in order to control the various biological disorders linked to the pathogenesis of a disease. (Figure 1) [17,18].

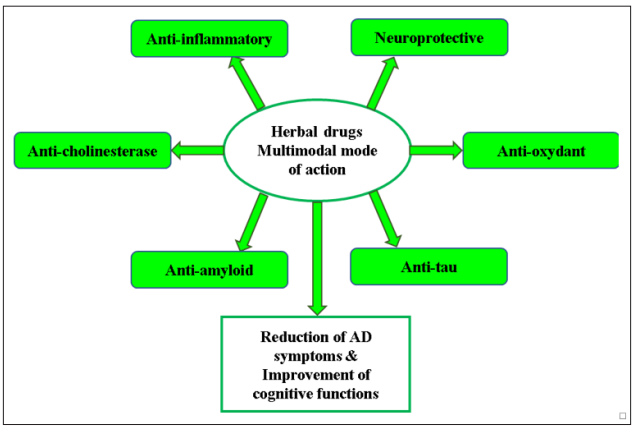

Figure 1: Multimodal approach of herbal medicines in Alzheimer's disease

Natural Products and Traditional Medicines

For thousands of years, the use of natural products as TM has increased. This has been through different types such as Traditional Chinese Medicine (TCM), Ayurveda and Unani in India, Kampo in Japan, and TM from Korea and Africa [17, 18]. TCM is a healing system based on the Chinese philosophy of the correspondence between nature and human beings [18]. Tracing the history of chemical and biological drug development, Western medicine (WM) has benefited greatly from TM with many WM drugs being derived from natural compounds used in TM. One of the most famous examples of this paradigm shift from TM to WM is Professor Youyou Tu's 2015 Nobel Prize in Medicine for her discovery of artemisinin in the treatment of malaria which saved millions of lives [19]. Many other therapeutic areas such as infectious diseases, oncology, hypertension and migraine have greatly benefited from natural products issued from TM experience. For example, galantamine is a natural alkaloid obtained from *Galanthus nivalis* for Alzheimer's [17, 20].

Many herbs and their extracts were developed in TM for AD and dementia [21, 22]. A meta-analysis and systematic review of 30 eligible randomised studies compared various oral TM formulations to AChEIs (donepezil = 28; donepezil, galantamine, and rivastigmine = 1) and memantine (= 1) on Alzheimer's Disease Assessment Scale - Cognitive subscale (ADAS-Cog) and Mini-Mental State Examination (MMSE) [23]. In 29 studies, the severity level of dementia was moderate, only being severe in the study with memantine. Overall, this analysis shows that the TM and donepezil groups had similar effects at 12 or 24 weeks on both scales showing significant improvements on MMSE within each treatment group over the course of 1 year. Looking at safety concerns, more AEs were reported in donepezil groups (RR 0.42; 0.28-0.63; $I^2 = 35\%$), with more dropouts than in TM groups (RR 0.57; 0.38-0.86; $I^2 = 0\%$). The authors concluded that, even if further well-designed studies are needed, the results suggested that the clinical benefits of TM were at least similar to those of donepezil with a better tolerability profile. Among the TM analysed in this systematic review, Neuro AiD (MLC901/MLC601) having a significant development program carried out according to international standards, we investigated its main biological properties and clinical profile for AD and other dementias.

A Multimodal Pathway to Alzheimer's Treatment: NeuroAiD (MLC901/601)

Development Programme at a Glance

MLC901 (NeuroAiD™II) is a natural formulation containing extracts from nine herbal components (*Radix astragali, Radix salviae mitorrhizae, Radix paeoniae rubra, Rhizoma chuanxiong, Radix angelicae sinensis, Carthamus tinctorius, Prunus persica, Radix polygalae and Rhizoma acori tatarinowii*). MLC901 is a simplified formulation of its parent formulation MLC601 (NeuroAiD™) registered as a TCM by the China Food and Drug Administration in 2001 [24]. Both MLC901 and MLC601 have similar neuroprotective and neuroregenerative properties, as demonstrated in animal models of cerebral ischemic and traumatic injuries [25-31]. Clinical translation of these biological properties has been confirmed in randomised double-blind clinical trials of Neuroaid efficacy, and biological and clinical safety, vs. placebo on post ischemic stroke recovery during mid-term and long-term, following its early administration within 72 hours after stroke onset [32-39]. In light of the biological and clinical effects observed on neuroprotection, neuroregeneration, functional and cognitive recovery in stroke and TBI, pharmacological and clinical studies were initiated in patients with mild to moderate AD, mild cognitive impairments (MCI), vascular cognitive impairment no dementia (VCIND), and vascular dementia (VaD) [40-50].

A large literature identified the reciprocal links between AD, stroke and TBI, both in terms of molecular and cellular modifications. The results found that they induce quite similar changes during the neurodegeneration process, alongside their epidemiological links, their cardiovascular risk factors, and their impact on cognition and memory functioning [51, 52].

Preclinical Development

First, we will review the pharmacological data provided by previous experience in stroke and TBI models evaluating the effects of NeuroAiD on mediators also involved in AD. Next, we will review the characteristics more directly related to AD, namely the APP expression and modulation of the Aβ production, and the mechanisms associated with the tau protein.

Neuroprotective and Neurorestorative Properties of NeuroAiD (MLC901/601)

Both MLC901 and its parent compound MLC601 have neuroprotective and neurorestorative properties after brain injuries [25-31].

Neuroprotection (Figure 2)

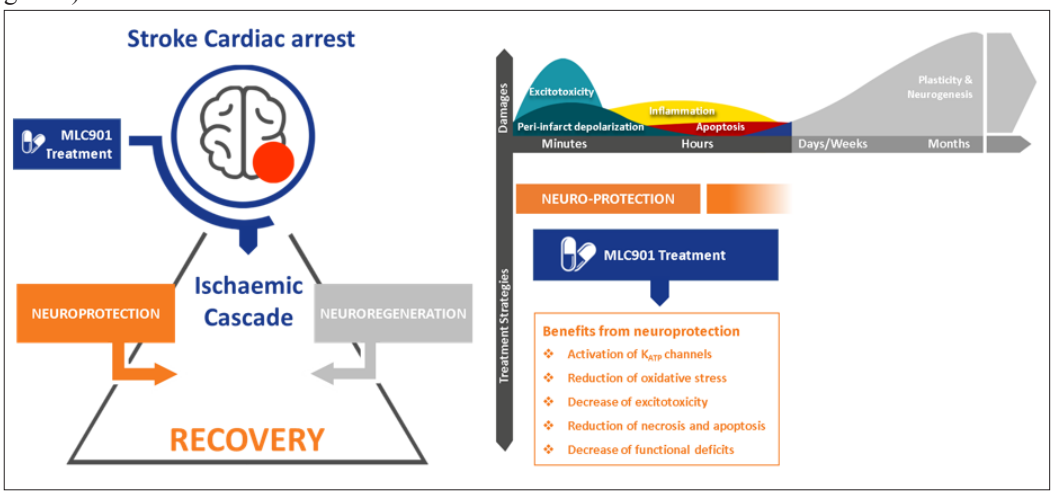

Figure 2: MLC901: Benefits from Neuroprotection

Peri-Infarct Depolarisation

In plasma and mitochondrial membranes, ATP-sensitive potassium (K_{ATP}) channels play major roles in modulating neuronal excitability, cell survival, and cerebral vascular tone. A study with the K_{ATP} opener diazoxide, showed an improvement of molecular, cytopathological, and behavioural alterations in a mice model of AD suggesting the potential in AD for drugs that activate K_{ATP} channels [53]. Electrophysiological experiments on mouse cortical neurons have demonstrated that MLC901 acts as an activator of K_{ATP} channels as potent as pinacidil - a classical K_{ATP} channel opener [28]. Hyperpolarization induced by MLC901 through K_{ATP} channel activation, particularly in neurons having suffered from energy deprivation, prevented the huge acute release of excitotoxic glutamate and the glutamate-triggered Ca^{2+} influx. This subsequently protected against glutamate excitotoxicity-induced cell death on cortical neurons in culture [25, 28]. Its neuroprotective and neurorestorative actions after brain trauma led to an improvement in the recovery of cognitive functions [25, 27, 42].

Excitotoxicity

The extreme vulnerability of neurons to hypoxic and excitotoxic lesions means that excitatory glutamatergic neurotransmission via the NMDA receptor (NMDAR) plays a key role in synaptic plasticity and neuronal survival in AD [54]. In contrast, abnormal NMDAR activity is associated with ischemic stroke and neurodegenerative disorders, such as AD. By decreasing amplified Ca^{2+} influx, MLC901 reduced oxygen glucose deprivation-induced excitotoxicity [25, 28].

Oxidative Stress

Improving mitochondrial function and reducing oxidative stress should be among the goals of AD treatment and prevention strategies [55]. The accumulation of malondialdehyde (MDA), a stable metabolite of lipid peroxidation and a biomarker of cellular oxidation status, is significantly increased in many neurological diseases [56]. *In vitro* tests in AD and VaD patients demonstrated that their levels of oxidative stress parameters were higher compared with controls, and higher in VaD than in AD patients [57]. They also showed that increased MDA concentration is negatively correlated with MMSE score, suggesting that MDA might be a biomarker for AD. It has been shown in a global ischemia model that the induced MDA production was drastically decreased by MLC901. This result indicates that MLC901 ingredients release active substances as antioxidants with neuroprotective properties via multiple mechanisms [27].

The p38-kinases are enzymes involved in a multitude of CNS-related physiological and disease states concerning cognitive functions [58]. Bax (Bcl-2-associated X-protein) is a protein-inducing apoptosis by opening mitochondrial permeability transition pores and allowing release of cytochrome complex (cyt. c) [59]. In AD brains, the phospho-p38 kinase concentration increases, and its activation enhances phosphorylation of Bax and its translocation into neuronal mitochondria. These changes contribute to elevated oxidative stress leading to neurodegeneration via apoptosis and NFTs [60]. The administration of MLC901 is associated with a decreased level of the Bax protein suggesting that the neuroprotection induced by MLC901 involves a decrease of apoptotic pathways, and prevents their neurodegenerative consequences [26, 27, 61].

Neuroinflammation

AD pathogenesis includes also strong interactions with immunological mechanisms in the brain [62]. At the crossroads of cellular and humoral immunity in the brain, microglia play an essential role in this inflammatory process by expressing themselves according to 2 different and opposite phenotypes [63]. A cytotoxic M1 polarization is accompanied by the release of destructive pro-inflammatory mediators such as pro-inflammatory cytokines (including TNFα, interleukin (IL) 1, IL-6, IL-12, and IL-18) with impaired phagocytic capacity due to downregulation of expression of Aβ phagocytosis receptors. M1 polarization also activates reactive oxygen species (ROS) and proteinases. A protective M2 polarization promotes neuronal tissue repair by the secretion of the anti-inflammatory cytokines (IL-4, IL10, IL-13, and TGF-β), with increased phagocytic capacity. MLC901 has been shown to inhibit astrocytes and microglia/macrophage activation, decreasing strongly the neutrophil invasion into the ischemic brain as well as by a negative regulation of pro-inflammatory mediator expression (cytokines, chemokines, matrix metalloproteinases) [29].

Functional and Morphological States

An *in vitro* study in humans compared serum levels of S100 beta (S100B) and neuron-specific enolase (NSE) in patients with mild, moderate and severe AD, as well as in healthy elderly people [64]. In patients with AD, serum S100B levels increased with disease severity, while decreased serum NSE levels were related to increased morphological brain damage. Therefore, the measurements of S100B and NSE could be used as biomarkers of the functional state of the brain and the morphological state of AD, respectively. MLC901 prevented the serum increase of S100B and the decrease of NSE - both also being potentially biomarkers to predict neurological outcomes in patients after TBI. Furthermore, an additional experiment in rats with cognitive deficits after TBI and temporal order memory suppression, showed that MLC901 improved the recovery of cognitive functions [30].

Neurorestoration (Figure 3)

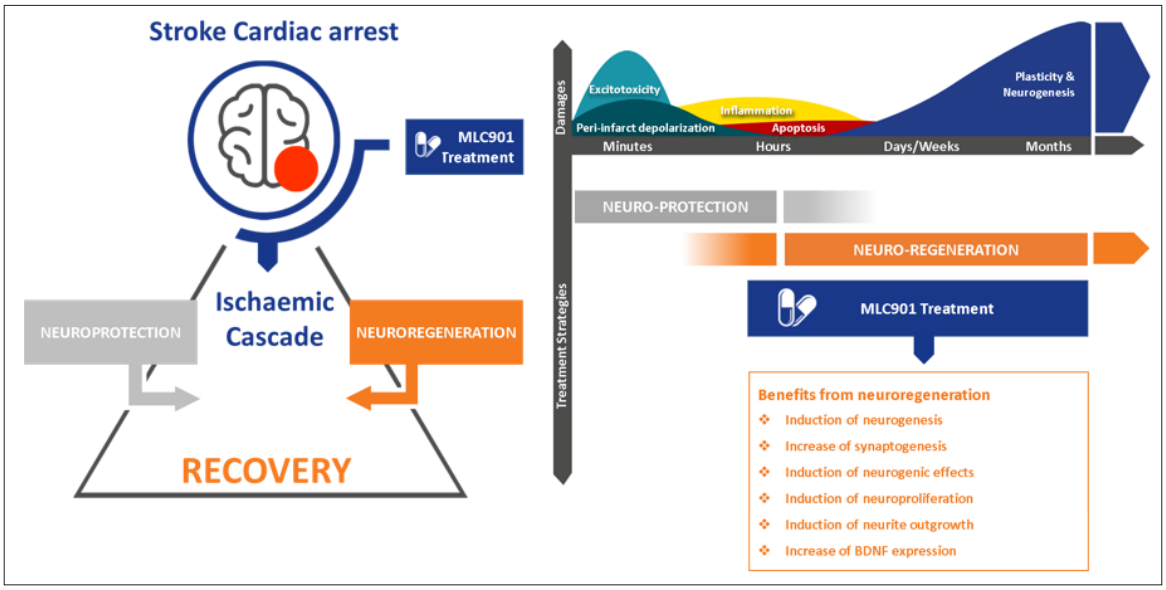

Figure 3: MLC901: Benefits from Neuroregeneration

As shown in animal models and human stem cell cultures, MLC901 stimulates neurogenesis and neuroplasticity, promoting cell proliferation, neurite growth, and the development of dense axonal and dendritic networks [25-27,30]. The healing of brain tissue damage depends on the effective stimulation of neuroregeneration processes - an important pathway relevant to AD [28].

Neuroplasticity

A cross-sectional study of 32 participants with early AD and 16 healthy control participants assessed plasticity in dorsolateral prefrontal cortex (DLPFC) by using paired associative stimulation with a memory test assessment [65]. Compared to controls, subjects with AD had significant deficits in DLPFC plasticity, with working memory performance significantly altered. The effect of MLC901 on neuronal plasticity results in increased neurogenesis, neurite growth, axonal growth, dendritic arborization and synaptogenesis. This effect is correlated with functional recovery seen in both local and global ischemia [25-27].

Neurogenesis

A recent review aimed to provide an answer to the question "*Is Alzheimer's disease a disorder of neurogenesis?*" [66]. The reviewed studies compared the adult hippocampal neurogenesis (AHN) between healthy elderly people and patients with MCI or AD, by labelling neuroblasts with doublecortin (DCX) in their dentate gyrus (DG). It shows that AHN is persisting during physiological as well as pathological ageing in humans [67,68]. It also shows that AHN, which is impaired prior to the onset of AD pathology, could be an underlying memory disorders' mechanism and another pathway to novel therapeutic strategies [69]. Studies in models of focal and global cerebral ischemia have demonstrated that MLC901 was able to: (i) promote basal neurogenesis, doubling the number of neuroblasts which differentiate into mature neurons in 3 weeks and (ii) to stimulate neurogenesis in the subgranular zone of DG after global ischemia [25, 27]. The administration of MLC901 increases the number of positive neuronal precursors for 5'-bromodeoxyuridine (BrdU)/DCX after ischemia compared to control arm [27]. Similar results in cortical neurons with MLC901 have also been observed in human embryonic stem cells. Altogether, these data suggest that MLC901 contains key molecules to set up a neurogenic niche and an enriched microenvironment - both stimulating development and differentiation of neural progenitors [25, 26].

Neuritogenesis and Synaptogenesis

Polygala tenuifolia is one of the 9 herbal ingredients of MLC901 [26]. In addition to its multiple neuroprotective effects (anti-Aβ aggregation, anti-Tau protein, anti-inflammation, antioxidant, anti-neuronal apoptosis, neuronal proliferation), *Polygala tenuifolia* has beneficial effects on neuritogenesis with the consolidation of dendrites and axons in AD [70]. *In vitro* experiments on cultured cortical cells have shown that MLC901 helps to develop a dense axonal and dendritic arborization, as shown by a large increase of DCX fluorescent labelling intensity and improved neurite outgrowth [26, 28]. With MLC901, the neurite network of cortical neurons densified with neurite elongation and more frequent branching, as well as increased expression of the growth-associated protein GAP43 in neurites. This, with a simultaneous increase of synaptogenesis visualized by the increase of synaptotagmin-1 expression, means that a synaptic vesicle protein having a critical role in both synaptogenesis and synapse functions [25, 26, 71, 72]. All these results highly suggest that MLC901, by its ability to promote neurogenesis, neurite outgrowth and synaptogenesis, has the potential to amplify the intrinsic brain properties for neuroplasticity, favouring subsequent neurological recovery after neurodegenerative lesions.

Brain-Derived Neurotrophic Factor (BDNF) Expression: Learning and Memory

During lifelong learning and memory activities, changes in brain plasticity occur during which the brain-derived neurotrophic factor (BDNF) plays an essential role [73]. The highly regulated expression of BDNF can lead to wide variability in BDNF levels with changes in its expression linked to both normal and pathological ageing. This, as well as with psychiatric diseases, features in structures particularly involved in memory processes such as the hippocampus. Since variations in the BDNF can be caused by many pathological conditions, it cannot be used as a biomarker for neurodegenerative or neuropsychiatric diseases. However, BDNF can be viewed as a marker relating specifically to the onset and/or progression of mnemonic symptoms common

to many pathological conditions, including AD. While ageing, AD and chronic stress could reduce BDNF levels, exercise, enriched environment and antidepressants could be used as potential treatments for cognitive impairments related to low BDNF levels. *In vitro* data with cultured cortical neurons showed that MLC901 increased the expression of BDNF in hippocampal Cornu Ammonis (CA1) pyramidal neurons - a key area for learning processes and associative memory [26, 27, 74].

Effects on Neuropathological Hallmarks of Alzheimer's Disease

An important pathological feature of AD is the presence of extracellular senile plaques in the brain which are composed of aggregations of small peptides forming Aβ [75]. The APP is a key molecule for memory in the healthy and pathologic brain. APP is a type 1 transmembrane protein expressed in many tissues and organs, including the central nervous system, and can go through two different cleavage processes [76]. Under physiological conditions, the non-amyloidogenic pathway with the APP cleavage by α- and γ-secretases results in releasing the non-pathogenic soluble APPα (sAPPα) and p3 fragments. Under pathological conditions such as AD, APP is preferentially processed via the amyloidogenic route, producing large amounts of Aβ by sequential cleavages first by β-secretase and then by the γ-secretase complex. APP may also be cleaved by other enzymes such as caspases as additional process to AD pathophysiology. Hence, in the search of new AD therapies, a better knowledge of APP processing remains critical. MLC601 is a potential modulator of APP processing. As shown in cultures of human neuroblastoma cells, MLC601 increases the level of sAPPα produced by the physiological cleavage of APP. Additionally, MLC601 decreases full-length APP levels, showcasing its modulatory effect on APP processing [40].

A recently concluded *in vitro* study shows that MLC901 also significantly reduced tau phosphorylation at various epitopes known to be associated with forming NFTs. It also increased phosphorylation of glycogen synthase kinase 3b along with concurrent decrease in the activation of cyclin-dependent kinase 5. This data supports that by reversing tau protein phosphorylation and APP processing. The multimodal action of MLC901 could have a disease modifying effect that makes MLC901 an attractive candidate for treatment of AD patients [40,41].

In a recent animal study, MLC901 showed positive effects on cognitive tasks in mice by promoting extinction in the passive avoidance and reversal learning in a Morris water maze, with improved performance in novel object recognition. Increased hippocampal neurogenesis with promoted proliferation, neuronal differentiation, and survival of young neurons, was also observed with MLC901. The neurogenesis effect is thought to have contributed to its precognitive effects [42]. This study shows that MLC901 improves memory performance and hence may delay the onset of AD dementia or disease progression.

Clinical Development

The clinical development programme of NeuroAiD in AD has been initiated with the parent formulation MLC601 in open studies to screen its effects on AD patients. At the time, the only registered drugs were the AChEIs and memantine for symptomatic treatment of AD. This clinical development is continuing with MLC901 and the publication of its first clinical results.

Comparative Studies vs. Acetyl-Cholinesterase Inhibitors (AChEIs) in AD

Study in AD Patients Who Failed Under Rivastigmine **(Table 2)**

The first study was conducted in 124 patients with mild to moderate AD. They were diagnosed according to DSM-IV criteria and failed on a 6-month treatment of rivastigmine at doses of 2 to 12 mg, due to either a lack of efficacy or bad tolerability [43]. They were switched to a MLC601 regimen of one capsule t.i.d. for up to 18 months. Two patients were lost to follow-up and 122 completed the 18-month treatment. Improved cognitive function was observed in the first 6 months of the regimen (ADAS-Cog = -3.1±10.1; MMSE = 1.2±3.0), and the stabilisation of cognitive decline was observed over the remaining 12 months (ADAS-Cog = -1.6±7.6; MMSE = 0.8±4.2). AEs were minor and predominantly gastrointestinal, occurring in 7.3% of patients.

Comparative study of MLC601 vs. AChEIs and Memantine (Table 2)

A multicentre, randomized controlled clinical trial has evaluated the effectiveness and safety of MLC601 in the treatment of mild to moderate AD when compared to the 3 approved AChEIs (donepezil, rivastigmine and galantamine) [44]. Over the course of 16 months, a total of 264 patients were randomly divided into 4 groups of 66 who either received one capsule t.i.d. of MLC601 or a standard dose schedule of one of the AChEIs according to clinical responses and recommended maximum dose schedule. The main results are summarized in Table 2. At baseline, the mean scores of ADAS-Cog and MMSE (~28 and ~17.6, respectively) in the study population corresponded to a rather mild severity level of cognition impairment. Both mean scores and their mean changes over 16 months are shown in Table 2. There were no significant differences between the treatment arms on ADAS-Cog and MMSE scores during the 16-month treatment period. Cognition scores improved over the first 8 months then declined slightly until month (M) 16 in all treatment groups. The 16-month mean changes in MMSE and ADAS-Cog for the MLC601 arm were –0.45 and 1.35 (± 5.3) points, respectively.

Safety and tolerability data are shown in Table 2. The total number of AEs per treatment groups and mean number per patient of AEs were significantly lower in the MLC601 group (34 and 0.51) compared to the AChEI arms, of which the corresponding results were as follows: donepezil (121 and 1.86), galantamine (157 and 2.37), and rivastigmine (189 and 2.86) [39]. In the MLC601 arm, this corresponds to 3.6 times less AE per patient than donepezil, 4.7 times than galantamine, and 5.7 times than rivastigmine. Overall, these results support that the benefit-risk ratio of MLC601 is significantly better than that of AChEIs.

Four-Year Treatment Study (Table 2 & Figure 4)

The 122 patients who completed the first study of MLC601 after failure of a 6-month treatment with rivastigmine, were offered to continue their treatment in a follow-up study with a visit every 4 months [43, 45]. All 122 were included and 105 (86%) completed follow-up to M48. The mean scores for both the ADAS-Cog and MMSE remained quite stable up to M30 and then changed gradually towards a worsening in disease severity. After 4 years of treatment and follow-up, the mean ± SD changes in ADAS-Cog and MMSE scores were -5.1±8.7 and 2.1±3.8, respectively. Repeated measure analysis revealed a statistically significant change in both scores with a significant greater change of ADAS-Cog scores than MMSE scores ($p<0.001$). During this 4-year safety follow-up period, there were 52 mild AEs with 3/4 of them being digestive symptoms. They were reported occurring in 18 (15%) patients with a mean number of AEs per patient of 0.50. No treatment withdrawals due to AEs related to MLC601 were reported. There were no abnormal values recorded in lab tests.

Table 2: MLC601 clinical studies vs. acetyl-cholinesterase inhibitors in Alzheimer's Disease

Study population	Study groups	Endpoints		Assessment visits				
Harandi et al, 2013 [43]				Baseline	Month 6	Month 12	Month 18	p value
- 124 patients with mild to moderate AD who failed on a 6-month treatment of rivastigmine - 122 (98%) completed the study	MLC601 one capsule t.i.d during 18 months - mean age ± SD = 65.3±6	ADAS-Cog: mean ± SD		23.2±8.1	20.0±8.3*	20.8±8.5	21.5±8.4	0.048*
		MMSE: mean ± SD		18.0±4.8	19.2±5.2	19.0±5.5	18.9±5.4	0.067
		Safety: - n AEs /mean of AEs per patient / % AE-related dropouts		Rivastigmine - 120 AEs (63% GI) / 1 per patient / 47% dropouts MLC601 - 17 AEs (11.3% GI) / 0.14 per patient / 0 dropout				
Pakdaman et al. 2015 [44]			Baseline	Month 4	Month 8	Month 12	Month 16	p value
	MLC601 one capsule t.i.d during 18 months - 59 (89%) completed the study - mean age ± SD = 71.8±5.7	ADAS-Cog: mean ± SD	27.51±4.19	27.06±6.52	26.75±5.96	27.55±6.15	28.45±6.04	0.87
		MMSE: mean ± SD	17.77±1.69	18.15±2.65	18.25±2.50	17.90±2.33	17.47±2.21	0.92
		Safety: - n AEs - mean of AEs per patient ± SD - % AE-related dropouts	- 34 AEs - 0.51±1.29 AE / patient - 0 dropouts					
	Donepezil - 57 (86%) completed the study - mean age ± SD = 71.8±5.5	ADAS-Cog: mean ± SD	27.80±6.56	28.10±8.58	27.78±7.67	28.27±7.87	28.87±7.67	0.87
		MMSE: mean ± SD	17.66±2.86	17.69±3.92	18.00±3.7	17.56±3.70	17.36±3.71	0.92
		Safety: - n AEs - mean of AEs per patient ± SD - % AE-related dropouts	- 121 AEs - 1.86±2.49 AE / patient - 2±3.0% dropouts					
	Rivastigmine - 53 (80%) completed the study - mean age ± SD = 73.2±4.7	ADAS-Cog: mean ± SD	29.69±7.78	30.12±9.39	29.42±9.30	28.62±7.91	29.37±7.63	0.87
		MMSE: mean ± SD	17.13±3.15	16.86±3.95	17.19±3.94	17.46±3.45	17.24±3.43	0.92
		Safety: - n AEs - mean of AEs per patient ± SD - % AE-related dropouts	- 189 AEs - 2.86±3.28 AE / patient - 5±7.6% dropouts					
	Galantamine - 56 (85%) completed the study - mean age ± SD = 71.8±5.5	ADAS-Cog: mean ± SD	27.37±5.02	26.98±6.45	27.6±7.48	28.40±7.36	29.14±7.65	0.87
		MMSE: mean ± SD	17.90±1.92	18.09±2.87	17.88±3.13	17.56±3.02	17.30±3.09	0.92
		Safety: - n AEs - mean of AEs per patient ± SD - % AE-related dropouts	- 157 AEs - 2.37±3.0 AE / patient - 4±6.1% dropouts					
Pakdaman et al. 2018 [45]			Baseline	Month 6	Month 12	Month 18	Month 24	Month 30
- Open follow-up of 112 patients with mild to moderate AD over 4 years - 105 (86%) completed the study	MLC601 one capsule t.i.d during 48 months in addition to a previous 18-month course of MLC601 with same dose schedule, i.e., a total of 66 months (5.5 years) of treatment. - mean age ± SD = 66.8±3	ADAS-Cog: mean ± SD	21.5±8.4	22.6±8.2	20.0±8.3	20.8±8.5	21.5±8.4	22.5±8.8
		MMSE: mean ± SD	18.9±5.4	18.4±4.7	19.2±5.0	19.1±5.3	18.9±5.2	18.3±5.5
			Month 36	Month 42	Month 48	p value		
		ADAS-Cog: mean ± SD	24.6±9.3	25.3±10.3	27.7±12.8	<0.001		
		MMSE: mean ± SD	17.2±5.4	16.8±5.1	16.3±4.8	<0.001		
		Safety: - n AEs - mean of AEs per patient ± SD - % AE-related dropouts	- 52 AEs in 18 patients - 2.9 AE / patient - 0 AE-related dropouts					

* Significant difference between the baseline and 6-month measurements of ADAS-Cog according to repeated measured analysis of all visits, AD, Alzheimer's disease; ADAS-Cog, Alzheimer's Disease Assessment Scale – Cognitive subscale; MMSE, Mini-Mental State Examination; AE, adverse event; SD, standard deviation

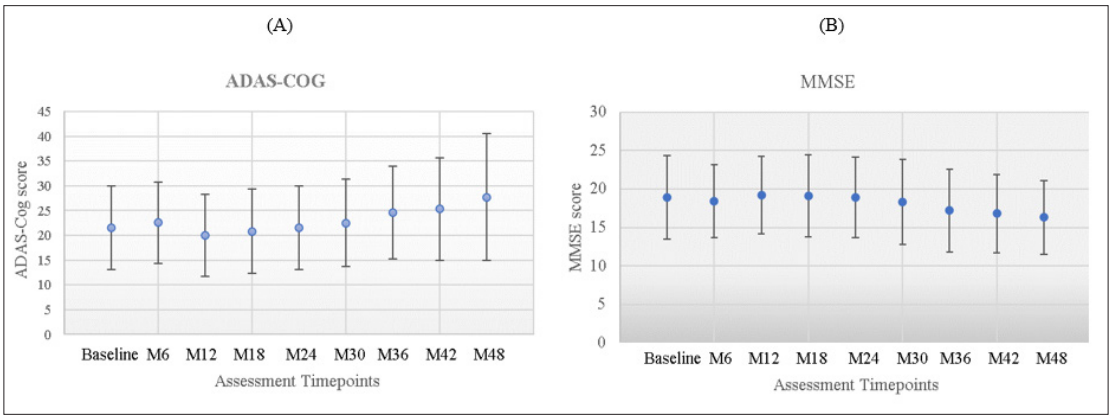

Figure 4: Efficacy of MLC601 assessed with (A) ADAS-Cog and (B) MMSE every 4 months from baseline to Month (M) 48

MLC901 vs. Placebo on Top of Standard Symptomatic Treatment in Alzheimer's Disease

A double-blind study, ATHENE (Alzheimer's disease Therapy with Neuroaid II) study (ClinicalTrials.gov reference: NCT03038035) was designed as a delayed-start study, randomizing patients to the same active treatment but started at different times to assess the safety and efficacy of MLC901. These were set at 2 capsules 3 times daily on top of the standard symptomatic treatment (AChEIs or memantine) [47]. A total of 125 patients (MLC901 n=62, placebo n=63) with mild to moderate probable AD were randomized at first in a double-blind, placebo-controlled phase from 0 to 6 months and was fully completed by 119 patients (95.2%; MLC901 n=59, placebo n=60). It was followed by an open-label delayed-start phase from 6 to 12 months that was completed by 101 patients (81%; early starters n=52, delayed starters n=49). Here, all patients on placebo switched to active treatments at M6, with the initial double-blind allocation being maintained up to the end of the study at M12. The study met its primary endpoint which was to test the safety of MLC901 as an add-on to standard symptomatic treatments. It showed no significant differences in the risk of Serious Adverse Event(s) (SAEs) and AEs between early and delayed starters throughout the 12-month study period. None of the SAEs were reported as related to the study treatment and 2 patients died whilst on MLC901 and 3 patients on placebo. None of the AEs led to study treatment withdrawal. As a secondary endpoint for efficacy, early starters did not differ significantly on ADAS-Cog from delayed starters at M6 and M12 on intention-to-treat (ITT) analysis. From M6, early starters improved on mean ADAS-Cog scores in the MLC901 arm at M9 in both ITT (-3.36; -5.64, -1.09) and per-protocol (PP; -3.66; -6.42, -0.89) analyses, and also at M12 in the PP analysis (-4.75; -8.92, -0.59). The difference between early- and delayed-starters' treatment groups on ADAS-Cog increased over time suggesting either a prolonged symptomatic effect of MLC901 or the slowing down of disease progression due to early treatment with MLC901. This treatment effect appeared most substantial at M12 in patients who were compliant to the study medication and in study completers as shown in the PP analysis. The minimal clinically relevant 3-point ADAS-Cog change was observed as statistically significance differences at M9 between early and delayed starters. The authors concluded that the ATHENE study showed no evidence of a significant increase in adverse events between MLC901 and placebo. This safety profile brings support for further studies on both efficacy and safety. There could be potential for MLC901 to slow down AD progression but this should be confirmed in larger and longer studies with AD biomarkers.

MLC901/MLC601 in Cognitive Declines Without or Before Dementia

It is well known that cognitive decline begins many years before the onset of AD and the subsequent functional decline. Between cognitive alterations of normal aging and severe dementia, the question arises of the diagnosis of intermediate stage of mild cognitive impairment (MCI), when clinical manifestations are subtle and hard to detect. Although the underlying neuropathological substrate of amnesic MCI often predicts pure AD, it may be underestimated that in many cases, cognitive impairment could be due to a vascular or mixed pathology related to brain injury associated with prior cerebrovascular disease [77]. At pre-dementia state, this vascular cognitive impairment (VCI) qualified as VCI no dementia (VCIND), can either progress to further cognitive decline up to vascular dementia (VaD) or regaining normal cognitive functions. Pilot studies have been conducted with MLC901 and MLC601 in subjects at the stages of MCI, VCIND and VaD.

A pilot randomised, double-blind study in 70 subjects with a diagnosis of MCI, assessed the effects of MLC601 vs. placebo on ADAS-Cog and MMSE [48]. General linear model, repeated-measures analyses showed a statistically significantly slower decline at 6 months on both scales in cognitive function in the MLC601 group compared to placebo.

A recently published study, NEURoaid II in cognitively Impaired not demenTED patients (NEURITES) is a randomized, double-blind, placebo-controlled exploratory study in 103 patients with Vascular Cognitive Impairment No Dementia (VCIND) [49]. MLC901 vs. placebo were given at a dose of 2 capsules 3 times daily for 24 weeks on top of standard post-stroke care. It was found to be safe with no significant differences in AEs (43.5% vs. 56.1%) or SAEs (13% vs. 22.8%) between placebo and MLC901 groups. Although the primary analysis did not detect a statistically significant difference between MLC901 and placebo, subgroup analysis reported in the article indicates a significant treatment effect on cognitive test (Colour Trail Test) at 12 weeks ($p<0.05$) in those patients exhibiting some impairment of executive functions at baseline. Overall, MLC901 multimodal action on brain neuroplasticity and neurogenesis led to increased functional recovery with 3 months of treatment in stroke patients with confirmed impairment. This effect extended to a greater proportion of the study population, from the 6th to the 24th month.

In a multicentre, pilot, randomised, double-blind trial, 82 patients with VaD diagnosed on DSM-5 criteria, received MLC601 or placebo capsules three times a day for 2 years [50]. Analyses with repeated measures confirmed on both MMSE and ADAS-cog that

scores were significantly better in the treatment group at 24 months ($p<0.001$). Safety analyses has reported 10 (24.4%) patients having declared predominantly transient gastrointestinal AEs, none of them being withdrawn due to AE. No clinically significant abnormalities on laboratory tests were reported. Overall, this pilot study showed the long-term safety and benefit/risk ratio of MLC601, well-tolerated and devoid of SAE, enabling the use of Neuroaid in patients affected by mixed dementia with symptoms of both AD and VaD.

Discussion

While research and development efforts are focusing more and more on finding drugs to prevent or delay the onset of Alzheimer's disease, it remains true that millions of people have already entered the symptomatic phase of this disease. Unfortunately, this will be the case for many years to come. The entry of aducanumab on the market remains conditional on the confirmation of its clinical efficacy and cost-effectiveness [78]. This will take several years, and its affordability will remain limited in many countries, so the growth of AD and other dementias will continue as the population ages. Therefore, it remains necessary to develop new therapeutic approaches aimed at safely slowing down the functional degradation of patients with symptomatic AD.

Another issue of early DMT in AD concerns diagnosis. The main aim is to do it with certainty in order to set up DMT as soon as possible. For this, it will be necessary to have reliable biomarkers before initiating a long-term treatment. This may induce tolerability and safety problems compromising treatment compliance and its benefit: risk ratio. This question is well illustrated by the results of the INSIGHT-AD cohort - where cerebral β-amyloidosis, a priori a well-recognized biomarker - did not solely record the progression from preclinical to prodromal AD stages [79]. Even at the prodromal stage of AD, nothing is already confirmed as many people with early and mild impaired cognitive and memory functions will never progress to AD or dementia. Furthermore, AD can pose diagnostic issues with other forms of dementia, such as VaD [52]. This is because AD can often follow cognitive disorders such as those of MCI or VCIND induced by an ischemic or traumatic cerebral event that occurred a few months or years earlier. The use of various biomarkers (alone or in combination) has long been the subject of various studies. These used blood or CSF concentrations of amyloid and phosphorylated tau proteins associated with amyloid-PET or tau-PET scans, as well as numerous blood biomarkers of neurodegeneration, like the ones we have reviewed [80,81]. In addition to the diagnosis, these biomarkers should provide information on disease progression and the potential for monitoring the treatment effects. But the main question remains when and how these biomarkers can be applied in clinical practice [81]. The same question arises when confirming the diagnosis of symptomatic AD and following its evolution and treatment with tools better adapted to daily practice.

Regarding the treatment of AD at the clinical stage, we undertook this review of other approaches such as that of traditional medicines and their multi-herbal formulations. Amongst these, MLC901 (NeuroAiD™II) appeared to be one of the most promising due to its extensive pharmacological and clinical development in the recovery of victims of cerebral lesions. This development has demonstrated its clinical benefits related to its neuroprotective and neuroregenerative properties in over 3000 patients. These were carried out in double-blind, placebo-controlled studies focusing on neurological diseases such as strokes, TBI, AD, VaD or VCIND. The efficacy results obtained with NeuroAiD in AD patients with impaired cognitive functions provided positive evidence of effective slowing down the disease which needs to be confirmed in further studies with longer follow-up and biomarkers [43-46]. In addition to a modulatory effect on APP processing preventing the generation of Aβ and a significant reduction in tau phosphorylation, NeuroAiD has shown a positive effect on cognitive functions assessed by ADAS-Cog and MMSE scales in patients with mild to moderate AD. This was at least comparable to that of standard treatments [44]. Successive 4-year and 8-year follow-up studies showed maintenance of favourable outcome with NeuroAiD for up to 30 months of treatment [45, 46]. Tolerability and safety are key success factors in patients with AD because the therapeutic effect is often compromised by the occurrence of adverse events and eventual discontinuation of treatment [3]. As demonstrated in the ATHENE one-year double-blind placebo-controlled study and in a 4-year long-term study in AD patients who failed on rivastigmine, these clinical benefits were achieved with promising safety and tolerability without side effects leading to treatment withdrawal. Alongside that, a comparative 16-month study vs. AChEIs, patients reported 3.6 to 5.7 times less AEs with MLC601 [44]. The accumulating evidence for all studied therapies in AD indicated that they should be implemented at the earliest phases of the disease. This is the same for NeuroAiD, which has potential to induce protective effects in the pre-clinical period of the disease but it should be further studied in long term follow-up clinical trials.

At the end of this review, we should keep in mind that alongside cognitive deficits, it is important to manage the decrease in functional, behavioural and self-care capacities. We should also consider the impact on the patient's quality of life and the burden on the caregiver, as well as the huge estimated socioeconomic consequences on the last decade [4, 82].

Conclusions

A growing consensus is emerging on the need for a multifactorial approach to the treatment of Alzheimer's disease, as well as of MCI, VCIND and VaD, and for the development of drug combinations. That has been the approach of traditional medicine for a long time. This works in the use of MLC901 (NeuroAiD™II), whose multi-ingredient formulation acts in a multimodal pathway combining neuroprotective and neuroregenerative properties. The clinical benefits and evidence-based long-term safety in frail patients with AD or brain lesions also offers a high benefit:risk ratio. This makes MLC901 a natural supplement that could be integrated safely with a disease-slowing effect both as first-line treatment in symptomatic patients, or in second-line on top of standard symptomatic treatments or after their failure, as well as in combination with other treatments needed in an increasingly frail elderly population.

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