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Ana Mafalda Gonçalves Gonçalo

Os efeitos da Trazodona na cognição humana: uma revisão sistemática/ The effects of Trazodone on human cognition: a systematic review

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NOME

Ana Mafalda Gonçalves Gonçalo

NÚMERO DE ESTUDANTE | E-MAIL

201506777 | mafaldagoncalo@gmail.com.

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The effects of Trazodone on human cognition: a systematic Review.

ORIENTADOR

Maria Augusta Vieira - Coelho

COORIENTADOR (se aplicável)

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Dedicatória

À minha família, em especial aos meus pais, pelo carinho, compreensão, aceitação, apoio incondicional, incentivo, paciência e palavra amiga certeira nos momentos de insegurança.

À Professora Maria Augusta, pela dedicação e encorajamento constantes. Uma palavra especial para a minha mãe, a minha melhor amiga, força da natureza, que personifica tudo aquilo que hoje me define e que me ensinou a colocar quanto sou no mínimo que faço.

The effects of Trazodone on human cognition: a systematic review

Ana Mafalda Gonçalves Gonçalo[1], Maria Augusta Vieira-Coelho[1,2]

[1]Department of Biomedicine. Pharmacology and Therapeutics unit, Faculty of Medicine, University of Porto, Porto, Portugal

[2]Department of Psychiatry and Mental Health, *University Hospital* Centre of *São João*, Porto, Portugal; ORCID number: 0000-0001-8968-1348

Corresponding author:

Ana Mafalda Gonçalves Gonçalo

Address:

Department of Biomedicine. Pharmacology and Therapeutics unit, Faculty of Medicine, University of Porto.

Rua Doutor Plácido Costa, 4200 – 450 Porto, Portugal

Email: mafaldagoncalo@gmail.com

TM: +351 919621957

Abstract

Trazodone is a widely used antidepressant, also useful in the control of agitation and insomnia in Alzheimer's disease. It is now recognized a new mechanism of action for trazodone, based on its effect on the Unfolded Protein Response (UPR) pathway, restoring protein translation and slowing neurodegenerative progression in mice. These mechanisms may be seen as promising in dementia modifying treatment.

To explore the effects of trazodone on human cognition and search for clinical evidence of its putative benefits in human's neurodegenerative diseases, a systematic review was conducted for studies that evaluated the effect of a minimum of 25 mg of trazodone daily, for at least one week, in the cognition of healthy or diseased patients with eighteen years or more. The search was run in MEDLINE, Web of Science, and CENTRAL from Cochrane databases, yielding a total of 16 studies, after selection. Overall, seven studies showed no effect of trazodone on cognition, five showed a beneficial effect by improving or reducing cognitive decline and four evidenced impaired cognitive function. Our analysis highlights the possibility of a dose-independent dual effect of trazodone on human cognition, with acute utilization associated with impaired cognitive function and long-term use with cognitive deterioration prevention. There was no evidence that trazodone could be used as an active treatment of neurodegenerative diseases itself, but the majority (n=12) of our findings indicate that it has no long-term cognitive detrimental effect. Future studies should explore trazodone's role in the UPR pathway and the implications in neurodegenerative diseases in humans.

Keywords: All Cognitive Disorders/Dementia; Alzheimer's disease; Memory; Executive function; Trazodone

Declarations

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The authors declare no funding for this study.

Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material

All data collected and analyzed are available in this article.

Code availability

Not applicable.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Introduction

Although being FDA approved only for use in the treatment of major depression, trazodone, a widespread drug created in the 1960s[1], is used off label to control agitation and insomnia in Alzheimer's disease (AD)[2], but also in anxiety, schizophrenia, bulimia, substance abuse, fibromyalgia[3], and post-traumatic stress disorder[1]. Additionally, it reduces the behavioural and psychological symptoms in AD and frontotemporal dementia[2].

It is now recognized a new mechanism of action for this drug, based on its effect in the UPR pathway. Trazodone acts downstream of eIF2α-P, preventing it from reducing levels of the ternary complex, allowing protein translation to occur[2] (Figure 2). Hence, trazodone precludes the effects of UPR overactivation observed in neurodegenerative diseases[2] and restores neuronal protein synthesis rates, preventing neurodegeneration in mice models[4]. As a result, and as stated by Halliday et al., it could be seen as a new disease-modifying treatment for neurodegenerative diseases in humans[2].

However, assuming that trazodone has a role in preventing cognitive decline, is that effect mediated through its action in the UPR pathway, through increasing the synaptic concentration of 5-hydroxytryptamine[5] or attributed to the sleep improvement this drug offers? To establish the effects of trazodone on human cognition would have a great effect on medical practice, given the increasing prevalence of neurodegenerative diseases and the extensive use of trazodone in this population of patients.

Thus, we aimed to establish the effects of trazodone on human cognition and to find out if there was evidence that it could be used in the treatment of neurodegenerative diseases in humans.

Methods

Following PRISMA guidelines, we conducted a systematic review without a meta-analysis of studies that evaluated the effect of trazodone on human cognition.

Our target population was healthy or diseased adults with eighteen years old or more. The diseased patients could have all types of illness, from atherosclerotic disease to psychiatric conditions. We excluded animal studies since we aimed to study the consequences in humans so that it was possible to assess the implications for clinical practice.

Our intervention consisted of a minimum of 25 mg of trazodone daily, for at least one week, to observe the chronic effects of this drug and not the effects obtained after a single dose. Consequently, we excluded studies that only tested the acute effects of trazodone by using it in a one-time-only fashion. The dose of 25 mg was chosen because it is the minimum dose that is used and that is capable of producing some effects of the drug observed in clinical practice. In terms of comparators, all comparators were accepted without restriction.

Our main outcome was to study the effect of trazodone on human cognition. To assess that, we analysed all instruments that measured the cognitive impairment that appeared in the studies included in the qualitative synthesis. These instruments encompassed: the Montreal Cognitive Assessment scale (MoCA), Mini-Mental State Examination (MMSE), the Digit Span subtest, the d2-test, the Wisconsin Card Sorting Test, Continuous Performance Test, N-back Test, Paired Associate Learning Test-Form I (short-term memory), Paired Associate Learning Test-Form II (long-term memory) of the Wechsler Memory Scale and Digit Span Test, Arithmetic, Letter-Number Sequencing, Digit Symbol-Coding, Symbol Search of the Wechsler Adult Intelligence scale (third edition, WAIS-III), Buschke Selective Reminding Test, the Brown-Peterson Memory Test, the Word Learning Test, the Memory Scanning Test, the Critical Flicker/Fusion frequency (CFF), the Critical Tracking Task, the Divided Attention Test, the Visual Vigilance Test, Rey's Verbal Memory test (RVM), the

Guild memory test, Trail Making Test, Free recall test, Corsi block test, Digit span test, Category generation, 'News' recall, Who's who? or Matching to sample. Our secondary outcome was to ascertain if trazodone could be included in the treatment of neurodegenerative diseases in humans. To assess that, we searched for beneficial effects of trazodone in cognitive decline and the mechanisms through which that can occur reported in the studies included in the analysis.

The studies included comprised of randomized controlled trials, non-randomized trials, retrospective, and prospective cohorts. No limits in language or publication year were applied.

The literature search was done in electronic databases from September 14 to September 22, 2020. The search was conducted in MEDLINE (1990-Present), Web of Science (1999-Present), and CENTRAL from Cochrane (1994-Present). The last search was run on November 2, 2020.

The following search terms were used to search in PubMed: trazodone; memory; memories; cognition; cognitions; cognitive; cognitively. The detailed search strategy is depicted in Table 1.

Studies were selected in two phases by two reviewers independently. In the first phase, articles were chosen by their title and abstract. In the second phase, the articles selected in the previous phase were read in full to search for inclusion. Data were collected manually by the two reviewers independently and synthesized in tables. Controversies between reviewers were decided with discussion and consensus. For each study included, the following information was collected: the study author(s), title, year of publication and design, the follow-up period, the study size, the population being studied, its age and sex, the intervention under study, the comparators used and the outcome measures (Table 2 and Table 3). The effects of trazodone on human cognition were synthesized using a table to

represent all scores of the cognitive evaluation scales obtained to reach a final result of the effect. The final result was divided into three categories: no effect (defined as neither improving nor impairing cognition), positive effect (defined as cognition improvement or delayed cognitive decline), and negative effect (defined as cognition impairment).

To ascertain the risk of bias, the two reviewers worked blindly and independently. For a formal risk of bias assessment, it was used the Cochrane risk of bias tool for randomized controlled trials and the ROBINS criteria for observational studies.

A level of significance of 0.05 was considered.

This review is registered in PROSPERO International prospective register of systematic reviews (Registration number: CRD42020172577) and can be accessed at https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=172577. However, due to the COVID-19 pandemic, the review protocol was published exactly as submitted.

Results

Study characteristics

A total of sixteen studies were included in the final qualitative analysis of the review, four of which were observational studies, and twelve were experimental studies. After the search in electronic databases, a total of 267 citations were found. However, after removing the duplicates, 218 studies remained and were screened. Of this, and based on out of context titles and abstracts, 186 were excluded and 32 full-text articles were assessed for eligibility. Sixteen articles were subsequently excluded because of the following reasons: there was no data on outcomes of interest (n=2), there was no follow-up period between trazodone use and effect assessment (n=10), the study comprised only a commentary on an article (n=1), an abstract (n=1) or a letter to the editors (n=1), or it was a randomized clinical trial in

recruiting phase (n=1). Sixteen studies met the inclusion criteria and were included in the final analysis (See Figure 1).

For each study, the information collected is presented in Tables 2 and 3. The minimum and maximum follow-up periods observed was eight days[6] and sixteen weeks[7], respectively, for experimental studies and twelve months[8] and thirteen years[9], respectively, for observational studies (Table 2 and Table 3).

Overall, the sixteen studies included 8646 participants in the final analysis, with sample sizes ranging from 8[10] to 6798 participants[9]. All studies assessed adult individuals with at least eighteen years of age, and the majority included males and females, with two studies including only males[11,6], one including only females[12], and one study not discriminating the sex[13]. The participants were either healthy or diseased patients with one or more of the following conditions: arteriosclerotic cerebral small vessel disease (ASVD), insomnia, HIV/AIDS, dementia, Alzheimer's disease, Frontotemporal dementia, and depression (Table 2 and Table 3).

The study designs englobed randomized trials (n=10), non-randomized trials (n=1), trials without reference to the randomization (n=1), retrospective cohorts (n=2), and prospective cohorts (n=2) published from 1990 to November of 2020. Only three randomized controlled trials did not use a placebo in the comparator arm[11,13,14]. Intervention groups consisted of trazodone (with doses ranging from 25mg to 40mg for a minimum of one week) alone or in association with sleep hygiene training (SHT) and mirtazapine (15mg) and of haloperidol (0.5mg), behaviour management techniques (BMT), buspirone (30mg), 5'-methyltetrahydrofolic acid (50 mg), amitriptyline (37.5 to 75 mg), sleep medications, zolpidem, benzodiazepines, tricyclic antidepressants or noradrenaline reuptake inhibitors. Only one cohort study reported the doses used in the intervention groups[15] (Table 3).

All studies evaluated the effects of continuous doses of trazodone on cognition as a primary or secondary outcome. All other outcomes can be consulted in Table 2 and Table 3.

Effects on cognitive function

The last column of Tables 2 and 3 displays the ultimate effect of trazodone in human cognition. Overall, from the sixteen studies included, seven showed no effect of trazodone on human cognition[16,13,17,18,14,8,6], while five showed a positive effect by improving or reducing cognitive decline[11,9,15,10,5] and four a negative effect by impairing cognitive function[12,19,20,7].

From studies with beneficial effects, Wang et al. demonstrated that trazodone improved concentration and recall abilities significantly in patients with ASVD and insomnia, with an increased concentration and recall scores on MoCA after trazodone treatment compared to those at baseline (pre/post-treatment mean (SD) score in concentration and recall, respectively: 4.41 (1.57)/ 5.33 (1.45) and 1.52 (1.03)/2.24 (1.12))[5]. Alikhani et al. also showed that sustained attention on d2-test was significantly improved from baseline to week six of treatment with trazodone alone or in combination with SHT[11]. Riedel et al. demonstrated that besides improving depressive symptoms during six weeks of treatment, trazodone lowered CNS arousal (CFF frequency was lower than during placebo periods: $F(1,7)\ 8{\cdot}26$, $p < 0{\cdot}025$) and improved delayed recall ($F(1,7)\ 25{\cdot}54$, $p < 0{\cdot}001$ in Word Learning Test), while having no change in tests that evaluated memory scanning, vigilance, tracking and divided attention[10]. Furthermore, La et al. demonstrated an association between trazodone and delayed cognitive decline[15], results also supported by Burke et al.[9]. La et al. demonstrated that in a follow-up period of four years on average, compared to trazodone users, trazodone non-users decreased 2.6-fold faster on the MMSE score[15].

Burke et al. also showed that, through a follow-up period of thirteen years, trazodone could potentially mitigate the risk of mild cognitive impairment (MCI) development, decreasing cognitive decline in patients with sleep disorders and normal cognition at baseline[9].

In contrast, Roth et al. documented modest cognitive and motor impairments associated with trazodone, with a decline in short-term memory (Brown-Peterson Memory Test: main effect, $F(1,15)=17.3$, post hoc $t=4.2$, $p<0.001$), and verbal learning (Selective reminding test: main effect, $F(1,15)=5.01$, $p=0.037$; post hoc $t=2.2$, $p=0.041$) with time[19]. In concordance to this findings, Teri et all. found a significant worsening in MMSE scores in trazodone treatment group (Change score means in MMSE ± SD: 1.97 ± 3.15) compared with BMT (Change score means in MMSE ± SD 0.05 ± 2.58) after sixteen weeks[7]. Sakulsripong et al. demonstrated that both amitriptyline and, although in lesser extent, trazodone caused impairments on memory tasks such as in free recall (recall trial 1; $F(2,20)= 15.6$; $P < 0.001$, and recall trial 2 ($F(2,20) = 8.6$; $P < 0.012$), short-term visual/spatial memory (Corsi block test: $F(2,19) = 6.79$, $P < 0.01$), short-term verbal memory (Digit span test: ($F(2.19) = 6.89$, $P < 0.01$) and visual matching-to-sample only at 2 hours testing ($F(1,20)= 10.5$, $P < 0.005$), since this effects decreased over the two week period ($F(2,19) = 4.5$, $P < 0.05$). Amitriptyline but not trazodone caused impairments in both immediate and delayed recall on ‘News’ recall after a single dose[20]. On that thought, Leng et al., demonstrated that very old women using trazodone over five years were three times as likely to progress to MCI and dementia, when compared to non-trazodone users (OR = 3.48; 95% CI = 1.12-10.81), even when excluding female participants with depressive symptoms (OR = 6.11; 95% CI = 1.16-32.21), and after adjusting for baseline cognition and depressive symptoms[12].

From studies with neutral effect in cognition, Sasada et al. showed no effect of acute or repeated doses of trazodone on driving performance or cognitive function[6]. Accordingly, Camargos et al. demonstrated a therapeutic effect of trazodone on sleep disturbances in patients with Alzheimer´s disease during two weeks, without showing any effect on cognition or functionality of this patients[16]. Lebert et al. demonstrated that during six weeks and compared to placebo, trazodone showed no difference in the variation of the MMSE score ($p=0.1$)[18]. Lawlor et al. demonstrated that, compared to placebo, both treatments with buspirone and trazodone for four weeks showed no significant change in free recall and in new learning measures on the Buschke Selective Reminding Task (no numerical values were presented)[17]. Besides that, Passeri et al. showed that although the score of immediate recall assessed in RVM increased from 20±7 to 23±8 ($p<0.01$) after eight weeks of treatment with 5'-methyltetrahydrofolic acid, it remained unchanged within the trazodone group (from 22± 9 to 22±9)[14]. For delayed recall, the score in the RVM test remained unchanged for both groups in the treatment period[14]. After four-week drug-free period, no changes were observed for both treatments in either immediate or delayed recall[14]. Fudge et al. evaluated the effect of fluoxetine and trazodone on the cognitive functioning of outpatients with depression, showing that neither drug affected cognitive skills on Guild memory test for six weeks (mean trazodone pair association (pair 1; pair 2) and digit span scores at baseline and after six weeks of treatment, respectively: baseline: 4.7+2.8; 4.3+3.9 and 12.2+2.4; after six weeks: 6.7+2.9; 6.3+3.3 and 13.0+2.5)[13]. At last, Pirker-Kees et al. showed that although MMSE declined over a 12 months follow-up period (MMSE: 21.2 ± 4 versus 19.7 ± 5, $p = 0.001$), the individual treatment groups (including trazodone) did not change significantly over the same period (20.0 ± 5 versus 19.9 ± 5)[8].

Other Effects

Most studies demonstrated the effects of trazodone on domains other than cognitive function. Wang et al., Alikhani et al., Camargos et al., and Roth et al. all showed that trazodone increases sleep quality and sleep parameters such as sleep efficiency[5], N3 sleep ratio[5], sleep continuity[5], daytime functioning[11] and night time percent sleep[16], while not inducing[11,16], or decreasing daytime sleepiness[19,5], lessening night time awakenings[19], minutes of Stage 1 sleep, and self-reports of trouble sleeping[19].

Lebert et al., Lawlor et al. and Pirker-Kees et al. also stated the beneficial role of trazodone in the treatment of behavioural disturbances in neurodegenerative diseases[17,8,18]. Teri et al. showed a modest reduction in agitation in patients with AD[7] while five studies revealed a decline and relief in symptoms of depression[11,10,14,13] or anxiety[5,11].

Roth et al. demonstrated small impairments in equilibrium and arm muscle endurance[19], and Sasada et al. demonstrated that trazodone did not affect driving performance in healthy volunteers[6].

Adverse events

Only five studies mentioned the adverse effects of drugs used[5,16,18,14,7]. Overall, all study participants tolerated trazodone well, and adverse events were mild and not a major cause for participants' drop-out. Wang et al. reported, in similarity with previous studies, insomnia deterioration, akathisia, nausea, loss of appetite, dizziness, and headache, all of the mild intensity and not statistically different between trazodone and placebo treatment groups[5]. Camargos et al. did not discriminate against the adverse events that occurred but stated that encountered no differences in frequency or severity rating of adverse events between trazodone and placebo groups[16]. Lebert et al. rated all adverse events as mild, leading to a drop-out in only one patient[18]. In the placebo period, fatigue and dizziness

were reported in three patients, while in the trazodone period, fatigue, dizziness, hypotension, and cold extremities were reported in 11 patients[18]. Passeri et al. reported blurred vision and vertigo in only one patient in the trazodone treatment[14]. Al last, Teri et al. reported agitation in the trazodone group (50%), unacceptable adverse effects not otherwise specified in the haloperidol group (43%), and increased agitation in the BMT group (35%) as the main reasons for patient drop-out[7].

Risk of bias

Table 4 displays the results from the quality assessment of experimental studies using the Cochrane risk of bias tool. Of the twelve studies included, six had a low overall risk of bias, five had a high risk, and one some concerns. For the high-risk studies, the main problems arose from the randomization process, deviations from the intended interventions, and the selection of the reported result. Detailed evaluation can be assessed in Table 4. For observational studies, Table 5 shows the results obtained using ROBINS-I criteria. Three studies presented an overall serious risk of bias mainly due to residual confounding and poor characterization of intervention, and in one study it was not possible to conclude about the risk of bias due to lack of information about the concomitance of the beginning of the intervention and the follow-up (Table 5).

Discussion

So far, studies evaluating the effects of trazodone on human cognition demonstrated diverse results, with some presenting a neutral or a positive effect, while others display an impairment on cognition, making it difficult to reach a final conclusion. Overall, in our systematic review, the majority of studies (n=12) reported no effect or a positive effect of trazodone on cognitive function, while four demonstrated a negative result (Table 2 and

Table 3). Although our main findings suggest that trazodone does not impair human cognition and may even have a beneficial effect, more studies are needed to confirm the overall effect of trazodone on cognitive function.

Wang et al., Alikhani et al., Riedel et al., La et al., and Burke et al. all demonstrated a beneficial effect of trazodone in cognition[11,5,15,10,9]. However, this positive result could be attributed to the effects of the improvement of sleep disturbances like insomnia[11,9,15,5] and depressive symptoms this drug offers[10]. It is known that insomnia contributes to the progression of neurodegenerative diseases since it is associated with cognitive deterioration[11,9,5]. For that reason, it seems plausible that a drug, like trazodone, that increases sleep continuity and slow-wave sleep (SWS) ratio could, consequently, improve cognitive function[5]. Depressive symptoms are also related to cognitive decline[20] since it was associated with poor sleep and anxiety[11].

On the other hand, it is also known that antidepressants with muscarinic receptor antagonism activity can induce cognitive dysfunction[10]. However, in the study conducted by Riedel et al. high doses of trazodone (100 to 400 mg/day) improved memory and cognitive function in outpatients with depression[10]. The main reason that could explain these findings is the absence of a pronounced antimuscarinic effect observed with trazodone, even at higher doses, since this drug has the least antimuscarinic effect within anticholinergic antidepressants[10]. On the other hand, Leng et al. evidenced an association of trazodone and increase risk of cognitive impairment in old women without cognitive disturbances after five years, even when adjusting for baseline cognition and depressive symptoms, and after excluding participants with high depressive symptoms[12], suggesting a negative effect on cognition that is independent of depression improvement. However, it is important to say that in this study, of the total number of participants (n=1234) only fifteen used trazodone alone. Besides that, since it only included older

Caucasian women, these results could not be generalized to a different age, sex, and race participants[12]. For that reason, future studies should evaluate if this association between trazodone use and cognitive decline is directly due to a negative effect of this antidepressant or if it is due to the detrimental effect depression exerts on cognitive function.

To sum up, all studies that obtained a positive effect of trazodone on cognitive functions evaluated participants with ASVD, sleep disorders, HIV/AIDS undergoing methadone maintenance therapy, major depression, AD or mild cognitive impairment, disorders frequently comorbid with insomnia and depression[11,5]. For that reason, the majority of these studies demonstrated a positive effect that is possibly not due to the effects of trazodone on cognition directly but is instead mediated through an improvement in sleep disorders and depressive symptoms. Despite this, none of these results showed a negative effect on cognitive deterioration as well.

On the other spectrum, Roth et al. demonstrated a mild decremental effect on short-term memory and verbal learning with short-term low doses of trazodone (50 mg) in patients with primary insomnia[19]. However, in concordance to studies mentioned above, trazodone improved cognitive function employing an indirect beneficial effect in the treatment of insomnia. Nevertheless, the results obtained in Roth et al. are still plausible since and as Wang et al., stated, acute sedation effects observed with trazodone treatment could impair cognitive function in initial phases, repercussions that decrease with continuous therapy[5]. Roth et al.'s study only evaluated the acute effects of trazodone treatment (seven days) thereby preventing a potential improvement on cognitive tests used. For that reason, and as the author's state, long-term treatment periods with this drug should be considered in future studies to better understand its true effects on human cognition. On that line, Teri et al.

conducted a study with a follow-up period of sixteen weeks[7]. In that study, efficacy in the treatment of behavioural disturbances did not differ between trazodone, the other treatments (haloperidol and BMT), and placebo, demonstrating an additional decline in MMSE score with trazodone when compared to BMT[7]. Even so, other studies have demonstrated that this drug can improve behavioural symptoms[18,17], making it not possible to exclude a positive effect of trazodone on cognitive function through a decrease in dementia behavioural disturbances. It is also important to state that despite not demonstrating a positive effect, none of these two studies evidenced either a sustained[19] or a direct negative effect[19,7] of trazodone in human cognition as well. At last, the negative memory effects observed in Sakulsripong et al. could be due to the timing of trazodone utilization: when given in the morning trazodone could impair memory tests due to sedation and daily functioning impairment[20].

In between these results, Sasada et al. demonstrated that 25 mg of trazodone did not affect cognitive function[6]. One could argue that this low dose could be insufficiently to have any repercussions on cognition however, other studies included in this systematic review[16,13,17,18,14] also demonstrated an absence of effect with higher doses, suggesting that there is not a dose-dependent effect of trazodone in cognition. We also said that acute sedation effects observed with trazodone treatment could impair cognitive function in initial phases[5]. However, in this study by Sasada et al., we also have a short follow-up period (eight days) with a relatively small population (nineteen participants), but the acute detrimental effects of trazodone are not shown. One reason that could explain this is the dose used in this study being half of the dose in Roth et al. study (that used 50 mg). Another reason is the younger and healthy population included in this study (healthy male volunteers with ages comprised between 26 and 49 years) compared to the older and diseased participants included in Roth et al. (patients with primary insomnia with ages

comprised between 18 to 65 years). These findings could suggest that acute impairments in cognition due to acute sedation effects observed with trazodone are related to diseased older individuals since “the elderly are more vulnerable to the side effects of pharmacological treatments” (Sasada et al., 2013). However, more studies are needed to better understand this acute effect.

Lebert et al., Lawlor et al., Passeri et al., and Fudge et al. all demonstrate that even medium-high doses of trazodone (at least 100 mg) can be used safely in the treatment of behavioural disturbances in FTD[18] and AD[17], in depressive symptoms in mild to moderate dementia[14] and in outpatients with depression[13] since no detrimental effects on cognition were shown, along with good tolerability and minimum adverse events. Nevertheless, in contrast with Riedel et al., Passeri et al. did not show an improved cognitive function through alleviation of depressive symptoms[14]. In comparison to Riedel et al., Passeri et al. had a long-term follow-up period (twelve weeks), included more participants (n=120) with older age (more than 65 years), and used lower doses of trazodone (100 mg). However, the authors state that do not completely understand why an improvement in depressive symptoms does not lead to a better score on cognitive function tests[14]. These results can be corroborated by Fudge et al.[13]. Furthermore, more studies with longer follow-up periods should be conducted to understand if the absence of effect on cognitive function that these studies demonstrate translates into additional cognitive deterioration prevention.

Another aspect worth mentioning is related to patients’ caregivers. Stabilization of cognitive decline in patients with dementia is not only relevant to the patient himself but also their caregivers since, as reported by Pirker-Kees et al., caregiver burden increases with

patients' cognitive decline[8]. Consequently, addressing cognitive decline also has a role in decreasing caregiver burden and preventing burnout.

A quite interesting result is shown in La et al. study which demonstrated an association between trazodone use and delayed cognitive decline in patients with normal cognition, AD, and mild cognitive impairment, suggesting a potential role for its use in the treatment of dementia itself, and not only in dementia's comorbidities such as insomnia and agitation[15]. However, the mechanism through which this was achieved was not explored[15]. In this study, trazodone use and the follow-up period was considerably longer than other studies included in this review that showed no effect or negative effects of trazodone on cognition. These results demonstrate a longitudinal beneficial effect with long-term trazodone use, not apparent after only a few weeks of utilization[15]. Thus, these results suggest that trazodone may possess a double-dose non-dependent effect on cognitive function: in acute use, it may impair cognitive function through its sedative acute effects. On the other hand, when used continuously for long-term periods, this drug may act on mechanisms that prevent deterioration in dementia, effects for which long periods of time are required to be felt[15]. These mechanisms could include improvement in SWS and prevention of UPR pathway overactivation as a result of both neurodegenerative diseases (Figure 2) and sleep deprivation[15].

It is also important to mention that we did not observe a dose-dependent effect of trazodone in cognition since studies with low and high doses demonstrated either a positive[11,9,15,10,5], negative[12,19,20,7] or no effect[16,6,21,17,18,14,13,8] of this drug on cognitive functions.

Limitations

This study has some limitations that should make the readers interpret our findings with caution. First, all but four studies[8,15,9,12] had small periods of trazodone utilization and follow-up, with less than a year, preventing it from assessing if the beneficial effects of trazodone could develop with continuous utilization, if they were truly persistent through time or if the absence of effects could translate into additional cognitive deterioration prevention. Second, the studies used a variety of different tests to evaluate trazodone´s effects on cognition, making it difficult to compare results across different studies. Third, most of the studies only evaluated the effect of trazodone in one or two cognitive domains, which difficult the possibility of concluding about an effect on human cognition as a whole. Fourth, three studies did not report the drug´s utilized doses[8,9,12]. Sixth, in one study, reporting of adverse effects was made by participants spontaneously, some of whom with dementia, which could lead to misrecognition of side effects and reporting bias[16]. Also, there was one experimental study in which participants were aware of the intervention they received[11], with a consequently possible influence in reporting the outcomes. Seventh, the majority of our experimental studies included few participants in each treatment arm. For that reason, future studies should incorporate a larger amount of participants. At last, of all studies included in this systematic review that demonstrated a positive or detrimental effect on cognition, none explains the mechanisms through which this occurs, making it difficult to understand if these effects are mediated through improvement in SWS, depression, and other behavioural disturbances, if they are attributable to a pharmacological action (such as acting in UPR pathway) or to a combination of this effects, opening doors for future larger and long-term prospective studies to answer this question.

Conclusion

In conclusion, trazodone is a widely used old molecule with multiple mechanisms of action, some of which are useful to treat depression, insomnia, agitation, and other behavioural disturbances[3], while others were recently discovered with benefits in the pathophysiological mechanism of neurodegeneration in mice[2]. Given its common use in the elderly population where some degree of cognitive decline is expected, the establishment of its effects on cognition is of paramount importance to prevent accelerated cognitive decline, patient quality of life deterioration, and caregiver burden. However, our results are not totally conclusive since trazodone cognitive consequences are complex and require more studies to fully understand its overall effect on human cognition. Despite that, twelve of sixteen clinical studies demonstrated a neutral or even a beneficial effect on cognition. This indicates that trazodone has no long-term detrimental effect on cognitive function, thereby suggesting that, despite being FDA approved only for the treatment of depression[3], trazodone can be used safely in the treatment of comorbid conditions of patients with dementia, such as insomnia, agitation and other behavioural symptoms.

Our results also highlight the possibility of a dose-independent dual effect of trazodone on human cognition, with acute utilization associated with impaired cognitive function and longitudinally long-term use with cognitive deterioration prevention. None of the studies evaluated its effects on the UPR pathway, and there was no evidence that trazodone could be used as an active treatment of neurodegenerative diseases itself, although it seems that it can integrate the therapeutic arsenal in these cases as a safe and well-tolerated adjuvant treatment for dementia comorbidities with minimal adverse events.

For these reasons and since only animal studies were conducted so far exploring trazodone effect on UPR pathway, future studies should privilege prospective double-blind large randomized controlled trials that focus on evaluating the long term repercussions of

trazodone in human cognition. This should be done in an ideally free depression and insomnia context, with the utilization of tests that represents all cognitive domains and exploring the role of trazodone on the UPR pathway. We consider that further study of trazodone, an old molecule with new perspectives, is crucial for a better understanding of neurodegenerative mechanisms that could open doors for potential sites of action of future antidementia drugs.

Table 1 Search strategies by database.

PubMed database Search strategy in advanced search	("Trazodone" [MeSH Terms] OR ("trazodone" [All Fields] OR "Trazodone" [MeSH Terms] OR "Trazodone" [All Fields])) AND ("Memory" [MeSH Terms] OR ("memories" [All Fields] OR "Memory" [MeSH Terms] OR "Memory" [All Fields] OR "memories" [All Fields]) OR "Cognition" [MeSH Terms] OR ("Cognition" [MeSH Terms] or "Cognition" [All Fields] OR "cognitions" [All Fields] OR "cognitive" [All Fields] OR "cognitively" [All Fields] OR "cognitives" [All Fields]))
Web of Science database Search strategy in advanced search	TS=((Trazodone) AND ((cognition) OR (memory)))
CENTRAL from Cochrane library database Search strategy in search manager	#1 (*trazodone) in Trials (Word variations have been searched) #2 (*cognition) in Trials (Word variations have been searched) #3 (*memory) in Trials (Word variations have been searched) #4 MeSH descriptor: [Trazodone] explode all trees #5 MeSH descriptor: [Cognition] explode all trees #6 MeSH descriptor: [Memory] explode all trees 7# ((#1 OR #4) AND (#2 OR #3 OR #5 OR 6#)

Table 2 Characteristics of trials included in systematic review.

Author(s)	Title	Year	Study design	Follow-up period	Study size (nº of participants included in final analysis) and gender	Population (age in years or mean age in years [a])	Intervention (dose; frequency)	Comparator(s)	Outcome(s)	Effect of trazodone in human´s cognition
Wang et al.	"Effects of Trazodone on Sleep Quality and Cognitive Function in Arteriosclerotic Cerebral Small Vessel Disease Comorbid With Chronic Insomnia"	2020	Randomized, double-blind, placebo-controlled pilot study	4 weeks	40 participants (30) 15 male participants	Patients with arteriosclerotic Cerebral Small Vessel Disease and insomnia from an outpatient clinic (40-70)	Trazodone tablets (50 mg; once daily)	Placebo pills in empty capsules	Primary outcome: the cognitive score on the Montreal Cognitive Assessment scale (MoCA) Secondary outcomes: sleep parameters measured with polysomnography (PSG) and the Pittsburgh Sleep Quality Index	Positive
Alikhani et al.	"Effects of treatment of sleep disorders on sleep, psychological and cognitive	2020	Randomized trial	12 weeks	75 participants (46)	Males outpatients with HIV/AIDS undergoing methadone	Trazodone (50 mg; once daily)	Trazodone (50 mg; once daily)	Primary outcome: the impact of three different sleep-improving interventions (trazodone;	Positive

	functioning and biomarkers in individuals with HIV/AIDS and under methadone maintenance therapy”				75 male participants	maintenance therapy at the Mehr Sina Clinic of the Kermanshah University of Medical Sciences (39,60)	Sleep hygiene training (SHT); Sleep hygiene + 50 mg of trazodone, once daily	Sleep hygiene training (SHT); Sleep hygiene + 50 mg of trazodone, once daily	sleep hygiene training; sleep hygiene training + trazodone) on sleep, psychological and cognitive functioning and biomarkers in males with HIV and undergoing methadone maintenance therapy. The cognitive testing evaluated the verbal working memory (with The Digit Span subtest) and sustained attention /with the d2-test)	
Sasada et al.	“Effects of repeated dosing with mirtazapine, trazodone, or placebo on driving performance and cognitive function in healthy volunteers”	2013	Double-blind, placebo-controlled three-way crossover trial	8 days	19 participants (19) 19 male participants	Healthy male volunteers with driving license for ≥5years and who regularly drove a car: minimum, 5000 km/year (26-49).	Mirtazapine (15 mg; continuous nocturnal doses) Trazodone (25 mg; continuous nocturnal doses)	Placebo in identical capsules	Primary outcome: the effects of repeated treatments with mirtazapine and trazodone on driving performance (road tracking, car following, and harsh braking) using a driving simulator, and	No effect

									cognitive function (using the Wisconsin Card Sorting Test, Continuous Performance Test, and N-back Test).	
Camargos et al.	"Trazodone Improves Sleep Parameters in Alzheimer Disease Patients: A Randomized, Double-Blind, and Placebo-Controlled Study"	2014	Double-blind, randomized and controlled trial	7 to 9 days at baseline and 2 weeks of treatment.	47 participants (30) 10 male participants	Patients with probable AD[b] and sleep disturbances from the Geriatric medical center of the university's general hospital (60 or older)	Trazodone (50 mg; once daily at 10:00 P.M.)	Placebo	Primary outcome: the effect of trazodone in sleep disorders in patients with AD[b] (total sleep duration, in minutes, during the nocturnal period) Secondary outcome: change from baseline in cognitive assessments (for that, it was used MMSE[c], Paired Associate Learning Test-Form I (short-term memory) and Paired Associate Learning Test-Form II (long-term memory) of the Wechsler Memory Scale and Digit Span Test,	No effects

									Arithmetic, Letter-Number Sequencing, Digit Symbol-Coding and Symbol Search of the Wechsler Adult Intelligence scale (third edition, WAIS-III))	
Roth et al.	"Cognitive, Psychomotor, and Polysomnographic Effects of Trazodone in Primary Insomniacs"	2011	Within-participants, randomized, double-blind, placebo-controlled design	3 weeks: Week 1 and 3 for study assessment, and week 2 for a washout period	63 participants (16) 4 male participants	Patients with primary insomnia recruited through media advertisements and outpatient clinics of Wake Forest University Health Sciences (18 to 65)	Trazodone capsules with methylcellulose (50 mg ; 7 days, 30 minutes before bedtime)	Placebo pills with methylcellulose	Primary outcome: to quantify the hypnotic efficacy of trazodone and subsequent daytime impairments in primary insomniacs (it was used Buschke Selective Reminding Test for assessing verbal learning and The Brown-Peterson Memory Test for evaluating short-term memory)	Negative

Lebert et al.	"Frontotemporal Dementia: A Randomized, Controlled Trial with Trazodone"	2004	Randomized, double-blind, placebo-controlled cross-over trial	Two 6-weeks periods	31 participants (26) 16 male participants	Patients with FTD[d] from the University Outpatient Memory Clinic of Lille and Bailleul, France, and the Department of Neurology, Heilig Hart Ziekenhuis, Roeselare, Belgium (61,70)	Trazodone (the dose was gradually increased: 50-100mg/day, 150mg/day, 300 mg/day)	Placebo	Primary outcome: the effect of trazodone on behavioural disturbances (assessed by the total Neuropsychiatry Inventory (NPI) score) Secondary variables included the Clinical Global Impression Improvement and the MMSE[c] score	No effect
Teri et all.	"Treatment of agitation in AD[b]: a randomized, placebo-controlled clinical trial."	2000	Randomized, placebo-controlled, parallel, multicenter trial	16 weeks	149 participants (91) 67 male participants	Patients with AD[b] and their caregivers (patients mean age: 74.80 ± 8.40)	Haloperidol (0,5mg; once daily) Trazodone (50-300 mg; once daily) BMT[e] ("eight weekly and three biweekly structured sessions")	Placebo	Primary outcome: the Alzheimer's Disease Cooperative Study Clinical Global Impression of Change (ADCS-CGIC) Secondary outcomes: Patient agitation and behavioural disturbance (it was used the Consortium to Establish a Registry for Alzheimer's Disease (CERAD)	Negative

									Behavioural Rating Scale for Dementia (BRSD), the Revised Memory and Behaviour Problem Checklist (RMBPC), the Cohen-Mansfield Agitation Inventory (CMAI) and the Agitated Behaviour Inventory for Dementia (ABID)). Patient functional disturbance (it was used the Physical Self- Maintenance (PSM) and Instrumental Activities of Daily Living (IADL) scales). Cognitive function (it was used the MMSE[c]). Caregiver burden and reactivity to disruptive behaviours (it was used the Screen for Caregiver Burden (SCB))	
Riedel et al.	"The Influence of Trazodone Treatment on Cognitive	1999	Single blind clinical trial	7 weeks	8 participants (8)	Outpatients diagnosed with	Trazodone gelatin capsules (from 100 to	Placebo gelatin capsules	Primary outcome: change in psychomotor and cognitive functions (for	Positive

	Functions in Outpatients with Major Depressive Disorder”				2 male participants	single or recurrent major depression and insomnia (43.50 ± 2.60)	400 mg/day; twice daily)	Matched control group (30 volunteers)	that it was used the Word Learning Test, the Memory Scanning Test, the Critical Flicker/Fusion frequency, the Critical Tracking Task, the Divided Attention Test and the Visual Vigilance Test), and depressive symptoms (for that it was used a psychiatric interview)	
Lawlor et al.	“A pilot placebo-controlled study of trazodone and buspirone in Alzheimer´s Disease”	1994	Pilot double-blind placebo-controlled, crossover study	12 weeks	10 participants (10) 7 male participants	Patients with AD[b] and behavioural complications (67.60 ± 7.24)	Trazodone (up to 150 mg; once daily (mean dose: 129±35mg a day) Buspirone (30 mg; once daily)	Placebo	Primary outcome: the effect on behavioural disturbances (assessed with the dementia mood assessment scale and brief psychiatric rating scale) Secondary outcome: the effect on cognition (assessed with a modified (six-word) selective reminding task-Buschke	No effect

									Selective Reminding Test)	
Passeri et al.	“Oral 5'-methyltetrahydrofolic acid in senile organic mental disorders with depression: Results of a double-blind multicenter study”	1993	Double-blind, randomized, controlled multicenter study	12 weeks	120 participants (96) 43 male participants	Normofolatemic elderly patients with mild to moderate dementia and depression (more than 65 years)	5'-methyltetrahydrofolic acid (50 mg; once daily, in the morning)	Trazodone (100 mg; once daily, 1 tablet in the morning and 1 tablet in the afternoon)	Primary outcome: the effect of 5'-methyltetrahydrofolic acid on depressive symptoms (assessed with the Hamilton Depression Rating Scale (HDRS)) and cognitive status (assessed with Rey's Verbal Memory test (RVM)), comparing to trazodone.	No effect

Sakulsripong et al.	"Does tolerance develop to the sedative and amnesic effects of antidepressants? A comparison of amitriptyline, trazodone and placebo"	1991	Double-blind, crossover study	2 weeks	12 participants (12) 9 male participants	Normal healthy volunteers (19 to 38 years)	Amitriptyline (37.5 mg once daily for the first 7 days of treatment and 75 mg once daily for the next 7 days of treatment) Trazodone (100 mg once daily for the first 7 days of treatment and 200mg once daily for the next 7 days of treatment)	Placebo Amitriptyline (37.5 mg once daily for the first 7 days of treatment and 75 mg once daily for the next 7 days of treatment)	Primary outcome: to examine whether tolerance develops to the sedative, anticholinergic, psychomotor and memory effects of trazodone and amitriptyline. Secondary outcome: the effect of amitriptyline and trazodone on memory tasks (evaluated using Free recall test, Corsi block test, Digit span test, Category generation, 'News' recall, Who's who? and Matching to sample)	Negative
Fudge et al.	"A comparison of the effect of fluoxetine and trazodone on the cognitive functioning of depressed outpatients"	1990	Double-blind, randomized, parallel-design clinical trial	Two periods: 1 week and 6 weeks periods	38 participants (31) There was no data on sex discrimination	Male and female voluntaries with major depressive disorder (more than 18 years)	Trazodone (from 100 to 250 mg/day for 3 weeks; 50 to 400 mg/day from week 4 to week 6)	Fluoxetine (20 mg/day for the first 3 weeks; 40 mg/day on week 4; 20, 40 or 60 mg/day on weeks 5 and 6)	Primary outcome: the effect of fluoxetine and trazodone, on immediate and short-term memory in outpatients with depression (for that it was used the Guild memory	No effect

test: digit span and paired associations)

Notes:

[a] The values for mean age in years are mean ± standard deviation or mean age only

[b] Alzheimer disease

[c] Mini Mental State Examination

[d] Frontotemporal Dementia

[e] Behaviour management techniques

Table 3 Characteristics of observational cohort studies included in systematic review.

Author(s)	Title	Year	Study design	Follow-up period	Study size (nº of participants included in final analysis) and gender	Population (age in years or mean age in years [a])	Intervention (dose; frequency)	Comparator(s)	Outcome(s)	Effect of trazodone in human´s cognition
Pirker-Kees et al.	"Effects of Psychotropic Medication on Cognition, Caregiver Burden, and Neuropsychiatric Symptoms in Alzheimer's Disease over 12 Months: Results from a Prospective Registry of Dementia in Austria (PRODEM[b])"	2019	Prospective cohort	12 months	309 participants (149) 142 male participants	Patients from PRODEM[b] diagnosed with possible or probable AD[c] who had undergone at least one follow-up visit (76.00 ± 9.00)	Psychotropic medication: Antidepressants: Serotonin Selective Reuptake Inhibition (--:--)[d], tricyclic antidepressants (--:--)[d], trazodone (--:--)[d], mirtazapine (--;-)[d], noradrenaline reuptake inhibitors (--;--)[d] Antipsychotics (--;--)[d] Benzodiazepine (--;--)[d]	No psychotropic medication	Primary outcome: the effects of psychotropic substances on cognition (assessed with Mini-Mental State Examination (MMSE[e])), behavioural symptoms (assessed with the Neuropsychiatry Inventory (NPI)) and caregiver burden (assessed with the Zarit caregiver burden interview)	No effect

La et al.	“Long-Term Trazodone Use and Cognition: A Potential Therapeutic Role for Slow-Wave Sleep Enhancers”	2019	Retrospective cohort	Trazodone users: 3.1 ± 1.9 years[f] Non-users: 5.1 ± 2.8 years[f]	347 participants (50) 28 male participants	Participants from the UCSF Memory and Aging Center cohort with sleep disturbances and AD[c], mild cognitive impairment or normal cognition (75.40 ± 7.50)	Trazodone (median dosage of 50 mg; once daily)	No trazodone use	Primary outcome: the change in MMSE[e] between baseline and final visits. Secondary outcomes: longitudinal changes in cognitive testing of visual and verbal episodic memory through 10-minute delayed recognition of the Benson Complex Figure and the California Verbal Learning Test (CVLT) and the CVLT Second Edition. Longitudinal performance on Modified Trail-Making B, Design Fluency, Calculations, Digit-Span Forward and Backward, phonemic and semantic Verbal Fluency, and Stroop Color-Naming and Interference. To evaluate if this effects can be translated to better	Positive

									disability scores through the Clinical Dementia Rating Scale Sum of Boxes	
Burke et al.	"Mild cognitive impairment: associations with sleep disturbance, apolipoprotein e4, and sleep medications"	2018	Retrospective cohort	13 years	8043 participants (6798) 2294 male participants	Participants from the National Alzheimer's Coordinating Center Uniform Data Set with sleep disturbance and/or apolipoprotein e4 alleles, but presenting normal cognition at baseline (71.62 ±9.97)	Sleep medication: A general category (doxepin, estazolam, temazepam, trazodone, triazolam, zaleplon, and zolpidem) (--;--)[d] zolpidem (--;--)[d] Trazodone (--;--)[d]	The general category, zolpidem users, trazodone users and sleep medication non-users	Primary outcome: the onset of mild cognitive impairment (assessed in Alzheimer's Disease Center by a consensus diagnosis or by a single clinician using 2011 National Institute on Aging/Alzheimer's Association guidelines)	Positive
Leng et al.	"Antidepressant Use and Cognitive Outcomes in Very Old Women"	2018	Prospective cohort	5 years	2732 participants (1234) 1234 female participants	Community-dwelling caucasian women enrolled in the Study of Osteoporotic Fractures with	Antidepressants users: Users of SSRIs alone (--:--)[d] Users of trazodone alone(--:--)[d]	Antidepressants users: Users of SSRIs alone	Primary outcome: cognitive status (assessed with the short-form MMSE[e], Trail Making Test (Part B; Trails B), the Modified MMSE (3MS), the California Verbal	Negative

	and without depression but with no cognitive impairment (83.20 ± 2.90)	Users of tricyclic antidepressants alone(--:--)[d] Users of any other or multiple antidepressants(--:--)[d]	Users of trazodone alone Users of tricyclic antidepressants alone Users of any other or multiple antidepressants Antidepressants non-users	Learning Test (Second Edition Short Form), Digit Span test and Category and Verbal Fluency tests)

Notes:

[a] The values for mean age in years are mean ± standard deviation or mean age only

[b] The prospective dementia registry in Austria

[c] Alzheimer disease

[d] No data in the article

[e] Mini Mental State Examination

[f] Mean ± standard deviation

Table 4 Risk of bias of individual studies utilizing Cochrane Risk of Bias tool.

Studies	Risk of bias					
	Randomization process	**Deviations from the intended interventions**	**Missing outcome data**	**Measurement of the outcome**	**Selection of the reported result**	**Overall risk of bias**
Wang et al.	Some concerns	Low risk	Low risk	Some concerns	Some concerns	Some concerns
Alikhani. et al.	Some concerns	Some concerns	Some concerns	Some concerns	Low risk	High risk
Sasada et al.	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Camargos et al	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Roth et al.	Some concerns	High risk	Low risk	Low risk	Low risk	High risk
Lebert et al.	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk

Teri et al.	Low risk	Low risk	Low risk	Low risk	High risk	High risk
Riedel et al.	High risk	High risk	Low risk	Low risk	Some concerns	High risk
Lawlor et al.	Low risk	High risk	Low risk	Low risk	Low risk	High risk
Passeri et al.	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Sakulsripong et al.	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Fudge et al.	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk

Table 5 Risk of bias of individual studies utilizing ROBINS-I criteria for cohort studies.

Studies	Risk of bias							
	Confounding	**Selection of participants**	**Classification of interventions**	**Deviations from the intended interventions**	**Missing data**	**Measurement of the outcome**	**Selection of the reported result**	**Overall risk of bias**
Pirker-Kees et al.	Moderate risk	Low risk	Serious risk	Low risk	Low risk	Low risk	Low risk	Serious risk
La et al.	Moderate risk	No information	Low risk	Low risk	Low risk	Low risk	Low risk	No information
Burke et al.	Moderate risk	No information	Serious risk	Low risk	Low risk	Low risk	Low risk	Serious risk
Leng et al	Moderate risk	Moderate risk	Serious risk	Low risk	Low risk	Low risk	Low risk	Serious risk

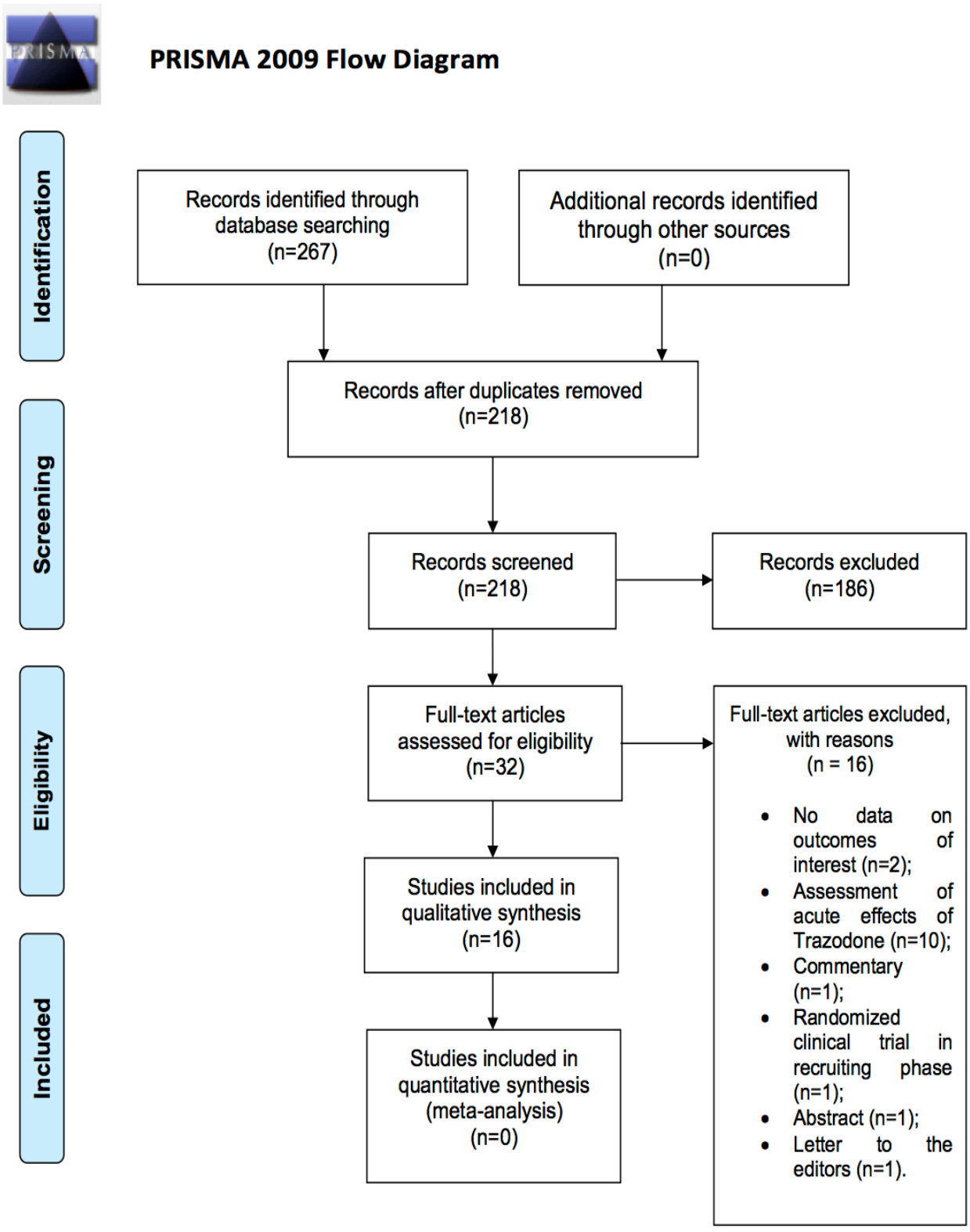

Fig. 1 Prisma 2009 Flow Diagram

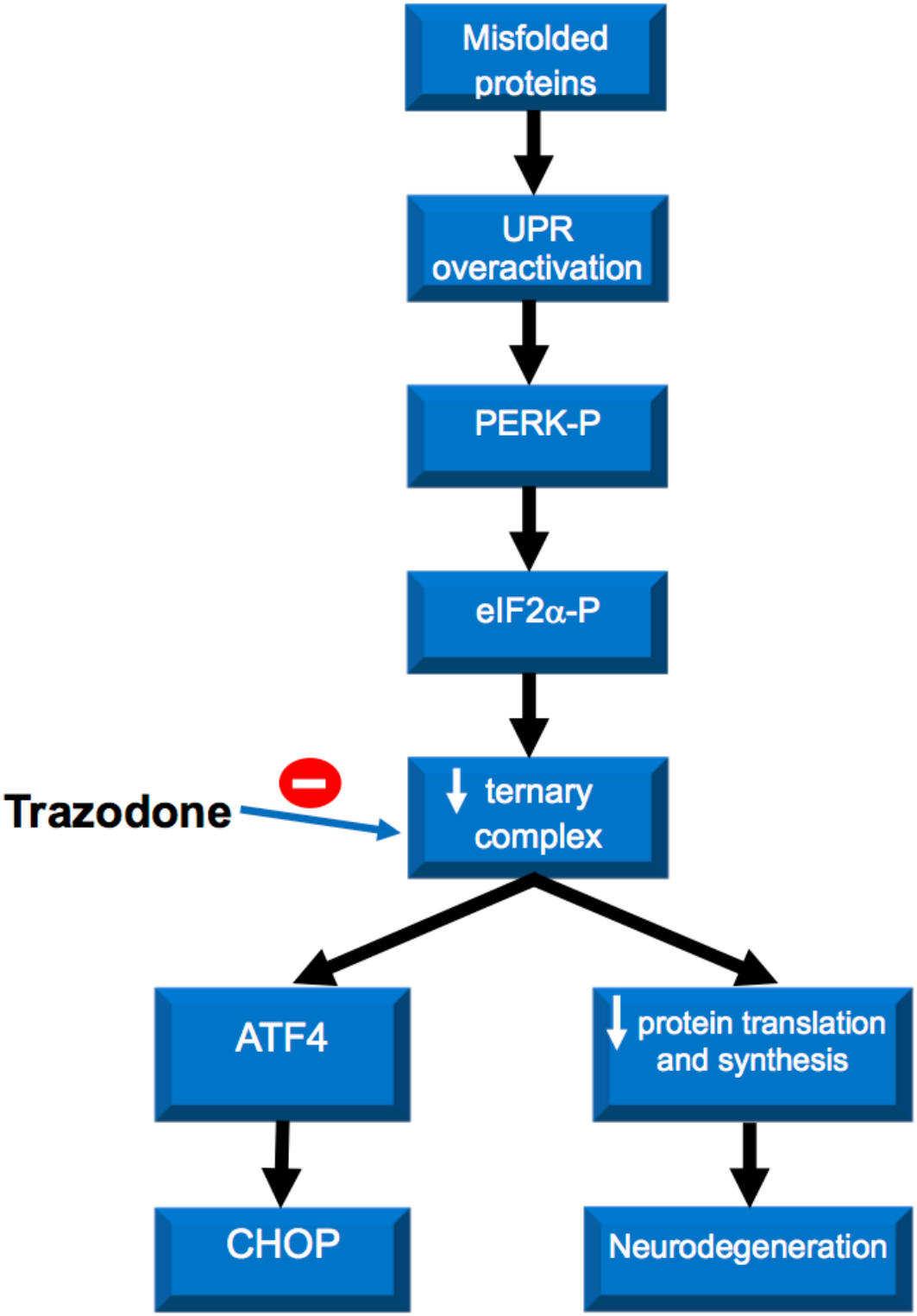

Fig. 2 UPR overactivation and trazodone site of action in the PERK branch of the UPR pathway. In protein misfolding disorders there is a disruption in protein homeostasis through

endoplasmic reticulum (ER) stress, leading to the activation and dysregulation of the UPR response [4]. UPR acts as a cellular mechanism for the regulation of protein homeostasis when there are misfolded proteins [4] and coordinates this process through three ER transmembrane proteins: PERK, inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). Thus, in the face of misfolded proteins, PERK dimerizes, autophosphorylates, and becomes activated. Subsequently, PERK phosphorylates the α-subunit of eIF2, averting the formation of the ternary complex, resulting in a consequent blockage of proteins crucial for learning, memory, synaptic maintenance, and neuronal survival [4]. On the other hand, PERK activation culminates with the translation of the activating transcription factor 4 (ATF4) which upregulates proteins that restore cellular homeostasis and CHOP [4].

Trazodone acts in the PERK branch of the UPR pathway downstream of eIF2α-P, preventing it from reducing levels of the ternary complex, allowing protein translation to occur [2], restoring neuronal protein synthesis rates, enabling a boost of memory and preventing neurodegeneration in mice models [4]. **UPR:** Unfolded protein response; **PERK-P:** phosphorylated RNA (PKR)-like ER kinase; **eIF2α:** α-subunit of eukaryotic initiation factor 2; **ATF4:** activating transcription factor 4; **CHOP:** CEBP homologous protein

Authors' contributions

Ana Mafalda Gonçalves Gonçalo contributed to the design and conceptualization of the study, and had a major role in the acquisition, analysis, and interpretation of the data.

Maria Augusta Vieira-Coelho, MD, Ph.D. contributed to the design and conceptualization of the study, had a major role in the acquisition, analysis, and interpretation of the data, and revised the manuscript for intellectual content.

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Anexos

Anexo I: PRISMA Reporting Guidelines

Anexo II: Normas da Revista: European Journal of Clinical Pharmacology

Anexo I: PRISMA Reporting Guidelines

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	"The effects of Trazodone on human cognition: a systematic review"	2
ABSTRACT			
Structured summary	2	"Trazodone is a widely used antidepressant, also useful in the control of agitation and insomnia in Alzheimer's disease. It is now recognized a new mechanism of action for trazodone, based on its effect on the Unfolded Protein Response (UPR) pathway, restoring protein translation and slowing neurodegenerative progression in mice. These mechanisms may be seen as promising in dementia modifying treatment. To explore the effects of trazodone on human cognition and search for clinical evidence of its putative benefits in human's neurodegenerative diseases, a systematic review was conducted for studies that evaluated the effect of a minimum of 25 mg of trazodone daily, for at least one week, in the cognition of healthy or diseased patients with eighteen years or more. The search was run in MEDLINE, Web of Science, and CENTRAL from Cochrane databases, yielding a total of 16	3

		studies, after selection. Overall, seven studies showed no effect of trazodone on cognition, five showed a beneficial effect by improving or reducing cognitive decline and four evidenced impaired cognitive function. Our analysis highlights the possibility of a dose-independent dual effect of trazodone on human cognition, with acute utilization associated with impaired cognitive function and long-term use with cognitive deterioration prevention. There was no evidence that trazodone could be used as an active treatment of neurodegenerative diseases itself, but the majority (n=12) of our findings indicate that it has no long-term cognitive detrimental effect. Future studies should explore trazodone's role in the UPR pathway and the implications in neurodegenerative diseases in humans."	
INTRODUCTION			
Rationale	3	"Although being FDA approved only for use in the treatment of major depression, trazodone, a widespread drug created in the 1960s, is used off label to control agitation and insomnia in Alzheimer's disease. It is now recognized a new mechanism of action for this drug, based on its effect in the UPR pathway. As a result, and as stated by Halliday et al., it could be seen as a new disease-modifying treatment for neurodegenerative diseases in humans. To establish the effects of trazodone on human cognition would have a great effect on medical practice, given the	5

		increasing prevalence of neurodegenerative diseases and the extensive use of trazodone in this population of patients.”	
Objectives	4	“we aimed to establish the effects of trazodone on human cognition and to find out if there was evidence that it could be used in the treatment of neurodegenerative diseases in humans.”	5
METHODS			
Protocol and registration	5	“This review is registered in PROSPERO International prospective register of systematic reviews (Registration number: CRD42020172577) and can be accessed at https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=172577. However, due to the COVID-19 pandemic, the review protocol was published exactly as submitted.”	8
Eligibility criteria	6	“Our target population was healthy or diseased adults with eighteen years old or more. The diseased patients could have all types of illness, from atherosclerotic disease to psychiatric conditions. We excluded animal studies since we aimed to study the consequences in humans so that it was possible to assess the implications for clinical practice. Our intervention consisted of a minimum of 25 mg of trazodone daily, for at least one week, to observe the chronic effects of this drug and not the effects obtained after a single dose. Consequently, we excluded studies that only tested the acute effects of trazodone by using it in a	6-7

		one-time-only fashion. The dose of 25 mg was chosen because it is the minimum dose that is used and that is capable of producing some effects of the drug observed in clinical practice. In terms of comparators, all comparators were accepted without restriction. Our main outcome was to study the effect of trazodone on human cognition. To assess that, we analysed all instruments that measured the cognitive impairment that appeared in the studies included in the qualitative synthesis. Our secondary outcome was to ascertain if trazodone could be included in the treatment of neurodegenerative diseases in humans. To assess that, we searched for beneficial effects of trazodone in cognitive decline and the mechanisms through which that can occur reported in the studies included in the analysis. The studies included comprised of randomized controlled trials, non-randomized trials, retrospective, and prospective cohorts. No limits in language or publication year were applied."	
Information sources	7	"The literature search was done in electronic databases from September 14 to September 22, 2020. The search was conducted in MEDLINE (1990-Present), Web of Science (1999-Present), and CENTRAL from Cochrane (1994-Present). The last search was run on November 2, 2020."	7

Search	8	"**CENTRAL from Cochrane library database-** Search strategy in search manager: #1 (*trazodone) in Trials (Word variations have been searched) #2 (*cognition) in Trials (Word variations have been searched) #3 (*memory) in Trials (Word variations have been searched) #4 MeSH descriptor: [Trazodone] explode all trees #5 MeSH descriptor: [Cognition] explode all trees #6 MeSH descriptor: [Memory] explode all trees 7# ((#1 OR #4) AND (#2 OR #3 OR #5 OR 6#)"	23
Study selection	9	"Studies were selected in two phases by two reviewers independently. In the first phase, articles were chosen by their title and abstract. In the second phase, the articles selected in the previous phase were read in full to search for inclusion. Data were collected manually by the two reviewers independently and synthesized in tables. Controversies between reviewers were decided with discussion and consensus. "	7
Data collection process	10	"Data were collected manually by the two reviewers independently and synthesized in tables."	7
Data items	11	"For each study included, the following information was collected: the study author(s), title, year of publication and design, the follow-up period, the study size, the population being studied, its age	7-8

		and sex, the intervention under study, the comparators used and the outcome measures (Table 2 and Table 3). The effects of trazodone on human cognition were synthesized using a table to represent all scores of the cognitive evaluation scales obtained to reach a final result of the effect. The final result was divided into three categories: no effect (defined as neither improving nor impairing cognition), positive effect (defined as cognition improvement or delayed cognitive decline), and negative effect (defined as cognition impairment)."	
Risk of bias in individual studies	12	"To ascertain the risk of bias, the two reviewers worked blindly and independently. For a formal risk of bias assessment, it was used the Cochrane risk of bias tool for randomized controlled trials and the ROBINS criteria for observational studies."	8
Summary measures	13	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise	
Synthesis of results	14	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise	

Section/topic	#	Checklist item	Reported on page #

Risk of bias across studies	15	“To ascertain the risk of bias, the two reviewers worked blindly and independently. For a formal risk of bias assessment, it was used the Cochrane risk of bias tool for randomized controlled trials and the ROBINS criteria for observational studies.”	8
Additional analyses	16	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise	
RESULTS			
Study selection	17	“A total of sixteen studies were included in the final qualitative analysis of the review, four of which were observational studies, and twelve were experimental studies. After the search in electronic databases, a total of 267 citations were found. However, after removing the duplicates, 218 studies remained and were screened. Of this, and based on out of context titles and abstracts, 186 were excluded and 32 full-text articles were assessed for eligibility. Sixteen articles were subsequently excluded because of the following reasons: there was no data on outcomes of interest (n=2), there was no follow-up period between trazodone use and effect assessment (n=10), the study comprised only a commentary on an article (n=1), an abstract (n=1) or a letter to the editors (n=1), or it was a randomized clinical trial in recruiting phase (n=1). Sixteen studies met the inclusion criteria and were included in the final analysis (See Figure 1).”	8-9

Study characteristics	18	“For each study, the information collected is presented in Tables 2 and 3. The minimum and maximum follow-up periods observed was eight days[6] and sixteen weeks[7], respectively, for experimental studies and twelve months[8] and thirteen years[9], respectively, for observational studies (Table 2 and Table 3). Overall, the sixteen studies included 8646 participants in the final analysis, with sample sizes ranging from 8[10] to 6798 participants[9]. All studies assessed adult individuals with at least eighteen years of age, and the majority included males and females, with two studies including only males[11,6], one including only females[12], and one study not discriminating the sex[13]. The participants were either healthy or diseased patients with one or more of the following conditions: arteriosclerotic cerebral small vessel disease (ASVD), insomnia, HIV/AIDS, dementia, Alzheimer’s disease, Frontotemporal dementia, and depression (Table 2 and Table 3). The study designs englobed randomized trials (n=10), non-randomized trials (n=1), trials without reference to the randomization (n=1), retrospective cohorts (n=2), and prospective cohorts (n=2) published from 1990 to November of 2020. Only three randomized controlled trials did not use a placebo in the comparator arm[11,13,14]. Intervention groups consisted of trazodone (with doses ranging from 25mg to 40mg for a minimum of one week) alone or in association with sleep hygiene training (SHT) and mirtazapine (15mg) and of haloperidol (0.5mg), behaviour	9-10, 24-37

		management techniques (BMT), buspirone (30mg), 5'-methyltetrahydrofolic acid (50 mg), amitriptyline (37.5 to 75 mg), sleep medications, zolpidem, benzodiazepines, tricyclic antidepressants or noradrenaline reuptake inhibitors. Only one cohort study reported the doses used in the intervention groups[15] (Table 3). All studies evaluated the effects of continuous doses of trazodone on cognition as a primary or secondary outcome. All other outcomes can be consulted in Table 2 and Table 3."	
Risk of bias within studies	19	"Table 4 displays the results from the quality assessment of experimental studies using the Cochrane risk of bias tool. Of the twelve studies included, six had a low overall risk of bias, five had a high risk, and one some concerns. For the high-risk studies, the main problems arose from the randomization process, deviations from the intended interventions, and the selection of the reported result. Detailed evaluation can be assessed in Table 4. For observational studies, Table 5 shows the results obtained using ROBINS-I criteria. Three studies presented an overall serious risk of bias mainly due to residual confounding and poor characterization of intervention, and in one study it was not possible to conclude about the risk of bias due to lack of information about the concomitance of the beginning of the intervention and the follow-up (Table 5)."	14, 38-40

Results of individual studies	20	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise	
Synthesis of results	21	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise	
Risk of bias across studies	22	“Table 4 displays the results from the quality assessment of experimental studies using the Cochrane risk of bias tool. Of the twelve studies included, six had a low overall risk of bias, five had a high risk, and one some concerns. For the high-risk studies, the main problems arose from the randomization process, deviations from the intended interventions, and the selection of the reported result. Detailed evaluation can be assessed in Table 4. For observational studies, Table 5 shows the results obtained using ROBINS-I criteria. Three studies presented an overall serious risk of bias mainly due to residual confounding and poor characterization of intervention, and in one study it was not possible to conclude about the risk of bias due to lack of information about the concomitance of the beginning of the intervention and the follow-up (Table 5).”	14, 38-40
Additional analysis	23	Não aplicável, uma vez que esta revisão sistemática não se acompanhou de meta-análise	
DISCUSSION			
Summary of evidence	24	“So far, studies evaluating the effects of trazodone on human cognition demonstrated diverse results, with some presenting a neutral or a positive effect, while others display an impairment on	14-19

		cognition, making it difficult to reach a final conclusion. Overall, in our systematic review, the majority of studies (n=12) reported no effect or a positive effect of trazodone on cognitive function, while four demonstrated a negative result (Table 2 and Table 3). Although our main findings suggest that trazodone does not impair human cognition and may even have a beneficial effect, more studies are needed to confirm the overall effect of trazodone on cognitive function. Wang et al., Alikhani et al., Riedel et al., La et al., and Burke et al. all demonstrated a beneficial effect of trazodone in cognition[11,5,15,10,9]. However, this positive result could be attributed to the effects of the improvement of sleep disturbances like insomnia[11,9,15,5] and depressive symptoms this drug offers[10]. It is known that insomnia contributes to the progression of neurodegenerative diseases since it is associated with cognitive deterioration[11,9,5]. For that reason, it seems plausible that a drug, like trazodone, that increases sleep continuity and slow-wave sleep (SWS) ratio could, consequently, improve cognitive function[5]. Depressive symptoms are also related to cognitive decline[20] since it was associated with poor sleep and anxiety[11]. On the other hand, it is also known that antidepressants with muscarinic receptor antagonism activity can induce cognitive dysfunction[10]. However, in the study conducted by Riedel et al. high doses of trazodone (100 to 400 mg/day) improved memory and cognitive function in	

		outpatients with depression[10]. The main reason that could explain these findings is the absence of a pronounced antimuscarinic effect observed with trazodone, even at higher doses, since this drug has the least antimuscarinic effect within anticholinergic antidepressants[10]. On the other hand, Leng et al. evidenced an association of trazodone and increase risk of cognitive impairment in old women without cognitive disturbances after five years, even when adjusting for baseline cognition and depressive symptoms, and after excluding participants with high depressive symptoms[12], suggesting a negative effect on cognition that is independent of depression improvement. However, it is important to say that in this study, of the total number of participants (n=1234) only fifteen used trazodone alone. Besides that, since it only included older Caucasian women, these results could not be generalized to a different age, sex, and race participants[12]. For that reason, future studies should evaluate if this association between trazodone use and cognitive decline is directly due to a negative effect of this antidepressant or if it is due to the detrimental effect depression exerts on cognitive function. To sum up, all studies that obtained a positive effect of trazodone on cognitive functions evaluated participants with ASVD, sleep disorders, HIV/AIDS undergoing methadone maintenance therapy, major depression, AD or mild cognitive impairment, disorders frequently	

		comorbid with insomnia and depression[11,5]. For that reason, the majority of these studies demonstrated a positive effect that is possibly not due to the effects of trazodone on cognition directly but is instead mediated through an improvement in sleep disorders and depressive symptoms. Despite this, none of these results showed a negative effect on cognitive deterioration as well. On the other spectrum, Roth et al. demonstrated a mild decremental effect on short-term memory and verbal learning with short-term low doses of trazodone (50 mg) in patients with primary insomnia[19]. However, in concordance to studies mentioned above, trazodone improved cognitive function employing an indirect beneficial effect in the treatment of insomnia. Nevertheless, the results obtained in Roth et al. are still plausible since and as Wang et al., stated, acute sedation effects observed with trazodone treatment could impair cognitive function in initial phases, repercussions that decrease with continuous therapy[5]. Roth et al.'s study only evaluated the acute effects of trazodone treatment (seven days) thereby preventing a potential improvement on cognitive tests used. For that reason, and as the author's state, long-term treatment periods with this drug should be considered in future studies to better understand its true effects on human cognition. On that line, Teri et al. conducted a study with a follow-up period	

		of sixteen weeks[7]. In that study, efficacy in the treatment of behavioural disturbances did not differ between trazodone, the other treatments (haloperidol and BMT), and placebo, demonstrating an additional decline in MMSE score with trazodone when compared to BMT[7]. Even so, other studies have demonstrated that this drug can improve behavioural symptoms[18,17], making it not possible to exclude a positive effect of trazodone on cognitive function through a decrease in dementia behavioural disturbances. It is also important to state that despite not demonstrating a positive effect, none of these two studies evidenced either a sustained[19] or a direct negative effect[19,7] of trazodone in human cognition as well. At last, the negative memory effects observed in Sakulsripong et al. could be due to the timing of trazodone utilization: when given in the morning trazodone could impair memory tests due to sedation and daily functioning impairment[20]. In between these results, Sasada et al. demonstrated that 25 mg of trazodone did not affect cognitive function[6]. One could argue that this low dose could be insufficiently to have any repercussions on cognition however, other studies included in this systematic review[16,13,17,18,14] also demonstrated an absence of effect with higher doses, suggesting that there is not a dose-dependent effect of trazodone in cognition. We also said that acute	

		sedation effects observed with trazodone treatment could impair cognitive function in initial phases[5]. However, in this study by Sasada et al., we also have a short follow-up period (eight days) with a relatively small population (nineteen participants), but the acute detrimental effects of trazodone are not shown. One reason that could explain this is the dose used in this study being half of the dose in Roth et al. study (that used 50 mg). Another reason is the younger and healthy population included in this study (healthy male volunteers with ages comprised between 26 and 49 years) compared to the older and diseased participants included in Roth et al. (patients with primary insomnia with ages comprised between 18 to 65 years). These findings could suggest that acute impairments in cognition due to acute sedation effects observed with trazodone are related to diseased older individuals since “the elderly are more vulnerable to the side effects of pharmacological treatments” (Sasada et al., 2013). However, more studies are needed to better understand this acute effect. Lebert et al., Lawlor et al., Passeri et al., and Fudge et al. all demonstrate that even medium-high doses of trazodone (at least 100 mg) can be used safely in the treatment of behavioural disturbances in FTD[18] and AD[17], in depressive symptoms in mild to moderate dementia[14] and in outpatients with depression[13] since no detrimental effects on cognition were shown,	

		along with good tolerability and minimum adverse events. Nevertheless, in contrast with Riedel et al., Passeri et al. did not show an improved cognitive function through alleviation of depressive symptoms[14]. In comparison to Riedel et al., Passeri et al. had a long-term follow-up period (twelve weeks), included more participants (n=120) with older age (more than 65 years), and used lower doses of trazodone (100 mg). However, the authors state that do not completely understand why an improvement in depressive symptoms does not lead to a better score on cognitive function tests[14]. These results can be corroborated by Fudge et al.[13]. Furthermore, more studies with longer follow-up periods should be conducted to understand if the absence of effect on cognitive function that these studies demonstrate translates into additional cognitive deterioration prevention. Another aspect worth mentioning is related to patients' caregivers. Stabilization of cognitive decline in patients with dementia is not only relevant to the patient himself but also their caregivers since, as reported by Pirker-Kees et al., caregiver burden increases with patients' cognitive decline[8]. Consequently, addressing cognitive decline also has a role in decreasing caregiver burden and preventing burnout.	

		A quite interesting result is shown in La et al. study which demonstrated an association between trazodone use and delayed cognitive decline in patients with normal cognition, AD, and mild cognitive impairment, suggesting a potential role for its use in the treatment of dementia itself, and not only in dementia's comorbidities such as insomnia and agitation[15]. However, the mechanism through which this was achieved was not explored[15]. In this study, trazodone use and the follow-up period was considerably longer than other studies included in this review that showed no effect or negative effects of trazodone on cognition. These results demonstrate a longitudinal beneficial effect with long-term trazodone use, not apparent after only a few weeks of utilization[15]. Thus, these results suggest that trazodone may possess a double-dose non-dependent effect on cognitive function: in acute use, it may impair cognitive function through its sedative acute effects. On the other hand, when used continuously for long-term periods, this drug may act on mechanisms that prevent deterioration in dementia, effects for which long periods of time are required to be felt[15]. These mechanisms could include improvement in SWS and prevention of UPR pathway overactivation as a result of both neurodegenerative diseases (Figure 2) and sleep deprivation[15].	

		It is also important to mention that we did not observe a dose-dependent effect of trazodone in cognition since studies with low and high doses demonstrated either a positive[11,9,15,10,5], negative[12,19,20,7] or no effect[16,6,21,17,18,14,13,8] of this drug on cognitive functions."	
Limitations	25	"This study has some limitations that should make the readers interpret our findings with caution. First, all but four studies[8,15,9,12] had small periods of trazodone utilization and follow-up, with less than a year, preventing it from assessing if the beneficial effects of trazodone could develop with continuous utilization, if they were truly persistent through time or if the absence of effects could translate into additional cognitive deterioration prevention. Second, the studies used a variety of different tests to evaluate trazodone´s effects on cognition, making it difficult to compare results across different studies. Third, most of the studies only evaluated the effect of trazodone in one or two cognitive domains, which difficult the possibility of concluding about an effect on human cognition as a whole. Fourth, three studies did not report the drug´s utilized doses[8,9,12]. Sixth, in one study, reporting of adverse effects was made by participants spontaneously, some of whom with dementia, which could lead to misrecognition of side effects and reporting bias[16]. Also, there was one experimental study in which participants were aware of the intervention they	19-20

		received[11], with a consequently possible influence in reporting the outcomes. Seventh, the majority of our experimental studies included few participants in each treatment arm. For that reason, future studies should incorporate a larger amount of participants. At last, of all studies included in this systematic review that demonstrated a positive or detrimental effect on cognition, none explains the mechanisms through which this occurs, making it difficult to understand if these effects are mediated through improvement in SWS, depression, and other behavioural disturbances, if they are attributable to a pharmacological action (such as acting in UPR pathway) or to a combination of this effects, opening doors for future larger and long-term prospective studies to answer this question."	
Conclusions	26	"In conclusion, trazodone is a widely used old molecule with multiple mechanisms of action, some of which are useful to treat depression, insomnia, agitation, and other behavioural disturbances[3], while others were recently discovered with benefits in the pathophysiological mechanism of neurodegeneration in mice[2]. Given its common use in the elderly population where some degree of cognitive decline is expected, the establishment of its effects on cognition is of paramount importance to prevent accelerated cognitive decline, patient quality of life	20-22

		deterioration, and caregiver burden. However, our results are not totally conclusive since trazodone cognitive consequences are complex and require more studies to fully understand its overall effect on human cognition. Despite that, twelve of sixteen clinical studies demonstrated a neutral or even a beneficial effect on cognition. This indicates that trazodone has no long-term detrimental effect on cognitive function, thereby suggesting that, despite being FDA approved only for the treatment of depression[3], trazodone can be used safely in the treatment of comorbid conditions of patients with dementia, such as insomnia, agitation and other behavioural symptoms. Our results also highlight the possibility of a dose-independent dual effect of trazodone on human cognition, with acute utilization associated with impaired cognitive function and longitudinally long-term use with cognitive deterioration prevention. None of the studies evaluated its effects on the UPR pathway, and there was no evidence that trazodone could be used as an active treatment of neurodegenerative diseases itself, although it seems that it can integrate the therapeutic arsenal in these cases as a safe and well-tolerated adjuvant treatment for dementia comorbidities with minimal adverse events. For these reasons and since only animal studies were conducted so far exploring trazodone effect	

		on UPR pathway, future studies should privilege prospective double-blind large randomized controlled trials that focus on evaluating the long term repercussions of trazodone in human cognition. This should be done in an ideally free depression and insomnia context, with the utilization of tests that represents all cognitive domains and exploring the role of trazodone on the UPR pathway. We consider that further study of trazodone, an old molecule with new perspectives, is crucial for a better understanding of neurodegenerative mechanisms that could open doors for potential sites of action of future antidementia drugs."	
FUNDING			
Funding	27	"The authors declare no funding for this study."	4

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Anexo II: Normas da Revista: European Journal of Clinical Pharmacology

SUBMISSION GUIDELINES

INSTRUCTIONS FOR AUTHORS

TYPES OF PAPERS

- Original research papers The number of printed pages per article including figures, tables and references should not exceed 8-10 printed pages (one printed page accounts to approx 650 words or 4300 characters, plain text without spaces; figures and tables have to be counted extra as half a page each).
- Editorials (without abstract)
- Letter to the Editor A commentary or a case report or otherwise a brief communication on a specific topic should have no more than 600 words main text (excepting the reference list) and contain no more than one table or one figure. The letter should not contain an abstract and should not be subdivided into sections.
- Review articles Review articles on various topics are welcome. Both invited and unsolicited submissions are published. Any submitted review will be peer-reviewed as other submissions. A word limit is not specified for reviews. The Journal welcomes "full-sized" reviews of up to 4,000 words (main text) as well as "condensed" reviews or "occasional updates" of around 1,000 words. If a meta-analysis is part of a review article, please refer to the section below "Reporting on Meta-Analyses". For unsolicited review articles, submissions should be accompanied by a cover letter which explains in detail: (i) whether the review is a systematic review (preferred) or a narrative review. If it is a narrative review, the reason why this procedure was favored needs to be given. (ii) the objective of the review. Specifically, it should be described what the review is expected to add. The author should also cite here the most recent and/or most similar published related manuscripts and briefly explain the gain of knowledge by the present manuscript as compared to the previous papers. (iii) a rationale for the methods selected, i.e. for the search strategy, for the method how relevant data was extracted, and for the analysis of the results. (iv) a brief description of the limitations of the review, accompanied by an explanation why the review is considered as valid despite the respective limitation. For instance, if just three articles were summarized, this should be emphasized here and it could be explained that there are no other high-quality data available and how this review still provides novel knowledge.
- Reporting Clinical Trials The Editors believe that it is important to foster a comprehensive, publicly available database of clinical trials. In compliance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), EJCP therefore requires that authors must register clinical trials before the first subject is enrolled. This policy goes into effect on June 1, 2007. Trials that were under way before that date and not registered and that are submitted to EJCP no later than June 1, 2008 will not be forced under the new guideline. For EJCP, clinical trial is defined as any research project that prospectively assigns human subjects to a pharmacological intervention or concurrent comparison or control groups to study the

cause-and-effect relationship between this intervention and a health outcome. The ICMJE policies on registration of clinical trials can be found at: http://www.icmje.org/clin_trialup.htm. EJCP does not advocate one particular registry. Appropriate registries (such as www.clinicaltrials.gov) must be (1) accessible to the public at no charge, (2) open to all prospective registrants, and (3) managed by a not-for-profit organization. There must be a mechanism to ensure the validity of the registration data, and the registry should be electronically searchable. An acceptable registry must include at minimum the data elements available at the ICMJE website listed above. The title page of a manuscript describing the results of a clinical trial must contain the name of the clinical trial registry and registration number of the trial. Any report of a clinical trial not containing such information will be returned to the corresponding author without review. Reports of randomized, controlled trials should follow the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) statement. See for the current CONSORT guidelines and checklist http://www.consort-statement.org/statement/revisedstatement.htm.

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  Comment: “I think this study would have more value if a higher dose of the perpetrator drug was studied”.

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Reference citations in the text should be identified by numbers in square brackets. Some examples:

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  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007

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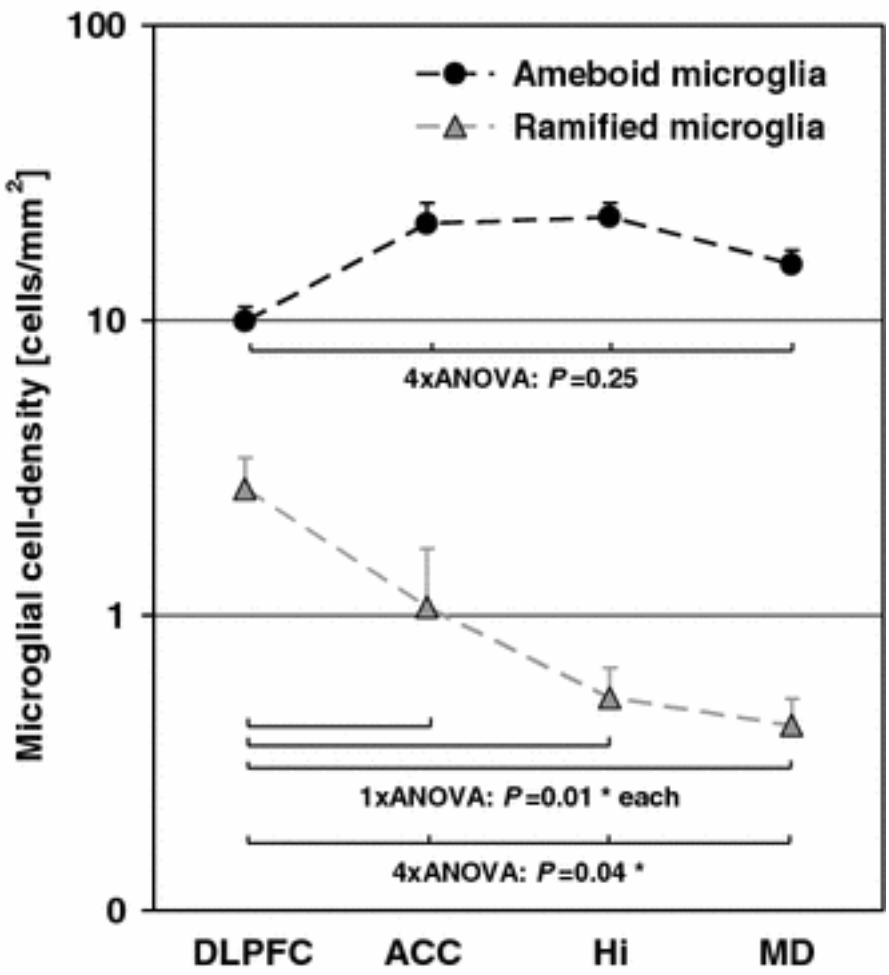

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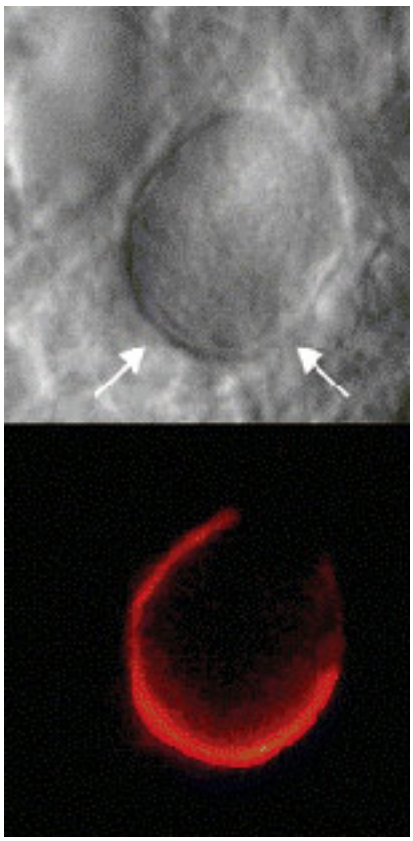

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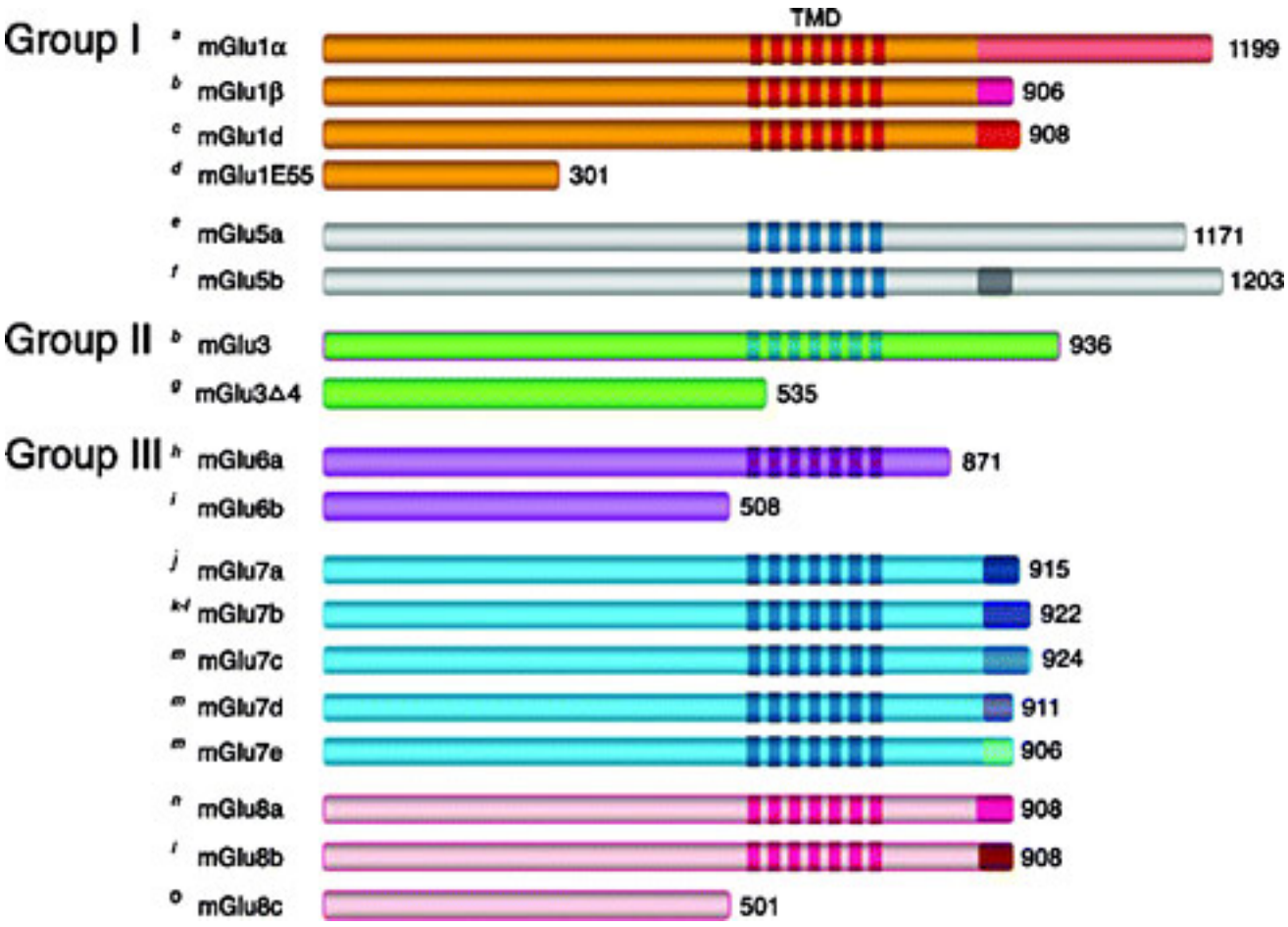

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Adding and/or deleting authors at revision stage are generally not permitted, but in some cases it may be warranted. Reasons for these changes in authorship should be explained. Approval of the change during revision is at the discretion of the Editor-in-Chief. Please note that journals may have individual policies on adding and/or deleting authors during revision stage.

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To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled "Compliance with Ethical Standards" when submitting a paper:

- Disclosure of potential conflicts of interest
- Research involving Human Participants and/or Animals
- Informed consent

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The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

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Authors are requested to disclose interests ***that are directly or indirectly related to the work submitted for publication***. Interests within the last 3 years of beginning the work (conducting the research and preparing the work for submission) should be reported. Interests outside the 3-year time frame must be disclosed if they could reasonably be

perceived as influencing the submitted work. Disclosure of interests provides a complete and transparent process and helps readers form their own judgments of potential bias. This is not meant to imply that a financial relationship with an organization that sponsored the research or compensation received for consultancy work is inappropriate.

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It is difficult to specify a threshold at which a financial interest becomes significant, any such figure is necessarily arbitrary, so one possible practical guideline is the following: "Any undeclared financial interest that could embarrass the author were it to become publicly known after the work was published."

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Please note that, in addition to the above requirements, funding information (given that funding is a potential conflict of interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is **not added** to the manuscript itself. Under ‘summary of requirements’ (see below) funding information should be included in the ‘**Declarations**’ section.

SUMMARY OF REQUIREMENTS

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Funding' and/or 'Conflicts of interests'/'Competing interests'. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.

Examples of statements to be used when funding has been received:

- Partial financial support was received from [...]
- The research leading to these results received funding from […] under Grant Agreement No[…].
- This study was funded by […]
- This work was supported by […] (Grant numbers […] and […]

Examples of statements to be used when there is no funding:

- The authors did not receive support from any organization for the submitted work.
- No funding was received to assist with the preparation of this manuscript.
- No funding was received for conducting this study.
- No funds, grants, or other support was received.

Examples of statements to be used when there are interests to declare:

- **Financial interests:** Author A has received research support from Company A. Author B has received a speaker honorarium from Company Wand owns stock in Company X. Author C is consultant to company Y.

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- **Financial interests:** The authors declare they have no financial interests.

  Non-financial interests: Author A is on the board of directors of Y and receives no compensation as member of the board of directors.

- **Financial interests:** Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.

  Non-financial interests: none.

- **Financial interests:** Author A and B declare they have no financial interests. Author C has received speaker and consultant honoraria from Company M and Company N. Dr. C has received speaker honorarium and research funding from Company M and Company O. Author D has received travel support from Company O.

Non-financial interests: Author D has served on advisory boards for Company M, Company N and Company O.

Examples of statements to be used when authors have nothing to declare:

- The authors have no relevant financial or non-financial interests to disclose.
- The authors have no conflicts of interest to declare that are relevant to the content of this article.
- All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.
- The authors have no financial or proprietary interests in any material discussed in this article.

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RESEARCH INVOLVING HUMAN PARTICIPANTS, THEIR DATA OR BIOLOGICAL MATERIAL

ETHICS APPROVAL

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

RETROSPECTIVE ETHICS APPROVAL

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

ETHICS APPROVAL FOR RETROSPECTIVE STUDIES

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

ETHICS APPROVAL FOR CASE STUDIES

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the individual (or parent or guardian if the participant is a minor or incapable) See also section on **Informed Consent**.

CELL LINES

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the NCBI database for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the International Cell Line Authentication Committee (ICLAC).

Authors should include a statement that confirms that an institutional or independent ethics committee (including the name of the ethics committee) approved the study and that informed consent was obtained from the donor or next of kin.

RESEARCH RESOURCE IDENTIFIERS (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt RRIDs when reporting key biological resources (antibodies, cell lines, model organisms and tools) in their manuscripts.

Examples:

Organism: ***Filip1*$^{tm1a(KOMP)Wtsi}$** **RRID:MMRRC_055641-UCD**

Cell Line: RST307 cell line **RRID:CVCL_C321**

Antibody: Luciferase antibody DSHB Cat# LUC-3, **RRID:AB_2722109**

Plasmid: mRuby3 plasmid **RRID:Addgene_104005**

Software: ImageJ Version 1.2.4 **RRID:SCR_003070**

RRIDs are provided by the Resource Identification Portal. Many commonly used research resources already have designated RRIDs. The portal also provides authors links so that they can quickly register a new resource and obtain an RRID.

Clinical Trial Registration

The World Health Organization (WHO) definition of a clinical trial is "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes". The WHO defines health interventions as “A health intervention is an act performed for, with or on behalf of a person or population whose purpose is to assess, improve, maintain, promote or modify health, functioning or health conditions” and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example www.clinicaltrials.gov or any of the primary registries that participate in the WHO International Clinical Trials Registry Platform.

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered’ should be included as the last line of the manuscript abstract.

Standards of reporting

Springer Nature advocates complete and transparent reporting of biomedical and biological research and research with biological applications. Authors are recommended to adhere to the minimum reporting guidelines hosted by the EQUATOR Network when preparing their manuscript.

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Checklists are available for a number of study designs, including:

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Case reports (CARE)

Clinical practice guidelines (AGREE) and (RIGHT)

Qualitative research (SRQR) and (COREQ)

Animal pre-clinical studies (ARRIVE)

Quality improvement studies (SQUIRE)

Economic evaluations (CHEERS)

SUMMARY OF REQUIREMENTS

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

• All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).

• This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).

• Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

• The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

• Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

• This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

• This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

• This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.

• The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

INFORMED CONSENT

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

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• Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.

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Consent and already available data and/or biologic material

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

Data protection, confidentiality and privacy

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered "informed". However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

CONSENT TO PARTICIPATE

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

CONSENT TO PUBLISH

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

here. (Download docx, 36 kB)

SUMMARY OF REQUIREMENTS

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Conflicts of interest/competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for **"Consent to participate"**:

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

Sample statements for **"Consent to publish"**:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

Images will be removed from publication if authors have not obtained informed consent or the paper may be removed and replaced with a notice explaining the reason for removal.

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Data availability

All original research must include a data availability statement. Data availability statements should include information on where data supporting the results reported in the article can be found, if applicable. Statements should include, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. For the purposes of the data availability statement, “data” is defined as the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article. When it is not possible to share research data publicly, for instance when individual privacy could be compromised, data availability should still be stated in the manuscript along with any conditions for access. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

1. The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

2. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

3. All data generated or analysed during this study are included in this published article [and its supplementary information files].

4. The datasets generated during and/or analysed during the current study are not publicly available due [REASON(S) WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.].

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6. The data that support the findings of this study are available from [THIRD PARTY NAME] but restrictions apply to the availability of these data, which were used under licence for the

current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [THIRD PARTY NAME].

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Data availability statements

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DATA CITATION

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FOR MORE INFORMATION:

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