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Abstract: Over the last two decades, the dissociative anaesthetic agent ketamine, an uncompetitive N-Methyl-D-Aspartate (NMDA) receptor antagonist, has emerged as a novel therapy for treatment-resistant depression (TRD), demonstrating rapid and robust antidepressant effects within hours of administration. Ketamine is a racemic mixture composed of equal amounts of (S)-ketamine and (R)-ketamine. Although ketamine currently remains an off-label treatment for TRD, an (S)-ketamine nasal spray has been approved for use in TRD (in conjunction with an oral antidepressant) in the United States and Europe. Despite the promise of ketamine, key challenges including how to maintain response, concerns regarding short and long-term side-effects and the potential for abuse remain. This review provides an overview of the history of ketamine, its use in psychiatry and its basic pharmacology. The clinical evidence for the use of ketamine in depression and potential adverse effects associated with treatment are summarised. A synopsis of some of the putative neurobiological mechanisms underlying ketamine's rapid acting antidepressant effects is provided before finally outlining future research directions, including the need to identify biomarkers for predicting response and treatment targets that may be used in the development of next-generation rapid-acting antidepressants that may lack ketamine's side-effects or abuse potential.

***Keywords*:** ketamine, (S)-ketamine, antidepressant, depression, mechanism of action

Introduction

Depression remains a leading cause of disability worldwide (WHO, 2017). It is a major contributor to the global burden of disease, associated with high rates of carer burden and rising socioeconomic and healthcare costs. There are two main limitations to current treatment strategies for depression, which predominantly focus on targeting deficits in monoaminergic neurotransmission. Firstly, there is a significant delay in the onset of therapeutic action (from weeks to months) and secondly, up to a third of patients fail to demonstrate an adequate response, with many developing persistent, treatment-resistant depression (TRD) (Al-Harbi, 2012).

At the turn of the millennium, there had been no new breakthrough pharmacological treatments for depression since the introduction of selective serotonin reuptake inhibitors (SSRIs) in the late 1980s. This changed with the discovery that ketamine, an uncompetitive N-Methyl-D-Aspartate (NMDA) glutamate receptor antagonist, could produce rapid and profound reductions in depressive symptoms following a single subanaesthetic infusion in individuals with major depressive disorder (MDD) (Berman et al., 2000). This finding has now been replicated and confirmed in several clinical trials in both unipolar and bipolar depression (including treatment-resistant individuals), resulting in a paradigm shift for depression research and treatment (Krystal et al., 2019).

Despite cautious optimism surrounding the potential of ketamine for depression, a number of uncertainties remain. These include understanding its underlying therapeutic mechanism of action, how best to maintain response, and concerns regarding potential short and long-term side-effects, including abuse potential. In this review we give a brief historical overview of the use of ketamine in psychiatry before addressing a number of key questions to provide the reader with an update on the current status of the use of ketamine for depression, before outlining areas for future consideration.

What is the history of ketamine use in psychiatry?

Ketamine, originally designated as CI-581, was first synthesized in 1962 by Calvin Stevens at the Parke Davis Lab. It is a structural analogue of its parent compound phencyclidine and was initially developed with the aim of finding an agent with similar anesthetic properties but that was shorter acting and lacked the prolonged emergence delirium associated with phencyclidine (Domino, 1980). In the first clinical study of ketamine, it was found to be a powerful anaesthetic and analgesic, producing a unique state of altered consciousness (Corssen and Domino, 1966). Subjects reported feeling as though they were ‘in outer space’, or ‘had no arms or legs,’ and the term “dissociative anaesthetic”, which is still in use today, was first coined (Domino, 2010). Following approval by the US Food and Drug Administration (FDA) in 1970, ketamine was used as a battlefield anaesthetic in the Vietnam War due to its fast onset and recovery period, ability to maintain or elevate blood pressure in trauma situations

and its minimal effects on respiratory drive. Because of these qualities it is still frequently used as an anaesthetic in situations where airway management is difficult or impossible (Kurdi et al., 2014).

As ketamine was being studied as an anaesthetic agent, its potential application to treat psychiatric and psychological conditions was also being considered. In Iran, ketamine as an adjunct to psychotherapy at subanaesthetic doses (0.4 - 0.6 mg/kg) was reported to be an effective abreactive agent in a number of conditions including depression, anxiety, obsessive-compulsive neurosis, conversion reaction and hypochondriasis with the authors highlighting the importance of the drugs, "mind expanding effects" (Khorramzadeh and Lotfy, 1973). At the same time in Argentina, ketamine was being examined as an adjunct for antidepressive psychotherapy to facilitate regression (Fontana, 1974), while in Mexico, others were exploring the use of ketamine in group settings as part of psychedelic psychotherapy sessions in patients with neurosis and personality disorders (Kolp et al., 2006). From 1985, Krupitsky utilised ketamine-assisted psychedelic therapy in a range of neurotic and personality disorders with particularly impressive results in the treatment of alcoholism (Krupitsky and Grinenko, 1997). Total abstinence for more than one year was observed in 65.8% (73 out of 111) alcoholic patients in the ketamine psychedelic therapy group, compared to 24% (24 out of 100) patients in the conventional treatment control group ($p < 0.01$).

Following from this exploratory work, it was in 2000 that the findings from the first randomised controlled trial (RCT) of ketamine in depression were reported (Berman et al., 2000). In this landmark study, Berman and colleagues used a subanaesthetic dose of intravenous (IV) ketamine (0.5 mg/kg infused over 40 min) in a randomized, crossover, double-blind design in eight medication-free patients with major depressive disorder (MDD) and one patient with bipolar disorder. Ketamine produced a significant antidepressant effect as soon as 4 hours after the infusion that increased progressively up to 72 hours (mean Hamilton Depression Rating Scale scores decreased by 14 ± SD 10 points vs. 0 ± 12 points, after ketamine and placebo treatment respectively) (Berman et al., 2000).

What is the basic pharmacology of ketamine?

Ketamine is a chiral arylcyclohexalamine and is classified as an uncompetitive NMDA receptor antagonist. However, ketamine has a complex pharmacological profile and also interacts with a range of other receptors and systems including γ-amynobutyric acid (GABA), dopamine, serotonin, opioid, and cholinergic receptors. With a few exceptions, including interactions with the dopamine receptor D_2 and nicotinic acetylcholine receptors by ketamine metabolites, the affinity for these other receptors is far weaker than the antagonism of the NMDA receptor. For an extensive review and summary of receptor binding studies see Zanos et al. (2018).

Ketamine refers to (R,S)-ketamine, a racemic mixture composed of two enantiomers, (S)- and (R)-ketamine (esketamine and arketamine respectively). Although most commercially available pharmacological preparations are made up of an equimolar mixture of the two, the separate enantiomers have also been investigated individually to varying degrees. (S)-ketamine binds to the NMDA receptor with three to four times the affinity compared with (R)-ketamine (Ebert et al., 1997) and is a more potent anaesthetic and analgesic (White et al., 1980; White et al., 1985). While both (S)-ketamine and (R)-ketamine appear to have rapid antidepressant effects (Muller et al., 2016), there has been limited clinical work investigating (R)-ketamine. However, accumulating preclinical evidence suggests (R)-ketamine may have more potent and longer lasting antidepressant effects than both (R,S)-ketamine and (S)-ketamine, with fewer side effects (Hashimoto, 2020; Jelen et al., 2020)

Ketamine is metabolized in the body to norketamine, hydroxynorketamines, hydroxyketamine and dehyronorketamine (Zarate et al., 2012a). In preclinical work, 2R,6R-hydroxynorketamine (2R,6R-HNK) has been reported to have antidepressant-like effects without ketamine-related behavioural side-effects (motor incoordination, pre-pulse inhibition deficits, ketamine-related discrimination responses or increased drug self-administration) (Zanos et al., 2016; Pham et al., 2018; Fukumoto et al., 2019), although the literature remains divided (Yamaguchi et al., 2018; Shirayama and Hashimoto, 2018; Yang et al., 2017). The (S)- metabolite (S)-norketamine has also been shown to have antidepressant-like effects that are similarly potent to its parent compound but with fewer associated side-effects (Yang et al., 2018a).

Several different routes of ketamine administration have been used in depression, including intravenous (IV), intramuscular, intranasal, sublingual, and oral routes each with their own advantages and challenges. IV administration provides the most reliable dosing with 100% bioavailability. For alternative routes approximate bioavailability values are as follows; intramuscular (93%), intranasal (45%), sublingual (30%) and oral (20%) (Zanos et al., 2018; Peltoniemi et al., 2016).

What is the evidence for the use of intravenous ketamine in depression?

In the two decades since the pivotal study from Berman and colleagues, the evidence base surrounding the use of ketamine in unipolar and bipolar depression has been growing (**Table 1**). An initial replication study was published six years later in a group of 18 patients with treatment-resistant MDD using an identical study design (Zarate et al., 2006). Here, similar results were reported with significant antidepressant effects emerging after 110 minutes after the infusion, that peaked after 1 day, before fading after 1 week (Zarate et al., 2006). The most common side-effect was acute dissociative symptoms, however in general these resolved within 80 minutes after the infusion. This group subsequently performed two further similar studies in patients with bipolar I or II depression

who were maintained on lithium or valproate (Diazgranados et al., 2010; Zarate et al., 2012b). In the first study, depressive symptoms significantly improved with 40 min and remained significant through day 3, with a response rate of 71% following ketamine compared to 6% for placebo (Diazgranados et al., 2010). In the second study, rapid and robust antidepressant effects were again seen within 40 min, remaining significant up to 3 days after the infusion, with response rates of 79% and 0% for ketamine and placebo respectively (Zarate et al., 2012b).

One of the limitations of these studies is the difficulty in maintaining the blind as ketamine produces clear dissociative symptoms. Other researchers have used midazolam, a benzodiazepine, as an active placebo, in an attempt to maintain the blind while using ketamine (Murrough et al., 2013; Murrough et al., 2015b; Fava et al., 2018). In a large study of 73 patients with treatment resistant MDD, it was demonstrated that IV ketamine treatment resulted in a significantly greater improvement in the MADRS score 24 hours after ketamine administration than the midazolam treated group, with response rates of 64% and 28% respectively. In another large multi-site parallel design dose-ranging trial, comparing IV ketamine doses of 0.1 mg/kg, 0.2 mg/kg, 0.5 mg/kg and 1.0 mg/kg with midazolam 0.045 mg/kg, only the standard dose (0.5 mg/kg) and high dose (1.0 mg/kg) were superior to the active placebo (Fava et al., 2018).

While many ketamine studies were carried out in medication-free MDD patients, other trials have examined adjunctive ketamine treatment in individuals also taking oral antidepressants. In a study of 27 individuals with MDD, maintained on their usual antidepressant medication, IV ketamine treatment was superior to placebo from days 1 through to day 7 (10/27 patients responded to ketamine, only 1/19 responded to placebo) (Sos et al., 2013). A further study examined single IV ketamine versus placebo augmentation to newly initiated escitalopram in 30 subjects with MDD (Hu et al., 2016). At the 4-week endpoint there was a significantly greater response in the ketamine + escitalopram group than the placebo + escitalopram group (92.3% and 57.1% respectively) with a significantly shorter time to response and remission.

Findings from studies examining the effect of a single dose of IV ketamine infusion in depression have also been summarised in meta-analyses. One meta-analysis examined nine studies (n=234) in patients with MDD and bipolar depression and demonstrated that ketamine reduced depression significantly more than placebo/active placebo beginning at 40 min, peaking at day 1 (Hedges' g = -1.00, 95% CI -1.28 to -0.73, $p < 0.001$), and losing superiority by days 10-12 (Kishimoto et al., 2016). A further meta-analysis examined the effects of a single dose of ketamine on depressed patients who had suicidal ideation at baseline and demonstrated that ketamine rapidly (within 1 day) reduced suicidal ideation significantly on both clinician-administered and self-report outcome measures, lasting up to 1 week

(Wilkinson et al., 2018). Reported effect sizes were moderate to large (Cohen's d=0.48-0.85) at all examined time points after dosing.

Although most of the studies of IV ketamine in depression have investigated racemic ketamine, others specifically exploring the antidepressant effects of the component (S)- and (R)- enantiomers have also been performed. In a first proof-of-concept study, IV (S)-ketamine at doses of 0.2 mg/kg and 0.4 mg/kg over 40 mins led to rapid and robust antidepressant effects in individuals with TRD (Singh et al., 2016a). However, dose-dependent treatment-emergent side-effects including headache, nausea and dissociation were still present. The authors suggest that the lower dose of (S)-ketamine may allow for better tolerability while maintaining efficacy, as improvements in depressive symptoms were not significantly different between the two tested doses. More recently, findings from a small open-label pilot study of (R)-ketamine in TRD have been published (Leal et al., 2020). Here, seven individuals received a single IV infusion of 0.5mg/kg (R)-ketamine over 40 mins. There was a significant reduction in mean MADRS scores from 30.7 at baseline to 10.4 at 1 day after the infusion with a 71% response rate at day 1 and 57% at day 7. Importantly, dissociative effects were nearly absent, alongside minimal haemodynamic parameter changes. Although very interesting, these results from a small open-label study should be interpreted with caution, and a larger RCT is needed to confirm these putative findings.

What is the evidence for alternative routes of administration?

While most of the RCTs of ketamine have investigated IV administration, it is possible to administer ketamine via a range of other routes including oral, intranasal, subcutaneous or intramuscular. Comparatively fewer RCTs have fully evaluated these options at this time.

Lapidus et al. (2014) examined the effect of a single dose of 50mg of racemic ketamine administered intranasally in 20 subjects with treatment resistant MDD. A significant antidepressant effect was detected as early as 40 min and after 24 h, 8/18 patients had responded to ketamine, while only 1/18 to placebo. Furthermore, there were minimal psychotomimetic or dissociative effects noted, without clinically significant changes in haemodynamic measures. In contrast, another group found that self-administered intranasal ketamine at 10 x 10 mg doses separated by 5 minutes was poorly tolerated, with significant cardiovascular, psychotomimetic and neurological side effects and significant inter-individual variation in pharmacokinetic parameters (Galvez et al., 2018). They concluded that this route of administration was not useful when using their device and dosing regime.

Following from the initial study of IV (S)-ketamine, a fixed-dose (S)-ketamine nasal spray has been developed and investigated in TRD. Results from several phase II and phase III RCTs demonstrated that

intranasal (S)-ketamine in addition to an oral antidepressant showed a significant benefit over placebo plus an oral antidepressant in individuals with TRD (Canuso et al., 2018; Daly et al., 2018; Popova et al., 2019; Daly et al., 2019), however other studies have failed to demonstrate positive results (Fedgchin et al., 2019; Ochs-Ross et al., 2020). The (S)-ketamine nasal spray, Spravato™, has now been approved for adults with TRD, when used in combination with an oral antidepressant, by the US Food and Drug Administration and subsequently by the European Medicines agency. Despite these regulatory approvals there are still some questions surrounding uncertainty of efficacy, long-term safety and potential for abuse which may limit widespread use (Kryst et al., 2020; Turner, 2019).

There have been two placebo controlled RCTs of adjunctive oral ketamine to standard antidepressant medications at doses of 50 mg a day over 6 weeks (Arabzadeh et al., 2018) and 1 mg/kg three times a week over 6 weeks (Domany et al., 2019). Results from both studies suggest that oral ketamine has significant antidepressant effects and is generally well tolerated, however the effects are not as rapid as those seen with IV ketamine (Arabzadeh et al., 2018; Domany et al., 2019). For subcutaneous and IM routes the evidence base is limited, in terms of placebo controlled RCTs. A small crossover study compared IV, SC and IM and found the antidepressant effects were comparable across the investigated routes of administration, although fewer adverse effects were reported with the SC route (Loo et al., 2016). A further multiple crossover RCT examined differing SC doses of ketamine in TRD ranging from 0.1 mg/kg to 0.5 mg/kg and reported that doses ≥ 0.2 mg/kg were significantly more effective than the active comparator midazolam (George et al., 2017).

How can response to ketamine be maintained?

Although the majority of studies have demonstrated a rapid and robust antidepressant response following a single dose of ketamine, in most patients the antidepressant effects following a single administration are not sustained beyond seven days (Kishimoto et al., 2016). Other studies have examined whether the antidepressant effects of ketamine could be extended with repeated administration. In the first double blind RCT of repeated ketamine administration, it was demonstrated that twice- or thrice-weekly administration of IV ketamine (0.5 mg/kg over 40 min) was sufficient to maintain antidepressant efficacy over 15 days in individuals with TRD (Singh et al., 2016b). In contrast, in a more recent double-blind RCT, where 26 individuals with TRD and current, chronic suicidal ideation were randomized to six ketamine infusions (0.5 mg/kg over 45 mins) or saline placebo over 3 weeks, at the end of the infusion phase differences in depression severity and suicidality were not significant between ketamine or placebo (Ionescu et al., 2019). The authors suggest that the traditional ketamine dose used may not have been sufficient to produce an improvement in their study sample that had high levels of chronicity, treatment-resistance and concomitant medications,

and that an increase in dose beyond 0.5 mg/kg may be required to achieve clinically significant effects in this group.

Although a large number of private clinics are offering ketamine infusion treatments across the United states (Ketamine-Clinics-Directory, 2020), the use of repeated IV ketamine infusions may not always be the most practical, considering the potential resources required that might limit capacity and scalability of services. Other routes including oral and intranasal may prove to be more feasible alternatives when it comes to repeat administrations and maintenance of response. MDD subjects receiving oral ketamine 50 mg/day as an adjunct to sertraline showed a significantly greater reduction in depressive symptoms at a 6 week time point compared to those randomised to receive placebo and sertraline (Arabzadeh et al., 2018). In another RCT of repeated oral ketamine for TRD, 33 individuals were randomised to receive 1 mg/kg oral ketamine or placebo thrice weekly for 21 days (Domany et al., 2019). The ketamine treated group had a significantly greater reduction in MADRS score on day 21 versus placebo, with 27.3% achieving remission compared to none of the controls. Importantly side-effects were mild and transient.

In the RCTs of intranasal (S)-ketamine in TRD (plus an oral antidepressant), utilising 28 - 84 mg twice-weekly dosing, significant reductions in depressive symptoms have been shown at 1 week (Daly et al., 2018), day 11 (Canuso et al., 2018) and up to 4 weeks (Popova et al., 2019), however other studies have failed to show significant differences at the 4 week time point (Canuso et al., 2018; Fedgchin et al., 2019; Ochs-Ross et al., 2020). In a large study examining intranasal (S)-ketamine (56 mg or 84 mg twice weekly) plus oral antidepressant treatment for 16 weeks followed by a randomised withdrawal phase (to continue with (S)-ketamine or switch to placebo), of the 297 adults with TRD who were randomized in the maintenance phase, those who continued treatment with intermittently administered (S)-ketamine nasal spray plus an oral antidepressant had a significantly delayed time to relapse compared to those treated with placebo nasal spray and oral antidepressant (Daly et al., 2019). This suggests that continued maintenance treatment with (S)-ketamine nasal spray plus an oral antidepressant can sustain antidepressant effects in individuals with TRD to a greater degree than oral antidepressant treatment alone.

What are potential adverse effects of ketamine?

Acute side-effects following subanaesthetic ketamine treatment are relatively common, especially when delivered intravenously (Short et al., 2018). However, most of these effects are mild and transient, occurring during the infusion period and resolving shortly after dose administration. Of note, these include elevated blood pressure (asymptomatic), nausea, headache, blurred vision,

perceptual disturbance, drowsiness, dizziness, dissociation and anxiety (Short et al., 2018; Acevedo-Diaz et al., 2020).

Blood pressure should be assessed before treatment with ketamine and individuals with comorbid hypertension should have their blood pressure management optimised before commencing ketamine. Blood pressure should be monitored after dosing until the blood pressure returns to acceptable levels. In an analysis of 684 infusions in 66 patients, with ketamine administered IV at 0.5 mg/kg over 40 min, the biggest increases in blood pressure were measured at 30 min (systolic 3.28 mmHg, diastolic 3.17 mmHg) (Riva-Posse et al., 2018). Although hypertensive patients had higher blood pressure peaks during the infusions, values returned to baseline during post-infusion monitoring at 70 min. For patients with congestive cardiac failure or history of cerebrovascular accident particular caution should be taken or alternative treatments considered (Short et al., 2018).

Another consideration is the effect of ketamine on cognition, particularly after repeated administration. An extensive review of the effects of acute ketamine on the memory of healthy volunteers and of repeated doses of ketamine in recreational users highlighted that non-chronic usage of ketamine impairs the manipulation of information in working memory and produces transient decrements in the encoding of information into episodic memory (Morgan and Curran, 2006). Further, chronic, frequent recreational use may be associated with more marked deficits in working and episodic memory (Morgan et al., 2012). These are important harms to be aware of, however, findings from studying cognitive side-effects in frequent or addicted recreational ketamine users should not be extrapolated to patients receiving carefully prescribed dose in a controlled clinical setting. In a clinical study examining the effects of repeated ketamine infusions in TRD , no patients reported any cognitive deficits in excess to that reported at baseline (aan het Rot et al., 2010). However, a limitation of this study and other studies of ketamine treatment in depression is a lack of formal cognitive testing (Short et al., 2018).

In chronic, frequent use in recreational users a major physical harm is ketamine induced urinary tract symptoms and ulcerative cystitis (Morgan et al., 2012). The aetiology of ketamine-induced ulcerative cystitis is unclear, however it appears to be particularly observed in individuals with a history of chronic, daily abuse use of the drug (Shahani et al., 2007). It should be noted that recreational ketamine consumption tends to be several orders of magnitude higher (one survey reported 34% of users reported use of 1 g or more in a typical session(Winstock et al., 2012)) than the doses prescribed in the clinical setting for depression. Although adverse effects of long-term ketamine use on the bladder would not necessarily be expected with the therapeutic dosing and frequency used in the treatment of depression, the lack of assessment of urinary symptoms has been another limitation in

RCTs of ketamine (Short et al., 2018). In the (S)-ketamine nasal spray clinical program, there were no reported cases of ulcerative or interstitial cystitis, however (S)-ketamine-treated patients had a higher incidence of lower urinary tract adverse events (FDA, 2019), and the recommendation to monitor for urinary tract and bladder symptoms during the course of treatment has appropriately been added to the summary of product characteristics (EMC, 2019).

Side-effects associated with intranasal (S)-ketamine administration appear similar to those seen with racemic ketamine (Swainson et al., 2019). However, there appears to be important differences in adverse effects between ketamine's enantiomers. In a healthy volunteer study while (S)-ketamine administration produced acute psychosis-like reactions, (R)-ketamine did not produce any psychotic symptoms but instead a state of relaxation and a feeling of well-being (Vollenweider et al., 1997). More recently, a small pilot trial of (R)-ketamine in TRD reported minimal dissociative symptoms without clinically significant changes in hemodynamic measures (Leal et al., 2020). A direct comparison study of the safety and efficacy of (R)-ketamine and (S)-ketamine in TRD is yet to be performed.

What is the abuse potential of ketamine?

Alongside its clinical applications ketamine is also widely used recreationally (Sassano-Higgins et al., 2016). At subanaesthetic doses users may experience psychedelic-like effects (including altered perceptions, synaesthesia, derealization and depersonalization), while at higher doses users may experience more pronounced out of body experiences including a loss of sense of space and time. Ketamine is commonly used recreationally by snorting the powdered form but may also be used intravenously or intramuscularly (Bokor and Anderson, 2014).

Ketamine, especially when used in large and frequent doses has the potential to lead to tolerance and addiction (Sassano-Higgins et al., 2016). The mechanisms underlying the reinforcing effects of ketamine may include pleasant sensations or temporary relief of negative emotions resulting from its dissociative effects, actions on dopaminergic (Kokkinou et al., 2018) and mu-opioid systems (Zanos et al., 2018; Williams et al., 2018), alongside reward-circuitry activation (Sterpenich et al., 2019). While there are reports of recreational ketamine users describing withdrawal symptoms including anxiety, shaking, sweating and palpitations when they stopped using (Morgan et al., 2012; Chen et al., 2014), the true prevalence, severity and duration of such symptoms is not known. Unlike opiate addiction, there is not definitive evidence to suggest physical dependence and a specific ketamine withdrawal syndrome has not yet been described. In fact, ketamine treatment delivered in controlled clinical settings is also being investigated as a treatment to reduce alcohol, cocaine or opioid dependence (Jones et al., 2018).

In an analysis of 11 healthy volunteer studies, involving repeated ketamine infusions in carefully monitored clinical research studies, there was no evidence of behavioural sensitization (Cho et al., 2005). Although this work suggests repeated low dose ketamine infusions can be used safely in clinical settings, it is important to note these studies were conducted in non-depressed rather than depressed patients that may have differences in reward-circuitry (Quevedo et al., 2017). In longer-term follow up data of depressed subjects receiving ketamine infusions in clinical trials there was no evidence of ketamine abuse, increased drug cravings or substance abuse (Wan et al., 2015). In the (S)-ketamine nasal spray randomised withdrawal study (Daly et al., 2019), no evidence of a distinct withdrawal syndrome was observed in individuals with TRD during the 2 weeks after cessation of (S)-ketamine (as assessed by the 20-item Physician Withdrawal Checklist) and no adverse events were reported by participants related to use or abuse of ketamine. While these findings are reassuring, there is still insufficient data examining repeated prolonged use in this population and some have argued for caution amongst clinicians until there is further data on ketamine's longer-term efficacy and risks (Schatzberg, 2014).

The need for careful monitoring of depressed patients receiving repeated ketamine treatment to ensure personal safety (for example dosage, side-effects and dependency) has been raised by patients and carers (Jilka et al., 2019). It has been argued as ketamine and intranasal (S)-ketamine become more widely and frequently prescribed, a multi-drug monitoring system should be in place to help ensure safer use, define less common side-effects and prevent abuse (McShane, 2019).

What are the mechanisms of ketamine's antidepressant action?

The antidepressant effects of ketamine may be mediated via blockade of NMDA receptors located on GABAergic inhibitory interneurons that normally act to suppress glutamate release from downstream glutamatergic neurons (Krystal et al., 2019). The preferential action of NMDA antagonists at GABAergic interneurons is supported by work that found NMDA receptor inhibition (via administration of the NMDA antagonist MK801) predominately reduces the activity of putative GABA interneurons and at a delayed rate increases the firing rate of pyramidal neurons (Homayoun and Moghaddam, 2007). This disinhibition hypothesis has been further supported by work demonstrating that ketamine enhances glutamatergic transmission, via pyramidal cells firing more action potentials, indirectly by reducing synaptic GABAergic inhibition (Widman and McMahon, 2018; Gerhard et al., 2020). This disinhibition of glutamatergic neurons results in an acute cortical glutamate surge (Moghaddam et al., 1997; Milak et al., 2016; Abdallah et al., 2018) and subsequent activation of post-synaptic α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid (AMPA) receptors appears to be a crucial step, leading to activation of neuroplastic signaling pathways and synaptogenesis (Lener et al.,

2017) (**Figure 1**). The critical role of AMPA activation is supported by findings from preclinical work demonstrating that AMPA receptor antagonist administration blocks ketamine's antidepressant effects (Maeng et al., 2008; Koike and Chaki, 2014; Koike et al., 2011).

One of the key intracellular pathways implicated in the antidepressant mechanism of ketamine is brain-derived neurotrophic factor (BDNF)- tropomyosin kinase B (TrkB) signaling, which has already been implicated in the pathophysiology of depression and the mechanism of action of currently prescribed antidepressants (Hashimoto et al., 2004; Duman and Monteggia, 2006). Preclinical work has demonstrated that the rapid antidepressant effects of ketamine are associated with rapid synthesis and upregulation of BDNF mediated by AMPA receptor activation (Autry et al., 2011; Zhou et al., 2014). Ketamine-mediated antagonism of post-synaptic NMDA receptors also leads to augmentation of BDNF synthesis through deactivation of eukaryotic elongation factor 2 (eEF2) kinase, reduced eEF2 phosphorylation and subsequent de-suppression of BDNF translation (Autry et al., 2011; Monteggia et al., 2013). Further animal work has shown that a TRkB antagonist was able to block the antidepressant effects of both of ketamine's enantiomers (Yang et al., 2015). Interestingly, (R)-ketamine induced greater effects on reduced dendritic spine density, BDNF–TrkB signalling and synaptogenesis compared with (S)-ketamine (Yang et al., 2015).

The mammalian target of rapamycin complex 1 (mTORC1) and extracellular signal-regulated kinase (ERK) are further signalling molecules implicated in the antidepressant mechanism of ketamine, with key roles in synaptic development and plasticity (Mendoza et al., 2011; Ignacio et al., 2016). Animal work has shown that ketamine administration rapidly activates the mTORC pathway leading to an increase in synaptic signalling proteins and spine density (Li et al., 2010). Moreover, administration of rapamycin, an mTORC1 inhibitor, has been demonstrated to block synaptogenesis and antidepressant- like effects induced by ketamine. (Li et al., 2010; Li et al., 2011). Considering ketamine's enantiomers, additional preclinical work has shown that the antidepressant-like effects of (S)-ketamine but not (R)-ketamine were blocked by mTORC1 inhibition and that the antidepressant-like effects of (R)-ketamine but not (S)-ketamine were blocked by an ERK inhibitor suggesting (R)-ketamine may cause a preferential activation of the ERK signalling pathway (Yang et al., 2018b).

mTORC signalling activation leads deactivation of glycogen synthase kinase-3 (GSK-3) and inhibition of GSK-3 has been shown to be required for the antidepressant-like effects of (R,S)-ketamine in a rodent model (Beurel et al., 2011). Further preclinical studies have demonstrated that ketamine administration in combination with lithium, a non-selective GSK-3 inhibitor, resulted in rapid activation of mTORC1 signalling, increased inhibition of GSK-3, increased synaptic spine density and

greater antidepressant-like responses (Liu et al., 2013), however clinical studies have not replicated these preclinical findings (Xu et al., 2015; Costi et al., 2019).

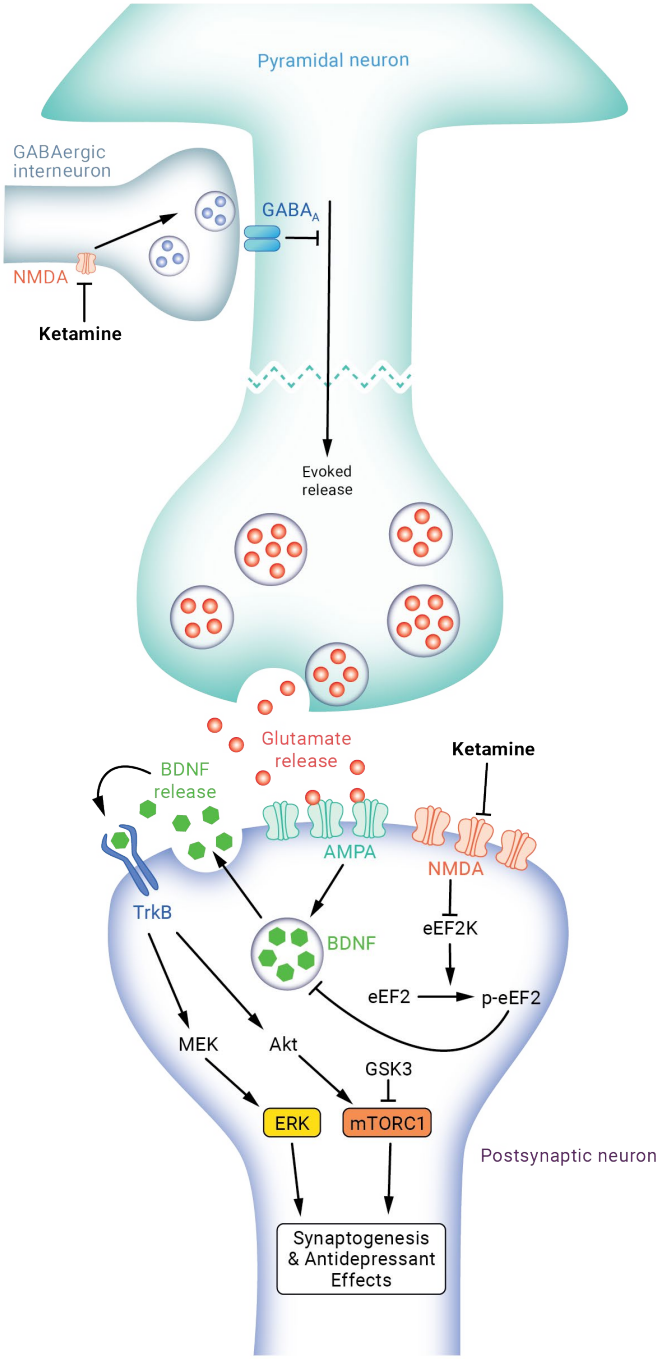

Figure 1: Proposed signalling pathways underlying antidepressant effects of ketamine. Ketamine selectively blocks NMDA receptors expressed on GABAergic inhibitory interneurons that synapse on the dendrites, cell body and axon initial segment of pyramidal neurons (Note, this simplified figure only shows an example GABAergic interneuron synapsing on the axon initial segment). This leads to disinhibition of pyramidal neurons, increased firing and evoked glutamate release. The resulting glutamate surge stimulates postsynaptic AMPA receptors leading to increased release of BDNF that activates TrkB and subsequent Akt/mTORC1 and MEK/ERK signalling pathways. This ultimately leads to increased synthesis of proteins required for synaptogenesis. Ketamine also suppresses resting postsynaptic NMDA receptor activity, deactivating eEF2 kinase, resulting in reduced eEF2 phosphorylation, augmentation of BDNF synthesis and subsequent TrkB-mTORC1 activation.

Monoaminergic systems may also play a role in the antidepressant mechanism of ketamine. Pre-clinical studies have shown that ketamine's antidepressant-like effects are blocked by 5-HT depletion (Fukumoto et al., 2016; Gigliucci et al., 2013) and that ketamine also inhibits serotonin transporter (SERT) function (Zhao and Sun, 2008). More specifically, increased 5-HT release in the medial prefrontal cortex (mPFC) through AMPA receptor signalling in the dorsal raphe nucleus (Pham et al., 2017; Chaki and Fukumoto, 2019) and subsequent 5-HT_{1A} activation with downstream convergence on mTORC1 signalling, appears to be another mechanism through which ketamine may exert its antidepressant effects (Fukumoto et al., 2018). A meta-analysis found ketamine administration at subanaesthetic doses in rodents to be associated with significantly increased levels of dopamine in regions including the cortex, striatum and nucleus accumbens across studies using microdialysis, high-performance liquid chromatography and electrochemical detection to measure extracellular and total dopamine, compared to control conditions (Kokkinou et al., 2018). Clinical work using PET imaging has also demonstrated that acute ketamine administration leads to increased dopamine release in the striatum in humans (Smith et al., 1998; Vollenweider et al., 2000). The exact role of the dopamine system in the antidepressant action of ketamine is yet to be fully elucidated, however recent preclinical work has suggested a key role for dopamine D_1 receptor activity in the mPFC (Hare et al., 2019).

Alongside the monoaminergic system, ketamine also interacts with opioid receptors, including mu, kappa and to a lesser extent, delta-opioid receptors (Zanos et al., 2018). Recent clinical work has suggested that opioid system activation may be required for the rapid-acting antidepressant effects of ketamine (Williams et al., 2018). In this study it was shown that pre-treatment with the opioid receptor antagonist naltrexone significantly blocked that antidepressant effects of ketamine in TRD. However, this study was limited by a small sample size and lack of a placebo control arm for the ketamine infusion. In contrast, another clinical study found that naltrexone pre-treatment did not reduce the antidepressant effects of ketamine in subjects with comorbid depression and alcohol use disorder (Yoon et al., 2019). While there has been preclinical work that demonstrated pre-treatment with naltrexone did not block the antidepressant-like effects of ketamine in a rodent model of depression (Zhang and Hashimoto, 2019), a further rodent study showed that treatment with an opioid receptor antagonist blocked ketamine's antidepressant-like behavioural effects and abolished ketamine's ability to reduce lateral habenula hyperactivity, while administration of the mu-opioid agonist, morphine, alone did not mimic either of these effects (Klein et al., 2020). This suggests that ketamine is not simply acting as a mu-opioid agonist, but that some mu-opioid receptor activity may be necessary for NMDA receptor antagonism.

The lateral habenula is a brain region involved in regulating reward and abnormal increases in neural activity in this region may signal down-regulation of monoaminergic firing resulting in depressive symptomatology including anhedonia and helplessness (Gold and Kadriu, 2019). It has previously been shown that inhibition of NMDA receptor-dependent activity in the lateral habenula facilitates ketamine's antidepressant-like actions in a rodent model of depression (Yang et al., 2018c). In brain regions including the habenula, opioid receptors and NMDA receptors are colocalized (Rodriguez-Munoz et al., 2012) and it is suggested that the glutamatergic and opioid systems may interact through direct 'crosstalk' (Chartoff and Connery, 2014).

The role of the opioid system in ketamine's antidepressant effects remains controversial. While some critics of ketamine therapy have claimed that ketamine seems to work mainly through stimulation of opioid receptors, with a high potential for abuse (Gøtzsche et al., 2019), evidence instead suggests that ketamine is not an opioid per se, and its interactions with the opioid system are more nuanced (Klein et al., 2020; Malinow and Klein, 2020). While cautious prescribing and monitoring of any drug with abuse potential is required, it is important that ketamine is not dismissed as merely an opioid and that we continue to explore the detailed nature of opioid signalling in terms of its rapid antidepressant effects as this could ultimately uncover novel pharmacological targets and antidepressant strategies.

Future directions

Although the discovery of ketamine's rapid acting antidepressant effects has brought a fundamental change in the treatment and understanding of the neurobiology of depression, there are still a number of unmet needs, both in terms of clinical and research directions. Most of the evidence for the antidepressant efficacy of ketamine and (S)-ketamine is via the IV and intranasal routes. Further research is needed to explore optimal dosing strategies, especially for routes where less evidence is available such as sublingual, oral and subcutaneous. Considering ketamine's transient antidepressant effects, these alternative routes of administration could prove to be more practical and effective maintenance strategies. Further, there is a need for careful monitoring and additional investigation into potential adverse effects associated with long-term use as well as more data on long-term efficacy and safety.

While the available evidence indicates that ketamine exerts rapid and robust antidepressant effects, it should be noted that not all patients respond to ketamine treatment, with most trials reporting between a 50-70% response rate in TRD (Wan et al., 2015). Further, other issues described in this review are that of tolerability (eg. dissociative or psychotomimetic effects) and safety (eg. CNS or urinary effects) with short and long-term administration. Considering the potential resources and

costs required for ketamine treatment (especially via the IV infusion route) and necessary surveillance post-administration, the identification of predictive clinical/ biomarkers of safety, efficacy and tolerability in response to ketamine could provide the opportunity to stratify subpopulations with TRD who are more likely to benefit from treatment (Rong et al., 2018). This could ultimately support a personalized medicine approach but also has implications in terms of cost-effectiveness of delivering ketamine treatment. While a full discussion is beyond the scope of this review, some candidate predictors of response to ketamine include clinical indicators (i.e., high BMI and family history of an alcohol use disorder) (Niciu et al., 2014), neuroimaging measures (i.e., anterior cingulate cortex activity (Salvadore et al., 2009), glutamatergic metabolite levels (Salvadore et al., 2012) and functional connectivity (Gartner et al., 2019)), genetics (i.e., Val66Met BDNF allele) (Laje et al., 2012), sleep (i.e., low baseline delta sleep ratio) (Duncan et al., 2013) and cognitive function (i.e., slow processing speed) (Murrough et al., 2015a). Alterations in terms of inflammation and metabolism in TRD are emerging as key areas of investigation and indeed a recent systematic review suggested that higher baseline interleukin-6 (IL-6) or C-reactive protein (CRP)/high-sensitivity-CRP (hsCRP) may predict response to medication with anti-inflammatory characteristics, including ketamine (Yang et al., 2019a). However, at this stage, none of these putative predictors have sufficient positive predictive validity (PPV) to inform clinical decision making.

In parallel to understanding potential predictors of response, further work to understand the specific mechanisms underlying ketamine's rapid antidepressant effects is needed. Not only will this further our knowledge of the neurobiological processes underpinning depression and antidepressant response to ketamine but will also help identify treatment targets for use in the development of next-generation rapid-acting treatments in depression that lack the dissociative and psychotomimetic effects or abuse potential (Jelen et al., 2018). Of particular note, (R)-ketamine and its metabolite (2R,6R)-HNK have demonstrated more potent and longer lasting antidepressant-like effects than (R,S)-ketamine and (S)-ketamine in preclinical models (and also lack dissociative effects and abuse potential in these models) (Yang et al., 2019b). Clinical trials of (R)-ketamine and (2R,6R)-HNK are either planned or underway ((Universal Trial Number: U1111-1241-1005), (ChiCTR1800015879), (Kraus et al., 2019)).

Conclusions

Ketamine has emerged as a novel antidepressant that has effects in individuals otherwise resistant to conventional antidepressants, with a remarkably rapid speed of onset. As such, it represents one of the most important breakthroughs in the treatment of depression in the last 50 years. However, maintenance of response remains problematic and longer-term risks associated with repeated administrations are less well characterised. Although ketamine remains an off-label treatment for

TRD, the use of (S)-ketamine, plus an oral antidepressant, has been approved for use in TRD in the United States and Europe. However, concerns regarding side-effects and abuse potential remain, highlighting the need for careful monitoring and further data on long-term safety. In the quest to understand ketamine's novel rapid acting-antidepressant mechanism, no one unique mechanism of action has emerged as of yet, rather multiple, potentially complementary, mechanistic pathways appear to exist. Further understanding of the specific underlying mechanisms of ketamine is critical as we seek to develop alternative rapid-acting treatments in depression that may not share the same dissociative effects or abuse potential.

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STUDY	DESIGN	PATIENT GROUP	(N)	DOSING REGIME	OUTCOME	ANTIDEPRESSANT EFFECTS
Single Administration						
(Berman et al., 2000)	Randomized, placebo-controlled, double- blind crossover study	MDD and BD	7	IV ketamine infusion (0.5 mg/kg over 40 min)	HAM-D	- Reductions in the HAM-D of 14 ± 10 *vs.* 0 ± 12, for ketamine and placebo respectively
(Zarate et al., 2006)	Randomized, placebo-controlled, double blind crossover study	TRD (MDD) HAM-D ≥ 18	18	IV ketamine infusion (0.5 mg/kg over 40 min)	HAM-D-21	- Very large effect size for ketamine at 24 h post-infusion, d=1.46 - 71% response rate at 24 h, 35% response at 1 week
(Diazgranados et al., 2010)	Randomized, placebo-controlled, double-blind, crossover, add-on study (to lithium or valproate)	TRD (BD) MADRS ≥ 20	18	IV ketamine infusion (0.5 mg/kg over 40 min)	MADRS	- Responses to ketamine and placebo were 71% and 6% respectively - Effect size of ketamine largest at day 2, d = 0.8
(Zarate et al., 2012b)	Randomized, placebo-controlled, double-blind crossover study, add on study (to lithium or valproate)	TRD (BD) MADRS ≥ 20	15	IV ketamine infusion (0.5 mg/kg over 40 min)	MADRS	- Response rate of 79% to ketamine vs. 0% for placebo
(Murrough et al., 2013)	Two-site, parallel-arm, randomized controlled trial of a single infusion of ketamine compared to an active placebo control, midazolam	TRD (MDD) IDS-C ≥ 32	73	IV ketamine infusion (0.5mg/kg over 40 min)	MADRS	- MADRS scores at 24 h post-infusion were 7.95 [95%CI, 3.21-12.71] lower in the ketamine vs. the midazolam group - Response rates of 64% for ketamine vs. 28% for midazolam - Large effect size of ketamine, NNT = 2.8
(Sos et al., 2013)	Randomized, placebo-controlled, double-blind crossover study in addition to usual antidepressant medication.	MDD MADRS ≥20	27	IV ketamine infusion (0.54 mg/kg within 30 min)	MADRS	- Ketamine was superior to placebo in all visits (day 1, 4, and 7) - Effect size of ketamine largest at day 1, d = 0.62
(Lapidus et al., 2014)	Randomized, placebo-controlled, double-blind crossover study, add on study (to stable dose of antidepressant)	TRD (MDD)	20	Intranasal ketamine 50 mg (5 x 10mg over 20 min)	MADRS	- MADRS scores at 24 h post-infusion were 7.6 ± 3.7 [95% CI, 3.9-11.3] lower in the ketamine vs. placebo group - Response rate of 44% to ketamine vs. 6% following placebo at 24 h
(Murrough et al., 2015b)	Randomized, placebo-controlled, double- blind crossover trial of a single infusion of ketamine compared to an active placebo control, midazolam	MDD, BD, PTSD ≥ 4 on suicide item of MADRS	24	IV ketamine infusion (0.5 mg/kg over 40 min)	BSI	- BSI score was not different between the treatment groups at 24 h (p = 0.32). However, there was a significant difference at 48 h (p = 0.047)
(Downey et al., 2016)	Two site, parallel-arm, randomized, double-blind, controlled study of a single infusion of ketamine compared to lanicemine	MDD	60	IV ketamine infusion (0.5 mg/kg over 60 min)	MADRS	- Neither drug improved mood rating scale scores more than saline infusion

(Hu et al., 2016)	Parallel arm, randomized controlled trial of a single infusion of ketamine and escitalopram 10mg/day compared to placebo and escitalopram 10mg/day	MDD (55.6% TRD) HAM-D ≥ 24	30	IV ketamine infusion (0. 5mg/kg over 40 min)	MADRS	- At 4 weeks greater response in ketamine + escitalopram vs placebo + escitalopram (92.3% v. 57.1%, p = 0.04)
(Singh et al., 2016a)	Parallel arm, randomized placebo-controlled, double- blind study	TRD (MDD) IDS-C ≥ 34	30	IV (S)-ketamine infusion 0.2 mg/kg or 0.4 mg/kg	MADRS	-The reduction in MADRS total score 24 hours after treatment, was significantly greater in both (S)-ketamine groups compared with the placebo group. Reductions in MADRS score of -16.8 for 0.2 mg/kg, -16.9 for 0.4 mg/kg and -3.8 for placebo.
(Su et al., 2017)	Parallel arm, randomized placebo-controlled, double- blind study	TRD (MDD) HAM-D ≥ 18	71	IV ketamine infusion (0.2 mg/kg or 0.5 mg/kg over 40 min)	HAM-D-17	- Significant dose-related ketamine effect on HAM-D scores. - The responder analysis also revealed a significant dose-related effect (saline: 12.5%, 0.2 mg/kg: 39.1% 0.5 mg/kg: 45.8%)
(Cao et al., 2018)	Parallel arm, randomized placebo-controlled, double- blind study	TRD (MDD) HAM-D ≥ 18	55	IV ketamine infusion (0.2 mg/kg or 0.5 mg/kg over 40 min)	HAM-D-17	- At 2h post-treatment 11/18 (61%) infused with 0.5 mg/kg showed significant response, 5/19 (26%) in the 0.2 mg/kg group and only 2/18 (11%) in the saline group
(Chen et al., 2018)	Parallel arm, randomized placebo-controlled, double- blind study	TRD (MDD)	24	IV ketamine infusion (0.2 mg/kg or 0.5 mg/kg over 40 min)	HAM-D-17	- Significant treatment response after 0.5 mg/kg ketamine infusion at 240 mins (37.5% vs. 0% vs. 0%, ×2 (df) = 6.86 (2), P = 0.032), and 1 day later (50% vs. 12.5% vs. 0%, ×2 (df) = 6.57 (2), P = 0.037) compared with 0.2 mg/kg ketamine and normal saline control groups
(Fava et al., 2018)	Six site, parallel arm, randomized placebo-controlled, double- blind study of four different doses of ketamine compared to active placebo control, midazolam	TRD (MDD) MADRS ≥ 20	99	IV ketamine infusion (0.1 mg/kg, 0.2 mg/kg, 0.5 mg/kg or 1.0 mg/kg over 40 min)	HAM-D-6	- Overall group × time interaction effect was significant for the primary outcome measure, the HAM-D-6 - Pairwise comparisons controlling for multiple comparisons, standard dose (0.5 mg/kg) and high dose (1 mg/kg) of intravenous ketamine were superior to active placebo
(Grunebaum et al., 2018)	Parallel arm, randomized placebo-controlled, double- blind study	MDD with suicidal ideation HAM-D ≥ 17	80	IV ketamine infusion (0.5 mg/kg over 40 min)	SSI	- Reduction in SSI score at day 1 was 4.96 points greater for the ketamine group compared with the midazolam group (95% CI =2.33, 7.59; Cohen's d = 0.75). -Proportion of responders at day 1 was 55% for the ketamine group and 30% for the midazolam group. NNT=4.0
(Nugent et al., 2019)	Randomized, placebo-controlled, double- blind crossover study	TRD (MDD) MADRS ≥20	35	IV ketamine infusion (0.5 mg/kg over 40 min)	MADRS	- MADRS scores were significantly lower post-ketamine infusion compared to placebo ($F_{1,77}=84.5$, $p < 0.001$)

Repeated Administration						
(Singh et al., 2016b)	Fourteen site, parallel arm, randomized placebo-controlled, double-blind study	TRD (MDD)	67	IV ketamine infusion (0.5 mg/kg over 40 min) 2 or 3 times a week over 4 weeks	MADRS	- In the twice-weekly dosing groups, the mean change in MADRS score at day 15 was -18.4 (SD=12.0) for ketamine and -5.7 (SD=10.2) for placebo; in the thrice-weekly groups, it was -17.7 (SD=7.3) for ketamine and -3.1 (SD=5.7) for placebo.
(Loo et al., 2016)	Multiple crossover, double-blind study with active placebo, midazolam	TRD (MDD) MADRS ≥20	15	Ketamine administered IV (n = 4), IM (n = 5) or SC (n = 6) injection. Dose titration commenced at 0.1 mg/kg increasing by 0.1 mg/kg up to 0.5 mg/kg, given in separate treatment sessions separated by ≥1 week, with one random placebo treatment	MADRS	- Twelve participants achieved response and remission criteria, achieved at doses as low as 0.1 mg/kg - IV, IM and SC routes resulted in comparable antidepressant effects - Fewest adverse effects were noted with the SC route
(George et al., 2017)	Randomized, active-placebo controlled (midazolam) double-blind, multiple-crossover study with a 6-month follow-up	TRD (MDD) Age ≥ 60 MADRS ≥ 20	14	Up to five subcutaneous doses of ketamine (0.1, 0.2, 0.3, 0.4, and 0.5 mg/kg) administered in separate sessions (≥1 week apart)	MADRS	- 7/14 RCT-phase completers remitted with ketamine treatment - 5 remitted at doses below 0.5 mg/kg - Doses ≥ 0.2 mg/kg were significantly more effective than midazolam
(Arabzadeh et al., 2018)	Parallel arm, randomized placebo-controlled, double-blind add on study to sertraline (150mg/day)	MDD HAM-D ≥ 20	81	Oral ketamine 50 mg/day (2 x 25mg /day for 6 weeks)	HAM-D-17	- Significant effect for time × treatment interaction on the HAM-D scores, with significant differences at all time points post treatment. - Early improvement was significantly greater in the ketamine group (85.4%) compared to the placebo group (42.5%).
(Daly et al., 2018)	Fourteen site, doubly randomized, placebo-controlled study	TRD (MDD) QIDS-SR16 ≥ 11	67	Intranasal (S)-ketamine 28 mg, 56 mg or 84 mg twice weekly for two 1-week periods followed by open label extension	MADRS	- Reduction in MADRS score (both periods combined) in all 3 (S)-ketamine groups was superior to placebo (28 mg: least-square mean difference = −4.2, SE=2.09, P = .02; 56 mg: −6.3, SE= 2.07, P = .001; 84 mg: −9.0, SE=2.1, P < .001). -Significant ascending dose-response relationship (P < 0.001)
(Canuso et al., 2018)	Eleven site, parallel arm, randomized placebo-controlled, double- blind study (in addition to standard-of-care treatment)	TRD (MDD) MADRS ≥ 22	68	Intranasal (S)-ketamine 84 mg twice weekly for 4 weeks	MADRS	-Significantly greater improvement in MADRS score was observed (S)-ketamine group compared with the placebo group at 4 h (least-square mean difference=−5.3, SE=2.10; effect size=0.61) and at 24 hours (least-square mean difference=−7.2, SE=2.85; effect size=0.65) -Difference not significant at day 25 (least-square mean difference=−4.5, SE=3.14; effect size=0.35).

(Popova et al., 2019)	39 site, parallel arm, randomized placebo-controlled, double- blind study in addition to newly initiated antidepressant	TRD (MDD) IDS-C ≥ 34	227	Intranasal (S)-ketamine 56 mg or 84 mg twice weekly for 4 weeks	MADRS	-Reduction in MADRS score with (S)-ketamine + antidepressant was significantly greater than with placebo + antidepressant at day 28 (least-square mean difference =-4.0, SE=1.69)
(Daly et al., 2019)	99 site, double-blind, randomized withdrawal study, in addition to oral antidepressant	TRD (MDD) MADRS ≥ 28	297	Intranasal (S)-ketamine 56 mg or 84 mg twice weekly for 16 weeks followed by randomized withdrawal phase (to continue (S)-ketamine or switch to placebo)	MADRS	- 176/297 in randomized maintenance phase achieved stable remission. 24 (26.7%) in the (S)-ketamine + antidepressant group and 39 (45.3%) in the placebo + antidepressant group experienced relapse (log-rank P = 0.003, NNT= 6) -121 achieved stable response. 16 (25.8%) in the (S)-ketamine + antidepressant group and 34 (57.6%) in the placebo + antidepressant group experienced relapse (log-rank P <0 .001, NNT= 4)
(Fedgchin et al., 2019)	Multicentre, parallel arm, randomized placebo-controlled, double- blind study, in addition to oral antidepressant	TRD (MDD) MADRS ≥ 28	346	Intranasal (S)-ketamine 56 mg or 84 mg twice weekly for 4 weeks	MADRS	- No significant difference in change in MADRS at 4 weeks between (S)-ketamine 84 mg + antidepressant compared with placebo + antidepressant (least-square mean difference –3.2, P = 0.088)
(Domany et al., 2019)	Parallel arm, randomized placebo-controlled, double-blind study add on to antidepressant treatment	TRD (MDD) MADRS ≥ 19	41	Oral ketamine 1 mg/kg 3 times a week over 6 weeks	MADRS	- The reduction in MADRS score on day 21 was 12.75 in the ketamine group versus 2.49 points with placebo (P < 0.001) - Six participants in the ketamine group (27.3%) achieved remission compared with none of the controls (P < 0.05) - NNT for remission was 3.7
(Ionescu et al., 2019)	Parallel arm, randomized placebo-controlled, double- blind study, add on to stable antidepressant treatment for ≥ 4 weeks	TRD (MDD) HAM-D ≥ 20	26	IV ketamine infusion (0.5mg/kg over 45 min) 2 times a week for 3 weeks	HAM-D-28	- No differences in depression severity or suicidal ideation between placebo and ketamine (P = 0.47 and P = 0.32, respectively) during infusion phase.
(Ochs-Ross et al., 2020)	Parallel arm, randomized placebo-controlled, double- blind study in addition to oral antidepressant	TRD (MDD) Age≥65 years	138	Flexibly dosed (28 mg, 56 mg, or 84 mg) (S)-ketamine nasal spray twice weekly for 4 weeks	MADRS	- No significant difference in primary outcome (change in MADRS at 4 weeks) between (S)-ketamine + antidepressant vs placebo + antidepressant

Table 1: Summary of placebo-controlled studies that have assessed the antidepressant effects of ketamine ((R,S)-ketamine) and (S)-ketamine.
Abbreviations: MDD: Major depressive disorder; TRD: Treatment-resistant depression; BD: Bipolar depression; HAM-D: Hamilton depression rating scale; MADRS: Montgomery Asberg Depression Rating Scale; BSI: Beck scale for suicide ideation; SSI: The Scale for Suicidal Ideation