Therapeutic Potential of Conjugated siRNAs for the Treatment of Major Depressive Disorder

Major depressive disorder (MDD) is a severe psychiatric syndrome with very-high socioeconomic impact worldwide (Global Burden of Disease Study 2013 Collaborators, 2015). This is attributable to three main factors: (i) MDD is a highly prevalent disorder in the general population, (ii) depressive episodes have long duration and occur during active periods of adult life, resulting in very large labor costs, and (iii) standard MDD treatments have limited efficacy, leaving a high percentage of patients with incomplete responses and poor quality of life, thus increasing suicide risk (Rush *et al*, 2006).

On the basis of clinical and pre-clinical studies, MDD may be associated with altered structural and synaptic plasticity, stress- or immune-related pathology, and genetic polymorphisms in brain circuits regulating mood and cognition (Ota and Duman, 2013). In this context, RNA interference (RNAi) may be a useful tool to examine the role of candidate genes in the pathophysiology and treatment of MDD. Hence, RNAi can be used as a therapeutic tool to silence targets involved in the disease process. However, despite its enormous potential, *in vivo* use of RNAi is limited due to the difficulty to deliver sequences of small RNAs (small interfering RNA-siRNA) to the desired neurons/circuits in mammalian brain. Our strategy has been to develop conjugate siRNA molecules (C-siRNA) in which the siRNA sequence was covalently bound to a selective serotonin reuptake inhibitor (SSRI-sertraline) in order to selectively accumulate it by the dense network of serotonin axon terminals in brain. The amounts (typically 0.5–2 nmol/day) of C-siRNA directed against 5-HT_{1A} receptors (5-HT_{1A}-R) or the serotonin transporter (SERT) were then administered intranasally to mice and C-siRNA sequences were localized into raphe serotonin neurons

(Bortolozzi *et al*, 2012; Ferrés-Coy *et al*, 2016). Using this strategy, we discerned the role of pre- and post-synaptic 5-HT_{1A}-R in response to stress, anxiety phenotype, and response to antidepressant treatments. Interestingly, the selective silencing of pre-synaptic 5-HT_{1A} autoreceptors was sufficient to elicit antidepressant-like effects in mice thanks to the increased capability of serotonergic neurons to release serotonin during stressful situations (Bortolozzi *et al*, 2012; Ferrés-Coy *et al*, 2013).

Likewise, we employed this approach to silence SERT expression/function. Intranasal administration of a C-siRNA targeting SERT (C-SERT-siRNA) evoked rapid and robust antidepressant-like responses in mice, including elevated forebrain serotonin levels, presynaptic 5-HT_{1A}-R desensitization, increased hippocampal neurogenesis and expression of trophic factors, and increased dendritic complexity. Further, C-SERT-siRNA reversed depressive-like behaviors in a mouse model of depression. C-SERT-siRNA evoked all these responses in 1 week, whereas SSRI fluoxetine required 1 month (Ferrés-Coy *et al*, 2016). In addition, we are using this strategy to knock down other genes potentially involved in stress resilience (eg, TASK-3 channels; Ferrés-Coy *et al* (2016), unpublished observations) and to target catecholamine neurons, by linking siRNA or antisense oligonucleotide sequences to the respective transporter inhibitors.

Despite the many advantages of siRNA to treat brain diseases, many challenges remain, including off-target effects, rapid degradation, immune response, and poor cellular uptake and selectivity, as well as *in vivo* delivery. Rational design strategies, predictive models based on second-generation algorithms, antibody and chemical modifications, and nanocarriers offer significant opportunities to overcome some of the above problems. Although it is still soon to know the impact of RNAi-based therapies on MDD treatment, our approach to deliver C-siRNA sequences to serotonin neurons through the intranasal route has proven successful in order to

elicit rapid and robust antidepressant-like actions in rodents, showing a high potential translational value.

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Error Processing in Depressive States: A Translational Opportunity?

Cognitive deficits are a core feature of depressive disorders, and have been linked to impaired daily functioning, worse clinical outcome, and treatment resistance. In particular, impairments in cognitive control (i.e., the ability to adaptively modulate action and thoughts based on internal goals and environmental demands) precede depression onset, persist after remission, and predict poor antidepressant responses (Pizzagalli, 2011). Moreover, among healthy participants, better cognitive control (as probed by an emotional Stroop task) predicted lower levels of pro-inflammatory cytokines in response to stress; thus, a better ability to modulate behavior when challenged by emotional information was associated with reduced inflammatory stress reactivity (Shields *et al*, 2016), highlighting a pathway by which cognitive control and immune function may directly influence one another. These findings suggest that cognitive control deficits might constitute markers of depression vulnerability and thus represent targets for new treatments. As recently emphasized, current pharmacological, psychological, and psychosomatic treatments often fail to ameliorate cognitive deficits despite improving mood (National Academies of Sciences, Engineering, 2015). Accordingly, novel interventions with pro-

cognitive effects may offer an innovative strategy to more effectively treat some of the most debilitating—yet often overlooked—symptoms of depression.

Challenges in developing treatments that target cognitive function are compounded by the use of different approaches in preclinical and clinical studies of depression, which unsurprisingly yields poor translation. Pre-clinical assays frequently involve end points such as time spent struggling in response to stress or latency to eat a novel food, neither of which matches the reality of the human condition or tap into high-level cognitive function. Clinically, cognitive control deficits often emerge when probing error processing. Specifically, individuals with depression show impaired post-error behavioral adjustments (i.e., lower accuracy immediately following an incorrect vs correct response)—a pattern associated with disrupted activation within frontocingulate pathways implicated in cognitive control (Pizzagalli, 2011). Building on these clinical findings, we reported that rats given corticotropin-releasing factor, a peptide that causes myriad signs of stress and depression in humans and laboratory animals, in an attention task showed similar reductions in post-error accuracy. This effect was attenuated by JDTic, a kappa-opioid receptor antagonist with antidepressant-like effects (Beard *et al*, 2015). These findings indicate that depression-like impairments in cognitive control can be recapitulated in rodents and are sensitive to classes of drugs under investigation for treating depression, opening exciting translational avenues.

Emerging evidence indicates that the same neural markers of cognitive control can be observed in rodents and humans, including potentiated post-error theta oscillations (Laubach *et al*, 2015) and feedback-related negativity after unsuccessful trials (Warren *et al*, 2015), which offer additional opportunities to align end points across species. These types of objective neurophysiological markers could be used to screen novel compounds, and prioritize those that normalize behavioral and EEG markers of cognitive control. Creating

a framework to study homologous end points in laboratory animals and humans should offer ways to more quickly and accurately predict clinical outcomes in humans, and is consistent with efforts at NIMH (e.g., 'Fast-Fail' trials: <http://www.nimh.nih.gov/research-priorities/research-initiatives/fast-fast-fail-trials.shtml>) to hasten the development of better therapeutics.

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Over the past 3 years, DAP has received consulting fees from Akili Interactive Labs, BlackThorn Therapeutics, Otsuka America Pharmaceutical, and Pfizer. WAC has a patent covering the use of kappa-opioid receptor antagonists for the treatment of depression, has received consulting fees from Cerecor, and currently serves as the Editor-in-Chief of this journal. This study was supported by 1UH2 MH109334 (DAP and WAC), 2R37 MH068376 (DAP), and 2R01 MH063266 (WAC). The author declares no conflict of interest.

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