Red/near-infrared irradiation therapy for treatment of central nervous system injuries and disorders

Melinda Fitzgerald [a, b], Stuart Hodgetts [a, c], Corinna Van Den Heuvel [d], Riccardo Natoli [e], Nathan S. Hart [b], Krisztina Valter [e], Alan R. Harvey [a, c], Robert Vink [d], Jan Provis [e], Sarah A. Dunlop [a, b]

Experimental and Regenerative Sciences [a], School of Animal Biology, The University of Western Australia [b], School of Anatomy, Physiology & Human Biology, The University of Western Australia [c], School of Medical Sciences, The University of Adelaide [d], ANU Medical School and John Curtin School of Medical Research, The Australian National University [e]

Corresponding Author

Asst Professor Melinda Fitzgerald: 61 8 6488 2353 lindy.fitzgerald@uwa.edu.au

Stuart Hodgetts: Assoc Professor, The University of Western Australia

Corinna Van Den Heuvel: Assoc Professor, University of Adelaide

Riccardo Natoli: Senior Scientist, Australian National University

Nathan Hart: Assoc Professor, The University of Western Australia

Krisztina Valter: Senior Lecturer, Australian National University

Alan Harvey: Winthrop Professor, The University of Western Australia

Bob Vink: Chair of Neurosurgical Research and Head of the School of Medical Sciences at the University of Adelaide.

Jan Provis: Associate Dean, Professor of Anatomy, ANU Medical School

Sarah Dunlop: Winthrop Research Professor, The University of Western Australia

Abstract

Irradiation in the red/near-infrared spectrum (R/NIR, 630 nm – 1000 nm) has been used to treat a wide range of clinical conditions including disorders of the central nervous system, with several clinical trials currently underway for stroke and macular degeneration. However, R/NIR irradiation therapy (R/NIR-IT) has not been widely adopted in clinical practice for CNS injury or disease for a number of reasons, as follows. The mechanism/s of action and implications of penetration ~~is~~have not been thoroughly addressed. ~~still not clear. Furthermore, t~~ The large range of treatment intensities, wavelengths and devices that have been assessed make comparisons difficult and a consensus paradigm for treatment has not yet emerged. Furthermore, the lack of consistent positive outcomes in randomised controlled trials, perhaps due to sub-optimal treatment regimens, has contributed to scepticism. This review provides a balanced précis of outcomes described in ~~synthesis of~~ the literature regarding treatment modalities and efficacy of R/NIR-IT for injury and disease in the CNS. We have addressed the important issues of specification of treatment parameters, penetration of R/NIR irradiation to CNS tissues and mechanism, providing the necessary detail to demonstrate the potential of R/NIR-IT for treatment of retinal degeneration, damage to white matter tracts of the CNS, stroke and Parkinson's disease. ~~We conclude with an assessment of the clinical application of R/NIR-IT for treatment of CNS injuries and disease.~~

Keywords: irradiation therapy, trauma – nervous system, retinal degeneration, Parkinson disease,

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1.1 Introduction

Irradiation in the red/near-infrared spectrum (R/NIR, 630 nm – 1000 nm) was developed as a therapeutic strategy for treatment of a range of injuries and diseases, following observations of beneficial effects on astronauts in space (Whelan et al., 2001). Therapeutic use of irradiation at these wavelengths is characterized by relatively low energy densities and is referred to as R/NIR-IT. It is distinct from high-energy ablative or thermocoagulatory laser treatments, or light dependent imaging techniques. Improvements following R/NIR-IT have been observed in a wide array of clinical conditions including wound healing (Whelan et al., 2003, Whelan et al., 2001, Yu et al., 1997), oral mucositis (Eells et al., 2004), cardial infarct size (Oron et al., 2001) and renal and hepatic complications during diabetes (Lim et al., 2009, Lim et al., 2010), although clinical efficacy is not always clear cut. Specific to the nervous system, beneficial effects have been reported following retinal degeneration (Albarracin and Valter, 2012b, Natoli et al., 2010), central nervous system (CNS) injury (Byrnes et al., 2005, Fitzgerald et al., 2010), stroke (Lapchak et al., 2007), peripheral nerve damage (Ishiguro et al., 2010, Rochkind et al., 2009) and for restless leg syndrome (Mitchell et al., 2011). However, R/NIR-IT has not been widely adopted in clinical practice for a number of reasons. Firstly ~~as~~ the mechanism/s of action ~~are~~ is/are still not clear and the impact of limited penetration of irradiation is unknown. ~~Furthermore~~Secondly, the large range of treatment intensities, wavelengths and devices that have been employed make inter-trial comparisons difficult and a consensus paradigm for treatment of CNS injury or disease has not yet emerged from preclinical studies yet alone clinical ones. Finally, the lack of a definitive positive effect to date in randomised controlled trials, perhaps due to sub-optimal treatment regimens, has contributed to scepticism.

Here we provide a synthesis of the literature regarding treatment modalities and efficacy of R/NIR-IT for injury and disease in the CNS. We begin by defining the principles of R/NIR-IT, and emphasise the importance of specifying parameters of wavelength, intensity, duration of treatment and the nature of the irradiation administered, ~~when reporting findings~~to enable comparison across different studies. R/NIR-IT cannot be effective without adequate penetration to the target tissue and to this end we have reviewed information describing penetration of R/NIR irradiation to CNS tissue and included additional calculations to aid interpretation. We briefly address mechanistic elements common to R/NIR-IT therapy across a range of disease and injury conditions and then comprehensively review the literature referring to retinal degeneration, damage to the optic nerve (ON) and visual cortex, spinal cord injury (SCI), traumatic brain injury (TBI), stroke and Parkinson's disease. Further, we provide a detailed tabulated comparison of the studies described, with information detailing: models used, irradiation source, intensity, wavelengths, duration of treatments and outcomes. We conclude by providing an assessment of the potential clinical application of R/NIR-IT for treatment of CNS injuries and disorders.

1.2 Parameters of R/NIR–IT

Despite positive results from preclinical research studies and successful clinical trials, as well as the obvious appeal of a relatively cheap and easily administered therapy, R/NIR-IT remains controversial and little used in mainstream medicine (Huang et al., 2011). This is in ~~large~~ part due to the lack of a standardized technical approach: search of the literature reveals a bewildering array of irradiation sources (laser or light-emitting diode), mode of delivery (pulsed or continuous), stimulation wavelength (630, 670, 780, 810, 830, 880, 904 nm), total dose (i.e. joules of irradiation per unit area), rate of delivery of the irradiation energy (watts

per unit area [note: watts = joules x time], also referred to as fluence), duration (length of exposure), timing (pre- or post insult) and frequency of treatment (Quirk and Whelan, 2011). This is confounded by the fact that dosages are usually specified in energy rather than quantal units; because photon energy varies with wavelength, an equal energy dose at different wavelengths will comprise different numbers of photons, and it is the number of photons interacting with a target photoacceptor that define the actual dose. While a range of treatment parameters have been trialled, we find few studies where variations in wavelength, total dose and dose rate have been tested in the same model. In short, while the empirical basis of R/NIR-IT for CNS injury and neurodegenerative disease is sound, its clinical application is hampered by uncertainty regarding treatment parameters. As an example of this variability, a comparison of parameters used in pre-clinical studies of R/NIR-IT for treatment of CNS injury or Parkinson's disease is provided in Table 1.

1.3 Penetration of irradiation in the human brain

The extent to which R/NIR irradiation can penetrate the brain is a key determinant of potential efficacy. Here we describe factors affecting penetration and give some biophysical examples to demonstrate that extremely low irradiation levels are sufficient to affect cells.

1.3.1 Extent of penetration (transmission)

When irradiation strikes biological tissue it is absorbed, scattered or transmitted. Optimal penetration within biological tissues occurs within a "therapeutic" or "optical window" with a wavelength range of 600 - 1000 nm (Parrish, 1981). The effective penetration depth of a given wavelength of irradiation is dependent upon the optical properties of the tissue, i.e. absorption and scattering (Cheong et al., 1990). Irradiation in the range of 600 - 1000 nm penetrates tissue because scattering by tissue inhomogeneities is dominant (Profio, 1989).

Scattering increases the distance travelled by photons thus diffusing the propagating irradiation. Absorption occurs predominantly by chromophores such as melanin and haemoglobin at short wavelengths and water and cytochrome c oxidase, a photoacceptor within the mitochondrial electron transport chain, at longer wavelengths (Karu, 1989, Sutherland, 2002).

Detailed characterisation of irradiation distribution in tissues is highly complex and ultimately requires the extrapolation of measurements that, for technical reasons that are discussed further below, must be made on samples that are considerably thinner than the tissue or organ of interest (Lenz, 1999). Issues such as tissue fixation, the limitations in availability and access to appropriate regions within living tissue as well as the limitation of the sensitivity of available equipment used to measure irradiation intensity, must all be considered. However, the theory of irradiation transmission through highly scattering media is well established and theoretical approximations commonly used to derive optical penetration depth (delta, δ) closely follow empirical measurements (Stolik et al., 2000). δ is the tissue thickness that causes irradiation to be attenuated to 37% its initial value (Muller and Wilson, 1986), i.e. not the maximum distance that irradiation will penetrate a tissue sample.

By way of example, we have used a diffusion model (Svaasand and Ellingsen, 1983) to calculate irradiation penetration to the centre of the human brain. We assumed brain dimensions of W140 mm × L167 mm × H93 mm, and a surface irradiance of 60 mW/cm^2 (e.g. the FDA-approved Vet 75 device at 670 nm, Quantum Devices Inc., Barneveld, WI) and that the head would be illuminated from the top, with the irradiation source positioned above the shaved scalp. We also assumed that the optical penetration depth of the skull is similar to

that of the overlying skin and the brain (Firbank et al., 1993), suggesting that all these tissues (and any other sub-cranial tissues and fluids) pass irradiation at any particular wavelength in a similar fashion, thereby simplifying calculations.

Calculated fluence rates just inside the cranium (assumed to be ~7 mm thick with a 3 mm thick skin covering to give a total thickness of 10 mm) were calculated to be 2.5 mW/cm^2 for 670nm irradiation (δ = 2.4 mm^{-1}) and 13 mW/cm^2 for 1064nm (δ = 4.0 mm^{-1}). Note that these two wavelengths are chosen as they are commonly used in R/NIR-IT; 670 nm irradiation is thought to change the oxidation reduction state of cytochrome c oxidase (see below) and 1064 nm irradiation is transmitted better through biological tissues (Karu, 1989, Sutherland, 2002). Indeed, greater transmission of irradiation at ~~increasing~~ longer wavelengths has been shown previously for a total human scalp and skull thicknesses of up to 13 mm (Wan et al., 1981). By contrast, and due to the exponential relationship between irradiation penetration and tissue thickness, fluence rates in the centre of the brain (10 mm skull and skin + 46 mm brain = 56 mm) were calculated to be very much lower at 1.2×10^{-11} W/cm^2 for 670 nm irradiation and 1.4×10^{-7} W/cm^2 at 1060 nm. The considerably greater (10^4) value for the penetration of longer wavelength irradiation calculated to reach the centre of the brain is in agreement with other studies comparing tissue penetration for a variety of wavelengths (Eichler et al., 1977, Lenz, 1999, Neupane et al., 2010).

To our knowledge, only two studies have directly measured the penetration of R/NIR irradiation in intact animals. Spectrophotometric and power transmission analyses in rat revealed that 6 % (9 mW) of transcutaneous 810 nm laser irradiation (power output 150 mW) was transmitted from the dorsal surface of the skin to the ventral side of the spinal cord (Byrnes et al., 2005). Similarly, a study in rat showed that when irradiation was directed at

the dorsal surface of the head (total R/NIR irradiance 252 W/m^2 550 - 750 nm), 0.7% (1.75 W/m^2) reached the ventral surface of the optic nerve, and 0.1% (0.3 W/m^2) the ventral surface of the braincase (Fitzgerald et al., 2010).

1.3.2 Factors affecting penetration

A number of factors affect R/NIR irradiation penetration of tissue. Haemoglobin and water are major chromophores, i.e. they absorb irradiation (Sterenborg, 1989), thus the extent of R/NIR irradiation penetration will presumably vary according to vascularisation and fluid balance. Although the absorption coefficient of water is low in the visible region, it is significant for R/NIR irradiation and the large volume fraction of water in biological tissue, together with haemoglobin, contributes significantly to absorption (Ankri et al., 2010). Indeed, in *ex vivo* human kidney and liver tissue, δ is greater for slices containing blood compared to those in which the blood had been removed (Eichler et al., 1977). Furthermore, δ values obtained from freshly resected human brain tissue were greater in malignant brain tumours that were more vascularised, compared to normal brain tissue (Muller and Wilson, 1986). Both vascularisation and water balance are profoundly influenced in neurodegenerative disease such as Alzheimer's and Parkinson's as well as stroke. For example, β-amyloid promotes angiogenesis and blood brain barrier permeability in Tg2576 AD mice (Biron et al., 2011) and in humans with cerebral amyloid angiopathy (Hartz et al., 2012) while integrin αvβ3, a marker for angiogenesis, is increased in human PD brains (Desai Bradaric et al., 2012). Aquaporin expression, indicative of changed water transport, is enhanced in AD brains (Moftakhar et al., 2010) and blood brain barrier permeability is increased following ischaemic stroke (Hom et al., 2011, Topakian et al., 2010). Not unexpectedly, vascular damage is also extensive following TBI both acutely (Iwamura et al., 2012) and with evidence for long term remodelling (Rodriguez-Baeza et al., 2003). Reactive

gliosis is a further contributor to changes in cellular architecture that may modulate penetration of R/NIR-IT (Sykova and Vargova, 2008). However, in these pathological conditions, it is unknown whether the increased blood supply and changed water balance would act as a more prominent substrate for the absorbance of R/NIR irradiation during therapy.

Oxygenation also influences penetration of irradiation. Compared to room air (21% O_2; 0.039% CO_2), carbogen breathing (95% O_2: 5% CO_2) in mice increased the penetration of R/NIR irradiation and improved the outcome of photodynamic therapy for tumour ablation (Mitra and Foster, 2004). Conversely, in situations where hypoxia occurs such as after traumatic and hypoxia-ischaemia induced brain injury (Howards, 2012, Oddo et al., 2011), any therapeutic use of R/NIR irradiation may require higher intensity irradiation to offset reduced tissue penetration. Myelination is also thought to influence penetration of irradiation with greater δ values in grey compared to white matter (Lenz, 1999). A study in bovine brain showed that irradiation penetration was greatest when irradiation was oriented parallel to white matter tracts (Hebeda et al., 1994), a finding that explains the increased incidence of lesions within the corpus callosum after photodynamic therapy in normal mouse brain (Sandeman, 1986).

1.3.3 Extremely low irradiation levels can affect cells

The foregoing calculations suggest that fluence/fluence rates at limited depths into the skull and brain are quite significant. A 20 minute exposure to a 60 mW/cm^2 670nm LED array (e.g. Vet 75) positioned above the head would give a total fluence (irradiation dose) of ~3 J/cm^2 at a fluence (dose) rate of 2.5 mW/cm^2 to the surface of the brain just under the cranium. This is within known effective dose / rate ranges (cf. (Huang et al., 2011)). Whether

or not the extremely low level of irradiation reaching the *centre* of the brain is 'effective' for irradiation therapy of CNS injury or disease remains to be determined.

Nevertheless, very low levels of irradiation can trigger significant biophysical phenomena. For example, at the threshold of useful vision - such as when we are able to detect the edges of large objects at night under a moonless, overcast sky - the ambient light intensity is such that (on average) each rod photoreceptor in the retina only absorbs a single photon of light or irradiation *every 84 minutes*. Even at dawn when there is enough light to see clearly, and objects appear coloured because cones are also active, rods are only capturing (on average) one photon every 5 seconds (Rodieck, 1998). However, because the retina contains around 90 million rods (Curcio et al., 1990), we are able to use this limited information to form an image and not just detect light. To put this into context, the intensity of the dimmest extended light source that can be seen by a human corresponds to a fluence rate of 1.5×10^{-14} W/cm^2, i.e. well below that reaching the centre of the brain in our modelled scenario above. Although the exact mechanism of R/NIR-IT remains to be elucidated, it is clear that the low levels of irradiation encountered are more than capable of triggering cellular signalling and probably sufficient to drive metabolic events.

1.4 Potential mechanisms of efficacy of R/NIR–IT

Several recent reviews have summarized existing knowledge regarding the potential mechanisms by which R/NIR-IT exerts its effects (Chung et al., 2012, Hashmi et al., 2010) and we therefore address this subject relatively briefly. Cytochrome *c* oxidase is proposed to act as a photoacceptor for irradiation at these wavelengths, with absorption spectra matching efficacious wavelengths and irradiation leading to changes in the oxidation reduction state of

the enzyme (Karu, 1999, Karu and Kolyakov, 2005, Karu et al., 2005, Karu et al., 2008). Increases in cytochrome *c* oxidase activity with R/NIR-IT are associated with increases in ATP content in treated tissues, indicating increased flux through the electron transport chain (Lapchak and De Taboada, 2010, Wong-Riley et al., 2005). While it has been proposed that these changes are associated with increased reactive oxygen species and resultant downstream signalling (Chung et al., 2012, Hashmi et al., 2010), it is important to note that studies linking various facets of oxidative metabolism to ROS/RNS production have been contradictory, largely conducted *in vitro*, and highly dependent on the timing of experimental observations and the conditions employed (Peng and Jou, 2010, Tretter et al., 2007). Indeed it is possible that increased flux of electrons through the electron transport chain may maintain mitochondrial membrane potential, reduce passage through the reverse electron transport chain, alter cAMP release and increase ATP synthesis, all of which may result in reduced leakage of free radical intermediates (Camello-Almaraz et al., 2006, Kowaltowski et al., 2009, Rojas et al., 2008). While direct deductions concerning the sequence of effects in mitochondria with R/RIR-IT are problematic with the existing information available to us, R/NIR-IT has been shown to improve indices of mitochondrial function following damage to the CNS in a range of model systems, many of which have been associated with improvements in function (Eells et al., 2004, Rojas et al., 2008). Further descriptions of reported effects of R/NIR-IT on cytochrome *c* oxidase and mitochondrial function in specific CNS injury and disease states are provided in the sections below.

While a significant proportion of the available data on mechanism of R/NIR-IT point towards cytochrome *c* oxidase as a primary photoacceptor, this does not preclude other potential modes of action. The chromophores melanin and haemoglobin may also play a role (Karu, 1989, Peoples et al., 2012a, Sutherland, 2002). Nitric oxide released from cytochrome *c*

oxidase may lead to downstream vasodilatation (Ball et al., 2011, Mason et al., 2006) and signal transduction, potentially also contributing to functional improvements. R/NIR-IT has been shown to modulate gene expression (Natoli et al., 2010), reduce apoptosis (Liang et al., 2008, Wong-Riley et al., 2005), alter cytokine release and modulate immune responses (Moreira et al., 2009)(Albarracin and Valter, 2012a)(Kokkinopoulos et al., 2012) (personal observation), and these outcomes may be up/down-stream or independent of modulation of cytochrome *c* oxidase activity.

1.5 R/NIR-IT for treatment of retinal degeneration

The studies discussed here employ R/NIR LED devices to deliver a therapeutic dose to the retina in an experimental setting, or to monitor effects on the aging retina. The high metabolic activity of photoreceptors renders the retina highly susceptible to oxidative damage (Winkler, 1981) (Yu et al., 1999). Oxidative damage to photoreceptors has been implicated in many forms of retinal degeneration including Age-Related Macular Degeneration (AMD) (Multipleauthors, 2001), retinitis pigmentosa (Shen et al., 2005), retinopathy of prematurity (Tsukahara et al., 2004) and in the later stages of all photoreceptor degenerations regardless of the initiating event (Stone et al., 1999). Arguably, amelioration of oxidative damage is the key to long-term survivability of the retina. Several laboratories have now used R/NIR irradiation to attenuate experimentally induced retinal degeneration, including models of AMD (Albarracin et al., 2011, Albarracin and Valter, 2012a, Albarracin and Valter, 2012b, Natoli et al., 2010, Qu et al., 2010), Parkinson's-related retinopathy (Peoples et al., 2012a) and methanol (Eells et al., 2003) and rotenone (Rojas et al., 2008) toxicity, with beneficial effects in normal aging (Kokkinopoulos et al., 2012). None of these studies report any adverse effects of 670nm irradiation on the retina.

Methanol is a potent mitochondrial toxin, which inhibits cytochrome oxidase activity (Seme et al., 1999). Eells *et al.,* (2003) reported the first direct link between mode of action of R/NIR irradiation in retinoprotection, using a methanol toxicity model. They found that mitochondrial damage in photoreceptors caused by methonal toxicity was reduced by exposure to 670nm irradiation. Mitochondria retained their normal structure in animals intoxicated with methanol and treated with 670 nm irradiation. Rod and cone response amplitudes (electroretinogram) were reduced up to 75% by methanol toxicity; however, when combined with 4 J/cm2 treatments of 670 nm irradiation at 5, 25 and 50 hrs of methanol intoxication, response amplitudes were reduced by only ~33%. The authors concluded that because formic acid (derived from breakdown of methanol) acts directly to inhibit cytochrome *c* oxidase, a key enzyme in mitochondrial metabolism, 670nm irradiation appeared to be directly modulating enzyme activity to reduce this toxic effect and promote mitochondrial function. In an *in vivo* study in the rat retina, 633nm irradiation was protective against Rotenone, a potent inhibitor of mitochondrial function, providing a further direct link between these organelles and R/NIR irradiation (Rojas et al., 2008). This link has also been noted in experiments using the mitochondrial dye JC-9, in which there is a shift in mitochondrial membrane potential in retinal pigment epithelial cells in direct response to 670nm irradiation (Kokkinopoulos et al., 2012).

Oxidative damage generated by excessive photo-oxidation of the rod outer segments is thought to be the initiating event in light-induced retinal damage (LD) (Demontis et al., 2002). However, it has also been shown in LD that retinal degeneration continues long after removal of the damaging stimulus (white light) (Rutar et al., 2010). This progressive

degeneration has been used to model the factors contributing to expansion of the degenerative area, as occurs in AMD, and appears to be largely mediated by inflammation (Hollyfield et al., 2008). Microarray analysis (Natoli et al., 2010) shows that expression of genes in pathways involved in inflammation, apoptosis and metabolism are down-regulated in LD retinas treated with 670nm irradiation. One of the most highly modulated genes identified in that study is Ccl2 - a potent chemokine involved in recruitment of macrophages to sites of tissue injury. Ccl2 has become a gene of interest from investigations in models of AMD and this chemokine family is now implicated in its pathogenesis (Rutar et al., 2011).

Several studies find that 670nm irradiation is protective against retinal degeneration in LD, citing histological, functional and molecular evidence (Albarracin et al., 2011, Albarracin and Valter, 2012a, Natoli et al., 2010, Qu et al., 2010). Treatment with 670nm irradiation before, during or after exposure to damaging white light attenuates retinal degeneration (Albarracin et al., 2011, Qu et al., 2010), protects photoreceptor function and reduces expression of stress markers in the retina, as well as microglial and macrophage invasion (Albarracin and Valter, 2012a). While treatment prior to exposure to bright light is most effective, animals treated with 670 nm *after* light damage recover photoreceptor function by one month post-exposure (Albarracin et al., 2011). LD induces upregulation of a number of markers of oxidative stress and these are downregulated by 670nm irradiation (Natoli et al., 2010). Two independent studies report downregulation of the pro - inflammatory cytokine TNF-alpha following treatment with 670nm irradiation. In the first, quantitative PCR was used to show a reduction in TNF alpha levels in the LD retina pre-treated with 670nm irradiation (Albarracin and Valter, 2012a). In the second, it was shown that treatment of aged mice with 670nm irradiation reduces TNF-alpha immunoreactivity, as well as recruitment of IBA1 positive macrophages to outer retina, and C3b and C3d immunoreactivity in Bruch's membrane

(Kokkinopoulos et al., 2012), all indicating downregulation of inflammatory responses. In addition, components of the 'classical' pathway of complement activation, as well as C3 are downregulated by pretreatment with 670nm irradiation in the LD model (unpublished observation).

Collectively these studies provide strong evidence that 670nm irradiation gives significant protection to the retina, and some studies indicate that cytochrome *c* oxidase is the most likely photo-acceptor. The observed effects can be explained by a theoretical model of 670 nm IT promoting effective mitochondrial function, resulting in a reduction of free radical production and oxidative damage. The downstream effects appear to be a downregulation of inflammatory processes. The LD model specifically, and the retina in general, are ideal models to explore the mechanisms, potential and limitations of R/NIR irradiation treatments, due to the relative ease of inducing damage, the known interactions between oxidative stress and inflammatory pathways, combined with accessibility for treatment.

1.6 R/NIR-IT for treatment of damage to the optic nerve and visual cortex

Evidence supporting the role of cytochrome *c* oxidase as a key photo-acceptor for irradiation in the R/NIR spectrum has also been generated in studies of neurons from the visual cortex. *In vitro* experiments assessing the effects of inhibitors on neurons from the postnatal rat visual cortex demonstrate that KCN inactivation of cytochrome *c* oxidase (by 100 μM KCN or less) is reversed by treatment with 670 nm irradiation delivered by LED array once or twice daily for 100 seconds (energy density = 4 J/cm^2, power density = 50 mW/cm^2) (Wong-Riley et al., 2001, Wong-Riley et al., 2005). Effects with twice daily treatments are more

pronounced for neurons lightly reactive for cytochrome c oxidase activity (Wong-Riley et al., 2005). Longer pretreatments with 670 nm irradiation (10 minutes prior to KCN exposure, equivalent to 30 J/cm^2) significantly reduce nuclear condensation (Wong-Riley et al., 2005), attributed to reduced apoptosis and associated with reduced oxidative stress (Liang et al., 2006). The effects of a range of wavelengths of irradiation delivered via LED array in the R/NIR spectrum on cytochrome *c* oxidase activity following blockade of voltage dependent sodium channels with tetrodotoxin, have also been compared in visual cortical neurons. 670 nm and 830 nm irradiation (energy density = 4 J/cm^2, power density = 50 mW/cm^2) restores cytochrome *c* oxidase activity and ATP content, whereas 728 nm, 770 nm and 880 nm irradiation are less effective (Wong-Riley et al., 2005). Effective wavelengths correlate positively with the known absorption spectra of oxidised cytochrome *c* oxidase (Carter and Palmer, 1982, Karu, 1999, Karu et al., 2008, Wong-Riley et al., 2005).

Cytochrome *c* oxidase activity in RGC somata has been linked to survival of these cells following complete ON transection (von Bussmann et al., 1993). While cytochrome *c* oxidase activity is higher in unmyelinated than myelinated regions of the human ON (Balaratnasingam et al., 2009), we have recently demonstrated *in vivo* that cytochrome c oxidase activity is increased in ON following partial ON transection and 670 nm irradiation treatment (LED, 30 minutes / day, 25 mW/cm^2), and that activity colocalised with oligodendrocytes, at least in the short term (unpublished observation). Increased cytochrome *c* oxidase activity in ON is associated with reduced oxidative stress, both in nerve homogenates and more specifically in astrocytes (Fitzgerald et al., 2010) and oligodendrocytes (unpublished observation). 670 nm irradiation (LED) treatment also decreases proliferation of oligodendrocyte progenitor cells (Fitzgerald et al., 2010) and reduces paranode elongation in injured ON vulnerable to secondary degeneration, associated

with later preservation of RGC numbers and restoration of visual function (Fitzgerald et al., 2010) (unpublished observation). Similarly, daily treatments of rat ON crush injuries with 630 nm irradiation (delivered by He-Ne laser, 10.5 mW) for 2 weeks significantly increases compound action potentials in ON *ex vivo* and postpones degeneration (Assia et al., 1989). For positive effects, treatment needs to be rapidly initiated (less than 5 hours after injury), maintained, and used on moderately rather than severely injured nerves (Assia et al., 1989).

1.7 R/NIR-IT for treatment of traumatic brain injury (TBI)

Neuropathological consequences of TBI include disruption in axonal transport leading to axonal swelling followed by secondary disconnection, extensive demyelination and Wallerian degeneration (Johnson et al., 2012, Tang-Schomer et al., 2012) (Brambilla et al., 2006). Alterations to mitochondria (including membrane permeability) influence axonal integrity as well as ionic imbalance, oxidative stress and lipid peroxidation (associated with both mitochondrial dysfunction and cytoskeletal degradation) which play a central role post-injury in both axonal degeneration and dysfunction of viable and intact axons (Buki et al., 1999, Johnson et al., 2012, Maxwell et al., 2003). Of the ten TBI studies presented here describing R/NIR-IT in mice (Ando et al., 2011, Khuman et al., 2012, Oron et al., 2007, Oron et al., 2012, Wu et al., 2012), rats (Moreira et al., 2009, Quirk et al., 2012) and humans (Naeser et al., 2011, Nawashiro et al., 2012), eight reported effects in acute (short term) or sub-acute, and only one in chronic (long term) (Naeser et al., 2011) contusive TBI. Of these ten studies, nine used lasers to deliver the R/NIR-IT. A further study using laser delivered R/NIR-IT (McCarthy et al., 2010) provides evidence that treatment with 808nm wavelength in the *uninjured* rat brain is safe at single and multiple doses (Table 1), with no treatment-related lesions, neoplasia or other toxicological abnormalities for up to 1 year after injury (McCarthy

et al., 2010). Most rodent model studies report statistically significant improvements in outcomes including: Neurological Severity Scores (NSS), evidence of increased axonal numbers and distance of re-growth, reduced lesion size, modulation of apoptotic and inflammatory responses, and pronounced anti-depressant effects.

Treatment of TBI generated by cortical impactor device in mice, with 808 nm Gs-As (gallium - arsenide) diode laser (10 and 20 mW/cm^2, 1.2 - 2.4 J/cm^2) for 2 minutes (at 4 hours post trauma) results in no significant improvements in NSS up to 48 hours after treatment. However, from day 5 - 28, NSS are reduced by about 27% in irradiated mice which also have smaller lesion sizes compared to controls (1.4% vs 12.1%) (Oron et al., 2007). Continuous wave (CW) GaAIAs (gallium - aluminium - arsenide) 780 nm or InGaAIP 660 nm low level laser irradiation following a 40 second cryoinjury to the brain results in immunomodulation of TNF-α, IL10, IL1β cytokine responses following R/NIR-IT (Moreira et al., 2009), although no functional recovery studies were performed in this study. Neuroprotective effects are also seen with 808 nm GaAIAs laser treatment at 50 mW/cm^2 (CW at 10 Hz and Pulsed wave (PW) at 100 Hz) for 12 minutes at 4 hours, following contusive TBI in mice. These neuroprotective effects, which are more pronounced after 10 Hz PW frequency treatment than 100 Hz CW, include improved behavioural recovery (NSS), reduced brain lesion volume and a pronounced anti-depressant effect at up to 4 weeks post TBI (Ando et al., 2011). A 2 - 7 minute exposure to 800 nm GaAIAs laser treatment at a variety of doses (250, 500 or 1000 mW/cm^2), at 60 - 80 minutes or 4 hours after contusive TBI in mice results in no effects on post-injury motor function (days 1 - 7), brain edema (24 hours), nitrosative stress (24 hours), or lesion volume (14 days), however there are improved cognitive outcomes and inhibition of microglia activation (Khuman et al., 2012).

Four weeks after a single 4 minute exposure at 4 hours post contusive TBI (36 J/cm^2 CW, 665 nm, 730 nm, 810 nm or 980 nm) in mice, there is improved behavioural recovery (NSS) and reduced brain "deficits", but only in 665 nm and 810 nm treated animals (Wu et al., 2012). Treatment of rats with 670 nm irradiation (at 50 mW/cm^2, 15 J/cm^2) for 2 x 5min per day for 72 hours or 10 days, post contusive TBI, results in functional (NSS) and morphological improvements, including decreased pro-apoptotic Bax expression and increased anti-apoptotic Bcl2 expression (Quirk et al., 2012). Finally Oron *et al* (2012) using an 808 nm GaAIAs laser to deliver 10 mW/cm^2 (1.2 J/cm^2) treatment (either CW or PW, at 100 or 600 Hz) for 2 minutes (at either 4, 6, or 8 hours post trauma) following contusive TBI in mice show improved neurobehavioural function (NSS) and an overall reduction in lesion size at 56 days (Oron et al., 2012). It has been proposed, based mainly on *in vitro* cortical neuron models (see above), that R/NIR-IT benefits recovery from TBI by inhibiting apoptosis whilst increasing mitochondrial activity, transcriptional activation, angiogenesis and neurogenesis (Chung et al., 2012)(Huang et al., 2012).

In humans, R/NIR-IT using 830 – 870 nm irradiation has been tested in a 40 year old male (2 x 30 min treatments per day for 73 days, applied 5mm from skin at 11.4 mW/cm^2, 20.5 J/cm^2 (Nawashiro et al., 2012) and in two females aged 52 and 59. In the latter case, R/NIR-IT using 633 / 870 nm was administered either (i) as treatments of 5 minutes duration and 10 seconds per area treated for 7 months, then 3 weeks per month at 25.8 mW/cm^2 (CW) or (ii) daily for 7 minutes duration per area for 1 month, increasing by 1 minute per area for each successive month at 22.2 mW/cm^2 (CW)) for treatment durations ranging from 3 months to 7 years (Naeser et al., 2011). In both studies, improved neurological outcomes (including executive function and memory) are reported, as well as reduced post-traumatic stress disorder and improved cerebral blood flow in chronic TBI (Naeser et al., 2011, Nawashiro et

al., 2012). Patients were able to eventually self-treat in the home, but benefits are reduced if the daily / weekly treatment frequency is not maintained.

1.8 R/NIR-IT for treatment of spinal cord injury (SCI)

Pathological changes following acute SCI are characterised by focal injury (Sekhon and Fehlings, 2001) followed by an expanding wave of secondary degeneration and cell death (Baptiste and Fehlings, 2006, Nashmi and Fehlings, 2001, Park et al., 2004), that is associated with oxidative damage (Keane et al., 2006, Liu et al., 1997, Profyris et al., 2004). Of the three SCI studies presented here, all describe R/NIR-IT using lasers in rats (Byrnes et al., 2005, Medalha et al., 2010, Wu et al., 2009). To date, no R/NIR irradiation treatments have been reported in humans with SCI. In all rat studies, treatment began either immediately (Medalha et al., 2010, Wu et al., 2009) or within 15 minutes (Byrnes et al., 2005) after SCI, which involved either; dorsal hemisection at T9 - 10 alone (Byrnes et al., 2005), dorsal hemisection at T9 or moderate contusion at T9 - 10 (10g dropped from 12.5 mm, NYU impactor) (Wu et al., 2009), or complete transection (Medalha et al., 2010) (Table 1).

R/NIR-IT using 810 nm irradiation (2 week treatment at 1,589 J/cm^2/day) results in increased axonal number and distance of regrowth, as well as immunomodulation and some aspects of functional recovery improvement (as measured by ladder footfall, run time and hindlimb paw placement - but no open field (Basso, Beattie and Bresnahan) assessment) (Byrnes et al., 2005). Similarly, 810 nm irradiation treatment (daily for 14 days at 2,997 seconds per day, 1,589 J/cm^2/day) results in increased axon length and number in both dorsal hemisection and moderate contusion SCI models (Wu et al., 2009). In the contusion model, improved open field (BBB) locomotion scores are reported with R/NIR-treated rats reaching BBB scores of 12 - 13 at 3 weeks after injury (ie. frequent to consistent weight supporting plantar steps with

frequent coordination between fore- and hind-limbs) compared to non-treated rats reaching BBB scores of 9 - 10 (ie. occasional weight-supported plantar steps but with no coordination) (Wu et al., 2009). More recently, 830 nm-treated rats show a "trend" toward improved hindlimb diaphysis, but no biomechanical or densitometric improvements following complete spinal cord transection (Medalha et al., 2010). No behavioural (locomotory) improvements as measured by BBB scoring are observed in any treatment group. It is of note however, that the 830 nm treatment was not actually applied directly to the spinal cord in this study, rather at two points on the hindlimb (Medalha et al., 2010).

1.9 R/NIR – IT for treatment of stroke

Although at least 1000 agents have been shown to be efficacious in preclinical ischaemic stroke evaluation, to date only intravenous tissue plasminogen activator (tPA) has been approved for clinical use in treating acute ischaemic stroke (Segura et al., 2008). However, the majority of stroke patients either do not meet the strict criteria for treatment with tPA or fail to receive adequate reperfusion. Therefore, additional new therapies are needed. Recently, non-invasive laser therapy has been applied to acute ischaemic stroke patients with positive results; specifically, R/NIR irradiation is applied to the scalp within 24 hours of stroke symptoms onset. The principle objective of this section is to evaluate the literature regarding R/NIR-IT delivered by laser, in experimental stroke models and recent clinical trials, demonstrating that the most advanced application for R/NIR-IT to date is in ischaemic stroke with promising pre-clinical and clinical results. This subject has recently been comprehensively reviewed by Lapchak (2012); as such, details are provided for purposes of comparison with other CNS injury and disease states.

1.9.1 Pre-clinical rabbit studies

Positive findings of the efficacy of R/NIR-IT in reducing lesion volume in myocardial infarction (Ad and Oron, 2001) prompted an investigation into whether a similar procedure would reduce stroke-related behavioural deficits due to the similarities between myocardial and cerebral ischaemia. A small clot embolic stroke model (RSCEM) was used in rabbits treated with transcranial R/NIR-IT, employing a continuous wave (CW) of irradiation at high power densities (25 mW/cm^2) for 10 mins. This initial exploratory study found that laser treatment significantly improves behavioural rating scores (reviewed in Lapchak et al., 2012). Of note, this therapy remainings effective when initiated within 6 hours post-occlusion, which is later than any other previously effective single therapy in the same model (Lapchak et al., 2002). Importantly, this beneficial effect is measurable up to 21 days after embolisation, with no short or long term detrimental effects of R/NIR-IT treatment. In a later study, the same group found that PWPulsed wave R/NIR-IT was more beneficial than CW R/NIR-IT, with statistically significant increases in clinical performance at 6 hrs post-treatment ($p < 0.05$), not seen with CW R/NIR-IT (Lapchak et al., 2007). This was, attributed to increased penetration of photons through the brain using the pulsed peaks (Lapchak, 2012). Lapchak and his colleagues also undertook investigations to assess the safety of combining R/NIR-IT with the only currently approved stroke therapeutic tPA. R/NIR-IT was found to be safe, with R/NIR-IT alone and R/NIR-IT administered following tPA not adversely affecting the haemorrhage rate, haemorrhage volume or survival after large clot embolism induced stroke in New Zealand white rabbits. As such the authors recommended that a clinical trial of the tPA (Alteplase) plus R/NIR-IT be conducted (Lapchak et al., 2008).

It is well established that the core ischaemic area following occlusion of a major cerebral artery experiences rapid loss of ATP and energy production with widespread neuronal depolarization (Streeter et al., 2004). Embolisation-induced decreases of cortical ATP are attenuated by subsequent treatment with either CW R/NIR-IT or PW R/NIR-IT, although PW R/NIR-IT is more effective (Lapchak and De Taboada, 2010). Indeed PW R/NIR-IT leads to increases in cortical ATP which are significantly higher than that seen in naïve animals. These results are compatible with the hypothesis that mitochondrial cytochrome *c* oxidase is a potent chromophore for 808 nm irradiation energy (Streeter et al., 2004) as described for other disease states in detail above.

1.9.2 Pre-clinical rodent studies

Given the therapeutic benefits for R/NIR-IT in the myocardial infarction setting, and its promising results after application in stroke models in rabbits, Oron and his colleagues studied the effects of transcranial R/NIR-IT initiated 4 and 24 hours after a middle cerebral artery occlusion (MCAO) stroke in rats (Oron et al., 2006). Using both CW and PW modes of R/NIR-IT at 7.5mW/cm^2 they found that R/NIR-IT significantly attenuates neurological deficits in CW laser-treated rats, without decreasing stroke lesion volume when administered at 24 hrs, but not 4 hrs after the onset of a stroke (Oron et al., 2006). Unlike the neuroprotective effects of R/NIR-IT demonstrated in the RSCEM, the authors found no early neuroprotective benefit of R/NIR-IT when administered at 4 hours after a stroke. Furthermore, both PW and CW R/NIR-IT have similar effects on motor outcome, although only the CW group reach significance in comparison to non-treated animals. This contrasted with an earlier study (Lapchak, 2010), the disparity perhaps attributable to the use of different experimental models and species. Interestingly, Oron *et al* proposed another potential

mechanism of action of R/NIR-IT, with immunocytochemical analysis of the brain post-treatment revealing increased markers of neurogenesis (Oron et al., 2006), which may increase the functionality of neuronal circuits and promote neuronal survival within this penumbral region (Lapchak, 2010). Others have agreed in principle with this finding, noting the 2 - 4 week delay in neurological outcome improvement evident post-stroke in rat models (Detaboada et al., 2006, Shen et al., 2008). Further investigations (Detaboada et al., 2006) revealed that the location of laser treatment does not affect its efficacy, with ipsilateral, contralateral or bilateral laser treatment (CW 808 nm, 7.5mW/cm^2) administered 24 hours post-stroke efficiently improving neurological outcome. Furthermore, marked and significant improvements in neurological deficits are evident at 14, 21 and 28 days post stroke. Additional proposed mechanisms of action of R/NIR-IT in preventing neuronal death include irradiation mediated upregulation of anti-apoptotic proteins (Liang et al., 2008), heat shock proteins and antioxidant enzymes, upregulation of the neuroprotective agent transforming growth factor beta 1 (TGF-β1) and suppression of the potentially neurotoxic agent nitric oxide synthase (NOS) (Leung et al., 2002). In summary, the beneficial effects of R/NIR-IT seen following stroke appear far reaching and likely achieved by attenuation of several processes in concert.

It is important to note that safety studies in rodents investigating the possible short and long-term adverse neurological effects of R/NIR-IT given at different power densities and frequencies have been conducted. A diode laser (808 nm wavelength) used to deliver power densities of (7.5, 75 or 750 mW/cm^2) in either CW or PW modes results in no discernible damage to tissue and no difference between laser-treated and control groups up to 70 days post-treatment. The only rats showing adverse neurological effects are those in the CW 750 mW/cm^2 group (about 100 fold increase over the current / optimal dose) (Ilic et al., 2006).

1.9.3 Acute ischaemic stroke clinical trials

Two randomized double-blind clinical trials with R/NIR-IT; NeuroThera ® Effectiveness and Safety Trial (NEST)-1 and NEST-2 have already been completed (Lampl et al., 2007, Zivin et al., 2009) and because pooled results indicate a clinical improvement, a third trial (NEST-3) is currently underway. The phase II NEST-1 was a prospective, randomized 2:1, double-blinded, placebo-controlled, international and multicenter trial involving 120 ischaemic stroke patients (79 in the active treatment group and 41 shams (Lampl et al., 2007)). Its primary aim was to assess the safety and efficacy of R/NIR-IT administered within 24 hours of onset of stroke symptoms. The low-energy lasers with a wavelength of 808 nm were applied at 20 locations on the scalp with 2 minutes of irradiation at each site, for a power density of 10 mW/cm^2 and energy density of 1.2 J/cm^2. Mean time to treatment was over 16 hours (ranging from 2 - 24 hours). The primary endpoint was the National Institutes of Health Stroke Scale (NIHSS) score collapsed into a binary outcome and the modified Rankin Scale (mRS). Briefly, patients receiving R/NIR-IT had a higher proportion of positive NIHSS and mRS outcomes than did sham-treated patients (Lampl et al., 2007) and therefore this trial provides some indication that R/NIR-IT is useful in treating the motor function deficits resulting from ischaemic strokes. Furthermore, no adverse outcomes could be attributed to the laser therapeutic procedure.

The larger phase III, NEST-2 trial was conducted in 660 stroke victims (Zivin et al., 2009). This was an acute ischaemic stroke study within 24 hours of stroke onset and excluded patients who had received thrombolytic therapy and patients with evidence of intracerebral haemorrhage. The trial results did not reach statistical significance ($p = 0.094$), and were

considered not to be positive when all patients were included. However, post-hoc subgroup analysis detects significant improvements at 90 days ($p < 0.04$) in the moderately impaired stroke patients ($n = 434$) that are not evident in severely impaired patients. Pooled analysis of the 778 patients from the NEST-1 and NEST-2 trials reveals a significantly improvement in those patients treated with laser therapy (Stemer et al., 2010).

The NEST-3 clinical trial design is very similar to the NEST-2 trial and proposes to include 1000 patients within 24 hours of a stroke with an NIHSS baseline of 7-17, which is the range where beneficial effects of R/NIR-IT are seen in the NEST-1 and NEST-2 trials (Lampl et al., 2007, Zivin et al., 2009). Patients have begun to be enrolled in the NEST-3 trial with the aim of demonstrating safety and efficacy of TLT with the NeuroThera® Laser System in the treatment of subjects diagnosed with acute ischemic stroke. The initiation of the R/NIR-IT procedure must be feasible for each subject between 4.5 and 24 hours of stroke onset. The earliest treatment time of 4.5 hours, is in agreement with the recently expanded 4.5 hour therapeutic window data for tPA (Lansberg et al., 2009). Patients with infarcts located exclusively in the brainstem, cerebellum or who have small deep infarctions or massive hemispheric strokes are excluded from this trial inferring that trial enrolment may be limited to stroke patients with only small superficial cortical infarcts perhaps owing to the limitations of the CW R/NIR-IT regimen (Lapchak, 2012).

Interestingly, both the NEST-1 (Lampl et al., 2007) and NEST-2 (Zivin et al., 2009) trials used a CW treatment regimen with a power density similar to preclinical rabbit studies (Lapchak and De Taboada, 2010). This CW method is also currently in use in the NEST-3 trial, and perhaps given the fact that the CW R/NIR-IT regimen was not effective in all

patients in the NEST-2 trial and that some preclinical studies demonstrated greater efficacy of the PW R/NIR-IT method, the CW method employed may not be optimal (Lapchak, 2012). A thorough review of the NEST trials concludes that, based upon the translational stroke results and experimental studies in other neurodegenerative conditions (De Taboada et al., 2011), laser devices in the future should incorporate PW modes to provide optimal photobiostimulation (Lapchak, 2012). Based upon the scientific justification presented in this review, there is little doubt that R/NIR-IT should be pursued as a potential non-invasive neuroprotective treatment for ischaemic stroke patients. However, the highly novel NEST trials have not taken into account many important factors such as PW utilization, dosimetry and adequate tissue coverage (Lapchak, 2012). Results from the NEST-3 trial are much anticipated and may pave the way for future studies potentially incorporating PW R/NIR-IT.

1.10 R/NIR-IT for treatment of Parkinson's disease

Parkinson's disease (PD) is currently the most common movement disorder worldwide, affecting 1% to 2 % of the population over the age of 60, and approximately 5% of people over the age of 85 (Alves et al., 2008). Interventions that can reduce this escalating disease burden are therefore urgently required. While regarded as a multifactorial disorder triggered by a combination of age, genetic, environmental and other factors, mitochondrial dysfunction has consistently been implicated in the disease and is widely considered as a potential unifying factor (Banerjee et al., 2009). Consistent with this, a number of experimental models of the disease are based on inhibition of mitochondrial respiratory chain function (using rotenone or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)), resulting in depletion of intracellular ATP levels and generation of free radicals. Accordingly, interventions targeted at improving mitochondrial function such as R/NIR-IT are highly attractive as potential

treatments in PD.

In a series of *in vitro* experiments, utilizing striatal and cortical neurones exposed to a variety of mitochondrial toxins (cyanide, rotenone and MPTP), Wong-Riley and colleagues (Liang et al., 2006, Liang et al., 2008, Ying et al., 2008) demonstrate that treatment with R/NIR irradiation (670 nm LED for 80 sec, 50 mW/cm^2; 4 J/cm^2) increases cytochrome *c* oxidase activity and reduces the production of reactive oxygen and nitrogen species. This is associated with a preservation of ATP content as well as a reduction in toxin-induced apoptosis. They also note in normal control tissue that the irradiation therapy increases cytochrome c oxidase activity and ATP content (Liang et al., 2008), consistent with the view that energy absorption by the mitochondrial chromophores will increase ATP production (Eells et al., 2003) by delivering protons across the mitochondrial membrane and generating a transmembrane proton-motive force (Lapchak, 2012). Increasing ATP content will presumably facilitate the ability of the neurons to combat the effect of the neurotoxins. Notably in the neurotoxin experiments, pretreatment is more effective than treatment during toxin exposure (Ying et al., 2008), presumably reflecting the advantage of having increased ATP stores prior to toxin exposure.

A subsequent series of experiments utilizing *in vivo* models of PD also demonstrate beneficial effects of R/NIR-IT on histological outcome measures. In a rapid onset model of MPTP challenge in mice, R/NIR-IT (670 nm LED for 90 sec, 50 mW/cm^2; 5 J/cm^2) administered immediately after the neurotoxin increases survival of dopaminergic neurons in the substantia nigra pars compacta by 35-45% (Shaw et al., 2010). Similar results are observed in a chronic MPTP model designed to replicate the slow progressive nature of human PD. Specifically, R/NIR-IT increases the survival of dopaminergic cells in the

substantia nigra pars compacta by up to 25% despite being administered during the 3 week survival period after the 5 week MPTP insult period (Peoples et al., 2012b). No effects are observed on other dopaminergic neurons in the periaqueductal grey matter or zona incerta-hypothalamus, suggesting that beneficial effects of R/NIR irradiation exposure are likely to improve motor function in PD rather than other non-motor abnormalities, such as sleep-wake cycles. Nonetheless, there are neuroprotective effects of R/NIR-IT on dopaminergic cells in the retina in both an acute and chronic MPTP based murine model of PD (Peoples et al., 2012a). In these experiments, R/NIR-IT *via* LED protects cells from degeneration when administered simultaneously with the toxin, as well as rescues cells when exposure is administered after the toxin (during the 3 week survival period after the 5 weeks of MPTP injections). The authors propose that the irradiation treatment stimulates release of melatonin, a well-known and powerful antioxidant that is localized to dopaminergic neurons.

Other reports suggest that the antioxidant action may also involve the normalization of levels of a number of antioxidant enzymes, including superoxide dismutase and catalase (Komel'kova et al., 2004). In their studies of PD patients, these authors also demonstrated that R/NIR-IT normalized blood levels of monoamine oxidase B, which catalyzes dopamine oxidation, in addition to superoxide dismutase and catalase. Whether the normalization of blood levels reflects the normalization of brain levels of these enzymes is unclear and requires further investigation.

While the effects of R/NIR-IT on antioxidant enzymes have been widely reported, other mechanisms of action may also play a role in improving outcome in experimental PD. R/NIR irradiation has been shown to promote the production of neurotrophic factors (Leung et al., 2002), as well as suppress inflammation, specifically the production of IL-1B, TNFαand TGF

(De Taboada et al., 2011). Whether these reported effects account for the positive effects of R/NIR-IT on neurogenesis is currently unknown (Oron et al., 2006), although both neurotrophic factors and inflammation are widely recognized as having modulatory effects on cell survival. These and the previously discussed mechanisms of action suggest that R/NIR-IT may thus represent a pleiotropic intervention that has been widely called for in the treatment of both acute and chronic conditions of the CNS.

Aside from the early studies of blood enzyme levels in PD (Komel'kova et al., 2004), few studies have investigated the effects of R/NIR-IT in a clinical setting for this disease. Nonetheless, several patents have been lodged over the past decade in an effort to facilitate such translation. A series of patents by Streeter and De Taboada (2012) (Streeter J, 2012) describe the use of R/NIR-IT to PD patients through the scalp and skull to the brain more generally, while Di Mauro and colleagues (Di Mauro TM, 2007, Toselli R, 2009) describe using an implantable probe to deliver R/NIR-IT locally to the substantia nigra. Which approach is the most efficacious is yet to be determined.

1.11 Clinical application of R/NIR-IT

It was originally thought that lasers (coherent, monochromatic) were essential to achieve therapeutic efficacy (Mester et al., 1985). However, the advent of LEDs (non-coherent & with wider bandwidth), a semiconductor irradiation source that releases energy in the form of photons, provided cheaper alternatives (Posten et al., 2005) and enabled rapid uptake in a large number of human studies and randomized controlled trials (RCTs). Nevertheless, lasers currently remain the predominant irradiation source for R/NIR-IT of stroke, SCI and TBI. At last count, 112 RCTs and clinical studies have been published since 1994 on an extraordinary

variety of conditions including osteoarthritis and rheumatoid arthritis (Christie et al., 2007, Hegedus et al., 2009) carpal tunnel syndrome (Tascioglu et al., 2010), oral mucositis for chemotherapy patients (Cauwels and Martens, 2011, Silva et al., 2011), neck pain (Chow et al., 2009) and leg ulcers (Kaviani et al., 2011). By contrast, only a small number of RCTs have been published for neurological conditions (Table 2). Efficacy has been reported for stroke (Lampl et al., 2007, Stemer et al., 2010, Zivin et al., 2009), as described above, along with 3 case reports for TBI (Naeser et al., 2011, Nawashiro et al., 2012) and a study on major depression (Schiffer et al., 2009). However, across the broad range of conditions that have been examined, clinical efficacy is not always clear cut with many reports showing no benefit, for example oral mucositis (Gouvea de Lima et al., 2012), leg ulcers (Kokol et al., 2005), stroke (Zivin et al., 2009), pain and joint disorders (Bjordal et al., 2003) and tinnitus (Teggi et al., 2009). Information from the far larger number of RCTs and clinical studies for non-neurological conditions may offer insights into published studies, current RCTs on neurological conditions and aid in the optimal design of low irradiation laser therapy treatments for a broader range of neurological dysfunctions.

Regardless of the irradiation source (laser or LED), dosimetry of R/NIR-IT is highly complex because a wide range of parameters can be altered, including wavelength, irradiance, pulse structure, coherence and polarization, as well as the actual dose delivered, which can involve variations in energy, energy density, irradiation time and treatment interval in addition to the site of the injury or disease (Chung et al., 2012). Highly variable dosimetry between studies makes direct comparison difficult if not impossible (Jenkins and Carroll, 2011) and has contributed to lack of consensus as well as scepticism regarding efficacy.

Indeed, for conditions that have been widely studied, 3 Cochrane Database Systematic Reviews reveal conflicting data for osteoarthritis (5 RCTs, 112 patients (Brosseau et al., 2003b)), some benefit for rheumatoid arthritis (5 RCTs, 222 patients (Brosseau et al., 2003a)) and insufficient data to draw conclusions for low-back pain (6 RCTs, 318 patients (Yousefi-Nooraie et al., 2008)). The Cochrane Reviews points to a lack of standardized, validated outcomes, lack of harmonized dose calculation and an absence of data on how effectiveness is affected by wavelength, treatment duration, dosage and site of application. The recent call to harmonise reporting of R/NIR-IT suggests mandatory inclusion of 8 beam parameters (wavelength, power, irradiation time, beam area, pulse parameters, anatomical location, number of treatments and the interval between treatments) as well as other details for the reporting of both clinical and laboratory studies (Jenkins and Carroll, 2011).

An interrelated dosimetry issue is that of the biphasic dose response in biological tissue which is observed both *in vitro* and *in vivo* and which is characterised by initial efficacy as irradiance and time are increased, followed by a decline, no effect or even inhibition (Chung et al., 2012, Huang et al., 2011). The mechanism underpinning the biphasic response is thought to involve reactive oxygen and nitrogen species that are normally produced at low levels in healthy cells and are key signalling transcription factors (Leonarduzzi et al., 2011, Shi and Gibson, 2007). Low R/NIR-IT doses result in cellular events such as proliferation, migration and neurite outgrowth *in vitro* as well as improvements in various conditions *in vivo* such as wound healing, cardiac infarction and arthritis. However, higher doses, which result in excessive ROS, show reduced effects or inhibition (reviewed (Hashmi et al., 2010, Huang et al., 2011)).

In this context and as described earlier, Huang *et al*., (Huang et al., 2011) have shown in a mouse pneumatic cortical impact model that delivering a single dose (36 J/cm^2 810 nm laser at 50 mW/cm^2) over 12 minutes is beneficial on the neurological severity score (NSS) but that a 10-fold greater dose (360 J/cm^2 810nm laser at 500 mW/cm^2) also delivered over 12 minutes results in worse outcomes compared to no treatment. Intriguingly, delivering the same low dose daily for 14 days starting at 4h after injury results in slight improvement at 4 days compared to the single dose but, between 14 - 28 days, NSS is no better than no treatment and shows a trend to worse outcome. The biphasic tissue response cautions that "more is not necessarily better". As we argue above, this conclusion is supported by the fact that only extremely low irradiation doses are sufficient to elicit biophysical changes at the cellular level. Nevertheless, the almost complete lack of adverse / serious adverse events reported in clinical trials argues for continued clinical investigation of R/NIR-IT.

In addition to relative safety, the non-invasive nature of R/NIR-IT has undoubtedly contributed to its relatively rapid uptake in clinical trials. For example, R/NIR laser therapy was developed and patented between 1997 and 2001, with the first clinical trial in acute ischaemic stroke published in 2007 (NEST-1) (Lampl et al., 2007). This was followed by NEST-2 (Zivin et al., 2009), an analysis of pooled data from NEST-1 and NEST-2 (Stemer et al., 2010) and with NEST-3 now underway (Lapchak, 2010). With respect to the future, there are 10 currently registered RCTs for R/NIR-IT in neurological conditions, one of which is published (NEST-2). The hope is that these will yield data that avoid the shortcomings highlighted by the Cochrane Reviews on R/NIR-IT in other conditions (Brosseau et al., 2003a, Brosseau et al., 2003b, Yousefi-Nooraie et al., 2008) and progress the field regarding the use of R/NIR-IT.

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Table 1 A summary of R/NIR-IT for treatment of neurotrauma and neurodegenerative disease.

Study	***In vitro*: cell type, species**	***In vivo:* model, species**	**Light source**	**Wave-length**	**Intensity, density**	**Treatment duration**	**Trial length**	**Key outcomes**
Retinal degeneration								
Eells *et al*., 2003 Proc Natl Acad Sci 18;100(6) p 3439	NA	Methanol toxicity, rat	LED Quantum Devices (Warp 10)	670nm	28 mW/cm^2 4J/cm^2	144 s, 5, 25, and 50 h – post Methanol	2 days	Photoreceptors protected from methanol toxicity (ERG & histology)
Kokkinopoulos *et al*, 2012 Neurobiol Aging [Epub ahead of print]	NA	Aging, mice	LED Quantum Devices (Warp 10)	670nm	40 mW/cm^2 3.6J/cm^2	5 x 90 s, every 7 hours (35hr total treatment time)	7 days	Shifted mitochond. potential, reduced inflammation in outer retina.
Albarracin *et al*., 2012 Jun 1;52(6):3582-92.	NA	Retinal light damage (1000 lux), rat	LED Quantum Devices (Warp 75)	670nm	60 mW/cm^2 9 J/ cm^2	176 s / day for 5 days, delivered pre- or post-light damage	13 days	Increased protection and survival of cone photoreceptors
Albarracin *et al*, Invest Ophthalmol Vis Sci. 2011 Jun 1;52(6):3582-92.	NA	Retinal light damage (1000 lux), rat	LED Quantum Devices (Warp 75)	670nm	60 mW/cm^2 9 J/ cm^2	176 s / day for 5 days, delivered pre-light damage	36 days	Increased long-term stability of the retina following acute light damage.
Qu *et al*., 2012: 664:365-74	NA	Retinal light damage (900, 1800, 2700 lux), rat	LED Quantum Devices (Warp 10)	670nm	50 mW/cm^2 90 J/ cm^2	1800 s, 3 hours before and 0, 24 and 48 hours post- light damage	2 days	Protective effect on retinal cells
Natoli *et al*., Mol Vis. 2010 Sep 3;16:1801-22.	NA	Retinal light damage (1000 lux), rat	LED Quantum Devices (Warp 75)	670nm	60 mW/cm^2 9 J/ cm^2	176 s / day for 5 days, delivered pre-light damage	6 days	Photoreceptor protection, modulation retinal gene expression.

Peoples *et al*, 2012 ISRN Neurol. [Epub ahead of print]	NA	Parkinson's (MPTP–100mg/kg), mice	LED Quantum Devices (Warp 10)	670nm	5 mW/cm^2 0.5 J/cm2	90 s, Delivered T0 and post-insult	56 days	Protection of retina from parkinsonian insult
Albarracin R *et al*, Photochem Photobiol. 2012 Feb 28.(Epub)	NA	Retinal light damage (1000 lux), rat	LED Quantum Devices (Warp 75)	670nm	60 mW/cm^2 9 J/ cm^2	176 s	13 days	Increased neuroprotection in retina, possibly *via* Müller cells
Rojas *et al*, J. Neurosci, Dec 10, 2008 28(50):13511	NA	Rotenone inhibition, rat	LEDtronics	633nm	2 mW/cm^2 3.6J/cm^2	1800 s	16 days	Neuroprotection against rotenone toxicity
Visual system								
Wong-Riley *et al*., 2001. NeuroReport 12 p3033	Visual cortex neurons, rat	NA	LED, Quantum Devices	670nm	50 mW/cm^2, 4J/cm^2	80 s / day	6 days	Increased COX activity
Wong-Riley *et al*., 2005. J Biol Chem,. 280(6): p4761	Visual cortex neurons, rat	NA	LED, Quantum Devices	670 nm 728 nm 770 nm 830 nm 880 nm	50 mW/cm^2, 4 J/cm^2	80 s / day, once or twice / day	6 days	670, 830nm increased ATP and COX with COX inhibitor KCN, but not tetrodotoxin
Liang *et al*., 2006. Neuroscience. 139(2): p639	Visual cortex neurons, rat	NA	LED, Quantum Devices	670nm	50 mW/cm^2, 30 J /cm^2	10 min	28 hrs	Decreased ROS and apoptosis induced by KCN
Fitzgerald *et al*., 2010. J Neurotrauma, 27(11): p2107	NA	Partial ON injury, rat	LED, Quantum Devices (Warp 10)	670nm	25 mW/cm^2, …	30 min / day	8-10 days	Reduced ox stress, limited OPC increases, restored visual function
Assia *et al*., 1989. Brain Res, 476(2): p205	NA	ON crush, rat	He-Ne laser	630nm	10.5 mW	2 min / day, for 4,7 or 14 days	14 or 21 days	Increased CAP; if moderate injury, early and continued

SCI								
Byrnes *et al*., 2005. Lasers Surg Med 36: p171	NA	SCI dorsal hemi-section T9, rat	Laser, Thor International	810nm	150mW, 1,589J/cm^2/ day (at skin)	50 min / day for 14 days (starting 15 min after surgery).	9wks	Improved axonal number, regrowth, and some function. Immunomodulation
Wu *et al*., 2009. Lasers Surg Med. 41: p36	NA	SCI contusionT10 dorsal hemi-section T9, rat	Laser, Thor International	810nm	150mW 1,589J/cm^2/ day (at skin)	50 min/day for 14 days (starting immediately after surgery).	3wks	Increased axon length and number, and open field (BBB) locomotion in contusion model
Medalha *et al* 2010. Photomed Laser Surg 28: p669	NA	SCI Complete transection T9-10, rat	Laser, Teralaser (MM Optics Ltd)	830nm,	30mW/cm, 250J/cm^2 (CW)	70 s/day 3x/wk at 2 points on hindlimb, (not on spinal cord)	4wks	No improvements. Note: treatment not applied directly to spinal cord
TBI								
Oron *et al*., 2007 J Neurotrauma 24: p651	NA	TBI acute cortical impactor, 94g, mice	Laser, Photothera Inc.	808nm	10 and 20m/W/ cm^2 (1.2-2.4J/cm^2)	2 min (at 4hrs post trauma)	4 wks	Improved neurobehavioural function (NSS) and reduced lesion size
Moriera *et al*., 2009. J Photochem Photobiol B. 97: p145	NA	TBI focal cryoinjury, rat	Laser Teralaser (MM Optics Ltd)	660nm 780nm	40mW 3-5J/cm^2 / point (CW)	Immediately and 3hrs after TBI	24hrs	Immunomodulation of TNF-α, IL10, IL1β cytokine responses
McCarthy *et al*., 2010. Photomed Laser Surg 30: p663	NA	Uninjured brain, rat	Laser ND	808nm	2230mW/cm^2 268 J/cm^2 or 10mW/cm^2, 1.2 J/cm^2	5 min on day 1, or on each of days 1, 3, 5	1 year	Single and multiple doses safe
Ando *et al*., 2011. PLoS One 6: e26212	NA	TBI acute, AMS 201 impactor, mice	Laser, DioDent Micro 810, (HOYA)	810nm	50 mW/cm^2, 36 J/cm^2, 10-Hz CW; 100-Hz PW	12 min at 4hrs post TBI	4wks	Improved NSS, reduced lesion volume, anti-depressant. 10-Hz PW better effect

Naeser *et al*., 2011. Photomed Laser Surg 29: p351	NA	Chronic TBI, Human (Females, 59yrs, 52yrs)	Laser, 2 devices, ND	633nm, 870nm (CW)	25.8mW/cm^2 *or* 22.2mW/cm^2	310 - 774s /wk 7 mths, then 3 wks/month *or* 7 - 10 min / day	7yrs 4month	Improved executive function, reduced post traumatic stress.
Nawashiro *et al*., 2012. Photomed Laser Surg 30: p231	NA	TBI, Human (Male 40yrs)	Laser, Sun-Mechatronics	830-870nm	299mW 11.4mW/cm^2, 20.5J/cm^2	2x30min / day, applied 5mm from skin.	73days	Some neurological improvement, increased cerebral blood flow
Khuman *et al*., 2012. J Neurotrauma. 29: p408	NA	TBI cortical piston, 3mm wide, 6m/sec, 100msec, depth 0.6mm, mice	Laser, Thor International	800nm	*Craniotomy*: 250-500 mW/cm^2 30-210 J/cm^2 *Transcranial*: 500mW/cm^2	*Craniotomy*: 2-7min, 60-80min post TBI *Transcranial*: 2 min, 60-80min or 4hrs post TBI	1wk	Improved cognition inhibition of microglia. No effect on motor function, edema, nitrosative stress, or lesion volume
Wu *et al*., 2012. Lasers Surg Med 44: p218	NA	TBI, acute Impactor, mice	Laser, Diomed Inc, or V-Raser, (Con-Bio)	665nm 730nm 810nm 980nm	36 J/cm2 (CW)	1x 4-min, at 4hr post-injury	4wks	Improved NSS, reduced brain "deficits" with 665nm and 810nm
Quirk *et al*., 2012. Photomed Laser Surg 30: p1	NA	TBI, cortical impactor, 6mm/sec, 3mm, rat	LED, Quantum Devices	670nm	50mW/cm^2, 15J/cm^2	2 x 5min/day for 72hrs or 10 days (top of head, 0.5cm from scalp)	10 days	Improved NSS, decreased Bax, increased Bcl2
Oron *et al*., 2012 J Neurotrauma 29: p401	NA	TBI cortical impactor, 94g mice	Laser, Photothera Inc.,	808nm	10m/W/ cm^2 1.2J/cm^2	2 min (4, 6, or 8 hrs post-trauma), PW 100 or 600Hz	56 days	Improved NSS reduced lesion size
Stroke								
Lapchak *et al*., 2004. Stroke 35 p1985-1988		RSCEM, rabbit	Acculaser PhotoThera Inc.	808nm	25mW/cm^{2}or 7.5mW/cm^2 (CW)	2 or 10 mins at 1 or 24 hrs post insult	Up to 21 days	Improved behavioural performance

DeToboada *et al*., 2006. Las. Surg. Med. 38 p 70-73		MCAO, rats	Ga-Al-As diode laser, PhotoThera	808nm	7.5mW/cm^2	2 min	Up to 28 days	Reduced neurological deficits
Oron *et al*., 2006. Stroke 37 p2620-2624		MCAO, rats	Ga-Al-As diode laser, PhotoThera Inc	808nm	7.5mW/cm^2 (CW and/or PW)	2 min at 4 or 24 hrs post-stroke	Up to 21 days	Improved neurological outcome with 24hr. No improvement in stroke volume
Lapchak *et al*., 2007 Neuroscience 148 p 907-914		RSCEM, rabbits	Acculaser, PhotoThera Inc.	808nm	7.5mW/cm^2 (CW or PW)	2 min at 6 or 12 hrs post-embolisation	48 hrs	Significant improvement with PW at 6 hrs
Lapchak *et al*., 2008. Stroke. 39 p307303078		RSCEM, rabbit	Acculaser, PhotoThera Inc.	808nm	10mW/cm^2	2 min at 90 min post-embolisation	24 hrs	Safe with tPA, haemorrhage and volume unaffected.
Lapchak & DeTaboada 2010. Brain Res. 1306 p100-105		RSCEM, rabbit	Ga-Al-As diode laser, PhotoThera Inc	808nm	7.5mW/cm^2 (CW) 37.5mW/cm^2 (PW1,100Hz) 262.2mW/cm^2 (PW2,100Hz)	5 min post-embolization CW-2 min PW1-2 min, 3.5mJ/pulse, PW2 -2 min 24.5mJ / pulse,	3 hrs	Increased cortical ATP content.
PD								
Ying *et al*., 2008. Brain Res. 1243 p 167-173	Cortical neurons; rotenone & MPTP rat	NA	LED, Quantum Devices	670 nm	50mW/cm^2; 4J/cm^2	80 s twice / day	2-4 days	Reduced apoptosis and increased ATP content
Liang *et al*., 2008. Neuroscience 153 p 963-974	neurons; rotenone & MPTP rat	NA	LED, Quantum Devices	670 nm	50mW/cm^2; 4J/cm^2	80 s twice / day	3-5 days	Increased ATP content, decreased neuronal apoptosis, reduced ROS and NO

Shaw *et al*., 2010. J Comp Neurol 518 p 25-40	NA	acute PD, 50-100 mg/kg ip MPTP over 30 h; mice	LED, Quantum Devices	670 nm	40mW/cm^2; 5.3 J/cm^2	90 s; 4 times over 30 h	30 h	Increased number of surviving dopaminergic cells in substantia nigra
Peoples et al., 2012. Parkinson Related Dis p 469-476.	NA	acute and chronic PD; 10x20 mg/kg ip over 5 wks; mice	LED, Quantum Devices	670 nm	40mW/cm^2; 5 J/cm^2	90 s; 10 times	3 - 8 weeks	Increased number of surviving dopaminergic cells in substantia nigra
Other								
Yan *et al*., 2011. J Peripheral Nervous System 16: p130	NA	Pain, rat	Irradia™	650nm 808nm	35mW 450mW	30s to each of 4 points		Decreased SSEP and CMAP amplitudes indicated potential pain relief

NA is not appropriate, ND is not described, abbreviations for laser type are combinations of the following elements: gallium (Ga), aluminium (AI) or arsenide (As). Abbreviations: Basso, Beattie and Bresnahan (BBB), cytochrome c oxidase (COX), continuous wave (CW), pulsed Wave (PW), Neurological Severity Score (NSS);. spinal cord injury (SCI); optic nerve (ON), traumatic brain injury(TBI), oligodendrocyte precursor cells (OPCs), compound action potentials (CAP), seconds (s), minutes (min), hours (hrs), weeks (wks), small clot embolic stroke model (RSCEM), middle cerebral artery occlusion (MCAO), somatosensory evoked potential (SSEP), comprehensive muscular activity profile (CMAP).

Table 2. Currently registered trials on the WHO International Clinical Trials Registry Platform Search Portal.

Title	**Registration date**	**Status**
Brain plasticity underlying back pain response to different acupuncture methods	5/2012	NR
Effects of LEDs on memory in TBI patients	5/2012	R
Transcranial laser therapy in the rehabilitation of hemiplegic patients from ischaemic stroke	3/2011	NR
Safety of Rt-PA + transcranial emission of low energy lasers for acute stroke recovery	10/2010	R
Efficacy and safety trial of transcranial laser therapy within 24hours from stroke onset (NEST-3)	5/2010	R
Brain effects of acupuncture 2: Laser acupuncture *vs* laser EMLA	4/2010	NR
Brain effects of acupuncture 2: Needle acupuncture *vs* laser acupuncture	4/2010	NR
Managing fatigue and sleep disturbance following traumatic brain injury	7/2008	R
Managing fatigue and sleep disturbance following traumatic brain injury	1/2008	NR
Effectiveness and safety trial of a new ischaemic stroke treatment within 24 hours from stroke onset (NEST-2)	1/2007	NR

R = recruiting; NR = Not recruiting