Stem Cell Treatments for Stroke: A Biopsychosocial Perspective

A thesis submitted for the Doctor of Philosophy/Master of Psychology (Clinical)

by

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LIST OF ABBREVIATIONS

Abbreviation	Full Description
ACA	anterior cerebral artery
ACT	acceptance and commitment therapy
CBT	cognitive behavioural therapy
CT	computerised tomography
CVA	cerebrovascular accident
G-CSF	granulocyte-colony stimulating factor
ICH	intracerebral haemorrhage
MCA	middle cerebral artery
MNC	mono-nuclear cell
MRI	magnetic resonance imaging
PCA	posterior cerebral artery
RCT	randomised controlled trial
SAE	serious adverse event
SAH	subarachnoid haemorrhage
SC	stem cell

ABSTRACT

Fifteen million people have a stroke each year. The majority survive due to improvements in medical care, although most experience ongoing physical, cognitive and psychological difficulties. Preliminary data suggest that stem cell (SC) therapy may improve stroke survivor outcomes but, in the absence of large-scale clinical trials, the overall effectiveness and medical and psychological risks remain unclear. Moreover, SC therapies administered throughout the acute, sub-acute and chronic phases of stroke are assumed to work differently due to differences in blood-brain-barrier permeability, however, the optimal treatments for each phase remain unknown due to current technological limitations.

Interest in the experimental SC treatments offered by private clinics throughout Asia, South America and Russia – known as 'stem cell tourism' – is increasing among some patient groups, although, whether this is true for stroke survivors is unknown. Additionally, whether patients with particular biopsychosocial and attitudinal characteristics are more likely to consider experimental SC treatments is undetermined. Patient educational resources have been developed to warn patients with neurodegenerative conditions about the risks associated with 'stem cell tourism', however, their content, format and design may impact upon their effectiveness with stroke survivors, due to differences in the physical, cognitive and psychological sequelae.

Four studies explored these issues. Two meta-analyses examined the safety and efficacy of SC therapies. Study 1 examined 11 SC therapies ($N_{studies}$ = 28) administered in the hyper-acute, acute, and sub-acute phases of stroke (≤ 90 days). Serious adverse events were observed following five therapies; improved neurological, functional and/or radiological outcomes were noted following six therapies. Study 2 analysed 17 SC therapies ($N_{studies}$ = 23) administered in the chronic phase of stroke (> 90 days). Safety concerns were identified for three therapies; four reported improved neurological and/or functional outcomes. Across

both meta-analyses, few studies tracked the SCs post-implantation, employed sham treatment control groups, performed psychological screening or evaluated participants' psychological wellbeing pre-/post-treatment.

Next, a cross-sectional survey of 183 Australian stroke survivors was conducted to assess patient attitudes toward experimental SC treatments (Study 3). Twenty-five percent were considering 'stem cell tourism'. Individuals with positive SC attitudes, longer post-stroke intervals, poorer physical functioning, and greater perceived caregiver burden were most likely to be considering treatment. Lastly, a randomised controlled trial (Study 4) was conducted to evaluate whether an online International Society for Stem Cell Research booklet or Stem Cell Network video deterred stroke survivors' (N = 112) from considering experimental SC treatments. Forty-five percent of participants were considering SC treatments at study commencement; significantly fewer were still considering SC treatments after reading the booklet. However, after 30 days, neither intervention was found to have altered participants' attitudes.

Overall, the findings suggest that whilst a small number of SC therapies may improve stroke outcomes, further large-scale, placebo-controlled clinical trials are required to clarify the medical and psychological risks. The level of interest in 'stem cell tourism' identified among patients with specific biopsychosocial and attitudinal characteristics suggests a potential role for clinicians to initiate discussions with higher-risk groups. The need for stroke-specific SC education resources, in conjunction with more proactive dissemination, is also highlighted.

DECLARATION

I, David Unsworth, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Chapter 4: Study 2

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Chapter 5: Study 3

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Chapter 6: Study 4

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Chapter 1: Stroke

1.1 Overview

Stroke is the second leading cause of death and the third leading cause of disability worldwide (Feigin, Norrving, & Mensah, 2017; Thrift et al., 2017). Of the 15 million people who experience a stroke each year, only 70% survive and, of those, only one in ten fully recover (Feigin et al., 2017; Jung, 2017; Mozaffarian et al. 2016). In global terms, this equates to approximately 33 million people who are currently living with stroke-related physical, functional, and/or cognitive disabilities (Hankey, 2017). Psychological problems, such as depression and anxiety are also common and affect one third of all stroke survivors (Cumming, Blomstrand, Skoog, & Linden, 2016; Mitchell et al., 2017). These consequences contribute to the significant economic and psychosocial burden of stroke.

The current chapter examines the biopsychosocial aspects of stroke, commencing with a discussion of the medical symptoms, epidemiology, and incidence and prevalence rates. The two major subtypes of stroke (i.e. ischaemic and haemorrhagic) are outlined and differences in the diagnosis, classification, and outcomes of each are described. Following this, the economic burden of stroke is highlighted and major risk factors for stroke are introduced. Lastly, a summary of the current recommended multidisciplinary treatments throughout the hyper-acute (≤ 24 hours post-stroke), acute (< 30 days post-stroke), sub-acute (< 90 days post-stroke), and chronic [> 90 days post-stroke]) phases is provided, with a focus on the role of clinical psychology at each stage.

1.2 Definition and General Characteristics of Stroke

A stroke, or cerebrovascular accident (CVA), occurs when cerebral blood supply is interrupted due to an obstructed (i.e. ischaemic stroke) or ruptured (i.e. haemorrhagic stroke) blood vessel (Sacco et al., 2013). Initial symptoms vary according to the type and location of

the stroke, but most commonly include: headaches/migraines, dizziness, loss of balance or coordination (ataxia), unilateral limb weakness (hemiparesis), paralysis (hemiplegia), facial droop, vision loss or double vision (diplopia), language and communication problems (aphasia, dysarthria), and swallowing difficulties (dysphagia) (Hankey & Blacker, 2015). Patients may also experience atypical symptoms including confusion, delirium, memory loss (amnesia), breathing difficulties (stridor), involuntary limb movement (Choi, 2016; Edlow & Selim, 2011), and a lack of insight into the nature and extent of their symptoms (anosognosia) (Byrd, Jablonski, & Vance, 2018). To be formally diagnosed as a stroke, the primary symptoms must persist for longer than 24 hours or result in death. The cause of the symptoms must be also visible on neuroimaging or at autopsy (Sacco et al., 2013).

1.3 Subtypes of Stroke

Ischaemic stroke is the first major subtype and accounts for approximately 85% of all CVAs (Mozaffarian et al., 2016). An ischaemic stroke occurs when a blood clot forms in an artery that supplies blood to the brain (thrombotic stroke) (Adams & Biller, 2015), or forms elsewhere in the body (e.g. the heart) before travelling through the blood stream and lodging in the brain (embolic stroke) (Hankey, 2017). Ischaemic strokes most commonly occur in the middle cerebral artery (MCA), which supplies blood to the lateral aspects of the frontal, temporal and parietal lobes; the anterior cerebral artery (ACA), which supplies blood to the medial portions of the frontal and parietal lobes; and the posterior cerebral artery (PCA), which supplies blood to the midbrain, thalamus, and temporal and occipital lobes (Kim, 2016). A small number of ischaemic strokes are also caused by cerebral small-vessel disease; a pathological condition that results in damage to the small (lacunar) blood vessels in the brain (Hankey, 2017; Shi & Wardlaw, 2016).

Haemorrhagic stroke is the second major subtype and accounts for the remaining 15% of CVAs (Mozaffarian et al., 2016). A stroke that results from bleeding within the brain is

referred to as an intracerebral haemorrhage (ICH). This type of stroke most commonly occurs following the rupturing of blood vessels that are tangled (arteriovenous malformations) or damaged from chronic high blood pressure (hypertension) (Keep, Hua, & Xi, 2012). ICHs may result in increased intracranial pressure and cerebral herniation, which may be fatal (An, Kim, & Yoon, 2017). The areas of the brain that are no longer receiving blood may also die, with secondary damage occurring throughout the areas around the haemorrhage due to the release of toxins present within the blood (An, Kim, & Yoon, 2017).

In contrast, a bleed that occurs within the tissue located between the brain and the skull (meninges) is referred to as a subarachnoid haemorrhage (SAH) (Miller, 2017). SAHs are commonly caused following the balloon-like swelling of weakened/damaged blood vessels ('berry' aneurysms) that subsequently rupture, or as a result of arteriovenous malformations that begin leaking blood into the subarachnoid space (Miller, 2017). Similar to ICHs, patients who experience a SAH may die as a result of the increased intracranial pressure (Kakami & Garg, 2016). Brain damage is common throughout the areas of the brain that are no longer receiving blood (Kakami & Garg, 2016), and/or within areas that were previously occupied only by cerebral spinal fluid (Miller, 2017).

1.4 Diagnosis

Ischaemic strokes may be provisionally diagnosed using the Face Arm and Speech Test (FAST) (Robinson, Reid, Haunton, Wilson, & Naylor, 2013) or tools such as the Recognition of Stroke in the Emergency Room (ROSIER) score (Nor et al., 2005). Where a stroke is suspected, neuroimaging is typically performed to confirm the diagnosis. Computerised Tomography (CT) scans are generally performed first, in order to identify whether a blockage or bleed has occurred within the brain (Malhotra & Liebeskind, 2017). However, as CT scans are less effective at identifying small or recent blockages, follow-up magnetic resonance imaging (MRI) scans may be conducted. Diffusion-Weighted MRIs are

the most effective method of detecting new ischaemic strokes and differentiating between strokes and other conditions with similar symptoms (e.g., seizures, tumours, encephalitis, multiple sclerosis, migraines) (Vilela & Rowley, 2017). More advanced MRI techniques, such as functional MRI, diffusion tensor imaging, magnetic resonance perfusion, magnetic resonance spectroscopy, and volumetric imaging, are also being developed to provide more detailed images of the brain (Almeida, Castellano, Vicentini & Min, 2018).

For haemorrhagic strokes, CT scans are highly effective at identifying recent intracranial and subarachnoid bleeds (Malhotra & Liebeskind, 2017), although MRI scans provide more detailed information about the aetiology and pathophysiological consequences of the stroke (Kakami & Garg, 2016). Digital Subtraction Cerebral Angiography, which involves the injection of contrast dye into the carotid artery via catheter, may also be used to determine the cause for an ICH or SAH. However, its use is limited, because it increases the risk of ischaemic stroke due to the potential for dislodging fatty plaques at the point of the catheter insertion (Malhotra & Liebeskind, 2017).

1.5 Classification

Once diagnosed, ischaemic strokes are classified according to the primary cause (aetiology) of the blockage, in order to inform treatment planning and to formulate the short- and long-term prognoses for a patient (Adams & Biller, 2015). A range of methods are used to classify ischaemic strokes; common examples include the Causative Classification System (CCS) (Arsave et al., 2010), the Atherosclerosis-Small vessel disease-Cardiac pathology-Other causes (ASCO) phenotyping system (Amarenco et al., 2013), and the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification system (Adams & Biller, 2015). While minor differences exist in the terminology used, each system broadly classifies the aetiology of an ischaemic stroke as: large artery atherosclerosis, small artery occlusion,

cardioembolism, other demonstrated cause, or undetermined (cryptogenic) (Adams & Biller, 2015; Amarenco et al., 2013; Arsave et al., 2010).

Haemorrhagic strokes are classified based on the location of the bleed. The majority of ICHs occur close to the surface of the brain (supratentorial) or within the deeper structures surrounding the cerebral ventricles (deep ICH) (Kakami & Garg, 2016). The severity of an ICH is then assessed based on the severity of the bleed and the associated mortality risk, which are determined from the ICH volume (mL) and affected locations, the person's age (≥ or < 80 years), and level of responsiveness (Kakami & Garg, 2016). When classifying a SAH, CT scans are used to calculate a Fisher Grading Scale score, which estimates the mortality risk based on the size (thin/thick) and location (one/both ventricles) of the haemorrhage (Miller, 2017). Additional information regarding the severity of a SAH is based on the alertness and orientation of the patient (ranging from mild confusion to comatose), or the presence of neurological deficits, neck stiffness (nuchal rigidity) and/or headache (Miller, 2017).

1.6 Outcomes

Approximately 90% of ischaemic stroke patients from developed countries survive, although the outcomes experienced depend on the location of the blockage (Mittal & Goel, 2017). Ischaemic strokes within the anterior circulation system (i.e. ACA, MCA) disrupt blood flow to the frontal, temporal and parietal lobes (Chandra, Li, Stone, Geng, & Ding, 2017), resulting in motor and sensory deficits, swallowing problems, language impairments, cognitive dysfunction, and impaired vision (Walcott et al., 2014). Depression is also common, particularly during the acute (24 hours to < 30 days post-stroke) and sub-acute phases (30 to 90 days post-stroke), when neuro-vegetative symptoms, such as, aboulia, anhedonia, akinetic mutism, insomnia, and agitation are most prevalent (Brust & Chamorro, 2016). PCA infarcts disrupt blood flow throughout the occipital lobe, inferomedial temporal

lobe and upper brainstem, causing hemiparesis, ataxia, oculomotor deficits, and sensory disturbances (Kim, 2016). The thalamus and limbic system may also be damaged, resulting in memory loss and increased emotional dysregulation (e.g. sadness, anger, fear, agitation, aggression) (Kim, 2016). Lacunar strokes cause damage to small arteries throughout the outer cortex, the cerebellum, and the deep encephalic areas (e.g. caudate nucleus, internal capsule, globus pallidus) (Norrving, 2016). Most lacunar infarcts fail to produce significant acute symptoms, although, over time the cumulative effects may cause cognitive decline (Vascular Neurocognitive Disorder), in addition to neuro-behavioural symptoms, such as, confusion, agitation and aggression (Makin, Turpin, Dennis, & Wardlaw, 2013).

Unlike ischaemic strokes, 30% to 40% of people who have a haemorrhagic stroke die within the first week (An, Kim, & Yoon, 2017). As ICHs predominantly occur throughout the cerebrum and cerebellum (Kase, Shoamanesh, Greenberg, & Caplan, 2016), sensorimotor and oculomotor deficits are common (An et al., 2017). Cognitive dysfunction, including aphasia, are also common following an ICH (An et al., 2017), as are symptoms of depression and anxiety, particularly among younger ICH survivors with poor health-related quality of life (e.g. severe pain, poor quality sleep, social isolation, unemployment) (Koivunen, Harno, Tatlisumak, & Putaala, 2015). The outcomes associated with SAH are highly variable and depend on the cause (e.g. aneurysm, trauma) and area of the haemorrhage. Nonetheless, unilateral limb weakness and visual problems are common (Suarez & Bershad, 2016), as are memory, attention, communication, concentration and problem solving deficits (Petridis et al., 2017). Depression and anxiety are also common following SAH, particularly among those with severe physical and functional disabilities (Petridis et al., 2017; Von Vogelsang, Thelin, Hakim, & Svensson, 2017).

1.7 Epidemiology

Incidence

Each year around 15 million people experience a new or recurrent stroke, which equates to an annual global incidence rate of 260 per 100,000 (Thrift et al., 2017). However, there is significant variability between countries. For instance, the 1-year incidence rate in the United States of America is slightly less than the global average of 243 per 100,000 (Mozaffarian et al. 2016). In Europe, the 1-year incidence rate ranges from 150 per 100,000 in the United Kingdom, France and Italy, and up to 300 per 100,000 throughout Eastern Europe and Russia (Béjot, Bailly, Durier, & Giroud, 2016). Similar variability is seen throughout Asia. China, India, South Korea and Malaysia have the lowest rates of stroke (70 to 220 per 100,000), while Japan, Taiwan, Pakistan, and Mongolia have the highest rates (250 to 420 per 100,000) (Venketasubramanian, Yoon, Pandian, & Navarro, 2017). The 1-year incidence rate of stroke in Australia (230 per 100,000) is also lower than the global average, but nonetheless remains a significant problem, with approximately 56,000 people diagnosed each year (Stroke Foundation, 2017).

Prevalence

Of the 15 million people who have strokes each year, approximately 30% die (Feigin et al., 2017). Of the 70% of people who survive, only 10% fully recover (i.e. have no residual symptoms) (Thrift et al., 2017). The remaining 60% of stroke survivors experience ongoing physical, functional and/or cognitive disabilities, in addition to psychological problems (Feigin et al., 2017; Hackett, Köhler, O'Brien, & Mead, 2014; Jung, 2017; Thrift et al., 2017). In global terms, this equates to an estimated 33 million people currently living with ongoing stroke-related disabilities (12-month prevalence rate: 50 per 100,000) (Feigin et

al., 2017); a number that increases by approximately 5 million people each year (Hankey, 2017).

The majority of stroke-related deaths occur in low-and-middle-income countries (71%) where specialised stroke treatments are less accessible (Feigin et al., 2017). Indeed, the mortality rate in countries throughout Southeast Asia and Eastern Europe is more than twice that of the United States of America, the United Kingdom and Australia (105 versus 60 per 100,000) (Feigin et al., 2017). However, as a result of having lower mortality rates, high-income countries have significantly more people living with stroke-related disabilities (12-month prevalence rates 1.5% to 2.6% versus < 1%) (Béjot et al., 2016; Mozaffarian et al. 2016; Rajsic et al., 2019). Despite the difference in prevalence rates, the number of healthy years lost due to stroke-related death or disability still remains significantly higher throughout developing countries (disability-adjusted life years: 1820 versus 980 per 100,000). This difference has been attributed to the fact that strokes occur earlier in life throughout developing countries (Feigin et al., 2017; Venketasubramanian et al., 2017).

Economic Cost

The current economic burden associated with stroke is estimated to be over US$73 billion per annum in the United States of America (Mozaffarian et al., 2016), €38 billion per annum throughout the European Union (Rajsic et al., 2019), and AUD$5 billion in Australia (Stroke Foundation, 2017). Concerningly, the economic burden experienced throughout these countries is expected to more than double by the year 2050 due to their ageing populations (Béjot et al., 2016; Rajsic et al., 2019; Stroke Foundation, 2017), at which time estimates suggest the cost may exceed US$1.5 trillion in the United States alone (Rajsic et al. 2019). The economic burden associated with treatment-related costs is greatest within the first five years after a stroke (Xu et al., 2018), during which time patients who have had more severe strokes, are older, and have more medical (e.g. diabetes, arthritis) and/or psychological

(e.g. depression) comorbidities are the most expensive to care for (Chinthammit, Coull, Nimworapan, & Bhattacharjee, 2017).

Risk Factors

Large-scale studies have identified a core group of risk factors that account for 90% of all strokes. These risk factors can be classified as modifiable - meaning that individuals can take measures to change them, or non-modifiable - indicating that they cannot be changed. In terms of modifiable risk factors, a history of hypertension (blood pressure > 160/90 mm Hg) is associated with the highest risk of ischaemic and haemorrhagic stroke, followed by current cigarette smoking, excessive alcohol consumption (> 30 drinks per month), obesity, poor diet, and prolonged stress (Feigin et al., 2016; O'Donnell et al., 2016). Individuals with coronary heart disease, diabetes mellitus, hypercholesteremia and carotid stenosis are also at increased risk of ischaemic stroke (Feigin et al., 2016; O'Donnell et al., 2016), as are people with major depressive disorder - due to its detrimental effect on neuroendocrine and immunological functioning (Lightbody et al., 2017). Lastly, while anticoagulant medications reduce the risk of ischaemic stroke, they increase the risk of haemorrhagic stroke due to their 'blood-thinning' properties (Batta, Kalra, & Khirasaria, 2019).

In terms of non-modifiable factors, older age increases the risk for ischaemic and haemorrhagic strokes, mainly due to the narrowing (atherosclerosis) and hardening (arteriosclerosis) of human arteries that occurs over time (Feigin et al., 2016; O'Donnell et al., 2016). Indeed, 90% of all CVAs occur in people over the age of 50 (Feigin et al., 2016). Women are also at a higher overall risk of stroke due to their longer life expectancy (Gibson & Attwood, 2016). Compared with men, women are also older, on average, at the time of their stroke (70 vs 66 years of age), which increases their disability and mortality risk (Gibson & Attwood, 2016). In terms of ethnicity, studies from the United States of America

suggest that people of Hispanic and African-American backgrounds are at increased risk of stroke, even after controlling for the effects of modifiable risk factors, such as hypertension, smoking and diabetes (Gardois, Booth, Goyder, & Ryan, 2014). Similarly, studies from Australia have found that Aboriginal people are more than twice as likely to have a stroke compared to non-Indigenous Australians and are also 1.4 times more likely to die as a result (Stroke Foundation, 2017). In addition, individuals with a family history of cerebrovascular disease are known to be at increased risk of stroke, which suggests that specific genes may also play a role (Anttila et al., 2018; Hankey, 2017).

1.8 Stroke Treatments and Rehabilitation

Medical Treatments

In the hyper-acute phase (< 24 hours) following an ischaemic stroke there are two approved emergency treatments, both of which are designed to break down/remove blockages from occluded blood vessels. The first, thrombolysis, involves the intravenous administration of tissue plasminogen activating medication to break down and disperse small-to-medium sized blood clots (Powers et al., 2019). Thrombolysis is most effective within the first 4.5 hours following a stroke and, historically, was not administered beyond this time due to the increased risk of ICH (Emberson et al., 2014; Powers et al., 2019). However, evidence from a recent clinical trial suggests that thrombolysis may be effective up to 9 hours after a stroke, when patients are identified via neuroimaging as having a large portion of salvageable brain tissue (Ma et al., 2019). Despite these advances, thrombolysis remains less effective at dispersing large-artery (e.g. carotid artery, MCA, basilar artery) occlusions (Evans, White, Cowley, & Werring, 2017), and is medically contraindicated where patients: are older than 80 years of age, had a seizure at stroke onset, have a history of/current haemorrhagic stroke, had head trauma or neurosurgery in the past 3 months, have a low platelet count, and/or take anti-coagulant medications (Hankey, 2017). Therefore, despite the effectiveness of thrombolysis

for small-to-medium sized ischaemic strokes, restrictions regarding its use mean that only 1% to 3% of all stroke patients currently receive this treatment (Emberson et al., 2014; Hankey, 2017).

Mechanical thrombectomy is the second of the approved emergency treatments for ischaemic stroke and involves the removal of the clot using a retractable mechanical device inserted via an artery (Evans et al., 2017). Thrombectomy is highly effective for large-vessel occlusions and, until recently, was only performed within 6 hours of a stroke due to the risk of ICH (Evans et al., 2017). However, the results of a recent trial suggest that patients with focal damage (i.e. small ischaemic cores) may benefit from having the procedure up to 24 hours post-stroke (Nogueira et al., 2018). Patients receiving thrombectomy in conjunction with thrombolysis also experience better functional outcomes than patients who receive just one treatment, therefore both treatments may be provided where indicated (Zhao & Willing, 2018).

Unfortunately, there are fewer hyper-acute treatments for haemorrhagic stroke and 30% to 40% of patients die within the first week (An et al., 2017). For large ICHs, an open craniotomy may be performed to remove the haematoma, minimise cerebral swelling and reduce intracranial pressure (Dastur & Yu, 2017). However, beyond reducing mortality, the clinical benefits of this procedure remain contentious, with 1 in 2 patients experiencing significant, ongoing physical disabilities as a result (An et al., 2017; Dastur and Yu, 2017). Therefore, non-surgical methods to reduce the size of the haematoma, including lowering blood pressure and providing blood clotting medications, are currently being examined (Dastur & Yu, 2017; Law, Salman, Bath, Steiner, & Sprigg, 2018). For SAH, surgery to clip a ruptured aneurysm, thereby stopping the haemorrhage, is commonly performed (Miller, 2017).

Multi-Disciplinary Rehabilitation

Where available, medically stable patients typically commence low-intensity physical rehabilitation five to 10 days post-stroke, including assisted walking and hand/arm training to improve mobility, restrengthen weakened limbs and improve fine motor skills (Dobkin & Dorsch, 2013; Jung, 2017). The benefits of commencing more intensive physiotherapy prior to this time have been examined, however, doing so was found to increase the risk of mortality (Bernhardt et al., 2015). In contrast, commencing speech therapy in the two weeks after a stroke has been found to benefit patients with severe communication (aphasia) and/or swallowing (dysphagia) difficulties (Coleman et al., 2017; Nouwens et al., 2017).

Clinical psychologists may also begin working with patients throughout the acute phase; a period where mood and adjustment disorders are present in 15% to 20% of patients (Mitchell et al., 2017). The initial interactions generally focus on validating any distress or frustration felt by the patient and affirming the therapist's supportive role throughout the rehabilitation process (Ownsworth, 2014). Following this, personalised information or 'psychoeducation' regarding the cause of the stroke and its expected sequelae, may be provided to the patient and family in order to reduce any feelings of worry and uncertainty (Hildebrand, 2015; Ownsworth, 2014). To assist with the communication of this information, memory aids such as written summaries and printed resources, may be used (Ni et al., 2018).

Following this, clinical psychologists may work with patients to identify short-term, achievable steps (e.g. walking with support for 5 minutes) that are aligned with their broader rehabilitation goals (e.g. regaining full mobility), as a means of boosting confidence and maintaining motivation throughout the rehabilitation process (Frost et al., 2018). A 'strengths-based approach' is generally utilised to achieve this, where the main focus is on providing positive feedback about the patient's current abilities, often combined with 'errorless-learning' tasks (Hildebrand, 2015). Where challenging/responsive behaviours are

present (e.g. physical and verbal aggression), clinical psychologists may work with staff and family members to implement behaviour management strategies with the goal of teaching patients more adaptive methods of coping with distress and frustration (Fisher, Bellon, Lawn, & Lennon, 2018).

The majority of stroke survivors enter the sub-acute phase (30 to 90 days post-stroke) with a myriad of ongoing disabilities (Mozaffarian et al., 2016). Intensive rehabilitation is not always available (Enderby et al., 2017), despite the sub-acute phase being recognised as the optimal time for undergoing rehabilitation (Cassidy & Cramer, 2017; Langhammer et al., 2015). During this time, the central nervous system begins to reallocate and reorganise neurons within the injured parts of the brain to recover motor functioning (Cassidy & Cramer, 2017). Furthermore, the benefits associated with sub-acute rehabilitation are enhanced by a range of natural endogenous repair processes that occur at this time ('spontaneous recovery'), due to the increased blood flow and reduced inflammation throughout the stroke-affected parts of the brain (Cassidy & Cramer, 2017; Jung, 2017).

In terms of specific physical rehabilitation techniques in the sub-acute phase, task-oriented, repetitive training approaches are commonly used to improve mobility and restrengthen weakened limbs, including treadmill walking, constraint-induced movement therapy, and robotic-assisted gait or arm training (Jung, 2017). Multimodal sensory stimulation may also be employed for the same purposes, using mirror therapy, action observation, motor imagery and virtual reality training (Jung, 2017). A variety of non-invasive cortical stimulation methods, such as repetitive transcranial magnetic stimulation and transcranial direct current stimulation, are also being investigated as ways of further augmenting physical and functional recovery, by altering the rate of neuronal firing throughout specific areas of the brain (Kang, Summers, & Cauraugh, 2016; Zhao & Willing, 2018).

Where multidisciplinary teams are present, other allied-health professionals may contribute to the rehabilitation process. For example, occupational therapists may commence work with patients to improve their level of independence, by teaching the necessary skills involved in performing self-care, leisure, and other 'activities of daily living' (Schiavi, Costi, Pellegrini, Formisano, Borghi, & Fugazzaro, 2018). Where modifications are required to the patient's home (e.g. grab bars, steps or ramps), occupational therapists may recommend and arrange the required changes (Fukumoto, Watanabe, Yasufuku, Furudate, & Momosaki, 2019). Speech therapists may also commence or continue to work with patients to treat communication deficits (aphasia, dysarthria) and swallowing problems (dysphagia) (Clarke & Foster, 2015), while social workers may help patients and their caregivers to prepare for the transition back into the community by helping to address any unmet needs (e.g. financial assistance, support groups) (Hughes, Woodward, Fritz, & Reeves, 2018).

The role of clinical psychology often continues throughout the sub-acute rehabilitation phase; a period where the number of patients exhibiting depressive symptoms increases to 40%, due to the increased level of insight gained by patients into the nature and severity of their disabilities (Dar et al., 2017; Mitchell et al., 2017). Notably, patients with damage throughout the frontal-subcortical-limbic networks may also experience 'Vascular Depression', a condition that presents with more prevalent neuro-vegetative symptoms (Aizenstein et al., 2016). Equally as problematic is anxiety, which is estimated to occur in up to one quarter of patients in the sub-acute period (Rafsten, Danielsson, & Sunnerhagen, 2018). Anxiety is typically due to increased worry about aspects of their recovery, including the risk of further strokes, and whether they will be able to return home, live independently, and return to work (Campbell-Burton et al., 2013; Rafsten et al., 2018).

Identifying and treating patients with depression and/or anxiety throughout the sub-acute phase is of critical importance because the presence of these affective symptoms can

lead to an increased risk of various medical complications, including death (Dar et al., 2017). Patients with low motivation, fatigue, and anxiety about falling, are also less likely to engage in physical rehabilitation, which directly impacts on their long-term functional recovery (Dar et al., 2017; Rafsten et al., 2018). Where symptoms are identified, anti-depressant medications, specifically, selective serotonin reuptake inhibitors (SSRIs), are commonly prescribed (Damsbo, Kraglund, Buttenschøn, Johnsen, Andersen, & Mortensen, 2019; Mead et al., 2013). However, in some situations, medications may not be appropriate due to side-effects, including headache, dizziness, tremor, fatigue, and insomnia (Deng et al., 2018).

Psychotherapeutic interventions, including cognitive behavioural therapy (CBT) and acceptance and commitment therapy (ACT), may also be provided by clinical psychologists in place of, or in addition to, taking medications (Graham, Gillanders, Stuart, & Gouick, 2015; Kneebone, 2016; Majumdar & Morris, 2019). There is now strong evidence in support of these treatment modalities for reducing depression and anxiety (Hildebrand, 2015; Wang et al., 2018), particularly when adapted or modified in line with the patient's specific cognitive or communication deficits (Kneebone, 2016). Initial data also support the use of complementary treatments for depression and anxiety, including relaxation therapy (Golding, Fife-Schaw, & Kneebone, 2018) and music therapy (Clements-Cortes & Haire, 2019).

Neuropsychological assessments may also be conducted during the sub-acute phase where concerns exist regarding the decision-making capacity of patients (Jokinen et al., 2015). These cognitive assessments also provide important information regarding the strengths and weaknesses of the patient, which may need to be considered when planning rehabilitation programs (Nakling et al., 2017). Increasingly, neuropsychologists are also being asked to provide cognitive interventions for attention, memory and executive functioning deficits (Loetscher, Potter, Wong, & das Nair, 2019; Sigmundsdottir, Longley, & Tate, 2016). There is preliminary support for the use of repeated activation and stimulation

tasks (e.g. repetitive drills, memory exercises) to improve attention and memory, in addition to interventions in the areas of problem solving, organisation and planning and organisational skills to improve executive functioning (De Luca, Calabrò, & Bramanti, 2018; Hill, House, Knapp, Wardhaugh, Bamford, & Vail, 2019).

Only 10% of stroke survivors achieve full physical, functional and cognitive recovery following sub-acute rehabilitation (Jung, 2017). For the remaining 90% percent, the rate of recovery begins to slow around the 90-day mark (i.e. the start of the chronic phase), and plateaus at the six-month mark (Bernhardt et al., 2017; Cassidy & Cramer, 2017). Some patients continue high-intensity rehabilitation post-discharge in an attempt to further enhance functional improvement (e.g., walk faster/for longer distances, improved fine motor skills) (Lee et al., 2015). However, most stroke survivors are discharged into the community at this time and the treatment focus switches from inpatient rehabilitation to adapting the home environment (e.g., installing ramps, grab rails, lifters) (Jung, 2017).

Unsurprisingly, clinical psychologists play an important role in supporting stroke survivors as they transition home from the rehabilitation environment. Indeed, the transition process is recognised as a period where symptoms of depression and anxiety may increase acutely (Chun et al., 2018; Mitchell et al., 2017). There are a myriad of reasons for this exacerbation of symptoms, including ongoing fears of further strokes or falls, increased insight into their overall loss of independence, concerns about burdening caregivers, financial difficulties, and social isolation (Cunningham, Blomstrand, Skoog, & Linden, 2016; Mitchell et al., 2017; Wright, Wu, Chun, & Mead, 2017). Although anti-depressant medications may again be prescribed, pharmacological approaches are not always effective (Damsbo et al., 2019) and the risk of unwanted side effects remains (Deng et al., 2018).

Psychotherapeutic interventions may aagain be used as an alternative to, or in conjunction with medication, to reduce the emotional distress experienced by patients

throughout the chronic phase of stoke (Mead et al., 2013; Wang et al., 2018). Specifically, research suggests that CBT is effective at helping patients change unhelpful thought processes which, in turn, impact their mood and/or quality of life, by challenging negative or unrealistic thoughts or by testing the accuracy of the thoughts through behavioural experiments (Kneebone & Jeffries, 2013; Kneebone, 2016; Wang et al., 2018). In addition, behavioural activation, whereby stroke survivors are encouraged and supported to resume participation in pleasurable activities (e.g. socialising, moderate exercise, old/new hobbies) (Ekers et al., 2014; Lee, Heffron, & Mirza, 2019), is commonly used to reduce depression by increasing levels of the neurotransmitter serotonin (Lee, Heffron, Mirza, 2019; Thomas, Walker, Macniven, Haworth, & Lincoln, 2013).

Clinical psychologists may also provide psychoeducation to patients and caregivers to promote awareness of, and potential solutions for, the range of longer-term medical (e.g. fatigue, chronic pain), functional (e.g. reduced independence, social withdrawal), cognitive (e.g. dementia) and emotional (e.g. loss of identity) challenges that may occur throughout the chronic phase (Hong et al., 2017). Although these educational programs may not always directly involve the patient, research suggests that they are critical to stroke survivor wellbeing, due to the fact that increased caregiver burden is associated with worse physical (e.g. mortality, illness), psychological (e.g. depression, anxiety), and social (e.g. isolation) outcomes for the patient (Haley, Roth, Hovater, & Clay, 2015; Wan-Fei et al., 2017).

1.9 Summary

Stroke remains as one of the leading causes of death and disability worldwide (Mozzafarian et al., 2016). Existing emergency stroke treatments are effective at saving lives and may reduce the severity of the associated disabilities (Hankey, 2017). However, the majority of patients do not receive the recommended treatments, due to medical contraindications and/or a lack of availability (Powers et al., 2019). Consequently, many

stroke survivors commence sub-acute rehabilitation with significant physical, functional, cognitive and psychological problems (Feigin et al., 2017; Mitchell et al., 2017; Thrift et al., 2017). An intensive period of sub-acute multidisciplinary rehabilitation may further improve the outcomes of stroke survivors (Enderby et al., 2017; Hankey, 2017). Although, this type of rehabilitation is not widely available (Jung, 2017). New and innovative treatments that improve the physical, cognitive and psychological outcomes of stroke survivors are therefore being developed to address this significant treatment gap.

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Chapter 2: Stem Cell Therapies for Stroke

2.1 Overview

A variety of novel treatments that involve the injection of SCs into the brain (intracranial), spinal cord (intrathecal), vein (intravenous), artery (intra-arterial), and/or skin (subcutaneous) of patients, are currently being developed in order to overcome the current limitations associated with existing stroke treatments and rehabilitation practices (Savitz, 2018). Although much of this research is still in its early stages, experimental SC treatments are now being offered by private clinics throughout Asia, South America and Eastern Europe (Cohen & Simana, 2018). Moreover, increasing numbers of patients are travelling overseas for the express purpose of receiving these risky and expensive treatments, a trend that is causing concern among government health agencies, clinicians and patient advocacy groups.

This chapter provides a detailed overview of SC therapy for stroke, including the main sources and types of SCs, primary methods of transplanting SCs, and hypothesised risks and benefits associated with each approach. Following this, current SC research guidelines, which were formulated to expedite the translational research process, are summarised and critiqued from a psychological perspective. Lastly, the practise of 'stem cell tourism' is discussed, including the associated medical and financial risks, and available interventions to dissuade patients from having these risky and experimental treatments.

2.2 Overview of SC Therapies

Definitions

SCs are cells that have the ability to produce copies of themselves and to reproduce or 'differentiate' into other cells (Giri, Alexander, Agrawal, & Saraf, 2019). 'SC therapy' or 'cell therapy' refers to the injection of SCs, SC derivatives (e.g. progeny cells obtained from SCs) and/or other cellular materials (e.g. granulocyte-colony stimulating factor [G-CSF],

exosomes) for the purposes of preventing or treating a medical condition (Dolati et al., 2019; Savitz, 2018). These terms are distinct from 'SC treatment' which is used more broadly in reference to non-evidence based experimental SC injections (Srivastava et al., 2016).

SC Sources

SCs are categorised according to their source. SCs harvested from the patient are referred to as autologous. SCs harvested from another human, including an embryo or fetus, are referred to as allogeneic. Finally, SCs harvested from a different species/animal are referred to as xenogeneic (Kenmuir & Wechsler, 2017; Zafar, Goswami, & Kumar, 2018). The distinctions between autologous, allogeneic and xenogeneic cells are important, from a treatment perspective, because SCs derived from foreign sources (i.e. allogeneic, xenogeneic) may be rejected by the recipient's immune system in the absence of immunosuppressant drugs (Boltze et al., 2015).

SC Types

SCs are categorised based upon whether they were derived from embryonic or post-natal adult cells, and the number of cell lines/lineages they can reproduce, referred to as their 'differentiation potential' (Mummery, Van de Stolpe, Roelen, & Clevers, 2014). Embryonic SCs only occur in the early stages following the fertilization of the ovum, during which time the zygote divides via the process of mitosis to form a blastocyst-stage embryo (Mummery et al., 2014). SCs that form following the initial splitting of the zygote (blastomeres) are totipotent and have the ability to reproduce into all embryonic and adult cells (Rossant & Tam, 2017).

Following this, totipotent SCs differentiate to form the blastocyst-stage embryo, at which time the SCs become pluripotent, meaning they can differentiate into the cell lineages required to form a human body (Trounsen & DeWitt, 2016). Human pluripotent SCs are

generally sourced from donated unused in-vitro fertilisation embryos (embryonic SCs) and, once obtained, can be replicated to create an ongoing line of 'daughter cells' (Romito & Cobellis, 2016). However, as a result of recent scientific breakthroughs, human pluripotent SCs, known as induced pluripotent SCs, can now also be made in labs by genetically reprogramming skin cells (Marei et al., 2018; Shi, Inoue, Wu, & Yamanaka, 2017).

Embryonic SCs are of significant interest to stroke researchers as they are thought to be a potential means of regrowing/replacing damaged brain tissue (e.g. neurons, oligodendrocytes, astrocytes), and if administered during the acute stage of stroke, may also support naturally occurring/endogenous repair processes through the release of anti-inflammatory cytokines and growth factors (Marei et al 2018; Zhao & Willing, 2018). However, there are significant risks associated with the use of totipotent and pluripotent cells, given that they may differentiate into tumours (Marei et al., 2018). Strong ethical concerns also remain regarding the harvesting and subsequent use of embryonic SCs, which was a key driver behind the research undertaken to create induced pluripotent SCs (Volarevic et al., 2018).

SCs obtained from post-natal human tissue are referred to as adult SCs (Visvadar & Clevers, 2016). Adult SCs are multipotent and, as such, only have the potential to differentiate into cells within the human body, including organs (e.g., heart, liver), tissue (e.g., bone, cartilage, fat), and blood (Giri et al., 2019). Multipotent SCs are harvestable from fat/adipose, dental and skin tissue (Dolati et al., 2019; Gancheva et al., 2019; Ojeh, Pastar, Tomic-Canic, & Stojadinovic, 2015; Ullah, Subbarao, & Rho, 2015). However, bone marrow is the most common source and provides both bone marrow mesenchymal SCs, a non-hematopoietic SC with the potential to differentiate into organs and tissue (Dolati et al., 2019), and bone marrow mononuclear cells, a collection of non-hematopoietic and hematopoietic cells (e.g. endothelial progenitor cells, CD34+ cells) with the potential to

differentiate into blood cells, organs and tissue (Boulais & Frenette, 2015; Gancheva et al., 2019). In addition, bone marrow mesenchymal and mononuclear cells are also thought to release trophic factors that provide neuroprotection and support naturally occurring (endogenous) repair processes in the brain following a stroke (Giri et al., 2019).

Umbilical cord blood, placental tissue and umbilical tissue (e.g. Wharton's jelly) are also a source of multipotent mesenchymal SCs and are of interest to stroke researchers given that they have the potential to repair tissue throughout artery walls (Li et al., 2016). SCs obtained from aborted human fetuses are also multipotent and form progenitor cells, which subsequently differentiate into the organs or tissue from which they were obtained (Mummery, Van de Stolpe, Roelen, & Clevers, 2014). Neural SCs, which are located within the dentate gyrus and subventricular zone of the human brain, are one such example and are of significant interest in the field of stroke because they have the potential to differentiate into brain tissue (Fuentealba et al., 2015). Notable examples of neural SCs that have been previously harvested then expanded to create an ongoing source for stroke research include the CTX (Kalladka et al., 2016) and LBS (Kondziolka et al., 2000) neural SC lines.

Broader 'Cell Therapies'

In addition to the injection of SCs and their derivatives, a small number of related cellular materials are also under investigation as potential 'cell therapies' for stroke. G-CSF, a naturally occurring hematopoietic growth factor used to treat white blood cell depletion (neutropenia) (Dale et al., 2015), is also thought to inhibit inflammation and promote regrowth of damaged brain tissue when administered during the early stages following a stroke (Cramer, 2018; Solaroglu, Digicaylioglu, Evren Keles, & Zhang, 2015). The synthetic equivalent of G-CSF, Filgrastim, has also been investigated as a possible adjunct to specific SC therapies ('combination SC therapies') and preclinical data suggest it may create a more receptive microenvironment for implanted SCs through the expression of pro-inflammatory

cytokines (Sarmah et al., 2018). Importantly, as G-CSF is naturally occurring, it does not require immunosuppressant drugs to be administered (Solaroglu et al., 2015). Instead, it passes through the intact blood-brain-barrier (i.e. the capillary wall that protects the brain from pathogens in the blood) and, as a result, is suitable for use throughout the acute, sub-acute and chronic stroke phases (England et al., 2016).

Exosomes, which are extracellular vesicles released by cells throughout the body to carry and transfer biomolecules, such as proteins, lipids and nucleic acids between cells, are also being investigated as a potential additional type of cell therapy for stroke (Chen & Chopp, 2018). Animal studies suggest that exosomes may mediate the effectiveness of transplanted mesenchymal SCs following a stroke by facilitating the transfer of beneficial biomolecules between the brain and its surroundings (Zhang, Buller, & Chopp, 2019). The stand-alone benefits of exosomes produced by mesenchymal SCs and injected into the bloodstream of rodents post-stroke have also been examined. The results suggest that exosomes are able to pass through an intact blood-brain-barrier, after which they promote neuronal regeneration, stimulate endogenous repair processes and reduce inflammation (Zhang et al., 2019). Although, preliminary clinical research has begun in the field of brain cancer, studies examining the benefits of exosomal therapy in stroke have yet to be conducted (Chen & Chopp, 2018; Zhang et al., 2019).

Methods of SC Transplantation

In addition to the broad range of SCs under consideration as treatments for stroke, a variety of transplantation methods have been examined, including intravenous, intra-arterial, intracranial, intrathecal and subcutaneous injections. The optimal transplantation methods remain unclear at present (Rodríguez-Frutos et al., 2016) and are hypothesised to differ based on: the type of stroke (ischaemic, haemorrhagic), phase of treatment (hyper-/acute, sub-acute, or chronic) (Bhatia, Gupta, Khurana, Sharma, & Khandelwal, 2018; Guzman et al., 2018;

Savitz et al., 2019), the permeability of the blood-brain-barrier (Daneman & Prat, 2015), the type of the SCs being administered, their expected mechanisms of action (e.g., replacement of damaged tissue, release of growth factors) (Rodríguez-Frutos et al., 2016), and the outcomes that are being measured (e.g. neurological, functional, radiological) (Wechsler, Bates, Stroemer, Andrews-Zwilling, & Aizman, 2018).

The least invasive transplantation methods (i.e. intravenous, intra-arterial, subcutaneous) have been examined predominantly throughout the hyper-acute and acute stages of stroke, a period of time when patients are at increased risk of treatment-related side effects (Boltze et al., 2015). Moreover, the blood-brain-barrier is more permeable throughout the early stages after a stroke, which allows SCs injected into the bloodstream to reach the brain (Daneman & Prat, 2015). Intravenous injections are one of the most common methods and are generally made into a vein of the forearm (Mays & Savitz, 2018). It was hoped that SCs administered in this way would migrate throughout the bloodstream toward the stroke-affected areas of the brain (Bhatia, Gupta, Khurana, Sharma, & Khandelwal, 2018; Savitz et al., 2019); however, most appear to divert to the other major organs (e.g., lungs, liver, spleen) where they are at risk of causing blood clots (Boltze et al., 2015; Rosado-de Castro et al., 2013). Although most SCs do not reach the brain, research suggests that intravenously administered cells may still exert neuroprotective effects indirectly, via the peripheral immune system (Mays & Savitz, 2018).

Intra-arterial SC injections are also relatively non-invasive and are most commonly injected into the MCA via femoral or carotid artery catheterisation (Guzman, Janowski, & Walczak, 2018). Intra-arterial delivery is thought to result in a larger number of cells reaching the brain, compared to intravenous injections, as the SCs bypass the major organs (Guzman et al., 2018). However, there is an increased risk of recurrent stroke due to either the SCs causing a blockage or as a result of arterial fat/plaque becoming dislodged in the

process and lodging in the brain (Boltze et al., 2015). Once located in the brain, the SCs may release trophic factors that aid endogenous repair processes and/or begin to replace lost neural connections (Rodríguez-Frutos et al., 2016).

Subcutaneous injections are the least invasive transplantation method and are generally made via the abdomen into the cutis (i.e. the skin layer directly below the dermis and epidermis) (Rodríguez-Frutos et al., 2016). G-CSF/Filgrastim is most commonly administered using this method, as it is thought to provide its neuroprotective effects via a range of paracrine mechanisms (i.e. bystander effects), rather than by directly restoring brain tissue (Gorthi, Prasad-Pathak, Dhull, & Nair, 2018; Solaroglu et al., 2015). In addition, given that G-CSF factor is able to bypass an intact blood-brain-barrier (Huang et al., 2017), subcutaneous injections are being investigated throughout all stroke phases (Gorth et al., 2018; Huang et al., 2017).

Two additional, but more invasive transplantation methods are also being examined, predominantly as a treatment throughout the chronic phase of stroke, at which time the blood-brain-barrier is no longer permeable (Daneman & Prat, 2015). Intracranial administration, which involves the stereotactic injection of SCs in-or-around the damaged areas of the brain via burr holes, is the first method (Steinberg et al., 2018). Despite being the most invasive form of SC transplantation (Boltze et al., 2015; Marei et al., 2018), intracranial injections are of particular interest because the cells can be directly implanted with a high degree of accuracy into the stroke-damaged areas of the brain (Neal et al., 2018; Savitz, 2018). Embryonic and neural SCs have been used most often in the hope that the SCs will regenerate and restore damaged areas (Kalladka et al., 2016; Wechsler et al., 2018). However, given that embryonic SCs have an increased risk of tumour formation (Boltze et al., 2015), intracranial neural SC and mesenchymal SC injections are thought to be potentially safer treatment options (Steinberg et al., 2018).

Intrathecal delivery, whereby SCs are injected into the spinal canal via lumbar puncture, is the last major method of transplantation. This method is again of significant interest in the field of stroke because the implanted cells are understood to reach the brain via the cerebral-spinal fluid, rather than intracranial injections (Zakerinia et al. 2018). Although primarily used in the chronic phase of stroke, intrathecal SC injections have been tested previously throughout the acute and sub-acute phases (Xue et al., 2014). Intraventricular injections are understood to work in a similar way to intrathecal injections (Rodríguez-Frutos et al., 2016); however, given the SCs are neurosurgically implanted directly into cerebral-spinal fluid within the subarachnoid space, it is considered to be more invasive (Al Fauzi, Suroto, Bajamal, & Machfoed, 2016). Embryonic SCs have again been used primarily for intrathecal and intraventricular injections given their potential to restore brain tissue (Borlongan, 2019). Their use is, however, associated with an increased risk of spinal and/or brain tumours (Marei et al., 2018).

2.3 Published Guidelines for SC Research

Given that SC research in the field of stroke is in the early stages, a range of recommended guidelines have been published to help expedite the translational research (bench-to-bedside) process. For preclinical research, this includes the Stroke Therapy Academic Industry Roundtable (STAIR) (STAIR collaborators, 1999; Albers et al., 2011; Fisher, 2003; Fisher, Hanley, Howard, Jauch, & Warach, 2007; Saver, Albers, Dunn, Johnston, & Fisher, 2009) and 'Stem Cell Therapies as an Emerging Paradigm in Stroke' (STEPS) guidelines (Boltze et al., 2019; Savitz et al., 2011; Wechsler et al. 2009). These guidelines suggest: (1) undertaking randomised, observer-blinded studies with multiple species, (2) documenting inclusion/exclusion criteria, (3) performing statistical power analyses, (4) investigating different administration routes, dosages and lengths of time post-stroke, (5) measuring multiple outcomes throughout each phase of stroke, (6) establishing

efficacy across multiple laboratories, (7) disclosing all conflicts of interest, (8) examining all stroke sub-types, (9) considering the age and medical comorbidities (e.g. diabetes, hypertension) of the recipients, and (10) reporting and sharing all data (positive, neutral, negative).

A further version of the STEPS document (Savitz et al., 2014) and the related 'Rigor Guidelines' (Lapchak, Zhang, Noble-Haeusslein, 2013) provide specific recommendations for clinical research. In addition to recommending that all data are published and any conflicts of interest are disclosed, these guidelines also advocate for: (1) the inclusion of sham treatment conditions to control for placebo effects, (2) justifying the type of SC, transplantation method and dosage based upon preclinical research, (3) screening for and documenting relevant medical comorbidities, (4) assessing pre-treatment functioning on two or more occasions to attain a stable neurological baseline, (5) performing neuroimaging of the stroke lesion/infarct size pre-and-post treatment, (6) labelling and tracking SCs to identify the mechanisms of action, (7) examining domain-specific outcomes, (8) providing physical rehabilitation in addition to the SC treatment, and (9) reporting serious adverse events beyond 12 months. The majority of the recommendations are intended for current clinical trials, although some recommendations, such as the labelling and tracking SCs post-implantation, remain aspirational due to current technological limitations (Mangin & Kubis, 2019). However, despite their apparent utility, the overall level of adherence by SC researchers has yet to be evaluated, therefore it is unclear whether particular aspects of research are being overlooked.

Further Psychological Considerations

Current SC clinical research guidelines were developed to help expedite the translational research process and, as such, are primarily focussed on confirming the medical safety and neurological, functional, and radiological efficacy of SC treatments for stroke

(Mangin & Kubis, 2019; Savitz et al., 2014). Unfortunately, the guidelines place much less emphasis on a range of potentially important psychological factors that may also influence both SC treatment outcomes and patient wellbeing. For instance, while it is recommended that all relevant medical comorbidities (e.g. diabetes, hypertension) are identified prior to SC treatment (Lapchak et al., 2013; Savitz et al., 2014), the routine assessment of depression and anxiety is not advocated for, despite patients with mood symptoms being at increased risk of treatment-related complications (e.g. mortality, infections, increased pain) and poorer long-term functional outcomes (Chun et al., 2018; Dar et al., 2017; Rafsten, Danielsson, & Sunnerhagen, 2018).

Furthermore, pre-operative anxiety, which is estimated to occur in up to 90% of patients who undergo invasive medical procedures, also places patients are greater risk of serious treatment-related complications and poorer treatment outcomes (Aust et al., 2018; Kumar, Dubey, & Ranjan, 2019; Majumdar et al., 2019; Strøm et al., 2018). Therefore, given the invasive nature of some SC therapy procedures, it is possible that pre-operative anxiety may also impact upon the safety and efficacy of those particular treatments. Moreover, patients who experience adverse treatment outcomes, including a perceived lack of effectiveness, are also at risk of longer-term depression and anxiety (Abu-Ruz, Alaloul, & Al-Dweik, 2018; Ghoneim & O'Hara, 2016), whereas patients who experience positive treatment outcomes generally experience an improvement in their mood (Mancuso et al., 2018; Switzer, Debru, Church, Mitchell, & Gill, 2016). These risks are again noteworthy in the current context, given that participants in the majority of SC trials are providing consent to receive treatments with largely unquantified risks and benefits.

Similarly, screening for the presence of a neurocognitive disorder (mild or major) is not explicitly stated in the available SC guidelines (Lapchak et al., 2013; Savitz et al., 2014). This is potentially problematic given that cognitive deficits are common following a stroke

and may impact upon the overall functioning of the patient (Hankey, 2017). In addition, SC therapies may also improve cognitive outcomes following a stroke, however, this is not examinable in the absence of pre-treatment screening and neuropsychological assessment (Giri et al., 2019).

2.4 Experimental SC Treatments and 'Stem Cell Tourism'

SC therapies that involve the injection of foreign cells (i.e. allogeneic, xenogeneic) to treat stroke and its related symptoms have yet to be approved for use in the United States of America, United Kingdom or Australia, due to ongoing concerns about the safety and effectiveness of these treatments (Sipp et al., 2018). However, autologous SC treatments, which involve the injection of the patient's own cells, are permitted under current regulations for stroke, stroke-related symptoms (e.g. chronic pain), and for certain neurological conditions, such as multiple sclerosis, Parkinson's disease, spinal cord injury and traumatic brain injury (Berger et al., 2016; McLean, Stewart, & Kerridge, 2015; Munsie et al., 2017; Turner, & Knoepfler, 2016). Autologous SC treatments are thought to be medically safer than the use of foreign SCs, due to a lower risk of immune rejection and tumour formation (Boltze et al., 2015), although recipients still face financial risks due to the significant expenses incurred when receiving these unproven treatments (Berger et al., 2016; Munsie et al., 2017).

In addition to the SC treatments offered by domestic clinics throughout the United States of America, United Kingdom and Australia, private clinics throughout Asia, South America and parts of Europe also offer experimental SC treatments that utilise both multipotent autologous and pluripotent foreign cell types (Cohen & Simana, 2018). The treatments provided by these clinics are largely unregulated and, as a result, the clinics are not required to disclose the source of their SCs or to provide empirical data to support the safety or efficacy of their treatments (Bauer Elsallab, & Abou-El-Enein, 2018; Srivastava et

al., 2016). In addition, the manufacturing processes used by these private clinics may lack the sophistication required to ensure high quality SCs, and in some cases, lack the capabilities to routinely test donor SCs for hepatitis or the Human Immunodeficiency Virus (Cohen & Simana, 2018; Mummery et al., 2014). In addition to these significant medical risks (Bauer et al., 2018), patients who undergo experimental SC treatments are at high risk of financial exploitation, with some patients reportedly paying up to US$100,000 to receive these non-evidence-based treatments (Sipp et al., 2017).

While little is currently known about the number of patients receiving autologous treatments in their home countries, travelling overseas for experimental SC injections, a practise known as 'stem cell tourism', is thought to have become increasingly popular over the past decade, particularly among patients with spinal cord injuries, multiple sclerosis, Parkinson's disease and amyotrophic lateral sclerosis (Rachul, 2011; Rai, Yuhasz, Julian, Salerno, & Imitola, 2019; Tanner, Petersen, & Munsie, 2017). Despite this concerning trend, only a small number of studies have examined the reasons why patients do so. A qualitative analysis of online blog posts from American, Canadian, Australian and Brazilian patients who had sought out SC injections for a range of neurological conditions (N = 32), found that dissatisfaction with the effectiveness of mainstream treatments, coupled with frustration regarding the perceived length of time taken for new treatments to become available in their home countries, were the main reasons for them having done so (Rachul, 2011). A subsequent Australian study, which interviewed 16 patients with a similar range of conditions, further identified that the notion of 'hope' along with a sense of having 'nothing to lose' as being strong motivating factors for them having travelled overseas to undergo experimental SC treatments (Petersen, Seear, & Munsie, 2014).

Preliminary research suggests that interest in 'stem cell tourism' may also be increasing among the stroke cohort (Cohen & Simana, 2018). This is extremely concerning,

given that there are published reports of stroke survivors experiencing brain tumours (Amariglio et al., 2009; Nakamura et al., 2016), spinal cord lesions, seizures and serious infections (Berkowitz et al., 2016; Hurst et al., 2013; Kawarai et al., 2011) following experimental SC treatments from clinics in Asia and Russia. The factors that specifically motivate stroke survivors to travel overseas for unproven SC treatments have yet to be identified. Although, a single South Korean study found that male stroke survivors who had more severe ongoing physical disabilities and positive expectations regarding the risks and benefits associated with SC treatments were more likely to consider them (Kim et al., 2013).

Theoretical Frameworks That May Further Predict Interest in 'Stem Cell Tourism'

A number of pre-existing theoretical frameworks may help to shed light on which patients are most likely to seek out experimental SC treatments. One such framework is the biopsychosocial model (Bolton, 2019). According to this model, which forms the basis for the World Health Organisation's International Classification of Functioning (Stucki, 2016), a patient's overall level of disability is not only based on biological factors (i.e. the medical model), but rather a complex interaction between biological, psychological and social factors (Wade & Halligan, 2017). When applied to stroke, the biopsychosocial model suggests that physical and functional disabilities, in addition to health-related quality of life (e.g. severity of pain, quality of sleep, presence of communication deficits), mental health, cognitive functioning, and social support should all be considered when measuring a patient's level of incapacity (Zhang et al., 2018). When considered in conjuction with previous research, which found that patients with severe ongoing physical disabilities were more likely to be considering experimental SC treatments (Kim et al., 2013), it appears possible that the biopsychosocial model may offer additional insight into which stroke survivors' are at greatest risk of participating in 'stem cell tourism'. However, despite its potential utility, it has yet to be applied in this context.

In addition to the biopsychosocial model, psychological research suggests that the attitudes and beliefs of a patient regarding a treatment or intervention may strongly influence whether they will consider undergoing it or not. This view, which is primarily associated with the Theory of Planned Behaviour model (Ajzen, 1991), proposes that a person's beliefs regarding the benefits of a particular intervention, the ease with which they believe they can access the intervention, and the broader perceived societal norms regarding the intervention, all influence the individual's intentions and subsequent actions (Ajzen, 1991). When applied to 'stem cell tourism', the Theory of Planned Behaviour posits that patients who perceive SC injections to be very safe, highly effective, easily accessible, financially affordable, and who feel that their family/friends support this treament, are more likely to consider and seek out these treatments (Sheeran et al., 2016). Preliminary evidence exists in support of the Theory of Planned Behaviour when assessing participation in other forms of 'medical tourism' (Seow et al., 2017; Sheeran et al., 2016), however, its utility has yet to be evaluated in the context of 'stem cell tourism'.

Interventions to Deter Patients from 'Stem Cell Tourism'

In recognition of the increasing level of interest in 'stem cell tourism' among various patient groups, and the significant risks associated with these unregulated treatments, a number of governments have implemented laws that prohibit experimental SC clinics from operating (Sipp & Okano, 2018). A prime example of this occurred in Germany in 2011, when the X-Cell Centre - one of Europe's largest SC clinics at the time - was shut down following the death of a boy following intracranial SC injections for autism (Bauer et al., 2018). The United States of America has also begun to review SC treatment practices, which has lead to a number of clinics closing, due to increased regulatory scrutiny regarding safety, efficacy, and treatment costs (Sipp, 2018; Sipp & Okano, 2018). Australia appears to have

been slower to act, however, and continues to have one of the highest percentage of SC clinics, per capita, in the world (Berger et al., 2016; Munsie et al., 2017).

In addition to regulatory changes, a plethora of patient education resources (e.g. written resources/handbooks, online videos, presentation slides, cartoons/comics) have been developed by SC research organisations, scientists and patient advocacy groups to dissuade patients from receiving unproven SC treatments, either in their home countries or abroad (Weiss, Turner, Levine, & Ikonomou, 2018). Although the type and level of detail varies considerably between these resources, most focus on the medical and financial risks associated with 'stem cell tourism' for neurological disorders, including multiple sclerosis, cerebral palsy, spinal cord injury, amyotrophic lateral sclerosis and Parkinson's disease, as opposed to stroke (Master, Robertson, Frederick, Rachul, & Caulfield, 2014).

A preliminary review of available SC educational resources designed for patients with neurodegenerative disorders highlighted strong concerns about the likely effectiveness of patient education as a stand-alone strategy against 'stem cell tourism' (Master et al., 2014). However, the extent to which patient education interventions effectively dissuade patients, including stroke survivors, from having experimental SC treatments has yet to be formally evaluated. This is particularly noteworthy in the current context because stroke survivors may require resources that have been developed with their specific disabilities, risk profiles, and psychological and cognitive comorbidities in mind for them to be effective (Du, Ma, & Li, 2016).

2.5 Summary

SCs, SC derivatives and cellular materials are being examined as potential treatments for stroke given they have the potential to restore and regenerate damaged brain tissue (Giri et al., 2019). A wide variety of SC sources, types and transplantation methods are being investigated; however, the safest and most efficacious SC therapies have yet to be determined

for each phase of stroke (Boltze et al., 2015; Giri et al., 2019; Kenmuir & Wechsler, 2017). Despite the availability of SC research guidelines to assist in the translational process (Lapchak et al., 2013; Savitz et al., 2014), their medical focus has lead to a number of important psychological considerations having been overlooked (Giri et al., 2019; Kenmuir & Wechsler, 2017). Very little is also currently known about the level of interest in 'stem cell tourism' among stroke survivors, and the reasons why patients may be considering these unregulated, experimental treatments is unclear. A number of biopsychosocial factors, including physical independence, health-related quality of life, cognition, depression, anxiety, social support, perceived caregiver burden, may provide important insights into this, but have yet to be examined. The Theory of Planned Behaviour (Ajzen, 1991) also suggests attitudinal factors may also influence whether stroke survivors will consider experimental SC treatments, however its utility has yet to be evaluated in this context. In the interim, SC research organisations and advocacy groups have created patient education booklets and videos warning patients about the medical and financial risks associated with 'stem cell tourism' (Weiss et al., 2018). However, whether existing patient education resources, which were primarily designed for patients with neurological disorders, are an effective means of dissuading stroke survivors from having risky treatments remains unknown.

2.6 Aims of the Current Research

As highlighted in this review, significant gaps remain in the treatment of stroke due to the inaccessibility and/or limitations associated with existing emergency interventions, varying availability of multidisciplinary rehabilitation, and a lack of safe and effective long-term restorative treatments (Emberson et al., 2014; Enderby et al., 2017; Jung, 2017). Moreover, while preliminary research suggests that SC therapies may improve the physical, functional and radiological outcomes of stroke survivors, the medical and psychochological risks, optimal SC types, administration methods, and dosages for each phase of stroke remain

unclear (Giri et al., 2019; Marei et al., 2018). Research also suggests that increasing numbers of patients with neurological conditions are travelling abroad to private SC clinics (Cohen & Simana, 2018). However, the number of stroke survivors that may be considering undergoing experimental SC treatments, and the reasons for doing so are undetermined. Patient education resources aimed at deterring patients with a range of conditions from participating in 'stem cell tourism' are available online, although the effectiveness of the existing resources for stroke survivors has yet to be examined.

The four studies that follow were designed to: (1) review and evaluate clinical research examining the safety and efficacy of SC therapy for stroke; (2) identify the extent to which psychological factors have been considered by existing SC researchers, (3) provide an estimate of the number of stroke survivors who are considering having experimental SC treatments; (4) clarify some of the biopsychosocial and attitudinal factors that increase the likelihood that stroke survivors will consider having experimental SC treatments; and (5) examine whether existing patient education resources deter stroke survivors from 'stem cell tourism'.

Objective 1 was examined through a systematic review and meta-analysis of published safety and efficacy data pertaining to use of SC therapy for stroke. Data were analysed according to whether the SC therapies were administered in the acute and sub-acute (Study 1; Chapter 3) or chronic (Study 2; Chapter 4) phases. The number of treatment-related serious adverse events was used to evaluate the safety of individual treatments. Efficacy was assessed across neurological, functional and radiological outcomes. The reporting quality of the included studies was also assessed using published reporting guidelines (e.g. Consolidated Standards of Reporting Trials [CONSORT]) (Moher et al., 2010) and recommended SC research guidelines (e.g., STEPS [Savitz et al., 2011; Savitz et al., 2014; Wechsler et al. 2009]). Studies 1 and 2 additionally examined the degree to which

psychological factors have been considered within the existing SC research literature (objective 2).

Objectives 3 and 4 were addressed by conducting a cross-sectional survey of stroke survivors regarding their beliefs and attitudes about experimental SC treatments (Study 3; Chapter 5). In addition to their beliefs and attitudes, participants provided demographic information, completed questionnaires detailing their current level of biopsychosocial functioning, and specified whether or not they were considering experimental SC treatments at the time of the survey (objective 3). From these data, a number of biopsychosocial (i.e. biological/medical, cognitive, and psychological) (Bolton, 2019) and attitudinal factors (based on the Theory of Planned Behaviour [Ajzen, 1991]) that increased the likelihood that a stroke survivor may consider experimental SC treatments were identified (objective 4). As part of the survey, participants also nominated their preferred SC types, administration methods, and treatment outcomes, which were then compared with the current focus of SC researchers to highlight inconsistencies that may be used to inform future research.

Lastly, the effectiveness of two patient education interventions for deterring stoke survivors from considering 'stem cell tourism' (objective 5) was examined in a small-scale, parallel-group RCT (Study 4; Chapter 6). Participants indicated whether they were considering 'stem cell tourism' prior to, immediately following, and 30-days after reading a booklet (intervention 1) or watching an online video (intervention 2). A wait-list control group (intervention 3) was included to provide further clarity about the effectiveness of the interventions over time. The proportion of stroke survivors who were considering 'stem cell tourism' at each time interval was compared to identify within-group changes and between-group differences. The attitudes of the groups toward SC treatments were also assessed at each point to measure the effect of the interventions on particular areas (e.g. safety, efficacy, affordability of SC treatments).

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Chapter 3: Study 1

Safety and Efficacy of Cell Therapies administered in the Acute and Sub-Acute Stages after Stroke: A Meta-Analysis.

This chapter consists of a published paper, however copyright restrictions prevent the reproduction of this paper in its published form. The details of this publication are:

Unsworth, D. J., Mathias, J. L., & Dorstyn, D. S. (2016). Safety and efficacy of cell therapies administered in the acute and sub-acute stages after stroke: A meta-analysis. *Regenerative Medicine*, *11*, 725-741. doi: 10.2217/rme-2016-0063

Please note:

- Tables and Figures are formatted in line with the Journal's requirements.
- Australian/British English spelling was used.
- The term 'cell therapies' is used in place of 'SC therapies', in line with the accepted vernacular, to describe treatments involving SCs, SC by-products, and SC-related materials.
- The lack of knowledge and research about the psychological variables had to be down-played in the article due to the medical focus of the journal.

3.1 Preface

This study provides a comprehensive overview of existing data pertaining to the safety and efficacy of SC therapy throughout the hyper-acute/acute and sub-acute stages of stroke (objective 1). A secondary aim was to identify the degree to which psychological factors had been considered in the published literature (objective 2). In doing so, this review extends upon the small number of previous reviews that focussed on specific study designs (e.g. RCTs, single-arm studies) and SC types (e.g. mesenchymal SCs) and combined data from patients with different types of stroke (i.e. ischaemic, haemorrhagic) and phases of recovery (e.g. acute, chronic) (Boncoraglio, Bersano, Candelise, & Reynolds, 2010; Cao & Li, 2015; Fan et al., 2015; Jeong et al., 2014; Lalu et al., 2012; Wang et al., 2016).

The current study analysed data solely from the hyper-/acute and sub-acute phases, and stratified SC therapies based upon the delivery route and SC type. The safety of the individual SC therapies was determined, based upon reported serious adverse events. Efficacy was assessed by comparing the neurological, functional, and/or radiological outcomes of: (1) patient groups who had received either SC therapy or standard care; and (2) the pre-/post-treatment outcomes of individual SC therapy treatment groups. The latter pre–/post-treatment changes were also compared with standard-care control group data from the included RCTs, to determine whether the observed changes exceeded those of current treatment practices. Data regarding the stroke type, region, and length of time post-stroke were also collated to characterise the treatment samples. Whether patients were screened for mood disoders prior to treatment, and whether mood was evaluated throughout the trials, was additionally noted.

3.2 Statement of Authorship

Title of Paper	Safety and efficacy of cell therapies administered in the acute and subacute stages after stroke: A meta-analysis
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
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Principal Author

Name of Principal Author (Candidate)	Mr David J. Unsworth		
Contribution to the Paper	Study design, data collection and analysis, writing manuscript.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	30/09/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Prof. Jane L. Mathias		
Contribution to the Paper	Study design, data interpretation, editing manuscript.		
Signature		Date	1/10/2019

Name of Co-Author	Dr. Diana S. Dorstyn		
Contribution to the Paper	Study design, data interpretation, editing manuscript.		
Signature		Date	30/9/2019

3.3 Abstract

Aims: To evaluate the safety and efficacy of cell therapies administered acutely/sub-acutely after stroke. **Methods:** Five databases were searched for studies examining the safety/efficacy of cell therapies administered ≤ 90 days post-stroke. Reporting quality and adherence to research guidelines were evaluated. Safety and efficacy were assessed using risk ratios/pooled incidence rates and Hedges' *g*, respectively. **Results:** 11 therapies ($N_{studies}$= 28) were trialled: reporting quality was high, but adherence to guidelines low. Serious adverse events were observed following five treatments; six improved outcomes. There was a trend toward larger treatment effects in non-blinded studies, younger participants, and higher dosages. **Conclusion:** Although a number of therapies appear effective, many studies did not control for normal recovery (standard-care). Long-term safety also needs to be established.

3.4 Introduction

Stroke is a leading cause of death and disability worldwide (Mozaffarian et al., 2015), with medical and functional outcomes being highly dependent on the treatments that are received in the first 90 days (Antonios & Sillman, 2013). Intravenous thrombolysis is the main treatment for acute ischaemic stroke but is used in less than 10% of cases due to the narrow recommended treatment window and potential for adverse effects (Reeves et al., 2005). For acute haemorrhagic stroke, surgery is typically performed to evacuate the hematoma and reduce intracranial pressure, although the impact is usually modest (NINDS, 2005). Beyond this, treatment has been limited to physical rehabilitation and the pharmacological management of psychological co-morbidities (e.g., depression, anxiety) (Belagaje & Butler, 2013). Given the restricted range and efficacy of existing treatments, combined with the significant medical and economic burden associated with stroke (Mozaffarian et al., 2015), cell therapies are increasingly being investigated as an alternative.

A variety of cell therapies have been trialed to promote structural and functional restoration after stroke, utilising a range of cell types (e.g., stem cells [SCs], mononuclear cells, growth factors), delivery routes (e.g., brain, spinal cord, vein, artery, abdomen) and cell sources (e.g., human, animal). Treatments involving cells derived from human tissue (e.g., bone marrow) and synthetic growth factors (e.g., G-CSF/Filgrastim) are the most common, and are typically injected via intravascular or subcutaneous routes where they are believed to stimulate neural repair through the release of trophic, growth and/or neuroprotective factors (Kitago & Marshall, 2015). Although less common, cells derived from embryonic or fetal matter (e.g., neural SCs [NSCs]) are usually administered via the intracranial or intrathecal route, after which they are thought to differentiate into specific brain cells, such as neurons and astrocytes (Liu et al., 2014). While cells are normally sourced from the patient (autologous) to prevent rejection, cells from other individuals (allogeneic) and animals (xenogeneic) are also being investigated in stroke patients (Boltze et al., 2015).

Cell therapies used in the acute/sub-acute phases of stroke are of particular interest because there are a number of changes that are specific to this period, including increased inflammatory markers and greater blood–brain barrier permeability, which have the potential to attract the injected cells to the injury site (Cramer, 2008; Detante et al., 2014). In addition, cells or growth factors administered in the early stages after a stroke (< 24 hours) may reduce secondary damage (e.g., necrosis, apoptosis) by releasing neuroprotective factors, which modify the inflammatory response (Savitz, 2015). However, patients are also at a higher risk of serious complications throughout this period (e.g., intracranial hemorrhaging, recurrent stroke) and, consequently, invasive cell therapies (e.g., intracerebral injections) may not be appropriate. Seizures, tumors and pulmonary emboli have also been observed following the injection of cells (Boltze et al., 2015), suggesting that some cell types, delivery methods and sources may be preferable to others. Given these potential benefits and risks, it is important to determine the safest and most effective form of cell therapy for acute/sub-acute stroke.

Early meta-analyses have focused on the efficacy of cell therapies for ischaemic stroke (Boncoraglio et al., 2010; Yuan, Zeng, & Wu, 2007) but were inconclusive, possibly due to the small number of randomised controlled trials (RCTs) and the fact that acute and chronic data were combined. A subsequent meta-analysis (Jeong et al., 2014) compared the pre- and post-outcomes of cell therapy recipients who were recruited into single-arm/cohort studies but, without controls, was unable to determine whether the observed improvements resulted from the cell therapy, spontaneous recovery and/or placebo effects, and whether they exceeded those seen as a consequence of standard care. Ischaemic and haemorrhagic strokes were also combined, therefore any differential efficacy for these stroke types remains unclear. Other meta-analyses have examined bone marrow mesenchymal SCs (BM-MSCs) (Cao & Li, 2015) or intravascular mesenchymal SC therapies (pediatric and adult) (Lalu et al., 2012) following ischaemic stroke, but they did not examine safety or efficacy relative to other treatments. Moreover, the impact of a number of clinical (patient age, treatment phase,

dosage) and methodological (randomisation) variables on efficacy remains uncertain, and an objective evaluation of the study reporting quality has yet to be undertaken. Thus, the optimal cell therapy for adult stroke patients in the acute/sub-acute phase remains unclear.

The current study therefore examined the safety and efficacy of cell therapies – broadly defined as SCs, cell-related materials and growth factors (Savitz, 2015) – administered within 90 days of ischaemic or haemorrhagic stroke. RCTs/non-RCTs and cohort/case–control studies were all included, and reporting quality assessed using the Consolidated Standards of Reporting Trials (CONSORT) (Moher et al., 2010) and Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Von Elm et al., 2008). All studies were additionally evaluated in terms of the Stem Cells as an Emerging Paradigm in Stroke (STEPS I–III) and RIGOR (Lapchak, Zhang, & Noble-Haeusslein, 2013; Reynolds, 2009; Savitz et al., 2011; Savitz et al., 2014) guidelines for cell therapy research. Therapies were stratified by cell type and delivery route: safety was assessed in terms of treatment-related serious adverse events (SAEs) and efficacy was assessed across neurological, functional and/or radiological outcomes. Control data from the RCTs were additionally used to determine whether the treatment effects reported by cell therapy recipients in the cohort/case–control studies exceeded the changes seen in non-treatment controls over an equivalent interval. Lastly, subgroup analyses examined whether efficacy differed according to patient age (< 60 vs ≥ 60 years), stroke type (ischaemic vs haemorrhagic), treatment phase (hyper-acute vs acute/sub-acute), dosage ($< 10^8$ vs $\geq 10^8$ cells) or study blinding (none vs single or double).

3.5 Methodology

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altmann, 2009).

Search Strategy and Inclusion Criteria

A comprehensive search of the PubMed, Embase, Web of Science, Cochrane and PsycINFO databases was completed (January 1960–April 2016) to identify research that administered cell therapies within 90 days of stroke onset (see Supplementary Appendix A for searches). Citation searches were additionally performed via Scopus and the reference lists of eligible studies and relevant reviews checked for references.

To be included, studies had to have assessed the neurological (e.g., National Institute of Health Stroke Scale [NIHSS]), functional (e.g., Barthel Index), radiological (e.g., MRI) and/or safety outcomes of adults (≥ 18 years) who had sustained an ischaemic or haemorrhagic stroke within 90 days of receiving cell therapy, and provided data that enabled the calculation of effect sizes (means and *SDs*, *t*- statistics, χ^2, exact *p*-values). Published studies that used randomised, non-randomised and observational (cohort/case–control) designs were all included, but case studies were excluded. Publications were not restricted to English. Study eligibility was assessed by the first author (DJ Unsworth) in consultation with the second author (JL Mathias).

The literature search identified 3940 articles (see flowchart, Supplementary Appendix B for details). Initial screening of the titles and abstracts reduced the number to 85, after which full-text versions were reviewed: two papers required information/data to be extracted by a fluent Mandarin Chinese speaker to enable inclusion (Chen et al., 2015; Meng et al., 2009). Thirty articles met the inclusion criteria; however, closer examination of the participant data revealed that nine had used overlapping samples, reducing the number to 25 independent studies (see Supplementary Appendix C for summary details). Of these, two examined multiple treatments in separate samples (Meng et al., 2009; Rosado-de-Castro et al., 2013), which were thereafter treated as five additional independent studies. Effectively, data from 28 independent studies were included in this meta-analysis (see Supplementary

Appendix C for details), including two for which graphical data were converted to numerical data (Moriya et al., 2013; Savitz et al., 2011) using GetData Digitizer software, Version 2 (Federov, 2013).

Data Extraction

Key background (sample size, age, gender, country), stroke (type, region) and treatment (delivery route, cell type/source/dosage, assessment point) data were extracted from each study by the first author (DJ Unsworth), as were outcome data. The corresponding authors were contacted if additional information was required. Data were analysed using the Comprehensive Meta-Analysis Software, Version 3 (CMA; 2014, Biostat, Inc., NJ, USA) and forest plots were generated using Meta Data Viewer (National Institutes of Health, 2015).

Assessment of Reporting Quality and Adherence to Research Guidelines

The 25-item CONSORT (Moher et al., 2010) and 22-item STROBE (Von Elm et al., 2008) guidelines were used to assess the reporting quality of the randomised and non-randomised/observational studies, respectively. A 13-item checklist (Supplementary Appendix D) was additionally constructed from the STEPS I–III (Reynolds, 2009; Savitz et al., 2011; Savitz et al., 2014) and RIGOR (Lapchak et al., 2013) guidelines to evaluate adherence to current recommendations for cell therapy research (e.g., placebo/sham treatments, assessment of co-morbidities, cell labeling/tracking).

For every study, information relating to each item was assessed as: 'Present' (2 points), 'Present with Limitations' (1 point), 'Not Present' (0 points) or 'Not Applicable' (n/a). Two scores were calculated: first, an overall quality score for each study, expressed as a percentage ([study score ÷ no. of applicable items] × 100) with scores < 50%/50%–75%/> 75% reflecting low/medium/high quality (Viswanathan et al., 2012) and second, the percentage of studies receiving scores of 2, 1 and 0 for each item (Viswanathan et al., 2012).

Effect size, Heterogeneity and Publication Bias analyses

Hedges' *g*, which takes into account the greater variance associated with small samples (Hedges, 1982), was calculated using a random-effects model (DerSimonian & Laird, 1986). The first set of analyses compared the post-treatment outcomes of cell therapy and standard-care stroke groups ('Post-treatment vs Control') using data obtained from the RCTs and non-RCTs (note: the cell therapy groups also received standard care; this comparison therefore controls for improvements due to normal recovery and rehabilitation). The second set of analyses compared the pre- and post-treatment outcomes of a single treatment group ('Pre-/post-treatment') using data extracted from all three study designs (RCTs, non-RCTs and cohort studies) in order to maximise the available data (note: not all RCTs/non-RCTs provided pre- and post-treatment data, consequently some could not be included here). Lastly, the latter pre–post changes were compared with those seen in a standard-care stroke control group over an equivalent interval ('Pre-/post-treatment vs Control') in order to determine whether they exceeded what would be expected to occur as a result of normal recovery and physical rehabilitation. Data for the standard-care stroke group were sourced from six of the eligible RCTs ($N_{controls}$ = 196; see Supplementary Appendix E for details) because they were thought to provide better quality data (Moher et al., 2010; Von Elm et al., 2008). Controls from non-RCTs were not suitable because they were often ineligible for cell therapy.

Cell therapies were stratified by delivery route, cell type and study design (Post-treatment vs Control, Pre-vs post-treatment) to enable comparisons between the individual treatments. Combined therapies were also included in order to assess whether they provided any additional benefits over and above those seen when used as mono-therapies (dela Peña & Borlongan, 2015). Risk ratios (RR) and pooled incidence rates (%) – including 95% CIs – were calculated to estimate the risks associated with each cell therapy. However, because the

definition of SAEs differed between studies, only instances of: death, cancer/tumor and major neurological (new stroke, seizure)/cardiac/renal disorders – which were common to all studies – were recorded. Moreover, SAEs were only included in the pooled incidence rates when they were 'possibly' or 'likely' to be related to treatment.

Mean effect sizes (Hedges' *g*) were calculated to assess changes in the neurological, functional, radiological and 'overall' (combined neurological, functional and radiological) outcomes for each treatment. The upper/lower bounds of *g* were estimated using 95% CIs and forest plots were used to assess statistical power, based on the width of the intervals (Boyles, Harris, Rooney, & Thayer, 2011; Valentine, Pigott, & Rothstein, 2010). A significant ($p < 0.05$) positive *g* indicates that cell therapy recipients experienced better outcomes than controls (Post-treatment vs Control and Pre-/post-treatment vs Control analyses) or improved pre- to post-treatment (Pre-/post-treatment analyses), with $g = 0.2$, 0.5, 0.8, 2.0 and 4.0 equating to small, medium, large, very large and extremely large treatment effects, respectively (Cohen, 1988; Hopkins, Marshall, Batterham, & Hanin, 2009). Fail-safe *N* statistics (*Nfs*) assessed the risk of publication bias by indicating the number of unpublished studies with small/non-significant effects required to reduce a finding to a small effect ($g = 0.2$) (Lipsey & Wilson, 2001). An *Nfs* greater than the number of studies examining a specific treatment ($N_{studies}$) was the criteria used to indicate a low risk of publication bias.

Unfortunately, we were not able to conduct heterogeneity analyses to determine whether the effect sizes for studies that examined the same therapy varied significantly ($N_{studies}$ for individual treatments too small) (Higgins, Thompson, Deeks, & Altman, 2003). Instead, a random-effects model was used to calculate the effect sizes in order to take into account clinical and methodological variability between the studies (Hopkins et al., 2009). In addition, *Q*-statistics and the I^2 index were calculated to measure the level of heterogeneity in

the treatment effects reported by studies included in: (1) the Post-Treatment vs Control and (2) the Pre-/post-treatment analyses. A significant *Q* indicates that treatment effects differed between studies, and I^2 index values < 50%, 50–75% and > 75% suggest low, medium and high levels of heterogeneity, respectively (Higgins et al., 2003). Subgroup analyses (with Bonferroni corrections [$p < 0.01$]) then examined potential source/s of heterogeneity (Xu, Platt, Luo, Wei, & Fraser, 2008) in order to determine whether the treatment effects differed according to: participants' age (< 60 vs ≥ 60 years), the type of stroke (ischaemic vs haemorrhagic), the cell dosage ($< 10^8$ vs $\geq 10^8$ cells), stroke phase (hyper-acute/acute [≤ 30 days] vs sub-acute [> 30–90 days]) and study blinding (none vs single/double).

3.6 Results

Study Characteristics

Details of the participants (age, sex, stroke type/region), cell therapies (time between stroke onset and therapy, type of cell therapy), outcomes assessed (safety, neurological, functional, radiological), study designs (RCT, non-RCT, observational) and countries of origin are summarised in Table 3-1. The Post-treatment vs Control analyses were based on 18 studies of 1273 participants ($N_{treatment} = 675$; $N_{controls} = 598$). Participants were typically middle-aged (mean age: 61.8 years) and had suffered ischaemic middle cerebral artery (MCA) strokes (45%). On average, cell therapies were administered in the acute phase (mean = 8.1 days post-stroke) with intravenous injections of G-CSF being most common ($N_{participants} = 193$). Seven mono- and two combined therapies were examined (see Table 3-1). All studies examined safety and most examined efficacy (94%), predominantly focusing on neurological and functional outcomes. Eleven studies were RCTs (Phase I or II) and most were conducted in China.

Table 3-1. *Descriptive and Categorical Data for the Study Participants*

	Post-treatment vs Control				Pre-/post-treatment			
	$N_{studies}$	$N_{participants}$	*M*	*SD*	$N_{studies}$	$N_{participants}$	*M*	*SD*
Total Studies & Participants:	18	1273	71	78.9	22	535	24	33.8
Treatment Group:	18	675	37	40	22	535	24	33.8
Age (years):	18	1273	61.8	10.6	22	535	62.3	11.6
Sex:								
Male	15	700 (55%)	-	-	19	316 (59%)	-	-
Female		573 (45%)				219 (41%)		
Stroke type:								
Ischemic	18	917 (72%)			22	530 (99%)		
Haemorrhagic		353 (28%)	-	-		5 (1%)	-	-
Stroke Region:								
Mixed / Not specified	11	347 (27%)	-	-	11	179 (33%)	-	-
MCA	8	575 (45%)	-	-	12	346 (65%)	-	-
ICH	3	346 (27%)	-	-	1	1 (<1%)	-	-
ACA	1	5 (1%)	-	-	2	9 (1.7%)	-	-
Onset to Therapy (days):	18	675	8.1	8.9	22	535	10.8	15.1
Cell Therapies:								
SubCut / G-CSF	7	136	-	-	6	122	-	-
IV / G-CSF	2	193	-	-	3	211	-	-
IV / BM-MNC	2	72	-	-	5	98	-	-
IV / BM-MSC	2	46	-	-	3	58	-	-
IV + SubCut / BM-MSC + G-CSF	1	30	-	-	-	-	-	-
IC + IT / BM-MSC	1	110	-	-	-	-	-	-
IC / BM-MNC	1	60	-	-	-	-	-	-
IT / NSC	1	20	-	-	-	-	-	-
IA / BM-MNC	1	10	-	-	3	37	-	-
IA / BM-CD34+	-	-	-	-	1	5	-	-
IA / UC-MSC	-	-	-	-	1	4	-	-
Outcomes Assessed:								
Safety	18	1273	-	-	22	535		
Neurological	17	1130	-	-	19	374	-	-
Functional	14	798	-	-	12	149	-	-
Radiological	6	488	-	-	6	96	-	-
	$N_{studies}$	Phase / Study type			$N_{studies}$	Phase / Study type		
RCT	11	I/II x 1	II x 2	IIa x 2	8	I/II x 1	II x 1	IIa x 1
		IIb x 2	n.s. x 4			IIb x 2	n.s. x 3	
Non-RCT	7	I/II x 1	I/IIa x 1	n.s. x 5	10	I x 5	I/II x 2	I/IIa x 1
Observational					4	Cohort x 4		
	$N_{studies}$	Study Origin			$N_{studies}$	Study Origin		
Asia	12	China x 8 South Korea x 1	India x 2	Japan x 1	12	China x 5 Japan x 3	India x 3 South Korea x 1	
Europe	6	U.K. x 2 Russia x 1	Germany x 1 Europe x 1	Spain x 1	6	U.K. x 2 Spain x 1	Germany x 2 Europe x 1	
Other					4	Brazil x 3	U.S.A. x 1	

ACA = anterior circulation artery; BM-CD34$^+$ = bone marrow CD34$^+$ cells; BM-MNC = bone marrow mononuclear cells; BM-MSC = bone marrow mesenchymal stem cells; G-CSF = granulocyte-colony stimulating factor; IA = intra-arterial; IC = intracerebral; ICH = intracerebral haemorrhage; IT = intrathecal; IV = intravenous; *M* = mean; MCA = middle cerebral artery; n.s. = not specified; NSC = neural stem cells; *Nparticipant*s = number of participants assessed; *Nstudies* = number of studies; RCT = randomised controlled trial; *SD* = standard deviation; SubCut = subcutaneous; UC-MSC = umbilical cord mesenchymal stem cells.

As seen in Table 3-1, the Pre-/post-treatment analyses were based on 22 studies of 535 participants (which included 11 studies/323 participants from the preceding Post-treatment vs Control analysis. The average age was 62.3 years, 59% were male and most suffered ischaemic MCA strokes (65%). Cell therapies were administered, on average, 10.8 days post-stroke (acute phase) and, of the seven treatments trialed (all mono-therapies), intravenous injections of G-CSF were again most common ($N_{participants}$ = 211). Safety was again examined by all studies and most assessed neurological outcomes (86%), with fewer considering functional (55%) and radiological (27%) outcomes. Most of the data originated from non-RCTs (Phase I or II), largely undertaken in Asia.

For the Pre-/post-treatment vs Control analysis, standard-care control data ($N_{participants}$ = 196; see Supplementary Appendix E for details) were additionally sourced from six RCTs that reassessed a non-treatment group after a comparable interval in order to determine whether the Pre-/post-treatment effects experienced by cell therapy recipients exceeded what would be expected as a consequence of normal recovery. The average age of the standard-care control group was 65.9 years, 58% were male and 88% had ischaemic strokes. Standard care commenced, on average, 2.3 days post-stroke.

Reporting Quality

The reporting quality of all 25 studies was assessed. Eleven RCTs were evaluated using the CONSORT reporting guidelines (Moher et al., 2010): the mean score was 79% (range: 40–97%), with nine (82%) equating to high quality reports (scored > 75%). Individual item data are summarised in Figure 3-1A, where it can be seen that the research objectives (item 2b: 100%), eligibility criteria (item 4a: 100%), baseline demographics (item 15: 91%), effectiveness (item 17a: 100%), risks (item 19: 100%) and funding sources (item 25: 82%) were all frequently reported. By contrast, sample size calculations (item 7a: 36%),

randomisation methods (items 9–10: 45-55%) and limitations/biases (item 20: 55%) were provided less consistently.

In total, 14 non-RCT/prospective cohort studies were evaluated using the STROBE guidelines (Von Elm et al., 2008), with a mean score of 79% (range: 62–93%) and the majority (86%) equating to high-quality reports. As Figure 3-1(b) shows, studies consistently provided information about their research objectives (item 3: 100%), study design (item 4: 100%), eligibility criteria (item 6a: 86%), baseline demographics (item 14a: 93%), missing data (item 14b: 100%), key findings (item 8: 100%), generalisability (item 21: 93%) and funding sources (item 22: 79%), but frequently omitted measurement information (item 8a: 29%), potential biases (item 9: 36%) and sample size calculations (item 10: 14%).

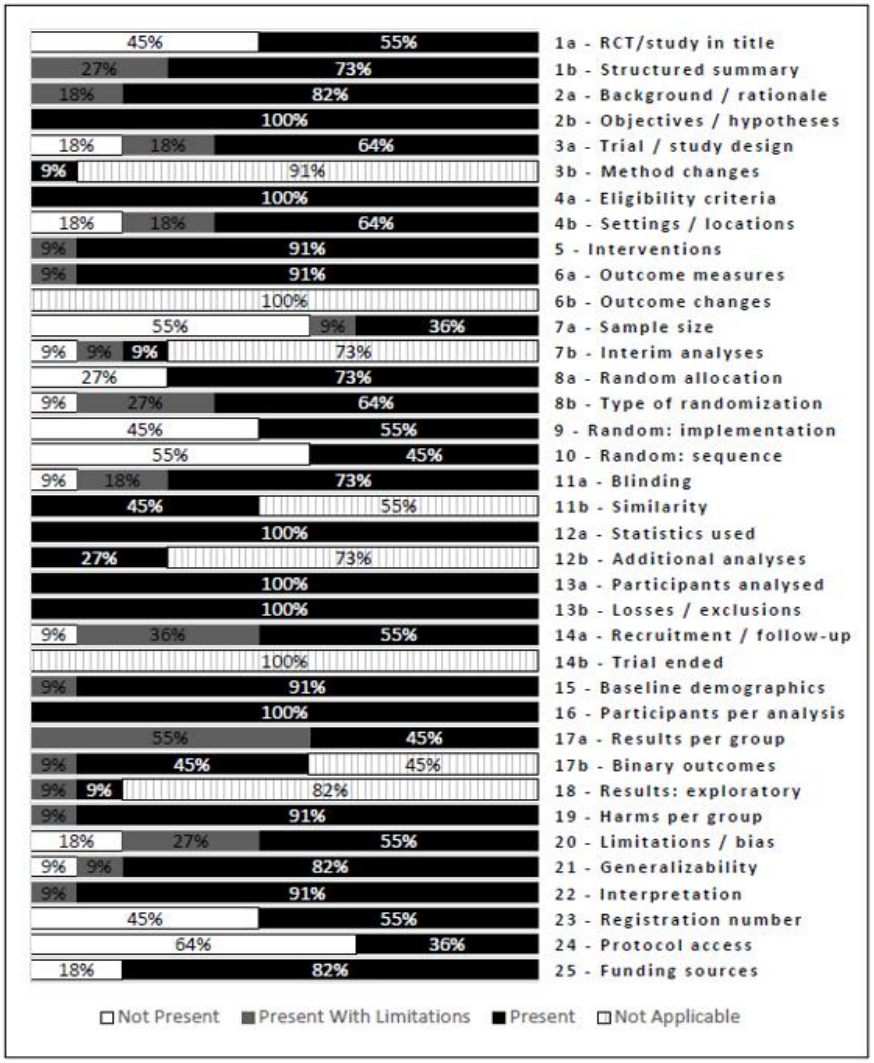

Figure 3-1(a)

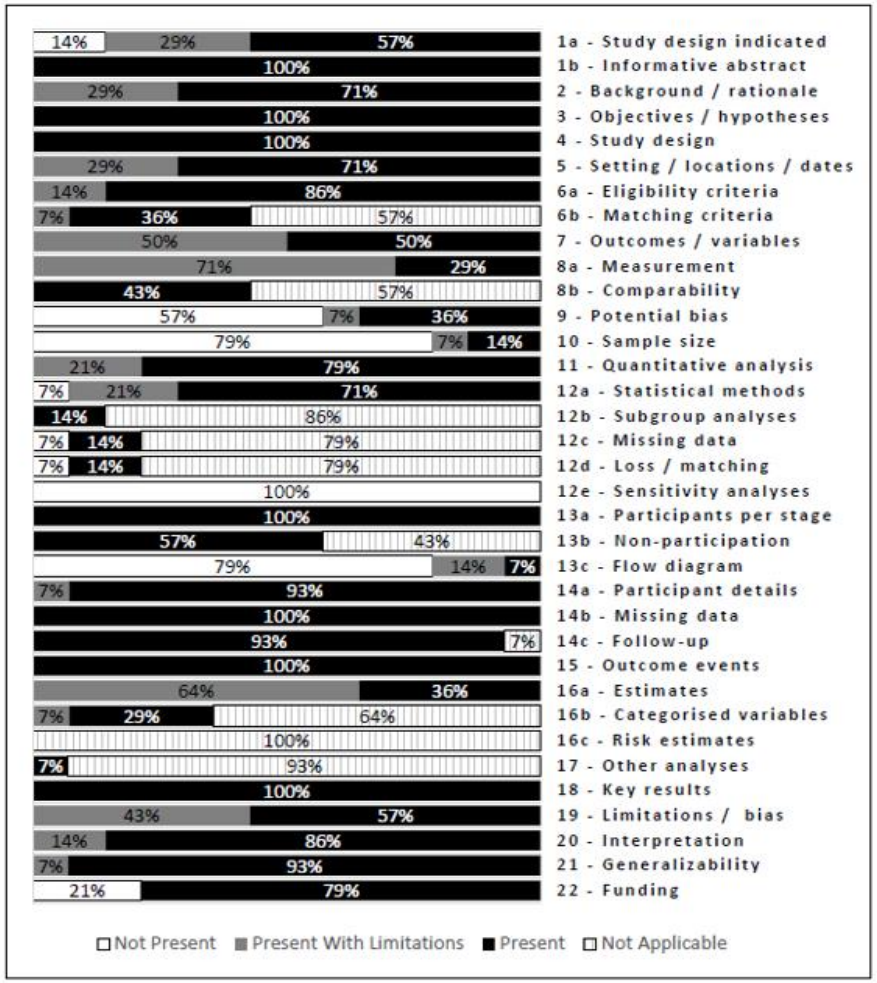

Figure 3-1(b)

Figure 3-1. Reporting quality assessment: (a) CONSORT evaluation of RCT study designs ($N_{studies}$ = 11) and (b) STROBE evaluation of non-RCTs and cohort study designs ($N_{studies}$ = 14).

Nstudies = number of studies; RCT = randomised controlled trial.

Adherence to Research Guidelines

All 25 studies were additionally evaluated against 13-key recommendations for cell-therapy research provided in the STEPS I–III (Reynolds, 2009; Savitz et al., 2011; Savitz et al., 2014) and RIGOR (Lapchak et al., 2013) guidelines. The average score was 43% (range: 19–77%), indicating relatively low adherence to the guidelines. As shown in Figure 3-2, very few studies used placebo/sham treatments (item 1: 20%), assessed psychological (item 4: 4%) or cognitive (item 5: 8%) comorbidities prior to inclusion, established stable neurological baselines (item 6: 8%) or labeled and tracked the cells (item 8: 8%). A higher number reported using physical rehabilitation with the cell therapy (item 9: 48%) and reported SAEs beyond 1 year (item 11: 32%), and most used neuroimaging to detect changes in the lesion/infarct size (item 7: 84%), reported significant and non-significant findings (item 12: 68%), and acknowledged conflicts of interest (item 13: 68%).

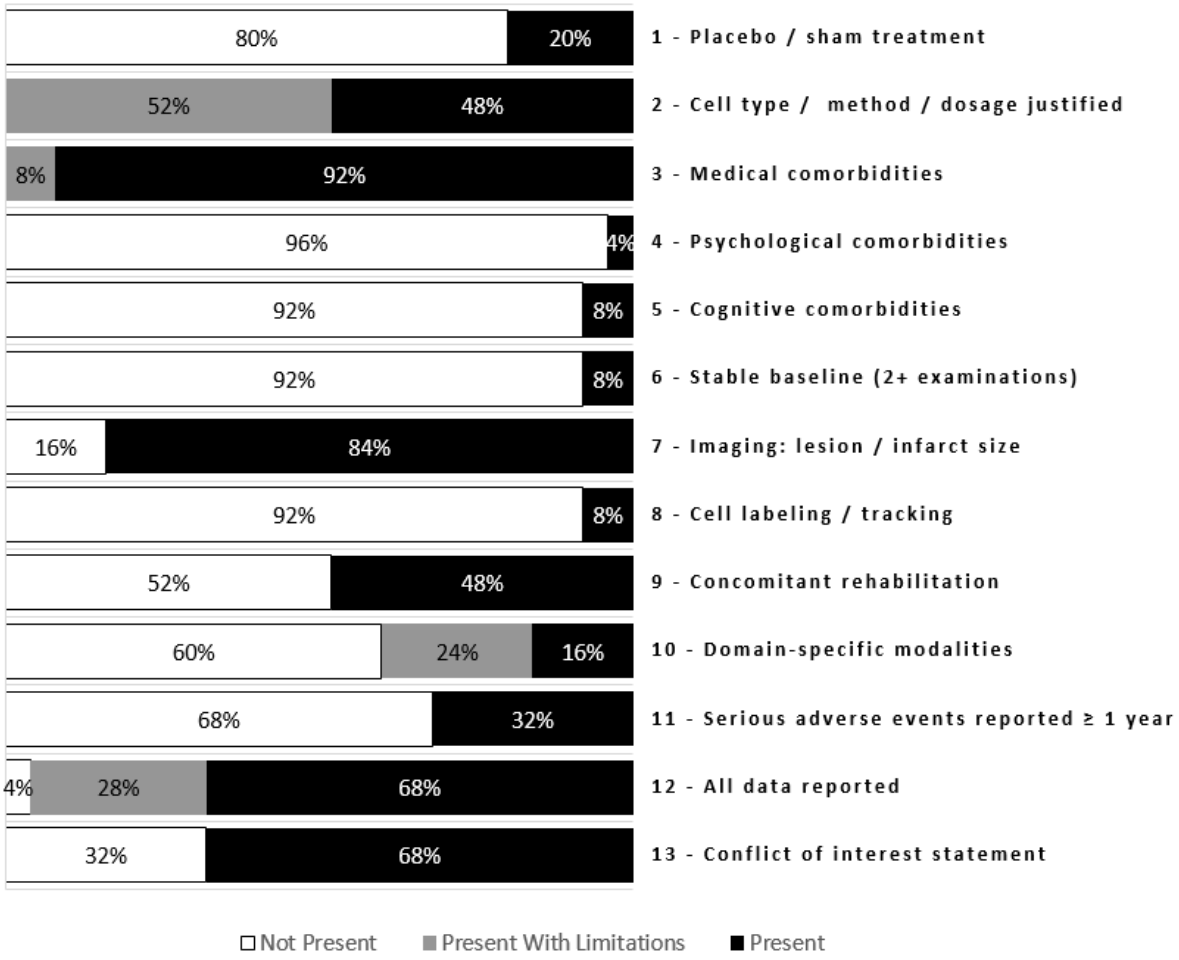

Figure 3-2. Percentage of studies ($N_{studies}$ = 25) meeting the STEPS (I-III) and RIGOR recommendations for cell-therapy research.

Safety and Efficacy of Cell Therapies

The safety and efficacy data for cell therapies administered in the hyper-acute, acute and sub-acute phases of stroke are summarised in Figure 3-3, with the Post-treatment versus Control and Pre-/post-treatment analyses grouped separately. For each of these analyses, the cell therapies are ordered (highest to lowest) according to their overall treatment effect, with individual therapies also being numbered (1 to 16, left-most column) to enable easy reference to specific findings. Study and participant details (age, stroke type/region, therapy/assessment timing), and safety and efficacy data are summarised for each treatment. Forest plots display the mean overall effect size and 95% CIs for each treatment. Mean differences in scores for the underlying measures (e.g., NIHSS) were additionally calculated when treatment effects were significant in order to provide clinically useful information (Cooper, 2008). Additional study information (e.g., number of injections and cells) is provided in Supplementary Appendix F.

	CELL THERAPY		STUDY DETAILS				TREATMENT GROUP						SAFETY		EFFICACY						FOREST PLOT
	Delivery Route	Cell Type	N Studies	Study Ref(s)	$N_{Participants}$ T/ment	Control	Age (M)	Stroke Type Isch	Stroke Type Hem	Stroke Region	Onset To Therapy (M Days / Range)	Assessment Point (Days)	Risk Ratio (95% CI)	Pooled Incidence (%) (95% CI)	Neuro g	p	Func g	p	Radio g	p	Overall g	p	Overall g + 95% CI
a	Post-treatment vs Control																						
1	IT	NSC	1	16	20	20	58.3	0	20	ICH	7 – 28	28	1.0 (0.0-48.1)		3.29	**			2.49	**	2.85	**	
2	IV + SubCut	BM-MSC + G-CSF	1	9	30	30	53.1	30	0	Mixed	23	180	1.0 (0.0-48.8)		3.19	**	1.97	**			2.56	**	
3	IV	BM-MSC	2	8, 9	35	55	54.2	35	0	Mixed	25	180 - 365	1.2 (0.9-1.7)		1.17		0.82	**	0.21	†	0.88	**	
4	IC	BM-MNC	1	15	60	40	56.3	0	60	ICH	6	180	0.7 (0.0-33.2)		0.45	*	0.47	*			0.46	**	
5	SubCut	G-CSF	7	9, 18-20, 22 - 24	134	97	62.0	128	6	Mixed	12	90 - 365	1.9 (0.7-5.2)		0.57		0.26	†	0.37	†	0.44	*	
6	IC + IT	BM-MSC	1	17	110	96	57.2	0	110	ICH	6	365	0.9 (0.0-43.6)		0.36	*	0.35	*			0.36	**	
7	IV	BM-MNC	2	1, 25	72	119	53.5	72	0	Mixed	16	180 - 365	1.9 (0.9-3.9)		0.39		0.25		0.12	†	0.24		
8	IV	G-CSF	1	2	124	128	69.1	124	0	MCA	≤ 9 hrs	30 - 90	1.2 (0.9-1.7)	†	-0.09				0.17		0.04		
9	IA	BM-MNC	1	13	10	10	66.9	10	0	MCA	5 – 9	180	5.0 (0.3-92.6)		0.01		0.00				0.00		
b	Pre-/post-treatment																						
10	IV	BM-MSC	3	6, 8, 9	47		55.5	47	0	Mixed	36	7 - 365	14%	† (7.4-25.7)	3.06	*	3.28	†	0.09	†	2.61	**	
11	SubCut	G-CSF	5	9, 18-20, 24	101		59.7	95	6	Mixed	12	90 - 365	1%	‡ (0.0-4.6)	2.56	**	3.10	† *	0.44	† *	2.46	**	
12	IV	G-CSF	2	2, 3	142		69.0	142	0	MCA	< 24 hrs	90	1%	† (0.0-4.1)	1.91	*	1.08	† **	-0.18	†	1.18		
13	IA	BM-CD34+	1	10	5		58.2	5	0	ACS	0 – 7	180	0% (0.0-43.5)		1.47	**			0.88		1.17	**	
14	IV	BM-MNC	4	4, 5, 12, 25	38		59.0	38	0	Mixed	13	30 - 180	11%	† (0.1-19.0)	1.08	**	1.51	**	-0.01	†	1.03	**	
15	IA	UC-MSC	1	11	4		49.0	3	1	MCA	26	180	0% (0.0-49.0)		0.72	*					0.72	*	
16	IA	BM-MNC	3	12 - 14	37		62.9	37	0	Mixed	18	180	11% (4.3-24.7)		0.62	**	0.70	† **			0.61	**	

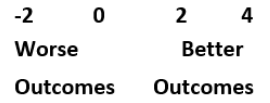

Figure 3-3. Safety and efficacy of cell therapy for hyper-acute, acute and sub-acute stroke, separated by delivery route and cell type for studies comparing (a) post-treatment and control outcomes and (b) pre- to post-treatment changes.

Notes: ** $p < .001$ * $p < .05$ The overall effect for standard care is shown by a dashed line on the forest plots for Treatments 10-16. †: $N_{studies}$ and $N_{participants}$ differs, refer to Appendix F for further details.

ACS = anterior circulation stroke; BM-CD34+ = bone marrow CD34+ cells; BM-MNC = bone marrow mononuclear cells; BM –MSC = bone marrow mesenchymal stem cells; CI = confidence interval; Func = functional; *g* = Hedges' g effect size; G-CSF = granulocyte-colony stimulating factor; Hem = haemorrhagic; hrs = hours; IA = intra-arterial; IC = intracerebral; ICH = intracranial haemorrhage; Isch = ischaemic; IT = intrathecal; IV = intravenous; *M* = mean; MCA = middle cerebral artery; Neuro = neurological; *Nparticipants* = number of participants; NSC = neural stem cells; *Nstudies* = number of studies; *p* = *p*-value; Radio = radiological; Ref = reference; SubCut = subcutaneous; T/ment = treatment; UC-MSC = umbilical cord mesenchymal stem cells.

Safety

As seen in Figure 3-3, the risk of experiencing an SAE varied considerably between the treatment and control groups (treatments 1–9; RR range: 0.7–5.0). Although none of the nine treatments resulted in a significantly higher risk of SAEs, some had very wide confidence intervals (treatments 1, 2, 4, 6, 9), indicating that these RR estimates were very imprecise and should be interpreted cautiously. Moreover, the risk of experiencing a SAE following intravenous BM-MNCs (treatment 7) approached significance ($p = 0.07$), based on 14 SAEs ($n = 8$ deaths; $n = 6$ seizures/strokes) in the treatment group, compared with 12 SAEs ($n = 5$ deaths; $n = 7$ seizures/strokes) in the control group (Prasad et al., 2014).

The pooled incidence rates for treatments 10–16 (Figure 3-3) indicate that SAEs were experienced by: 14% of patients who received intravenous BM-MSCs (treatment 10: $n = 8$ out of 58), 11% who received intravenous BM-MNCs (treatment 14: $n = 11$ out of 89), 11% who received intra-arterial BM-MNCs (treatment 16: $n = 4$ out of 37), 1% who received subcutaneous G-CSF (treatment 11: $n = 2$ out of 156) and 1% who received intravenous G-CSF (treatment 12: $n = 3$ out of 211). Of these SAEs, seizures and recurrent strokes were most common. No SAEs were reported following intra-arterial BM-CD34+ or UC-MSC injections (treatments 13 and 15). However, the CIs for each treatment were again found to be wide, which suggest these statistics lack precision and warrant caution.

Post-treatment versus Control Groups

When the treatment and control groups were compared (Figure 3-3: treatments 1–9), patients undergoing cell therapy experienced significantly better overall outcomes after treatment with: (1) intrathecal NSCs (treatment 1: resulting from very large improvements in neurological and radiological outcomes); (2) combined intravenous BM-MSC and subcutaneous G-CSF injections (treatment 2: resulting from very large improvements in neurological and functional outcomes); (3) intravenous BM-MSCs (treatment 3: resulting

from large improvements in functional outcomes); (4) intracerebral BM-MNCs (treatment 4: resulting from medium improvements in neurological and functional outcomes); (5) subcutaneous G-CSF (treatment 5: no significant effects for individual outcome areas); and (6) combined intracerebral and intrathecal BM-MSCs (treatment 6: resulting from small to medium improvements in neurological and functional outcomes). However, as the findings relating to treatments 4 and 6 were found to be at risk of publication bias (see Supplementary Appendix F), these findings should be interpreted tentatively.

In clinical terms, patients who received NSCs via intrathecal injection (treatment 1) scored an average of 6.3 points (95% CIs: 5.2–7.5) lower/better on the NIHSS and were observed to have mean hematoma volumes 12.8 ml (95% CIs: 9.7–16.0) smaller than the standard-care group. Patients treated with combined intravenous BM-MSC and subcutaneous G-CSF injections (treatment 2) scored an average of 25.7 points (95% CIs: 21.7–27.2) lower/better on the Fugl-Meyer Motor test and 21.7 points (95% CIs: 16.2–27.2) higher/better on the Functional Independence Measure (FIM). Patients who received intravenous BM-MSCs (treatment 3) scored an average of 8.9 points (95% CIs: 3.5–14.3) higher/better on the FIM and patients treated with intracerebral BM-MNCs (treatment 4) scored an average of 4.3 points (95% CIs: 0.5–8.0) lower/better on the NIHSS and 10.5 points (95% CIs: 1.6–19.4) higher/better than controls on the Barthel Index (note: these data were not available for treatments 5 and 6). No significant improvements were observed following intravenous BM-MNCs (treatment 7) or G-CSF (treatment 8) or intra-arterial BM-MNCs (treatment 9).

Pre-/post-treatment change

When the Pre-/post-treatment outcomes of cell therapy recipients were compared for treatments 10–16 (Figure 3-3), participants were found to have experienced significantly

better overall outcomes after treatment with: (1) intravenous BM-MSCs (treatment 10: resulting from very large improvements in neurological and functional outcomes); (2) subcutaneous G-CSF (treatment 11: resulting from large to very large improvements in neurological and functional outcomes and medium improvements in radiological outcomes); (3) intra-arterial BM-CD34+ cells (treatment 13: resulting from large improvements in neurological outcomes); (4) intravenous BM-MNCs (treatment 14: resulting from large improvements in neurological and functional outcomes); (5) intra-arterial UC-MSCs (treatment 15: resulting from large improvements in neurological outcomes); and (6) intra-arterial BM-MNCs (treatment 16: resulting from medium to large improvements in neurological and functional outcomes). Large neurological and functional improvements were also observed following intravenous G-CSF injections (treatment 12); however, the overall effect was non-significant. *Nfs* statistics confirmed a low risk of publication bias for each of these results ($N_{fs} > N_{Studies}$; see Supplementary Appendix F).

Lastly, changes in the standard-care control group (see Supplementary Appendix E) were assessed in order to evaluate the changes that occur as a result of normal recovery and physical rehabilitation. Standard care was associated with large to very large and significant improvements in overall (g = 1.44), neurological (g = 1.74) and functional (g = 1.27) outcomes. By contrast, there were only small and non-significant changes in radiological measures (g = -0.14). Notably, however, when the above Pre-/post-treatment findings were benchmarked against the changes seen in these standard-care controls in order to control for the effects of spontaneous recovery and physical rehabilitation, no cell therapy showed significantly better outcomes than standard care (note: the overall effect for standard care is shown as a dashed line in Figure 3-3 forest plots for treatments 10–16). However, there was a trend toward patients who received subcutaneous G-CSF (treatment 11) or intra-arterial BM-CD34+ cells (treatment 13) having better radiological outcomes (p = 0.08 and 0.05,

respectively) and for patients who received intra-arterial BM-MNCs (treatment 16) to show moderate neurological improvements ($p = 0.05$). No significant improvements were observed following intravenous BM-MSCs, G-CSF or BM-MNCs (treatments 10, 12, 14), and the neurological outcomes of patients who received intra-arterial delivery of umbilical cord MSCs were not significantly better than those seen in untreated patients (treatment 15). Nonetheless, these results should be interpreted cautiously because the standard-care control group had significantly more haemorrhagic strokes and/or were significantly older than some of the treatment groups (treatments 10, 11, 12, 14, 15).

Heterogeneity and Subgroup analyses

Given the small number of studies that examined individual treatments ($N_{studies} = 1–7$), it was not possible to perform individual heterogeneity analyses to evaluate the variability in the effect sizes from different studies (Higgins et al., 2003). However, significant variability was observed when examining the overall treatment effects for the Post-treatment vs Control ($Q[16] = 214.8$; $p < 0.001$; $I^2 = 92.6$ [95% CI: 89.6–94.7]) and Pre-/post-treatment ($Q[18] = 479.5$; $p < 0.001$; $I^2 = 96.3$ [95% CI: 95.2–97.1]) study designs, possibly due to methodological and/or sampling differences between studies. Subgroup analyses were therefore performed to determine whether study blinding, age, dosage, stroke-type or treatment timing contributed to this heterogeneity (see Table 3-2). As seen in Table 3-2, none of the subgroups differed significantly after Bonferroni corrections ($p > 0.01$), possibly due to the small number of studies in each analysis. However, a number of the results approached significance, suggesting there was a trend toward: (1) blinded studies reporting smaller treatment effects than non-blinded studies ($p = 0.02$); (2) younger patients (< 60 years) experiencing better outcomes than older patients (≥ 60 years; $p = 0.06$); and (3) higher dosages (≥ 10^8 cells) being more effective than lower doses (< 10^8 cells; see Supplementary Appendix F for study doses; $p = 0.09$).

Table 3-2. *Subgroup and Heterogeneity Analyses*

SUBGROUP			ANALYSIS	EFFICACY				HETEROGENEITY				
				$N_{studies}$	*g*	95% CIs	*p*	*Q*	*df*	*p*	I^2	95% CIs
(1)	Study Blinding:	None	Post-treatment vs Control	7	1.23	0.70 to 1.77		61.6	6	**	90.3	82.5 to 95.6
		Single / Double		10	0.37	-0.09 to 0.82		30.5	9	**	70.5	43.6 to 84.6
	Study Blinding:	None	Pre-/post-treatment	11	1.82	1.08 to 2.55		152.3	10	**	93.4	90.1 to 95.6
		Single / Double		8	1.54	0.70 to 2.39		127.5	7	**	94.5	91.3 to 96.5
(2)	Age:	< 60 years	Post-treatment vs Control	10	1.03	0.56 to1.49		90.5	9	**	90.1	83.9 to 93.9
		≥ 60 years		7	0.29	-0.29 to 0.86		7.2	6		17.4	0.0 to 61.3
	Age:	< 60 years	Pre-/post-treatment	10	2.18	1.40 to 2.96		158.8	9	**	94.3	91.5 to 96.2
		≥ 60 years		9	1.20	0.40 to 2.00		133.6	8	**	94.0	90.7 to 96.2
(3)	Treatment	< 10^8 cells	Post-treatment vs Control	10	0.46	-0.02 to 0.94		35.4	9	**	74.6	52.5 to 86.4
	Dosage:	≥ 10^8 cells		7	1.09	0.54 to 1.64		69.0	6	**	91.3	84.7 to 95.1
	Treatment	< 10^8 cells	Pre-/post-treatment	12	1.72	1.03 to 2.41		189.8	11	**	94.2	91.6 to 96.0
	Dosage:	≥ 10^8 cells		7	1.66	0.75 to 2.56		84.9	6	**	92.9	87.9 to 95.9
(4)	Stroke Type:	Ischaemic	Post-treatment vs Control	13	0.69	0.23 to 1.16		88.2	12	**	86.4	78.4 to 91.4
		Haemorrhagic		3	1.11	0.18 to 2.04		29.1	2	**	93.1	83.3 to 97.2
	Stroke Type:	Ischaemic	Pre-/post-treatment	17	1.83	1.23 to 2.44		268.7	16	**	94.0	91.9 to 95.7
		Haemorrhagic		0	-	-		-	-	-	-	-
(5)	Stroke Phase:	Hyper/Acute	Post-treatment vs Control	16	0.74	0.36 to 1.12		119.4	15	**	87.4	81.2 to 91.6
		Sub- Acute		1	0.50	-1.15 to 2.16		0.0	0		0.0	0.0 to 0.0
	Stroke Phase:	Hyper/Acute	Pre-/post-treatment	15	1.82	1.20 to 2.44		287.4	14	**	95.1	93.3 to 96.5
		Sub- Acute		4	1.23	0.02 to 2.44		3.9	3		22.	50.0 to 89.0

Note: ** $p < .001$, * $p < .05$.

CI = confidence interval; *df* = degrees of freedom; *g* = Hedges' *g* effect size; I^2 = I^2 index; $N_{studies}$ = number of studies; *Q* = *Q* statistic.

3.7 Discussion

The present study evaluated the reporting quality and findings of 25 studies that used cell therapies within 90 days of stroke onset. Based on the CONSORT (Moher et al., 2010) and STROBE (Von Elm et al., 2008) reporting guidelines, the quality of reporting was generally very good. Similar strengths were observed across all study designs, with the research objectives, eligibility criteria, baseline demographics and key results all consistently provided. However, statistical power, methods of randomisation and study limitations/biases were provided less consistently, highlighting potential areas for improvement. Given that these were Phase I and II trials, which focus on safety, statistical power may be a lower priority; but this also impacts on any evaluation of efficacy (Von Elm et al., 2008). Randomisation details, on the other hand, are needed to identify potential sources of bias in participant selection and group allocation (Moher et al., 2010).

When the studies were evaluated against STEPS (Reynolds, 2009; Savitz et al., 2011; Savitz et al., 2014) and RIGOR (Lapchak et al., 2013) recommendations for cell therapy research, compliance was found to be quite low. Most notably, few studies labeled and tracked the cells. Although aspirational, given currently available technologies, cell labeling and tracking is an important to our understanding of the mechanisms that underpin treatment effects (Savitz et al., 2014). Indeed, preliminary research suggests that cell uptake in the brain may be minimal, compared with other major organs (liver, lungs, spleen, kidneys), especially when delivered via intravascular routes (Boltze et al., 2015), highlighting the importance of labeling/tracking cells. As more cost-effective tracking techniques (e.g., optical imaging) are refined for use in clinical studies (Gavins & Smith, 2015), *in vivo* migration, localisation and differentiation of cells will increasingly be monitored, thereby enabling a more comprehensive examination of different cell types, delivery routes and dosages.

Similarly, few studies used placebo/sham controls. Although not surprising, given ethical constraints, such controls are needed to definitively rule out placebo effects (Savitz et al., 2014).

Controls are particularly important in the current context because treatment effects may be mediated by patient expectations and/or the research/hospital setting (Wager & Atlas, 2015), with invasive surgical procedures (e.g., intracerebral injections) potentially being more prone to placebo effects than other treatments (Diederich & Goetz, 2008). Sham stereotactic procedures, involving the injection of saline solutions, have been used previously (Li et al., 2013; Zhu et al., 2013), but are controversial and may also not meet ethics regulations. At a minimum, an untreated stroke group would control for improvements attributable to standard care, normal recovery and any practice effects that may arise from the repeated administration of an outcome measure (Savitz et al., 2014).

Lastly, contrary to STEPS and RIGOR recommendations, few studies considered psychological and/or cognitive co-morbidities as part of their inclusion/exclusion criteria. Given that patients with depression or dementia commonly experience poorer outcomes after stroke (Cramer, 2008), the failure to screen for these conditions may undermine the study findings (Savitz et al., 2014). Similarly, changes to psychological and cognitive outcomes were rarely evaluated; consequently the broader impact of cell therapies on patients' functioning and wellbeing remains to be determined. Anti-depressant medications and cognitive rehabilitation both improve stroke outcomes (Langhorne, Bernhardt, & Kwakkel, 2011; Mead et al., 2013), raising the possibility of combined treatment approaches.

When evaluating the safety and efficacy of the cell therapies, treatment-related SAEs were observed after five of the eleven treatments and six were associated with significantly better outcomes than standard care alone. For haemorrhagic stroke, the most effective therapy involved the intrathecal delivery (lumbar puncture) of NSCs (two injections of 4.0×10^8 cells for 2 weeks) (Xue et al., 2014), with significant improvements evident in motor/sensory/speech functions and a reduction in lesion size. However, given outcomes were assessed after only 28 days, it is unlikely that neural regeneration would explain these improvements (Janowski, Wagner, & Boltze, 2015). Instead, preclinical research suggests that the transplanted cells may have prevented further damage by secreting a range of growth (VEGF, NGF and neurotrophic [e.g., BDNF]) factors (Horie et al.,

2011; Ramos, Cabrer, Justicia, Widermann, & Hoehn, 2010). Although no SAEs were observed, an accurate determination of the risks was not possible using the available data and, as such, the long-term safety has yet to be determined.

For ischaemic stroke, the data indicated that combined subcutaneous G-CSF (Filgrastim; one injection of 150 μg/kg) and intravenous BM-MSCs (one injection of 2.97×10^9 cells [mean dosage]) are most effective (Meng et al., 2009), with recipients experiencing better motor/sensory functioning and greater physical independence at 6 months. Preclinical research suggests that G-CSF promotes neuroprotection by activating anti-apoptotic and anti-inflammatory signaling pathways (Solaroglu, Digicaylioglu, Keles, & Zhang, 2015) and, when combined with BM-MSCs, mobilises cells to the lesion where they stimulate endogenous repair (angio/neurogenesis) (dela-Peña & Borlongan, 2015). Interestingly, recipients of the combined BM-MSC/G-CSF therapy were also found to have experienced significantly better outcomes than patients receiving BM-MSCs or G-CSF treatments, challenging the findings of recent preclinical research (Balseanu et al., 2014). Determining the optimal dosages (Wagner et al., 2014) and timing of the combined treatments (Pösel et al., 2014) will further clarify the extent of any benefits. Preliminary findings also suggest that the treatment is relatively safe (based on the available 6-month data) and, given autologous BM-MSCs are typically used, may be preferable to allogeneic alternatives (requiring immunosuppression) in older patients.

Smaller treatment effects were also observed for persons with a haemorrhagic stroke who received an intracerebral injection of BM-MNCs (one injection of 3.79×10^9 cells [mean dosage]) or combined intracerebral and intrathecal injections of BM-MSCs (one injection per route of 8.47×10^7 cells [mean dosage]) (Li et al., 2013; Zhu et al., 2010). Despite the risks often associated with these procedures, no complications were observed. Importantly, experimental research indicates BMMNCs are smaller than NSCs and MSCs, which may lower the risk of further blockages to the cerebral arteries or ventricles (Fischer et al., 2009). More research is needed to assess the efficacy of the remaining five cell therapies (Valentine et al., 2010), with treatments involving the intra-

arterial delivery of BM-CD34+ cells warranting particular attention, given the observed reduction in lesion size. In addition, the long-term risks of all cell therapies require monitoring as few studies reported SAEs beyond 1 year. Although initial findings suggest intravenous delivery of BM-MNCs and BM-MSCs may pose the greatest risk, more data are needed.

The subgroup analyses yielded some additional interesting information. First, there was a trend toward non-blinded research yielding larger treatment effects, highlighting the need for double-blind, randomised, placebo-controlled trials, wherever possible. Second, there was also a trend toward younger patients (< 60 years) having better outcomes, possibly due to their better general health and/or greater engagement in rehabilitation (Kitago & Marshall, 2015), or because these therapies augment the greater neuroplasticity seen in younger patients (Cramer, 2008). Additional research is therefore needed to determine whether there is an optimum age for cell therapies or an age beyond which efficacy is reduced. Third, there was a trend toward higher doses ($\geq 10^8$ cells) being more effective, although this may impact on the safety and tolerability of these therapies. By contrast, it was unclear whether cell therapies were more effective following haemorrhagic or ischaemic strokes, or when administered in the hyper/acute (< 1 month) or sub-acute phases (1–3 months), largely due to the small number of studies that could be compared in these analyses.

The aforementioned findings must be tempered by a number of limitations. First, the treatment effects calculated by the current meta-analysis were largely based on the short-term findings (< 365 days) of small treatment groups (individual studies: $N_{participants}$ = 4–252; individual treatments: $N_{participants}$ = 4–211), making it difficult to accurately evaluate their safety and efficacy. A conservative random-effects model was used to account for the anticipated heterogeneity in treatment effects (DerSimonian & Laird, 1986); however, a high level of variability remained, highlighting the need for research that is based on more homogenous samples (e.g., same stroke type/region, narrow age range) and assessment points (Higgins et al., 2003; Xu et al., 2008). Second, the controls that were used to evaluate the Pre-/post-treatment effects of cell therapy

recipients were not matched with the treatment groups in these analyses and, consequently, sometimes differed in terms of age and stroke type. Matched control groups (sham or non-treatment/standard-care controls) provide a more reliable assessment of treatment efficacy but were not always available. Although not ideal, this solution was adopted to reduce the likelihood that treatment effects would be overestimated. Finally, potentially relevant studies were excluded because the information they provided was insufficient for current purposes: ten authors were contacted, but only one responded (Moniche et al. 2014).

Conclusion

The current findings suggest that a range of mono/combined cell therapies significantly improve acute stroke outcomes; however, a number resulted in treatment-related SAEs. Most notably, the data suggest that the intrathecal delivery of NSCs and combined subcutaneous G-CSF and intravenous BM-MSCs appear very promising for the treatment of acute haemorrhagic and ischaemic stroke, respectively. However, additional adequately powered double-blinded placebo-/sham-/non-treatment-controlled research that screens for psychological/cognitive co-morbidities, assesses a broader range of outcomes (neurological, functional, radiological, cognitive, psychological) and tracks cell migration *in vivo,* is now needed to provide a more definitive evaluation of these emerging treatments. In terms of safety, the data suggest intravascular approaches are riskier than standard care, although the long-term risks of all treatments require continued monitoring. The origin of the cells, the cell sorting process and the standards for cell transplantation safety should all be reported in future trials, given the risks associated with uncontrolled transplantation procedures (Amaraglio et al., 2009; Berkowitz et al., 2016; Hurst et al., 2013). Some patients may also benefit more than others, depending on their age, and alternative dosages or combined approaches may also prove effective, but need further investigation.

As the safety and efficacy of cell therapies for acute/sub-acute stroke becomes clearer, the next challenge will be to integrate these treatments into existing paradigms of care (e.g., intravenous/chemical thrombolysis, mechanical thrombectomy, decompressive surgery). Although

the current findings suggest that cell therapies administered in the hyper-acute phase may reduce secondary damage and that cells administered in the acute/sub-acute stages may improve neurological, functional and radiological outcomes, some treatments resulted in seizures, tumors and further strokes. While it is likely that treatments administering non-immunogenic (autologous) cells via intravenous, intra-arterial and subcutaneous routes will be the easiest to implement within clinical settings, the current findings suggest non-invasive procedures still involve significant risks and, as such, ongoing caution should be exercised.

Future Perspective

Given the ever-increasing medical and economic burden associated with stroke, continued research into emerging treatments is needed. In excess of 50 clinical trials are currently underway (as registered on clinicaltrials.gov) and, as the safety of individual treatments are more definitively established, larger-scale Phase III trials will occur. The refinement of cell labeling and tracking techniques should improve the *in vivo* monitoring of cells, elucidating the mechanisms of action. Alternate pluripotent cell sources (e.g., CTX0E03 cells; induced pluripotent SCs) will help to overcome the ethical and practical concerns regarding embryonic/fetal NSCs, and the modification of BM-MSCs (Steinberg et al., 2016) may provide a more accessible source of multipotent cells for use in the hyper-acute/acute phases of stroke. Less invasive delivery methods also need to be investigated (e.g., intranasal) to mitigate the risks associated with acute/sub-acute stroke. Cell therapies may also be augmented through the harnessing of glial cells, blood–brain barrier permeabilisers, biobridges, extracellular scaffolds and psychological/cognitive rehabilitation.

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* *Denotes studies that were meta-analysed*

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APPENDIX A

Search Terms used to create Database Specific Logic Grids (Study 1 & 2)

AND → AND → AND

Stroke	Stem cell therapy	Delivery method
Stroke*	Stem cell	Stereotaxic
OR	Autologous	Stereotactic
Cerebrovascular accident*	Allogenic	Neurotransplant
Cerebral vascular accident*	Mesenchymal	Transplant*
Ischem*		Neurosurg*
Ischaem*		
Infarct		

Note: Truncating terms with an asterisk results in variations of the term (including the plural forms) being included in the search.

PubMed Logic Grid

Stroke	Stem cell therapy	Delivery method
stroke[mh] or stroke[tw] or cerebrovascular accident*[tw] or cerebro-vascular accident*[tw] or cerebral-vascular accident*[tw] or cva[tw] or brain ischaemia[tw] or brain ischemia[tw] or infarct*[tw]	stem cell transplantation[mh] or stem cell*[tw] or bone marrow transplantation[mh] or bone marrow transplant*[tw] or mesenchymal*[tw]	neurosurg*[tw] or neurotrans*[tw] or stereotaxic techniques[mh] or stereotaxic[tw] or stereotactic[tw] or transplant*[tw]

Cochrane Library Logic Grid

Stroke	Stem cell therapy	Delivery method
Stroke	Stem cells	-

Embase Logic Grid

Stroke	Stem cell therapy	Delivery method
‘stroke’:de,ti,ab or ‘brain ischemia’/syn or ‘cerebrovascular accident’/syn or ‘cerebro vascular accident’:de,ti,ab or ‘cerebro vascular accidents’:de,ti,ab or ‘cerebrovascular accident’:de,ti,ab or ‘cerebrovascular accidents’:de,ti,ab or ‘cva’:de,ti,ab or ‘infarct’:de,ti,ab	‘stem cell transplantation’/syn or ‘stem cell’/syn or ‘stem cells’:de,ti,ab or ‘mesenchymal’:de,ti,ab or ‘autologous’:de,ti,ab or ‘allogenic’:de,ti,ab	‘stereotaxic surgery’/syn or ‘sterotactic’:de,ti,ab or ‘neurosurgery’/syn or 'transplant':de,ab,ti or ‘neurotransplant’:de,ti,ab

Web of Science Core Collection Logic Grid

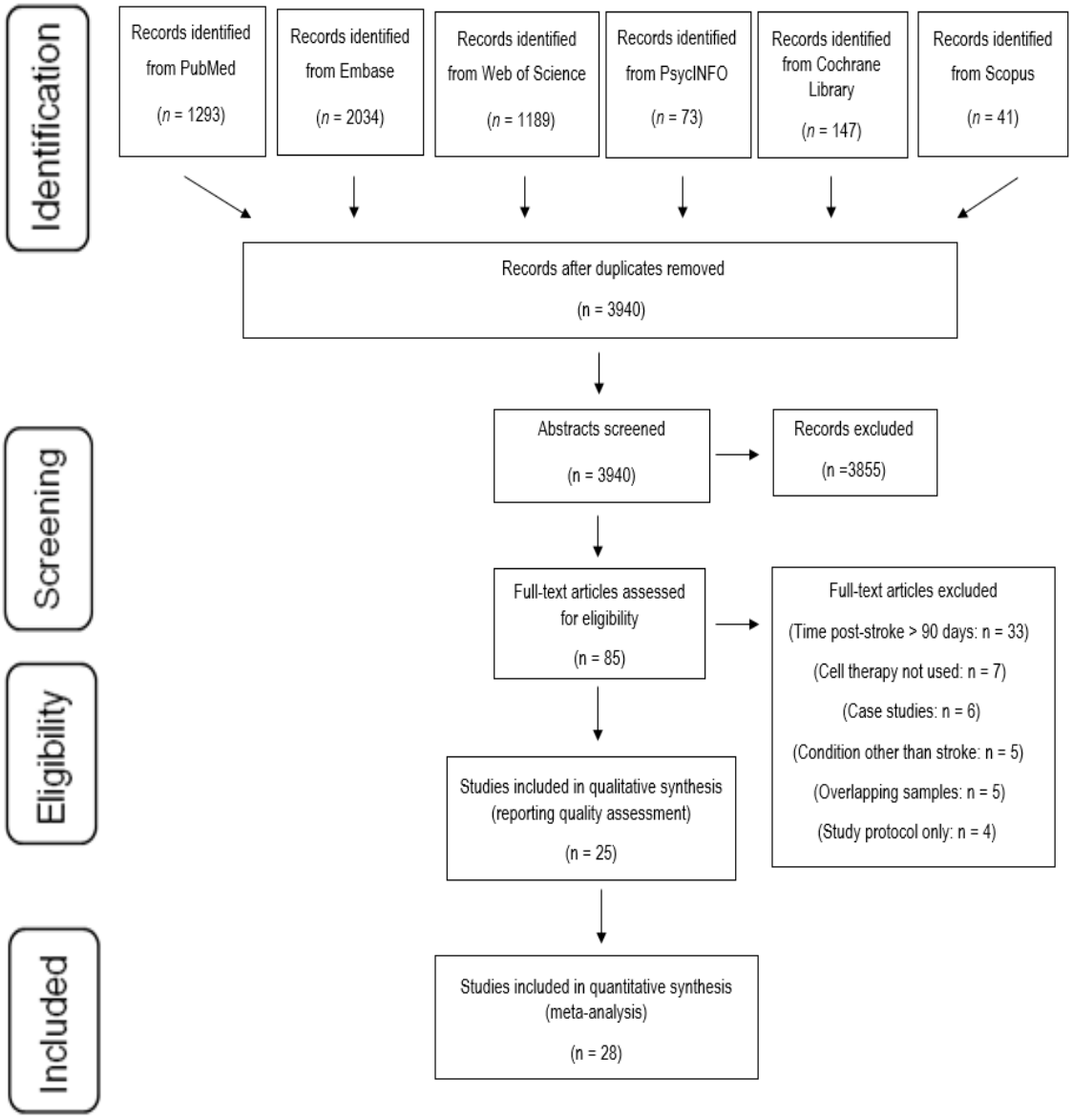
Stroke	Stem cell therapy	Delivery method
TS=(stroke* OR cerebrovascular accident OR cerebro-vascular accident* OR cerebro vascular accident* OR cerebral-vascular accident* OR cerebral vascular accident OR cva OR infarct* OR ischem* OR ischaem*)	TS=(stem cell* OR autologous OR allogenic OR mesenchymal)	TS=(neurosurg* OR stereotaxic OR stereotactic or transplant*)

PsycINFO Logic Grid

Stroke	Stem cell therapy	Delivery method
stroke.TW OR exp cerebral ischemia OR exp cerebrovascular accident OR cerebrovascular accident*.TW OR cerebro vascular accident*.TW OR cerebralvascular accident*.TW OR cerebral vascular accident*.TW OR cva.TW OR exp cerebral ischemia OR infarct*.TW	exp stem cells OR autologous.TW OR allogenic.TW OR mesenchymal.TW	exp neurosurgery OR exp stereotaxic techniques OR sterotactic.TW OR transplant*.TW

APPENDIX B

PRISMA Flow Chart, detailing Study Selection Process for Study 1



APPENDIX C

Publication Details of the Studies used in the Meta-Analysis for Study 1

Study reference	First Author	Last Author	Publication Year	Country of Origin	Study Type	Publication Source
1	Prasad, K.	Nityanand, S.	2014	India	RCT – Phase II	Stroke
2	Ringelstein, E.B.	Berrouschot, J.	2013	Europe	RCT – Phase IIb	Stroke
3	Moriya, Y.	Takizawa, S.	2013	Japan	Non-RCT – Phase I	Journal of Cerebrovascular Diseases
4	Prasad, K.	Mishra, N.K.	2012	India	Non-RCT – Phase I	Indian Journal of Medical Research
5	Savitz, S.I.	Grotta, J.C.	2011	U.S.A.	Observational -Prospective Cohort	Annals of Neurology
6	Honmou, O.	Kocsis, J.D.	2011	Japan	Observational - Prospective Cohort	Brain
7	Schabitz, W.R.	Schneider, A.	2010	Germany	RCT – Phase IIa	Stroke
8	Lee, J.S.	Bang, O.Y.	2010	South Korea	RCT – Phase I / II	Stem Cells
	Bang, O.Y.	Lee, G.	2005	South Korea	RCT – Phase I / II	Annals of Neurology
9	Meng, X.G.	Li, D.	2009	China	Non-RCT–Phase n.s.	Journal of Clinical Rehabilitative Tissue Engineering Research
10	Banerjee, S.	Chataway, J.	2014	U.K.	Non-RCT – Phase I	Stem Cells Translational Medicine
11	Jiang, Y.	Liu, X.	2013	China	Observational - Prospective Cohort	Cell Transplantation
12	Rosado-de-Castro, P.H.	Barbosa da Fonseca, L.M.	2013	Brazil	Non-RCT – Phase I	Regenerative Medicine
	Battistella, V.	Andre, C.	2011	Brazil	Non-RCT – Phase I	Regenerative Medicine
	Barbosa da Fonseca, L.M.	Freitas, G. R.	2010	Brazil	Non-RCT – Phase I	Experimental Neurology
13	Moniche, F.	Gil-Peralta, A.	2014	Spain	Non-RCT – Phase I/II	Stroke
	Moniche, F.	Gonzalez, A.	2012	Spain	Non-RCT – Phase I/II	Cell Transplantation
14	Friedrich, M.A.G.	De Freitas, G.R.	2012	Brazil	Observational - Prospective Cohort	Cell Transplantation
15	Li, Z.M.	Wang, L.X.	2013	China	Non-RCT– Phase n.s.	Clinical Neurology and Neurosurgery
	Zhu, J.X.	Guo, C.J.	2010	China	Non-RCT– Phase n.s.	Journal of Clinical Rehabilitative Tissue Engineering Research
16	Xue, Y.Z.	Hao, P.L.	2014	China	RCT – Phase n.s.	Genetics and Molecular Research
17	Zhu, J.X.	Geng, F.	2015	China	Non-RCT – Phase n.s	Stem Cells International
18	England, T.J.	Bath, P.M.W.	2012	U.K.	RCT – Phase IIb	Stroke
19	Prasad, K.	Mishra, N.K.	2011	India	RCT – Phase n.s.	Stroke Research and Treatment
20	Chen, Y.H.	Liu, X.L.	2011	China	RCT – Phase n.s.	Journal of Clinical Rehabilitative Tissue Engineering Research
21	Boy, S.	Bogdahn, U.	2011	Germany	Non-RCT– Phase I/II	PLoS ONE
22	Alasheev, A.M.	Isakova, T.M.	2011	Russia	RCT – Phase II	Translational Stroke Research
23	Sprigg, N.	Russell, N.	2006	U.K.	RCT – Phase IIa	Stroke
24	Shyu, W.C.	Li, H.	2006	Taiwan	RCT – Phase n.s.	Canadian Medical Association Journal
25	Taguchi, A.	Nagatsuka, K.	2015	Japan	Non-RCT–Phase I/IIa	Stem Cells and Development

Note: Brackets indicate where pairs of studies reporting on the same patient group were combined for parts of the analysis.

n.s. = not specified; RCT = randomised controlled trial.

APPENDIX D

Stem Cell Therapy Research Guidelines (STEPS I-III, RIGOR) Assessment Checklist

1. Placebo / sham treatment employed
2. Cell therapy (cell type, delivery method and dosage) justified by previous research
3. Medical comorbidities considered prior to inclusion
4. Psychological comorbidities considered prior to inclusion
5. Cognitive comorbidities considered prior to inclusion
6. Stable baseline established based on 2 or more examinations
7. Imaging employed to assess lesion/infarct size
8. Cell tracking / labelling undertaken to observe mechanisms of action
9. Concomitant physical rehabilitation undertaken by participants
10. Domain-specific modalities (e.g., sensory, motor, visual, cognitive functions) assessed with validated measures
11. Serious adverse events (SAEs) reported ≥ 1 year
12. All statistical data reported (including non-significant findings)
13. Conflict of interest statement provided

APPENDIX E

Study 1: Baseline Characteristics of the Control Group for Treatment vs Control: Pre-/Post-Analysis (*N* = 196)

Age *M (SD)*	Male Gender (%)	Stroke Type		Stroke Area	Onset to randomisation *M days (SD)*	NIHSS baseline *M (SD)*	Received Physical Rehabilitation (%)
		Isch	Haem				
65.9 (9.17)	58	173	23	MCA = 156 ICH = 20 Anterior = 12 Lacunar = 5	2.3 (3.86)	13.4 (4.5)	13

Haem = haemorrhagic; ICH = Intracerebral Haemorrhage; Isch = ischaemic; MCA = Middle Cerebral Artery; *M* = mean; *SD* = standard deviation.

Study 1: Publication Details of the Studies used for the Control Group for Treatment vs Control: Pre-/Post-Analysis

Study reference	First Author	Last Author	Publication Year	Country of Origin	Study Type	Publication Source
2	Ringelstein, E.B.	Berrouschot, J.	2013	Europe	RCT – Phase IIb	Stroke
16	Xue, Y.Z.	Hao, P.L.	2014	China	RCT – Phase n.s.	Genetics and Molecular Research
18	England, T.J.	Bath, P.M.W.	2012	U.K.	RCT – Phase IIb	Stroke
19	Prasad, K.	Mishra, N.K.	2011	India	RCT – Phase n.s.	Stroke Research and Treatment
20	Chen, Y.H.	Liu, X.L.	2011	China	RCT – Phase n.s.	Journal of Clinical Rehabilitative Tissue Engineering Research
24	Shyu, W.C.	Li, H.	2006	Taiwan	RCT – Phase n.s.	Canadian Medical Association Journal

RCT = randomised controlled trial.

APPENDIX F

Treatment Dosages per Cell Therapy and Notes relating to Figure 3-1

	CELL THERAPY		DOSAGE		SAFETY		OUTCOMES				NOTES
	Delivery Route	*Cell Type*	*No. of Injections*	*No. of Cells (Range)*	*Risk Ratio (95% CI)*	*Pooled Incidence (%)*	*Neuro g p*	*Func g p*	*Radio g p*	*Overall g p*	
a	Post-treatment vs Control										
1	IT	NSC	8	4×10^8							
2	IV + SubCut	BM-MSC + G-CSF	1 1	29.7×10^8 150 ug/kg							
3	IV	BM-MSC	1 2	3×10^7 $1 - 5 \times 10^7$					0.21[a]		a: N*studies* = 1, T/ment = 5, Control = 25, Study ref = 8
4	IC	BM-MNC	1	3.79×10^9							
5	SubCut	G-CSF	1 – 5	10 - 150 ug/kg				0.26[b]	0.37[c]		b: N*studies* = 6, T/ment = 114, Control = 77, study refs = 9, 18, 19, 22 - 24 c: N*studies* = 3, T/ment = 30, Control = 16, study refs = 18, 22, 24
6	IC + IT	BM-MSC	2	8.47×10^7							
7	IV	BM-MNC	1	$2.5 – 3.4 \times 10^8$					0.12[d]		d: N*studies* = 1, T/ment = 32, Control = 27, study ref = 1
8	IV	G-CSF	1	135 ug/kg	1.2 (0.9-1.7)[e]						e: N*studies* = 2, N*participants* = 193, study refs = 2, 7
9	IA	BM-MNC	1	1.59×10^8							
b	Pre-/post-treatment										
10	IV	BM-MSC	1 2	29.7×10^8 0.5×10^8		14%[f]		3.28[g]	0.09[h]		f: N*studies* = 3, N*participants* = 58, study refs = 6, 8, 9 g: N*studies* = 2, N*participants* = 35, study refs = 8, 9 h: N*studies* = 1, N*participants* = 12, study ref = 6
11	SubCut	G-CSF	1 – 5	10 - 150 ug/kg		1%[i]		3.10[j]	0.44[k]		i: N*studies* = 8, N*participants* = 156, study refs = 9, 18 – 24 j: N*studies* = 3, N*participants* = 41, study ref = 9, 19, 24 k: N*studies* = 1, N*participants* = 14, study ref = 18
12	IV	G-CSF	1 - 5	135 - 450 ug/kg		1%[l]		1.08[m]	-0.18[n]		l: N*studies* = 3, N*participants* = 211, study refs = 2, 3, 7 m: N*studies* = 1, N*participants* = 18, study refs = 3 n: N*studies* = 1, N*participants* = 18, study refs = 3
13	IA	BM-CD34$^+$	1	2.2×10^6							
14	IV	BM-MNC	1	$0.09 – 3.4 \times 10^8$		11%[o]			-0.01[p]		o: N*studies* = 5, N*participants* = 98, study refs = 1, 4, 5, 12, 25 p: N*studies* = 2, N*participants* = 21, study refs = 4 - 5
15	IA	UC-MSC	1	2×10^7							
16	IA	BM-MNC	1	$1.59 – 3.2 \times 10^8$				0.70[q]			q: N*studies* = 2, N*participants* = 17, study refs = 12, 13

*Note: **p < .001, *p < .05.*

BM-CD34+ = bone marrow CD34+ cells; BM-MNC = bone marrow mononuclear cells; BM –MSC = bone marrow mesenchymal stem cells; CI = confidence interval; Func = Functional; G-CSF = granulocyte -colony-stimulating factor; IA = Intra-arterial; IC = Intracerebral; IT = Intrathecal; IV = Intravenous; kg = kilogram; Neuro = Neurological; *Nparticipant*s = number of participants assessed; NSC = neural stem cells; *Nstudies* = Number of studies; Radio = Radiological; Ref = reference; SubCut = Subcutaneous; T/ment = treatment; UC-MSC = umbilical cord mesenchymal stem cells; ug = microgram.

Chapter 4. Study 2

Cell therapies administered in the chronic phase after stroke: A meta-analysis examining safety and efficacy.

This chapter presents a published paper, however copyright restrictions prevent the reproduction of this paper in its published form. The details of this publication are:

Unsworth, D. J., Mathias, J. L., & Dorstyn, D. S. (2017). Cell therapies administered in the chronic phase after stroke: A meta-analysis examining safety and efficacy. *Regenerative Medicine*, *12*, 91-108. doi: 10.2217/rme-2016-0082

Please note:

- Tables and Figures are formatted in line with the Journal's requirements.
- Australian/British English spelling was used.
- The term 'cell therapies' is used in place of 'SC therapies', in line with the accepted vernacular, to describe treatments involving SCs, SC by-products, and related materials.
- The lack of knowledge and research about the psychological variables had to be down-played in the article due to the medical focus of the journal.

4.1 Preface

This study extends on Study 1 (Chapter 3), using meta-analytic techniques to evaluate the safety and efficacy of SC therapies administered in the chronic phase of stroke. The need to examine beyond the acute and sub-acute phases was deemed important due to differences in the way that SC therapies are thought to work once the blood-brain-barrier is no longer permeable (Savitz, 2018). That is, given that SCs administered intravenously or intra-arterially are no longer thought to reach the stroke-affected regions of the brain during the chronic phase of stroke, the emphasis is on intracranial and intrathecal delivery methods (Marei et al., 2018). Key outcomes explored in the current study included the safety and neurological and functional efficacy of SC therapies, stratified by SC type and administration route. Notably, data for radiological outcomes was not routinely reported, hence not explored in this review. As with Study 1 (Chapter 3), this review considers the degree to which psychological comorbidities were screened for and/or psychological or cognitive outcomes were measured. Indeed, given that mood symptoms often worsen during the chronic phase of stroke (Chun et al., 2018; Mitchell et al., 2017), identifying research gaps in these areas was thought to be of critical importance.

4.2 Statement of Authorship

Title of Paper	Cell therapies administered in the chronic phase after stroke: A meta-analysis examining safety and efficacy.
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Unsworth, D. J., Mathias, J. L., & Dorstyn, D. S. (2017). Cell therapies administered in the chronic phase after stroke: A meta-analysis examining safety and efficacy. Regenerative Medicine, 12, 91-108.

Principal Author

Name of Principal Author (Candidate)	Mr David J. Unsworth		
Contribution to the Paper	Study design, data collection and analysis, writing manuscript.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	30/09/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Prof. Jane L. Mathias		
Contribution to the Paper	Study design, data interpretation, editing manuscript.		
Signature		Date	1/10/2019

Name of Co-Author	Dr. Diana S. Dorstyn		
Contribution to the Paper	Study design, data interpretation, editing manuscript.		
Signature		Date	30/9/2019

4.3 Abstract

Aims: To assess the safety and efficacy of cell therapies for chronic stroke. **Methods:** Five databases were searched for treatments administered > 90 days post-stroke. Reporting quality, adherence to research guidelines, treatment safety (risk ratios/pooled incidence rates), and neurological/functional efficacy (Hedges' *g*) were all evaluated. **Results:** Twenty-three studies examined 17 treatments. Reporting quality scores were medium-high, but adherence to recommended guidelines was lower. Three treatments resulted in serious adverse events; four improved outcomes more than standard care. However, many studies were under-powered and individual patients varied in their response to some treatments. **Conclusions:** Preliminary findings suggest that some cell therapies may be relatively safe and effective, but larger double-blinded placebo-controlled studies are needed to determine the long-term risks and benefits.

4.4 Introduction

Stroke is the third most common cause of disability (Feigin et al., 2014; Murray et al., 2012). Globally, an estimated 33 million stroke survivors currently have ongoing disabilities (Corbyn, 2014), and a further five million are permanently disabled each year (Mozaffarian et al., 2015). Following acute medical care, rehabilitation – involving physiotherapy, repetitive task training, speech therapy, and psychotherapy – is commonly initiated in order to prevent further physical and cognitive deterioration, improve functional independence, and reduce psychological distress (Clarke & Forster, 2015; Wade, 2016). However, patients vary in their response to rehabilitation (Langhorne, Bernhardt, & Kwakkel, 2011; Pinter & Brainin, 2012) and recovery often plateaus during the chronic phase (> 90 days) (Richards, Malounin, & Nadeau, 2015), leaving a significant number of people with residual disabilities (Richards et al., 2015; Teasell, 2013). Beyond rehabilitation, there are currently no approved treatments that improve the long-term neurological (motor, speech, vision) and functional (mobility, bathing, grooming) outcomes of stroke patients, highlighting the need for safe and effective alternatives (Azad, Veeravagu, & Steinberg, 2016).

A variety of pharmacological (e.g., selective-serotonin re-uptake inhibitors [SSRIs]), neuromodulatory (e.g., cortical/cerebellar stimulation) and neuroprotective (e.g., mild hypothermia, postsynaptic density-95 protein) interventions are being tested (see Azad et al., 2016 for a review); as are a range of cell therapies, which involve the transplantation of neural stem cells (NSCs), mesodermal-derived SCs (e.g., bone marrow mesenchymal SCs [BM-MSCs]), hematopoietic/endothelial cells (e.g., umbilical cord MSCs [UC-MSCs], peripheral-blood SCs [PBSCs]), and/or cell-related products (e.g. bone marrow mononuclear cells [BM-MNCs]), and growth factors (e.g., granulocyte-colony stimulating factor [G-CSF]). Preliminary research suggests that cells obtained from either the patient (autologous) or from foreign sources (non-autologous/allogeneic: human or animal) may offer significant

neurorestorative benefits (Borlongan, Jolkkonen, & Detante, 2015; Savitz, 2015), although the latter source may require immunosuppression to prevent rejection (Boltze et al., 2015).

To date, trials undertaken throughout the chronic phase of stroke have largely focused on the intracerebral (stereotactic) delivery of cells (commonly NSCs) because it is thought to provide a greater chance of cellular regeneration (neuro- or glio-genesis) due to the higher concentration of cells in and around the infarct (Reyes, Tajiri, & Borlongan, 2015; Tang et al., 2015). Intracerebral injections circumvent the blood-brain-barrier, permitting cells to penetrate the lesion and penumbra, where they are thought to repair and restore stroke-related damage through the formation of new neurons, astrocytes and oligodendrocytes (Azad et al., 2016). However, these benefits need to be balanced against the complex and invasive nature of intracerebral transplantation (Reyes et al., 2015), and an increased risk of complications (e.g., haemorrhages, seizures, infections) and tumors/graft overgrowth due to the strong proliferative capacity of NSCs (Boltze et al., 2015).

A range of less invasive approaches are also being investigated. Intrathecal delivery involves the injection of cells – commonly NSCs – into the subarachnoid space via lumbar puncture, which results in a diffuse spread of cells throughout the central nervous system (including the lesion site), purportedly resulting in angiogenesis, neovascularisation and increased synaptic plasticity (Boltze et al., 2015; Sharma et al., 2014). This method may therefore provide a safer and more accessible form of cell transplantation (Sharma et al., 2014), although lumbar-sacral damage can occur (Hurst et al., 2013) and, in the case of NSCs, spinal tumors may develop (Amariglio et al., 2009; Saad et al., 2016).

Intravenous and subcutaneous injections of BM-MSCs, UC-MSCs, PBSCs, BM-MNCs and G-CSF have also been examined; with these cell types and delivery methods considered to be safer, more readily available, and less likely to raise ethical concerns than human embryonic/fetal NSCs (Janowski, Wagner, & Boltze, 2015). However, cell survival

and migration may be compromised when these approaches are used in the chronic phase of stroke, due to diminished inflammatory signaling (Tang et al., 2015) and decreased blood-brain-barrier permeability (Tajiri et al., 2016). Instead, neurorestoration is thought to occur as a consequence of 'bystander' effects, resulting from the release of growth factors (dela Peña, & Borlongan, 2015; Liu et al., 2014).

To date, research examining cell therapies in the chronic phase of stroke has largely consisted of early-phase clinical trials and observational studies. A few meta-analyses have compared efficacy, based on randomised control trials (RCTs) (Boncoraglio et al., 2010; Yuan, Zeng, & Wu, 2007) or single-arm studies (Jeong et al., 2014), but they did not examine acute/sub-acute and chronic treatments separately, despite pathophysiological differences (e.g., blood-brain-barrier permeability) that may impact upon efficacy. Moreover, in the absence of a control group, it was not possible to determine whether the changes observed in single-arm studies were due to the cell therapy, spontaneous recovery and/or placebo effects (Jeong et al., 2014). Other meta-analyses have examined MSCs (Cao & Li, 2015; Lalu et al., 2012; Wang et al., 2016) and G-CSF (Fan et al., 2015) in isolation, but were not able to compare the safety and efficacy of these treatments with other cell therapies. Moreover, the impact of various clinical (e.g., patient age, treatment timing, cell source/dosage) and methodological (e.g., study blinding) variables on efficacy, and the reporting quality and adherence to cell-therapy guidelines, have yet to be evaluated.

The current study therefore meta-analysed the data from all studies – RCTs, non-RCTs, case-control and cohort designs – that have treated patients with cell therapies in the chronic phase after stroke (> 90 days) in order to evaluate their relative safety and efficacy. It extends on the findings of a recent study, which examined cell therapies administered in the acute and sub-acute phase (Unsworth, Mathias, & Dorstyn, 2016). Reporting quality was assessed using the Consolidated Standards of Reporting Trials (CONSORT) (Moher et al.,

2010) and Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (Von Elm et al., 2008), and adherence to current cell-therapy research recommendations was evaluated using the Stem Cells as an Emerging Paradigm in Stroke (STEPS I-III) and RIGOR guidelines (Lapchak, Zhang, & Noble-Haeusslein, 2013; Reynolds, 2009; Savitz et al., 2011; Savitz et al., 2014). Treatments were stratified by delivery route and cell type, with safety being evaluated in terms of the number of treatment-related serious adverse events (SAEs), and efficacy measured in terms of improvements to neurological, functional and 'overall' (combined) outcomes. Subgroup analyses were additionally performed to determine whether efficacy differed according to patient age (</≥ 60 years), cell source (autologous vs allogeneic), dosage (</≥ 10^8 cells), treatment timing (</≥ 1 year), reporting quality score (low vs medium/high), and study blinding (none vs single/double).

4.5 Methodology

This study adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altmann, 2009).

Search Strategy and Inclusion Criteria

A comprehensive search of the PubMed, Embase, Web of Science, Cochrane, and PsycINFO databases was conducted (January 1960–June 2016) to locate published research that administered cell therapies > 90 days after ischaemic/haemorrhagic stroke onset (see Supplemental Appendix A for searches). To be included, studies had to have assessed the safety (SAEs), neurological (e.g., National Institutes of Health Scale [NIHSS], modified Rankin Scale [mRS]) and/or functional outcomes (e.g., Barthel Index [BI]) of adult (≥ 18 years) stroke survivors, and provided data that permitted the calculation of standardised mean differences (Hedges' *g*) *(*e.g., means and *SDs*, *t*-statistics, exact *p*-values). Experimental

(RCTs, non-RCTs) and observational (case-control, cohort) study designs were included, but case studies were excluded. The search was not restricted to English publications. Study eligibility was determined by the 1st author (DJ Unsworth) in consultation with the 2nd author (JL Mathias). Scopus citation searches were performed for the included studies in order to find any additional relevant research.

The literature search identified 3,941 articles (see flowchart, Supplemental Appendix G for details). Initial screening of titles and abstracts reduced the number to 86, after which full-text versions were reviewed: five required information/data to be extracted by a fluent Mandarin Chinese speaker to enable inclusion (Huang et al., 2009; Li et al., 2007; Wang et al., 2007; Yang et al., 2007; Zhang et al., 2006). Twenty-five articles met the inclusion criteria, however closer examination of the participant data revealed that eight used overlapping samples, reducing the number to 21 independent studies (Supplemental Appendix H). Of these, two examined multiple treatments in separate samples (Bhasin et al., 2013; Chen et al., 2013), which were therefore treated as two additional independent studies. Effectively, data from 23 independent studies were included in the meta-analysis (refer to Supplemental Appendix H), including one for which graphical data was converted to numerical data (Qiao et al., 2014) using GetData Digitizer software, Version 2 (Federov, 2013).

Data Extraction

Key background (sample size, age, gender, country), stroke (type, region), treatment (delivery route; cell type, source and dosage; time post-stroke; assessment point/s) and outcome (neurological and/or functional) data were extracted from each study by the first author (DJ Unsworth). All analyses were conducted using the Comprehensive Meta-Analysis

Software, Version 3 (CMA; 2014, Biostat, Inc., Englewood, NJ, USA) and forest plots were generated using Meta Data Viewer (National Institutes of Health, 2015).

Assessment of Reporting Quality and Adherence to Research Guidelines

The CONSORT (Moher et al., 2010) and STROBE (Von Elm et al., 2008) guidelines were used to assess the reporting quality of the RCTs and non-RCTs/case-control/cohort studies, respectively. A 13-item checklist (Supplemental Appendix D) was also formulated from the STEPS I-III (Reynolds, 2009; Savitz et al., 2011; Savitz et al., 2014) and RIGOR (Lapchak et al., 2013) cell-therapy research guidelines to evaluate adherence to current recommendations (e.g., placebo/sham treatments, cell labeling/tracking, reporting conflicts of interest). For both the CONSORT/STROBE and STEPS/RIGOR evaluations, information relating to each item was judged to be: 'Present' (2 points), 'Present with Limitations' (1 point), 'Not Present' (0 points), or 'Not Applicable' (n/a). Two percentage scores were then calculated: (1) one measuring the overall reporting quality of *each study* ([study score ÷ no. of applicable items] x 100), with scores < 50%/50%-75%/> 75% reflecting low/medium/high quality (Viswanathan et al., 2012); and (2) one measuring the quality of reporting for *individual checklist items* (i.e. % studies that reported, reported with limitations, or did not report each item).

Hedges' g, Heterogeneity and Publication Bias analyses

Hedges' *g* was calculated using the inverse variance method (Hedges, 1982) to measure the standardised mean difference in: (1) the post-treatment outcomes of cell therapy and standard-care stroke groups ('Post-treatment vs Control') using data obtained from the RCTs, non-RCTs and case-control studies; and (2) the pre- and post-treatment outcomes of a single treatment group ('Pre-/post-treatment') using data extracted from all four study designs (RCT, non-RCT, case-control & cohort studies) (note: not all RCTs/non-RCTs provided pre-

and post-treatment data). In addition, the latter pre-post differences were also compared to those seen in a standard-care stroke group over an equivalent interval ('Pre-/post-treatment vs Control') in order to determine whether they exceeded what would be expected to occur as a result of spontaneous/normal recovery, practice and/or placebo effects. Data for the standard-care stroke group were sourced from four of the eligible RCTs/non-RCTs ($N_{controls}$ = 53; see Supplemental Appendix I for further details) because these were thought to provide the most reliable source of control data (Moher et al., 2010; Von Elm et al., 2008).

Cell therapies were stratified by delivery route and cell type to allow for comparisons between treatments. Risk ratios (RR) and pooled incidence rates (%) were calculated to assess the safety/risks associated with each therapy. The definition of a SAE varied between studies, consequently it was restricted to: death, cancer/tumor, stroke, seizure, and major cardiac/renal disorders. SAEs were only included if they were deemed to be possibly/probably/definitely related to the treatment. Hedges' *g* statistics were computed to determine the neurological, functional and 'overall' (all outcomes combined) efficacy of each cell therapy. Ninety-five percent confidence intervals (95% CIs) indicate the upper/lower bounds of *g* and forest plots were used to assess statistical power (Boyles, Harris, Rooney, & Thayer, 2011; Valentine, Pigott, & Rothstein, 2010). A significant ($p < .05$) positive *g* indicates that cell-therapy recipients experienced better outcomes than controls (Post-treatment vs Control and Pre-/post-treatment vs Control analyses) or improved pre- to post-treatment (Pre-/post-treatment analyses), with g = .2, .5, .8 and 2.0 equating to small, medium, large and very-large standardised mean differences, respectively (Cohen, 1988; Hopkins, Marshall, Batterham, & Hanin, 2009). Fail-safe *N* statistics (N_{fs}) assessed the risk of publication bias by estimating the number of unpublished studies with non-significant effects that would be required to reduce a finding from the current study to a small standardised mean difference (g = .2) (Lipsey & Wilson, 2001). Where N_{fs} exceeded the

number of studies examining a particular treatment ($N_{studies}$), publication bias was considered unlikely to pose a serious threat to the findings.

As an emerging area of research, the total number of studies was small, preventing us from performing heterogeneity analyses to determine whether the data from studies that examined the same treatment could be validly combined (Higgins, Thompson, Deeks, & Altman, 2003). Instead, a random-effects model was used to calculate Hedges' *g* in order to take into account clinical and methodological variability between the studies (DerSimonian & Laird, 1986). As an additional step, *Q*-statistics and the I^2 index were used to provide an overall indication of the heterogeneity in treatment effects reported by the studies in both the Post-treatment vs Control and the Pre-/post-treatment analyses. A significant *Q* indicates that effects differed between studies and I^2 index values < 50%, 50-75%, and > 75% suggest low, medium, and high levels of heterogeneity, respectively (Higgins et al., 2003). Following on from this, subgroup analyses (with Bonferroni corrections [$p < .008$]) examined potential source/s of heterogeneity (Xu, Platt, Luo, Wei, & Fraser, 2008) in order to determine whether the reported effects for individual studies differed according to: age (</≥ 60 years); cell source (autologous [incl. G-CSF] vs allogeneic); dosage (</≥ 10^8 cells); treatment timing (</≥ 1 year); reporting quality score (low vs medium/high); and study blinding (none vs single/double).

4.6 Results

Study Characteristics

Details of the participants (e.g., age, stroke type/region), cell therapies, outcomes assessed, study designs (e.g., RCT, non-RCT) and countries of origin are summarised in Table 4-1. The Post-treatment vs Control analyses were based on eight studies of 206 participants ($N_{treatment}$ = 98, $N_{controls}$ = 108) who had a mean age of 52.3 years. Most (74%)

were male and had ischaemic strokes (88%) affecting mixed/non-specified regions. On average, cell therapies were administered 1.5 years post-stroke: six therapies were examined, with intravenous injections of BM-MNCs being the most common ($N_{participants}$ = 26). All studies assessed safety, six examined neurological outcomes and seven assessed functional outcomes. Three studies were RCTs and most ($N_{studies}$ = 4) were conducted in India.

Table 4-1. *Descriptive and Categorical Data for the Study Participants*

	Post-treatment vs Control				Pre-/post-treatment			
	$N_{studies}$	$N_{participants}$	*M*	*SD*	$N_{studies}$	$N_{participants}$	*M*	*SD*
Total Studies & Participants:	8	206	21	9.7	17	279	16	15.9
Treatment Group:	8	98	12	5.0	17	279	16	15.9
Age (years):	8	206	52.3	9.8	15	262	54.6	14.9
Sex:								
Male	7	73 (74%)	-	-	17	182 (65%)	-	-
Female		25 (26%)				97 (35%)		
Stroke type:								
Ischemic	7	182 (88%)			14	160 (75%)		
Hemorrhagic		24 (12%)	-	-		52 (25%)	-	-
Stroke Region:								
Mixed / Not specified	5	140 (68%)	-	-	11	210 (76%)	-	-
MCA	2	48 (23%)	-	-	2	13 (4%)	-	-
BGS	1	18 (9%)	-	-	5	56 (20%)	-	-
Onset to Therapy (years):	8	98	1.5	0.7	16	272	3.6	3.5
Cell Therapies:								
IV / BM-MNC	2	26	-	-	-	-	-	-
IV / BM-MSC	2	12	-	-	-	-	-	-
SubCut / G-CSF	1	21	-	-	-	-	-	-
SubCut + IC / G-CSF + PBSC	1	15	-	-	-	-	-	-
IC / NT2-D1	1	14	-	-	2	26	-	-
IT / NSC	1	10	-	-	4	142	-	-
SubCut + IT / G-CSF + BM-MNC	-	-	-	-	1	24	-	-
IC / NSC	-	-	-	-	1	21	-	-
IC / SB623	-	-	-	-	1	18	-	-
IV / UC-MSC	-	-	-	-	1	10	-	-
SubCut + IT / G-CSF + BM-CD34+	-	-	-	-	1	8	-	-
IC / OEC	-	-	-	-	1	7	-	-
IC / BM-MSC	-	-	-	-	1	5	-	-
IC / LGE	-	-	-	-	1	5	-	-
IV + IT / NPC + BM-MSC	-	-	-	-	1	5	-	-
IC / OEC + NPC	-	-	-	-	1	4	-	-
IC + IT / OEC + NPC	-	-	-	-	1	4	-	-
Outcomes Assessed:								
Safety	8	98	-	-	17	279	-	-
Neurological	6	67	-	-	13	150	-	-
Functional	7	83	-	-	15	256	-	-
	$N_{studies}$	Phase / Study type			$N_{studies}$	Phase / Study type		
RCT	3	II x 2	n.s. x 1		1	II x 1		
Non-RCT	4	n.s. x 4			2	I x 1	I/IIa x 1	
Observational	1	Case-Control x 1			14	Prospective Cohort x 13	Case-Control x 1	
	$N_{studies}$	Study Origin			$N_{studies}$	Study Origin		
Asia	5	India x 4	China x 1		11	China x 10	India x 1	
Europe	2	Germany x 1	Russia x 1		1	Russia x 1		
Other	1	U.S.A x 1			5	U.S.A x 4	Cuba x 1	

ACA = anterior circulation artery; BGS = basal ganglia stroke; BM-CD34$^+$ = bone marrow CD34$^+$ cells; BM-MNC = bone marrow mononuclear cells; BM-MSC = bone marrow mesenchymal stem cells; Cl = control group; G-CSF = granulocyte-colony stimulating factor; IC = intracerebral; IT = intrathecal; IV = intravenous; LGE = porcine fetal cells; *M* = mean; MCA = middle cerebral artery; NSC = neural stem cells; *Nparticipant*s = number of participants assessed; NPC = neural progenitor cells; n.s. = not specified; *Nstudies* = number of studies; NT2-D1 = Ntera 2.cl.D1; PBSC = peripheral blood stem cells; RCT = randomised controlled trial; SB623 = modified BM-MSCs; *SD* = standard deviation; SubCut = subcutaneous; Tmt = treatment group; UC-MSC = umbilical cord mesenchymal stem cells.

The Pre-/post-treatment analysis was based on data from 17 studies of 279 participants (including 2 studies/24 participants [Kondziolka et al., 2005; Rabinovich et al., 2005] from the previous analysis) (see Table 1). The average age was 54.6 years, 65% were male and 75% had ischaemic strokes. On average, cell therapies were administered 3.6 years post-stroke and, of the 13 treatments examined, intrathecal NSCs were most common. Safety was again examined by all studies; 13 and 15 of which examined neurological and functional outcomes, respectively. The majority used prospective cohort designs and were undertaken in China.

As noted, we additionally extracted standard-care control data ($N_{participants}$ = 53; see Supplemental Appendix I) from four RCTs/non-RCTs to supplement the Pre-/post-treatment vs Control analyses; enabling us to evaluate whether improvements in the Pre-/post treatment outcomes of cell-therapy recipients exceeded the changes seen as a consequence of spontaneous/normal recovery, test practice and/or placebo effects. The average age of the standard-care control group was 47.4 years (*SD* = 11.5), 79% were male, and 96% had ischaemic strokes. Standard care – which included physical rehabilitation – commenced, on average, 1.2 years post-stroke.

Reporting Quality

The reporting quality of all 21 studies was assessed. The three RCTs were evaluated against the CONSORT reporting guidelines (Moher et al., 2010), with a mean score of 76% (range: 68%-82%). Two studies had scores indicating high reporting quality (scored > 75%) and one was of medium quality (68%). Individual item data are summarised in Figure 4-1a, where it can be seen that the title (i.e. included RCT), rationale, objectives, study design and eligibility criteria (Items 1a, 2a, 2b, 3a, 4a) were consistently provided (100%), as were details of the interventions, outcome measures, blinding, statistics, losses, harms,

generalisability, interpretation, and funding sources (Items 5, 6a, 11a, 12a, 13b, 19, 21, 22, 25). Details regarding the settings/locations (Item 4b: 33%), randomisation methods (Items 8-10: 0%-33%), recruitment/follow up (Item 14a: 33%) and protocol access (Item 24: 33%) were all reported less frequently.

The remaining 4 non-RCTs, 1 case-control and 13 cohort studies were evaluated using the STROBE guidelines (Von Elm et al., 2008): the mean reporting quality score for these studies was 71% (range: 46% - 90%); six studies (33%) were assessed as high-quality, 11 studies (61%) were medium-quality and 1 study (6%) was rated in the low-quality category. As Figure 4-1(b) shows, all studies provided details of the research objectives (Item 3: 100%), number of participants at each stage of analysis (Item 13a: 100%), amount of missing data (Item 14b: 100%), outcome events (Item 15: 100%), unadjusted results (Item 16a: 100%), and key results (Item 18: 100%), and most confirmed the study design (Item 4: 94%), quantitative analyses (Item 11: 88%), and generalisability of the findings (Item 21: 83%). However, details regarding the methods of assessment (Item 8a: 11%), potential biases (Item 9: 11%), sample size calculations (Item 10: 0%), sensitivity analyses (Item 12e: 0%), and funding sources (Item 22: 50%) were frequently omitted.

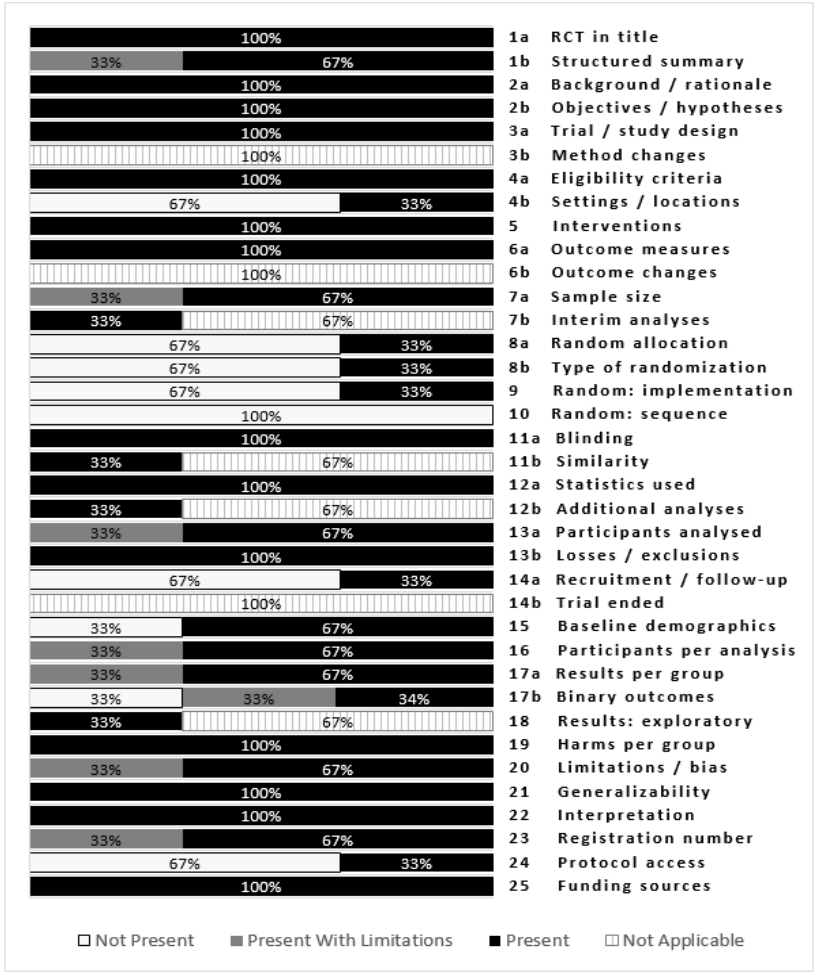


Figure 4-1(a)

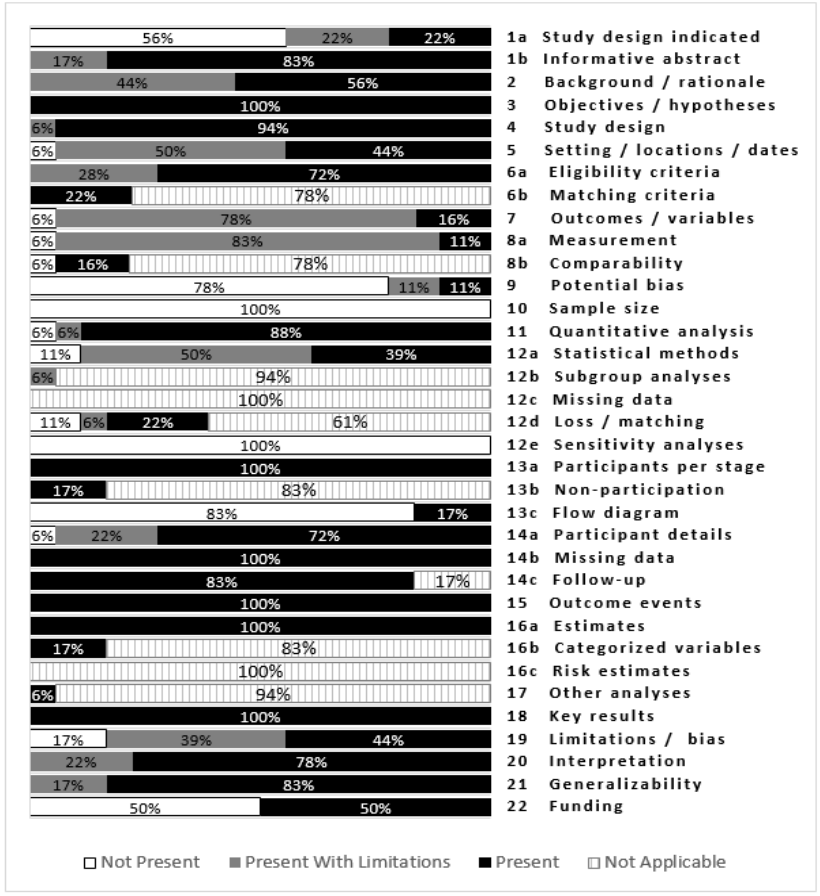


Figure 4-1(b)

Figure 4-1. Reporting quality assessment: (a) CONSORT evaluation of RCT study designs ($N_{studies}$ = 3) and (b) STROBE evaluation of non-RCTs and cohort study designs ($N_{studies}$ = 18).

Nstudies = number of studies; RCT = randomised controlled trial.

Adherence to Research Guidelines

All 21 studies were additionally evaluated against the 13 cell-therapy research recommendations provided in the STEPS I-III (Reynolds, 2009; Savitz et al., 2011; Savitz et al., 2014) and RIGOR (Lapchak et al., 2013) guidelines (see Figure 4-2). The average score for all studies was 45% (range: 15%-73%), indicating relatively low adherence to these guidelines. Notably, very few studies used placebo/sham treatments (Item 1: 5%), assessed psychological (Item 4: 19%) or cognitive (Item 5: 24%) comorbidities prior to inclusion, established stable neurological baselines (Item 6: 10%), labeled and tracked the cells (Item 8: 5%), or assessed domain-specific outcomes (e.g., sensory, motor, visual, cognitive; Item 10: 33%). More justified the cell type/method/dosage (Item 2: 43%), used neuroimaging to detect changes in the lesion/infarct size (Item 7: 42%), confirmed the use of concomitant physical rehabilitation (Item 9: 48%), reported SAEs ≥ 1 year (Item 11: 57%), and acknowledged conflicts of interest (Item 13: 52%). Most assessed medical comorbidities (Item 3: 90%) and reported significant and non-significant findings (Item 12: 67%).

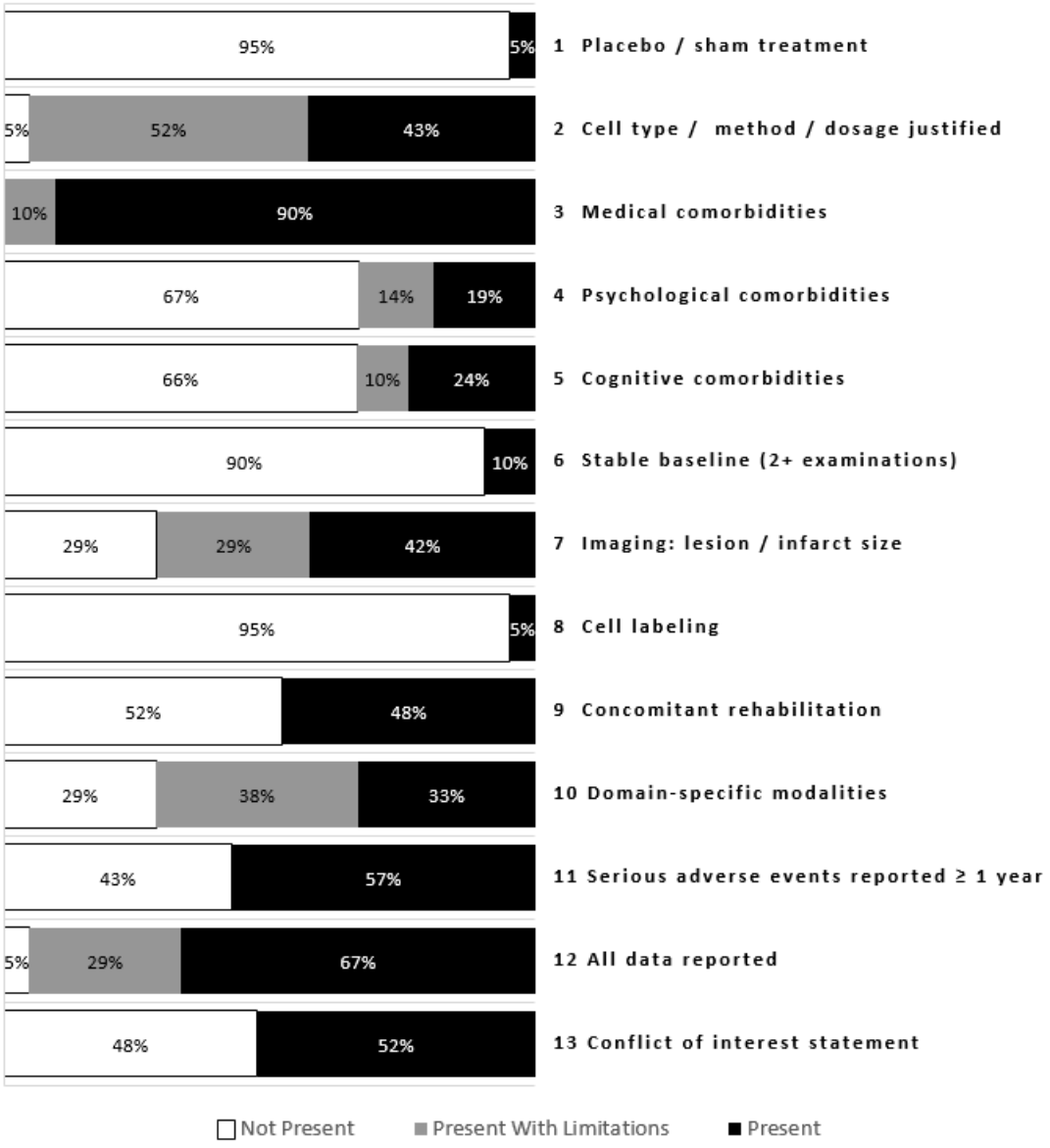


Figure 4-2. Percentage of studies ($N_{studies}$ = 21) meeting the STEPS (I-III) and RIGOR recommendations for cell-therapy research.

Safety and Efficacy of Cell Therapies

The safety and efficacy data for cell therapies administered in the chronic stages of stroke are summarised in Figure 4-3, where treatment data are grouped according to study design (Post-treatment vs Control or Pre-/post-treatment) and cell therapies ordered according to the associated Hedges' *g* (highest to lowest: 1 to 19). Summary study and participant details (mean age, stroke type/region, time from stroke-onset to therapy, timing of assessment) are provided, in addition to the safety (risk ratios, pooled incidence), efficacy (neurological, functional, overall) and publication bias (N_{fs}) data. Forest plots display the overall Hedges' *g* statistic and 95% CIs for each therapy. The comparable Hedges' *g* statistic for the control group (Pre-/post-treatment vs Control analysis) is shown as a dashed line on the forest plots for treatments 7-19. Additional study information (e.g., dosages) is provided in Appendix J (supplementary materials).

	CELL THERAPY		STUDY DETAILS				TREATMENT GROUP						SAFETY		EFFICACY				FOREST PLOT
	Delivery Route	Cell Type	N Studies	Study Ref(s)	$N_{Participants}$ T/ment	Control	Age (M)	Stroke Type Isch	Hem	Stroke Region	Onset To Therapy (M Years/Range)	Assessment Point (Months)	Risk Ratio (95% CI)	Pooled Incidence (%) (95% CI)	Neuro g p	Func g p	Overall g p	Nfs	Overall g + 95% CI
A	Post-treatment vs Control																		
1	IT	NSC	1	[58]	10	11	46.1	7	3	Mixed	1.1	6	1.1 (0.0-50.4)			2.82 **	2.82 **	13	
2	SubCut + IC	G-CSF + PBSC	1	[50]	15	15	50.1	15	0	MCA	2.7	12	1.0 (0.0-47.4)		1.31 **		1.31 **	6	
3	IC	NT2/D1	1	[53, 61]	14	4	58.5	6	8	BGS	1 - 5	6	0.6 (0.1-4.8)		1.13	1.11	1.12 *	5	
4	IV	BM-MSC	2	[45, 47, 49]	12	26	43.5	10	2	Mixed	0.8	6 – 42	2.1 (0.0-99.0)		0.30	0.64	0.44	2	
5	IV	BM-MNC	2	[45,48]	26	32	46.0	25	1	Mixed	0.8	6	1.2 (0.0-59.6)		0.10	0.74 *	0.41 *	2	
6	SubCut	G-CSF	1	[52]	21	20	66.4	21	0	Mixed	1.0	1.3	1.0 (0.0-46.0)			0.28	0.28	0	
B	Pre-/post-treatment																		
7	IV	UC-MSC	1	[55]	10		35-75	6	4	Mixed	1.9	3		0% (0.0-27.8)	2.10 **	2.26 **	2.18 **	10	
8	IC	BM-MSC	1	[62]	5		51.4	3	2	Mixed	5.0	12		0% (0.0-43.5)	1.53 **	2.93 **	2.09 **	9	
9	IV + IT	NPC + BM-MSC	1	[57]	5		56.0	5	0	Mixed	0.6	24		0% (0.0-43.5)	1.14 *	1.16 *	1.15 **	5	
10	IC	SB623	1	[60]	18		61.3	18	0	Mixed	1.8	12		17% (5.8-39.2)	0.97† **		0.97 **	4	
11	IC	NSC	1	[51]	21		44.6	21	0	BGS	3.4	6		0% (0.0-15.5)		0.84 **	0.84 **	3	
12	SubCut + IT	G-CSF + BM-MNC	1	[17]	24		57.0	14	10	Mixed	3.4	30		0% (0.0-13.8)		0.58 *	0.58 *	2	
13	SubCut + IT	G-CSF + BM-CD34+	1	[42]	8		41.5	8	0	Mixed	2.6	12		0% (0.0-32.4)	0.42	0.60 *	0.51 *	2	
14	IC	OEC + NPC	1	[50]	4		51.8	2	2	BGS	4.5	18		0% (0.0-49.0)	0.40	0.55	0.47 *	1	
15	IC	LGE	1	[59]	5		39.8	5		BGS	4.9	48		40% (11.7-76.9)	0.43		0.43	1	
16	IT	NSC	4	[43, 44, 58, 63]	142		55.1	73 (50 n.s.)	29	Mixed	4.1	6		0% (0.0-2.6)	0.12†	0.54 *	0.35 *	3	
17	IC	OEC	1	[40]	7		40-87	2	2	Mixed	1 – 14	1		0% (0.0-35.4)	0.22	0.16	0.19	0	
18	IC + IT	OEC + NPC	1	[46]	4		60.0	3	1	Mixed	8.6	18		0% (0.0-49.0)	0.05	0.06	0.05	0	
19	IC	NT2/D1	2	[53, 54, 56, 61]	26		59.7	18	8	BGS	2.9	6		8% (2.1-24.1)	0.25	-0.32†	0.00	0	

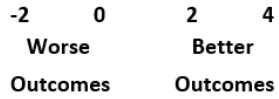


Figure 4-3. Safety and efficacy of cell therapy for chronic stroke, separated by delivery route and cell type for studies comparing (a) post-treatment and control outcomes and (b) pre- to post-treatment changes.

Notes: ** $p < .001$, * $p < .05$. The overall effect for standard care is shown by a dashed line on the forest plots for Treatments 7-19.
†: *Nstudies* and *Nparticipants* differs, refer Appendix J for further detail.

BGS = basal ganglia stroke; BM-MNC = bone marrow mononuclear cells; BM –MSC = bone marrow mesenchymal stem cells; CI = confidence interval; Func = functional outcomes ; G-CSF = granulocyte-colony stimulating factor; Hem = haemorrhagic; IC = intracerebral; Isch = ischaemic; IT = intrathecal; IV = intravenous; LGE = lateral ganglionic eminence cells; *M* = mean; MCA = middle cerebral artery; Neuro = neurological outcomes; *Nfs* = Fail-safe *N*; *Nparticipants* = number of participants; NPC = neural progenitor cells; n.s. = not specified; NSC = neural stem cells; *Nstudies* = number of studies; NT2/D1 = Ntera 2/cl.D1; OEC = olfactory ensheathing cell; PBSC = peripheral blood stem cells; SB623 = modified BM-MSCs; SubCut = subcutaneous; T/ment = treatment; UC-MSC = umbilical cord mesenchymal stem cells; yrs = years.

Safety

As seen in Figure 4-3, the risk of experiencing a SAE (death, tumor, stroke, seizure), varied considerably between the treatment and control groups (Treatments 1-6). No statistically-significant increases in risk were observed for any of the six treatments; however, most data were based upon small samples and/or short follow-up periods, which limits the reliability of these findings. The pooled incidence rates (Figure 4-3: Treatments 7-19) indicate that 40% (n = 2 out of 5: 4 year follow-up) of patients who received intracerebral porcine fetal cells (Treatment 14), 17% (n = 3 out of 18: 1 year follow-up) of patients who received intracerebral SB623 (modified BM-MSCs) cells, and 8% (n = 2 out of 26: 6 month follow-up) of patients who received intracerebral NT2/D1 (human embryonic carcino-derived) cells (Treatment 18), suffered SAEs. No SAEs were reported for the other 10 cell therapies (Treatments 7-13, 15-17), although – with few exceptions (e.g., Treatments 10, 11, 15, 18) – the small sample sizes and short follow-up periods again limit the reliability of these findings.

Post-treatment vs Control Group comparisons

When the (post-treatment) outcomes of the treatment and control groups were compared (Figure 4-3: Treatments 1-6), cell-therapy recipients were found to have significantly better overall outcomes after treatment with: (1) intrathecal NSCs (Treatment 1: very large difference in functional outcomes); (2) combined subcutaneous G-CSF and intracerebral PBSCs (Treatment 2: large difference in neurological outcomes); (3) intracerebral NT2/D1 cells (Treatment 3: large overall difference); and (4) intravenous BM-MNCs (Treatment 5: large difference in functional outcomes). Treatments 1 to 3, but not 5, were additionally found to be at low risk of publication bias (see Figure 4-3).

Further analyses revealed the extent of these improvements. Specifically, patients receiving NSCs via intrathecal injection (Treatment 1) scored an average of 30.3 points (95% CIs: 20.9 to 39.7) higher/better on the Karnovskii Functional Activity Score than controls; patients receiving

combined subcutaneous G-CSF/intracerebral PBSC injections (Treatment 2) had lower/better scores than controls on the NIHSS (mean = -3.2 points, 95% CIs: -1.8 to -4.6), European Stroke Scale (ESS; mean = 9.0 points, 95% CIs: -3.1 to -14.9), European Motor Scale (EMS; mean = 8.8 points, 95% CIs: -2.6 to -15.0), and mRS (mean = -0.6 points, 95% CIs: -0.3 to -0.9); and patients receiving intravenous BM-MNCs (Treatment 5) scored higher/better than controls on the modified BI (mean = 8.2 points higher, 95% CIs: 2.7 to 13.7), although – as noted – this finding should be interpreted cautiously because it is vulnerable to publication bias. These same analyses were not performed for intracerebral NT2/D1 recipients (Treatment 3) because significant differences were only observed for the overall/combined data, not for individual measures. No significant differences were observed following treatment with intravenous BM-MSCs (Treatment 4) or subcutaneous G-CSF (Treatment 6).

Pre-/post-treatment changes

When differences in the pre- and post-treatment outcomes of cell-therapy recipients were compared (Figure 4-3, Treatments 7 to 19), significant overall improvements were observed after treatment with: (1) intravenous UC-MSCs (Treatment 7: very large differences in neurological and functional outcomes); (2) intracerebral BM-MSCs (Treatment 8: very large differences in neurological and functional outcomes); (3) combined intravenous NPCs and intrathecal BM-MSCs (Treatment 9: large differences in neurological and functional outcomes); (4) intracerebral SB623 cells (Treatment 10: large difference in neurological outcomes); (5) intracerebral NSCs (Treatment 11: large difference in functional outcomes); (6) combined subcutaneous G-CSF and intrathecal BM-MNCs (Treatment 12: medium difference in functional outcomes); (7) combined subcutaneous G-CSF and intrathecal BM-$CD34^+$ cells (Treatment 13: medium difference in functional outcomes); (8) combined intracerebral OECs/NPC injections (Treatment 14: medium difference in overall outcomes); and (9) intrathecal NSCs (Treatment 16: medium difference in functional outcomes). Treatments 7 to 13 were all at low risk of publication bias, but the findings for Treatments 14 and 16 should be interpreted cautiously (see Figure 4-3).

Lastly, differences in the Pre-/post-treatment outcomes of the standard-care control group (see Appendix I) were assessed, with large to very large improvements observed in neurological (g = 0.95), functional (g = 2.09), and overall (g = 1.54) outcomes. The overall Hedges' g statistics for the Pre-/post-treatment and control groups were then compared, which indicated that no cell therapy led to significantly better outcomes than standard care (see Figure 4-3, forest plots for Treatments 7-19: overall Hedges' g for standard care shown as dashed line); although the improvement in neurological outcomes following intravenous UC-MSCs (Treatment 7) approached statistical significance (p = .06). However, caution should again be exercised when interpreting these results because the standard-care control group proved to be significantly younger, had fewer haemorrhagic strokes and was assessed earlier ($p < .05$).

Heterogeneity and Subgroup analyses

Although the heterogeneity of individual therapies could not be assessed due to the small number of studies examining each treatment (range: 1 to 4 studies) (Higgins et al., 2003), medium to large and significant variability was observed when all of the Post-treatment vs Control (Q[7] = 23.1, $p < .001$, I^2 = 70.3 [95% CI: 38.5 to 85.7]) and Pre-/post-treatment (Q[16] = 107.58, $p < .001$, I^2 = 85.13 [95% CI: 77.6 to 90.1]) studies were analysed together. Subgroup analyses therefore explored whether patient age, cell source, dosage, treatment timing, reporting quality score and study blinding contributed to this heterogeneity. As seen in Table 4-2, treatment effects were significantly larger for studies with low reporting quality scores (i.e. studies that provided information for < 50% of assessable items) and higher dosages ($\geq 10^8$ cells) (see Appendix J for study dosages). Non-significant differences were observed for the remaining subgroup analyses (possibly due to the small $N_{studies}$); however, there was a trend toward allogeneic cells being more effective than autologous cells (Post-treatment vs Control: p = .01), blinded studies (single/double) reporting smaller treatment effects than non-blinded studies, and younger patients (< 60 years) experiencing better outcomes than older patients (≥ 60 years). Whether therapies administered within 1 year after a stroke were more effective than later treatments remained unclear.

Table 4-2. *Subgroup and Heterogeneity Analyses*

SUBGROUP			ANALYSIS	EFFICACY				HETEROGENEITY				
				$N_{studies}$	*g*	95% CIs	*p*	*Q*	*df*	*p*	I^2	95% CIs
(1)	Reporting Quality Score:	Low	Post-treatment vs Control	1	2.82	1.55 to 4.09	**	0.0	0		0.0	0.0 to 0.0
		Medium / High		7	0.57	0.22 to 0.92	**	7.9	6		24.1	0.0 to 66.5
		Low	Pre-/post-treatment	1	2.05	0.99 to 3.10	**	0.0	0		0.0	0.0 to 0.0
		Medium / High		16	0.54	0.32 to 0.75	**	51.4	15	**	70.8	51.5 to 82.4
(2)	Treatment Dosage:	$< 10^8$ cells	Post-treatment vs Control	7	0.57	0.22 to 0.92	**	7.9	6		24.1	0.0 to 66.6
		$\geq 10^8$ cells		1	2.82	1.55 to 4.09	**	0.0	0		0.0	0.0 to 0.0
		$< 10^8$ cells	Pre-/post-treatment	15	0.46	0.27 to 0.64	**	35.3	14	**	60.3	30.1 to 77.4
		$\geq 10^8$ cells		2	2.10	1.41 to 2.79	**	0.0	1		0.0	0.0 to 0.0
(3)	Cell Source	Allogeneic	Post-treatment vs Control	2	1.94	0.97 to 2.91	*	4.2	1	*	75.9	0.0 to 94.5
		Autologous		6	0.53	0.09 to 0.97	*	6.8	5		26.7	0.0 to 69.5
		Allogeneic	Pre-/post-treatment	14	0.58	0.32 to 0.84		58.5	13	**	77.8	63.1 to 86.6
		Autologous		3	0.81	0.22 to 1.40		5.6	2		64.5	0.0 to 89.8
(4)	Study Blinding	None	Post-treatment vs Control	5	0.82	0.09 to 1.56		16.3	4	**	75.5	39.9 to 90.0
		Single / Double		3	0.87	-0.05 to 1.79		4.7	2		57.8	0.0 to 88.0
		None	Pre-/post-treatment	15	0.67	0.43 to 0.92		63.8	14	**	78.1	64.3 to 86.5
		Single / Double		2	0.03	-0.76 to 0.81		0.5	1		0.0	0.0 to 0.0
(5)	Age	< 60 years	Post-treatment vs Control	7	0.93	0.33 to 1.54		19.0	6	**	68.3	30.0 to 85.7
		≥ 60 years		1	0.28	-1.16 to 1.73		0.0	0		0.0	0.0 to 0.0
		< 60 years	Pre-/post-treatment	12	0.61	0.35 to 0.87		43.9	11	**	74.9	55.8 to 85.8
		≥ 60 years		3	0.39	-0.13 to 0.91		5.9	2		66.1	0.0 to 90.4
(6)	Treatment Timing	< 1 year	Post-treatment vs Control	4	0.42	-0.30 to 1.14		1.8	3		0.0	0.0 to 78.1
		≥ 1 year		4	1.24	0.51 to 1.98		15.2	3	**	80.2	47.7 to 92.5
		< 1 year	Pre-/post-treatment	1	1.15	0.02 to 2.27		0.0	0		0.0	0.0 to 0.0
		≥ 1 year		15	0.62	0.37 to 0.87		62.5	14	**	77.6	63.4 to 86.3

Note: ** $p < .001$, * $p < .05$.

CI = confidence interval; *df* = degrees of freedom; *g* = Hedges' *g* effect size; I^2 = I^2 index; *Nstudies* = number of studies; *Q* = *Q* statistic.

4.7 Discussion

The present study evaluated the reporting quality of 21 studies – 3 RCTs, 4 non-RCTs, 1 case-control and 13 prospective cohort designs – that administered cell therapies in the chronic phase (> 90 days) after stroke. Based on the CONSORT (Moher et al., 2010) guidelines, the reporting quality of the three RCTs was found to be medium to high, with details regarding the study objectives, participants, interventions, key results and funding sources frequently provided. However, important information relating to the treatment setting (e.g., hospital, clinic), randomisation procedures (e.g., randomisation implementation, sequence) and trial protocols were frequently omitted, making it difficult to evaluate the generalisability of the findings, the potential for selection bias, and whether the analyses and findings were reported accurately and completely (Moher et al., 2010).

Although a number of similar strengths were observed when evaluating the reporting quality of the 18 non-RCT/case-control/cohort studies using the STROBE (Von Elm et al., 2008) guidelines, the quality of reporting was found to be lower for these studies; largely due to the fact that a number of important limitations were frequently overlooked when interpreting the data. Most notably, observer bias was rarely acknowledged throughout the non-/single-blinded studies, which may have led to the overestimation of treatment effects by clinicians (Hróbjartsson et al., 2014); and sample-size calculations were seldom provided, which meant that some studies may have lacked the statistical power to accurately assess the safety and/or efficacy of the treatment (Von Elm et al., 2008).

When evaluating all 21 studies against the STEPS I-III (Reynolds, 2009; Savitz et al., 2011; Savitz et al., 2014) and RIGOR (Lapchak et al., 2013) research recommendations for cell-therapies, adherence was also found to be relatively low. Most notably, few studies incorporated sham-treatment groups, which are necessary to rule out placebo effects (Diederich & Goetz, 2008), or tracked the cells post-transplantation (Savitz et al., 2014). Although the *in vivo* monitoring of cells

remains difficult at present (Gavins & Smith, 2015), research that tracks the survival and migration of cells is needed in order to better understand the mechanisms of action underpinning different cell therapies (Savitz et al., 2014).

In addition, few studies screened for psychological (e.g., depression, anxiety) and cognitive (e.g., dementia) problems at each stage of the treatment process; thus, little is currently known about the impact of psychological and cognitive comorbidities on treatment outcomes or patient wellbeing following cell therapies. Greater consideration of psychological/cognitive factors is important because depression and anxiety are very common following stroke (Ayerbe et al., 2013; Campbell-Burton et al., 2013), and depression and cognitive decline have both been observed in stroke patients who have undergone cell therapies (Suárez-Monteagudo et al., 2009).

In terms of safety, it proved difficult to assess the risks associated with specific cell therapies due to the small number of patients who underwent these treatments and the fact that most were only followed up within the first year after undergoing treatment. However, based on the available research, intracerebral procedures appeared to involve the most risk, with a seizure and cortical vein occlusion observed following the transplantation of porcine fetal cells (Treatment 14 [Savitz et al., 2005]); a seizure, subdural hematoma and case of pneumonia observed after SB623 injections (Treatment 10 [Steinberg et al., 2016]); and a seizure and subdural hematoma experienced following the delivery of NT2/D1 cells (Treatment 3/18 [Kondziolka et al., 2000; Kondziolka et al., 2005]). Although these results must be treated as preliminary, it is important to note that each of these SAEs was attributed to the surgery itself and that no cell-related consequences were reported during follow-up (4 years, 1 year and 6 months, respectively). The remaining 14 therapies appeared to be relatively safe; however the long-term risks (> 1 year) have yet to be examined in many cases, which is problematic because some SAEs (e.g. teratoma formation) may not be evident in the short term (Boltze et al., 2015).

The limited amount of data also made it difficult to definitively establish the efficacy of the various treatments, although the findings provisionally indicate that a few cell therapies (Treatments 1-3, 5) may produce significantly better outcomes than standard care. The largest improvements were reported following the intrathecal delivery of human-fetus-derived NSCs (1-2 injections of 2.0 x 10^8 cells), with very-large differences observed when comparing the post-treatment functional outcomes of cell therapy and control groups following ischaemic and haemorrhagic stroke (Rabinovich et al., 2005). Pre-clinical research suggests that NSCs delivered via lumbar puncture migrate to the lesion site via the cerebral ventricles, where they promote endogenous repair and angiogenesis through the release of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) (Janowski et al., 2015). However, given the mechanisms of action have yet to be established in a clinical setting, and smaller improvements were observed when analysing the Pre-/post-treatment data, further research is needed to more accurately determine the benefits of this treatment.

Promising changes were also seen in recipients of combined subcutaneous G-CSF (Filgrastim; 5 injections of 15 μg / kg) and intracerebral PBSCs (1 injection of 3.0-8.0 x 10^6 cells) (Chen et al., 2014), where large neurological differences were observed between treatment and control groups following middle cerebral artery (MCA) stroke. In addition, despite being based on a relatively small sample ($N_{treatment}$ = 15, $N_{control}$ = 15), structural improvements were observed throughout the stroke-affected areas when brain imaging was performed at 12 months, providing additional support for the efficacy of this treatment. When administered as a combined treatment, preclinical research suggests G-CSF may enhance the regenerative benefits of the PBSCs (dela Peña, & Borlongan, 2015; Detante et al., 2014; Solaroglu et al., 2015), with the later also supporting endogenous repair by allowing host SCs to reach the lesion site through the creation of a 'biobridge' (Sullivan et al., 2015).

Large overall differences were also observed between recipients of intracerebral delivery of NT2/D1 cells and controls following ischaemic and haemorrhagic stroke. Further examination of

this treatment appears warranted given NT2/D1 cells are derived from an immortalised teratocarcinoma cell line, rather than human-embryonic/fetal cells, and the results suggested new synapses were formed (Kondziolka et al., 2000; 2005; Takagi, 2016). Moreover, preclinical research suggests efficacy may be further enhanced via extracellular scaffolds (Cohen & Jensen, 2015) or through the harnessing of glial cells within the microenvironment (Xiao, Saiki, & Ide, 2014).

Large functional differences were also observed when comparing the outcomes of intravenous BM-MNC recipients ($N_{participants}$ = 26, majority ischaemic strokes) and controls, which suggests that this approach may be an effective method of facilitating neural regeneration (Bhasin et al., 2013). Further examination of this treatment also appears warranted, given preclinical research suggests intravenous treatments for chronic-phase stroke may be augmented through the use of blood-brain-barrier permeabilisers (e.g. mannitol), which may allow more cells to reach the infarct (Tajiri et al., 2016).

Lastly, the Pre-/post-treatment comparisons also indicated that intravenous UC-MSCs may improve the neurological and functional outcomes of ischaemic and haemorrhagic stroke patients; although, when compared to standard care – in order to control for spontaneous physical recovery, rehabilitation, practice and/or placebo effects – the benefits were only borderline (p = .06). None of the remaining cell therapies were found to have produced significantly larger pre/post treatment differences when compared with standard care, although the findings are not definitive given the control group was younger, had fewer haemorrhagic strokes and were assessed earlier than many of the treatment groups.

The subgroup analyses indicated that studies with low reporting quality scores (i.e. provided information for < 50% of assessable items) found significantly larger effects than studies with higher reporting quality scores (i.e. provided information for > 50% of assessable items), and that higher dosages (> 10^8 cells) were significantly more effective than lower dosages (< 10^8 cells).

These findings highlight the importance of comprehensive reporting by future trials, particularly in relation to the number of injections/cell dosages that are administered, given they may impact upon the efficacy. No further significant differences were observed, although there was a trend toward allogeneic cells (e.g., NSCs) being more effective than autologous cells (e.g., BM-MSCs), non-blinded research reporting larger treatment effects than single-/double-blinded research, and younger patients (< 60 years of age) having better outcomes. Although non-significant, the later findings reinforce the importance of: developing viable and practical sources of pluripotent allogeneic cells (e.g., CTX0E03 cells; induced pluripotent SCs [iPSCs]) (Cefalo et al., 2015; Kalladka et al., 2013), minimising the impact of observer bias throughout future trials (Hróbjartsson et al., 2014), and examining whether younger patients show greater improvements, possibly due to differences in the rate of neurological recovery and/or neuroplasticity (Cramer, 2008; Popa-Wagner et al., 2015).

The aforementioned findings should, however, be interpreted in light of the following limitations. First, cell therapies are an emerging field and, as such, many of the current findings are based on small samples and short follow-up intervals. Moreover, despite the broad range of cell therapies under investigation, heterogeneity analyses could not be performed for individual treatments due to the limited number of studies (Xu et al., 2008). Although Hedges' *g* statistics and a random-effects model were used in order to reduce the impact of the small samples and methodological variability (Hedges, 1982; DerSimonian & Laird, 1986), the confidence intervals were typically wide, suggesting that safety and efficacy was quite variable. Therefore, rather than providing definitive conclusions, the current study standardised and compared preliminary data from a broad range of treatments, in order to inform future research. Second, reporting quality was assessed on the basis of the information provided in the study publications. It is possible that some studies may have met additional CONSORT or STROBE criteria, but failed to report this information. Third, cell therapy recipients were, on average, younger than the majority of stroke patients (< 65 years of age) (Mozaffarian et al., 2015), which may limit the generalisability of the

findings (Cramer, 2010). Fourth, the control group used in the final analysis differed from the treatment groups in potentially important ways (e.g., age, stroke type, post-injury interval). Although matched controls (sham/placebo or non-treatment controls) would provide a more reliable comparison, they were not typically available. The current comparison was therefore undertaken in order to reduce the likelihood that the effects would be overestimated. Lastly, although the impact of dosage was crudely assessed in a subgroup analysis (</> 10^8 cells) in order to examine potential sources of variability in the data, this issue needs to be examined further by comparing the outcomes (safety and efficacy) of groups who receive different dosages of the same treatment, while also controlling for other confounding variables (e.g., age, stroke type, treatment onset).

Conclusion

Serious adverse events were relatively uncommon amongst the cell therapies that were examined, and a small number of treatments (intrathecal NSCs, combined subcutaneous G-CSF and intracerebral PBSCs, intracerebral NT2D1 cells, and intravenous BM-MNCs) resulted in better treatment outcomes than current approaches. However, given the majority of findings were based on small, non-blinded, observational studies, with short assessment periods, these results should be considered provisional. Adequately-powered double-blinded placebo-controlled research that screens for psychological and cognitive comorbidities, tracks cell migration *in vivo*, and evaluates treatment outcomes beyond 1 year is now needed to provide a more definitive assessment of these emerging treatments. More comprehensive reporting is also required, guided by the relevant reporting guidelines.

Future Perspectives

Given the medical and economic burden associated with stroke, and the limited availability of long-term treatments, there is a need for safe and effective alternatives. With increasing evidence of safety, larger studies are likely, some of which may examine combined treatments (e.g., SCs, growth factors, SSRIs, cortical/cerebellar stimulation, psychological/cognitive rehabilitation),

in conjunction with blood-brain-barrier permeabilisers, biobridges, and extracellular scaffolds. Clarifying the role of glial cells may also augment current treatments, and the development/refinement of cell labeling and *in vivo* tracking techniques should lead to an improved understanding of the underlying mechanisms of action.

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* *Denotes studies that were meta-analysed*

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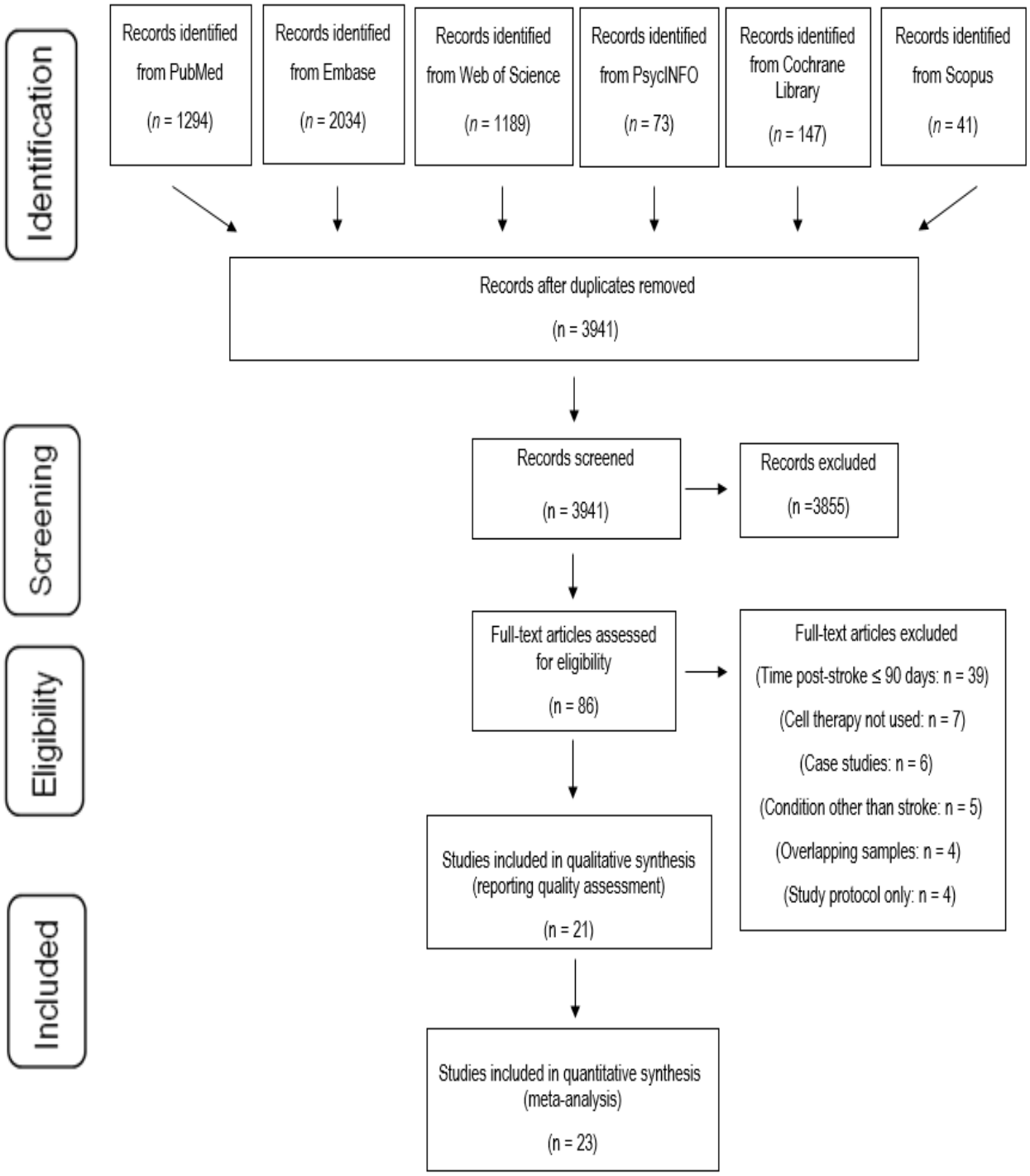
APPENDIX G

PRISMA Flow Chart detailing Study Selection Process for Study 2



APPENDIX H

Publication Details of the Studies used in the Meta-Analysis for Study 2

Study reference	First Author	Last Author	Publication Year	Country of Origin	Study Type	Publication Source
1	Chen, D.C.	Shyu, W.C.	2014	China	RCT – Phase II	Cell Transplantation
2	Lu, W.	Zeng, Y.	2013	China	Observational – Prospective Cohort	Neurosurgery Quarterly
3	Chen, L.	Xiao, J.	2013	China	Observational - Prospective Cohort	Cell Transplantation
4	Suarez-Monteagudo, C.	Bergado, J.A.	2009	Cuba	Observational – Prospective Cohort	Restorative Neurology and Neuroscience
5	Huang, H.	Zhang, F.	2009	China	Observational – Prospective Cohort	Chinese Journal of Reparative and Reconstructive Surgery
6	Savitz, S.I.	Caplan, L.R.	2005	U.S.A.	Observational – Prospective Cohort	Cerebrovascular Diseases
7	Kondziolka, D.	Teraoka, J.	2005	U.S.A	RCT – Phase II	Journal of Neurosurgery
	Stilley, C.S.	Wechsler, L.	2004	U.S.A	RCT – Phase II	Neurology
8	Meltzer, C.	Jacobs, A.	2001	U.S.A	Non-RCT– Phase I	Neurosurgery
	Kondziolka, D.	Bynum, L.	2000	U.S.A	Non-RCT– Phase I	Neurology
9	Bhasin, A.	Bose, S.	2013	India	Non-RCT–Phase n.s.	Clinical Neurology and Neurosurgery
10	Bhasin, A.	Bose, S.	2012	India	Non-RCT–Phase n.s	Journal of Stem Cells & Regenerative Medicine
11	Bhasin, A.	Airan, B.	2011	India	Non-RCT–Phase n.s	Cerebrovascular Diseases
	Bhasin, A.	Kumaran, S.S.	2013	India	Non-RCT–Phase n.s	Cerebrovascular Diseases
12	Li, J.B.	Duan, Y.L.	2007	China	Observational – Prospective Cohort	Journal of Clinical Rehabilitative Tissue Engineering Research
	Man, Y.	Ma, J.	2006	China	Observational – Prospective Cohort	Neural Regeneration Research
13	Sharma, A.	Badhe, P.	2014	India	Observational – Prospective Cohort	Stroke Research and Treatment
14	Wang, L.	Wu, M.	2013	China	Observational – Prospective Cohort	ISRN Neurology
15	Yang, Q.C.	Ma, D.F.	2007	China	Observational – Prospective Cohort	Journal of Clinical Rehabilitative Tissue Engineering Research
16	Zhang, R.Y.	An, Y.H.	2006	China	Observational – Prospective Cohort	Chinese Journal of Clinical Rehabilitation
17	Yang, Q.C.	Li, H.W.	2005	China	Observational – Prospective Cohort	Chinese Journal of Clinical Rehabilitation
18	Rabinovich, S.S.	Taraban, V.Y.	2005	Russia	Observational – Case-Control	Cell Technologies in Biology and Medicine
19	Qiao, L.Y.	Geng, T.C.	2014	China	Observational – Prospective Cohort	Cell Transplantation
20	Floel, A.	Schäbitz, W.R.	2011	Germany	RCT – Phase II	Plos One
21	Steinberg, G.K.	Schwartz, N.E.	2016	U.S.A	Non-RCT – Phase 1/2a	Stroke

Note: Brackets indicate where pairs of studies reporting on the same patient group were combined for parts of the analysis.

n.s. = not specified; RCT = randomised controlled trial.

APPENDIX I

Study 2: Baseline Characteristics of the Control Group for Treatment vs Control: Pre-/Post-Analysis (*N* = 53)

Age *M (SD)*	Male Gender (%)	Stroke Type		Stroke Region		Onset to randomisation *M yrs (SD)*	NIHSS baseline *M (SD) N = 15*	Barthel Index baseline *M (SD) N = 15*	Received Physical Rehabilitation (%)
		Isch	Haem						
47.4 (11.53)	79	51 (96%)	2 (4%)	Large Artery:	19	1.2 (1.1)	9.6 (1.3)	47.6 (9.7)	100%
				MCA:	15				
				Small Vessel:	6				
				Cardioembolic:	4				
				Not specified:	9				

Isch = ischaemic; Haem = haemmorhagic; *M* = mean; MCA = Middle Cerebral Artery; NIHSS = National Institutes of Health Stroke Scale; *SD* = standard deviation.

Study 2: Publication Details of the Studies used for the Control Group for Treatment vs Control: Pre-/Post-Analysis

Study reference	First Author	Last Author	Publication Year	Country of Origin	Study Type	Publication Source
1	Chen, D.C.	Shyu, W.C.	2014	China	RCT – Phase II	Cell Transplantation
9	Bhasin, A.	Bose, S.	2013	India	Non-RCT – Phase n.s.	Clinical Neurology and Neurosurgery
10	Bhasin, A.	Bose, S.	2012	India	Non-RCT Phase n.s.	Journal of Stem Cells & Regenerative Medicine
11	Bhasin, A.	Airan, B.	2011	India	Non-RCT Phase n.s.	Cerebrovascular Diseases

n.s. = not specified; RCT = randomised controlled trial.

APPENDIX J

Treatment Dosages per Cell Therapy and Notes relating to Figure 4-1

	CELL THERAPY		DOSAGE		EFFICACY				PUBLICATION BIAS		NOTES
	Delivery Route	Cell Type	No. of Injections	No. of Cells	Neuro g	Neuro p	Func g	Func p	Overall g	Overall p	N Studies	Overall Nfs	
	Post-treatment vs Control												
1	IT	NSC	1 – 2	2×10^8							1	13	
2	SubCut + IC	G-CSF + PBSC	5 1	15 ug/kg $3 – 8 \times 10^6$							1	6	
3	IC	NT2/D1	1	$5 - 10 \times 10^6$							1	5	
4	IV	BM-MSC	1	$5 - 6 \times 10^7$							2	2	
5	IV	BM-MNC	1	$5 - 6 \times 10^7$							2	2	
6	SubCut	G-CSF	10	10 ug/kg							1	0	
	Pre-/post-treatment												
7	IV	UC-MSC	6	1×10^8							1	10	
8	IC	BM-MSC	1	$1.4 – 5.5 \times 10^7$							1	9	
9	IV + IT	BM-MSC + NPC	1 [a] 3 [b] 3 [c]	0.5×10^6 5×10^6 6×10^6							1	5	a: IV / BM-MSCs b: IT / BM-MSCs c: IT / NPC
10	IC	SB623	1	$2.5 – 10 \times 10^6$	0.97 [d] **						1	4	d: $N_{studies}$ = 1, $N_{participants}$ = 16, study ref = 21
11	IC	NSC	1	3×10^7							1	3	
12	SubCut + IT	G-CSF + BM-MNC	1	G-CSF: n.s. body weight (kg) x 10^6							1	2	
13	SubCut + IT	G-CSF + BM-CD34+	5 4 – 5	5 ug/kg $0.8 - 3.3 \times 10^7$							1	2	
14	IC	OEC + NPC	1 1	1×10^6 2×10^6							1	1	
15	IC	LGE	1	10×10^6							1	1	
16	IT	NSC	1 – 2	$.04 – 2 \times 10^8$	0.12 [e]						4	3	e: $N_{studies}$ = 2, $N_{participants}$ = 60, study refs = 16, 18
17	IC	OEC	1 – 2	1×10^6							1	0	
18	IC + IT	OEC + NPC	1 2	$1 - 2 \times 10^6$ $2 - 5 \times 10^6$							1	0	
19	IC	NT2/D1	1 – 3	$2 - 10 \times 10^6$			-0.32 [f]				2	0	f: $N_{studies}$ = 1, $N_{participants}$ = 12, study refs = 8

Notes: $^{**}p < .001$, $^{*}p < .05$.

BM-MNC = bone marrow mononuclear cells; BM –MSC = bone marrow mesenchymal stem cells; Func = functional outcomes; G-CSF = granulocyte -colony-stimulating factor; IC = intracerebral; IT = intrathecal; IV = intravenous; kg = kilogram; LGE = lateral ganglionic eminence cells (porcine fetal cells); Neuro = neurological outcomes; n.s. = not specified; *Nfs* = fail safe *N* statistic; *Nparticipant*s = number of participants; NPC = neural progenitor cells; NSC = neural stem cells; *Nstudies* = number of studies; NT2/D1 = Ntera 2/cl.D1 (human embryonic carcino-derived cell line); OEC = olfactory ensheathing cell; PBSC = peripheral blood stem cells; Ref = reference; SB623 = modified BM-MSCs; SubCut = subcutaneous; UC-MSC = umbilical cord mesenchymal stem cells; ug = microgram.

Chapter 5. Study 3

Stroke Survivor Attitudes Toward, and Motivations for, Considering Experimental Stem Cell Treatments

This chapter presents a published paper, however copyright restrictions prevent the reproduction of this paper in its published form. The details of this publication are:

Unsworth, D. J., Mathias, J. L., Dorstyn, D. S., & Koblar, S. A. (2019). Stroke survivor attitudes toward, and motivations for, considering experimental stem cell treatments. *Disability and Rehabilitation*, 1-9. doi: 10.1080/09638288.2018.1517193

Please note:

- Tables and figures are formatted in line with the Journal's requirements.
- Australian/British English spelling was used.

5.1 Preface

Studies 1 (Chapter 3) and 2 (Chapter 4) highlighted a range of concerns regarding the safety and efficacy of SC therapies for stroke. Most notably, there is a lack of large-scale, placebo-controlled clinical trial data that confirm the effectiveness and examine the long-term treatment-related risks associated with individual SC therapies. Despite these concerns, unregulated, experimental SC treatments are increasingly being sought by patients with a variety of neurodegenerative disorders, particularly those who are dissatisfied with mainstream treatments and have unrealistic views about the probable treatment outcomes (Cohen & Simana, 2018; Petersen, Seear, & Munsie, 2014; Rachul, 2011 Sipp et al., 2017).

To date, however, little research has been undertaken to assess the general level of interest in 'stem cell tourism' among stroke survivors, or to examine the reasons why patients may consider having risky and experimental SC treatments. A cross-sectional, nation-wide survey of Australian stroke survivors was therefore conducted to address this research gap. The number of patients who were considering experimental SC treatments, in addition to some of the biopsychosocial (i.e. medical, cognitive, psychological) and demographic (i.e. age, sex) factors that may increase the likelihood of a stroke survivor considering experimental SC treatments, were considered in order to address objectives 3 and 4. In addition, patients' attitudes regarding the safety, effectiveness, accessibility, affordability, and perceived social norms regarding SC treatment were examined, consistent with the Theory of Planned Behaviour (Ajzen, 1991).

5.2 Statement of Authorship

Title of Paper	Stroke survivor attitudes toward, and motivations for, considering experimental stem cell treatments
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Unsworth, D. J., Mathias, J. L., Dorstyn, D. S., & Koblar, S. A. (2018). Stroke survivor attitudes toward, and motivations for, considering experimental stem cell treatments. Disability and Rehabilitation, 1-9.

Principal Author

Name of Principal Author (Candidate)	Mr. David J. Unsworth		
Contribution to the Paper	Study design, preparation of ethics application, participant recruitment, data collection and analysis, manuscript preparation.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	30/09/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Prof. Jane L. Mathias		
Contribution to the Paper	Study design, preparation of ethics application, data interpretation, editing manuscript.		
Signature		Date	1/10/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr. Diana S. Dorstyn		
Contribution to the Paper	Study design, preparation of ethics application, data interpretation, editing manuscript.		
Signature		Date	30/9/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Prof. Simon A. Koblar		
Contribution to the Paper	Study design, preparation of ethics application, data interpretation, editing manuscript.		
Signature		Date	9/10/2019

5.3 Abstract

Purpose: Interest in stem cell (SC) treatments is increasing among some patient groups, but it is unclear whether this holds true for stroke survivors. This study examined stroke survivor attitudes toward SC treatments and identified a number of variables that may increase the likelihood that patients will consider these treatments. **Methods:** Adult stroke survivors (*N* = 183) were recruited (stroke advocacy/support groups, outpatient register) for a cross-sectional study. Attitudes to SC treatments were surveyed, guided by the Theory of Planned Behaviour. Demographic information was collected, and a number of self-report medical, cognitive and psychological measures completed. **Results:** Twenty-five percent (*n* = 46) of respondents indicated they were considering undergoing SC treatments, although most were unsure about the safety/effectiveness and accessibility/affordability. Stroke survivors with positive attitudes toward SC treatments, longer post-stroke intervals, poorer physical functioning, younger age, and greater perceived caregiver burden were more likely to be considering experimental treatments (odds ratios = 1.22, 1.08, .95, .96, 1.07; respectively). **Conclusions:** Stroke survivors may consider undergoing experimental SC treatments despite uncertainty regarding the risks/benefits. Clinicians should be mindful of the factors that may increase the likelihood of patients considering these treatments, and intervene, where appropriate, to clarify any misconceptions regarding the medical/financial risks.

5.4 Introduction

Over 15 million people suffer a stroke annually (Thrift et al., 2014) and, despite improvements in acute care (Wang & Wang, 2018) and multidisciplinary rehabilitation (Anderson et al., 2017), one in three patients experience ongoing physical disabilities, cognitive impairments (Mozaffarian et al., 2016), and/or emotional problems (Hackett et al., 2014). The extent to which a patient recovers is largely dependent upon the speed with which medical treatment is received, but is also contingent upon the type of stroke (ischaemic, haemorrhagic), extent of the damage, and severity of the initial impairments; in addition to, the patient's age, premorbid health/functioning, ongoing mental health, and level of social support (Richards, Malouin, & Nadeau, 2015). SC treatments (i.e. intracranial, intrathecal, intra-arterial, intravenous, or subcutaneous injections of SCs/cell-related matter), are being examined as a potential means of reducing stroke-related disability, and preliminary data suggest that some treatments may improve neurological (motor, speech,) and functional (mobility, self-care) outcomes, by augmenting endogenous repair processes and restoring damaged brain tissue (Nagpal et al., 2017; Unsworth, Mathias, & Dorstyn, 2016; 2017).

However, the majority of data originate from early-phase clinical trials or observational studies, few of which controlled for placebo effects or tracked the injected cells. Moreover, treatment-related adverse events, including brain and spinal tumours, seizures, and further strokes have been observed (Nagpal et al., 2017; Unsworth, Mathias, & Dorstyn, 2016; 2017). Phase II/III clinical trials are currently being conducted (NCT02448641; NCT02961504; NCT03545607; NCT03004976; NCT01716481 [clinicaltrials.gov]) but, even if proven to be safe and effective, it will be some time before these treatments are approved for clinical use (Jolkkonen & Kwakkel, 2016). Thus, SC treatments are not currently approved for use with stroke patients in the United States, United Kingdom or Australia.

Despite uncertainty regarding their risks and benefits, expensive (≤ US$100,000) experimental SC treatments are currently offered for stroke by private clinics throughout Asia, Russia and South America (Sipp et al., 2017). These treatments often involve unregulated, non-standardised administration practises, non-disclosure of cell sources and manufacturing processes, and a lack of empirically-based treatment data (Srivastava et al., 2016). Although research suggests that there is a growing interest in experimental SC treatments among people with multiple sclerosis, Parkinson's disease and amyotrophic lateral sclerosis (Mikati et al., 2014), it is not yet known whether stroke survivors show a similar interest in, and expectations of, these treatments. Moreover, little is known regarding why patients seek unproven SC treatments, with the limited data suggesting that being male, having severe long-term physical disabilities (Kim et al., 2012), and dissatisfaction with existing/available treatments (Petersen, Seear, Munsie, & 2014; Rachul, 2011) may increase the chance that a person will consider or undergo experimental SC treatments.

Given that the stroke sequelae differs from that of the neurodegenerative disorders that have been studied (e.g., sudden onset of severe, persistent disabilities), a number of additional variables may need to be considered when examining which patients are most likely to consider SC treatments (Richards et al., 2015). Indeed, the stroke literature identifies multiple factors that contribute to physical disability; including age, time-post stroke, health-related quality of life, cognitive functioning, mental health and social support (Stucki et al., 2016). In addition, the attitudes of a stroke survivor and their family/friends toward a treatment (i.e. the perceived risks/benefits/accessibility/affordability), may influence whether or not they consider it. This is consistent with the Theory of Planned Behaviour model (Ajzen, 1991; Sheeran et al., 2016), an empirically validated socio-cognitive framework that has been used to predict treatment-seeking behaviours. Thus, stroke survivors who believe SC treatments are safe, effective, accessible and affordable, and whose

family/friends support them having these treatments, may also be more likely to consider having them (Seow et al., 2017).

The current study therefore surveyed a group of Australian stroke survivors who had not previously undergone SC injections in order to: (1) evaluate how many were considering having SC treatments; (2) explore their attitudes toward, and expectations of, these treatments; (3) identify what demographic, medical, cognitive, psychological and attitudinal variables may increase the likelihood of a stroke survivor considering SC treatments; and (4) construct an integrated model to help identify which stroke survivors may be most likely/unlikely to consider such treatments.

5.5 Method

This study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines (Von Elm et al., 2007) (see Supplementary Material for STROBE checklist).

Participant Eligibility and Recruitment

Participants were eligible if they were adults (≥ 18 years) at the time of their stroke (ischaemic or haemorrhagic), had not previously undergone experimental SC treatments, and could complete a paper-based, online or telephone survey (with or without assistance). Eight Australian stroke advocacy organisations and stroke support groups (see acknowledgements) promoted the survey among their members, which resulted in 114 responses. The Australian Stroke Clinical Registry, an outpatient registry, also mailed 500 paper-based surveys to members, from which 69 responses were received (13.8% response rate). Of the 183 responses that were received in total, 173 were completed via mail or online, and 10 were completed via telephone interview with the first author (DJU). Informed consent was obtained from each participant prior to completion of the survey. Recruitment occurred from September 2016 to January 2018.

SC Treatment Survey

A 14-question survey (see table 1) was constructed to: (1) assess the number of stroke survivors who were considering experimental SC treatments (yes, no, or unsure) (item 1); and (2) explore respondents' familiarity with, and main sources of information about, SC treatments (items 2-4), the perceived risks and benefits of SC treatments (items 5, 6), their preference for different treatment types (items 7, 8), their desired outcomes (item 9), the perceived accessibility and affordability of these treatments (items 10-13), and their main concerns (item 14). SC treatments were defined as those that are unproven and experimental in nature, and that involve the injection of SCs/cell-based materials sourced from the patient, other people, embryos/foetuses or animals, into the brain, spinal cord, vein, artery or stomach by an overseas clinic (Srivastava et al., 2016). Prior to conducting the survey, the content was piloted with a separate group of 10 stroke survivors, who were sourced from Stroke SA, to ensure that the survey wording and length was acceptable.

Table 5-1. *Survey Questions Exploring Attitudes and Expectations of Stroke Survivors concerning Experimental Stem Cell Treatments, together with Summary Findings (N=183)*

		n (%)		*n* (%)
Interest in stem cell treatments	(1) Are you considering experimental stem cell treatments?			
	Yes	46 (25.1%)		
	No	97 (53.0%)		
	Unsure	40 (21.9%)		
Familiarity with stem cell treatments and main sources of information	(2) How much do you know about stem cell treatments for stroke?		(3) From what source have you heard about them most?	
	Nothing	105 (57.4%)	Media	88 (48.1%)
	A little	64 (35.0%)	No source	74 (40.5%)
	Quite a bit	10 (5.5%)	Doctors/Nurses	7 (3.8%)
	A lot	4 (2.1%)	Empirical research	7 (3.8%)
			Socially	7 (3.8%)
	(4) How many stem cell therapy information booklets have you read?			
	None	165 (90.2%)		
	One or more	18 (9.8%)		
Perceived risks and benefits	(5) How safe do you think they are? [a]		(6) How effective do you think they are?[a]	
	Unsure	114 (62.3%)	Unsure	123 (67.2%)
	Very safe	6 (3.3%)	Very effective	15 (8.2%)
	Quite safe	56 (30.6%)	Moderately effective	28 (15.3%)
	Quite unsafe	6 (3.3%)	Slightly effective	14 (7.7%)
	Very unsafe	1 (0.5%)	Not effective	3 (1.6%)
Preferred treatment types and outcomes	(7) Are there any cell sources that you would not consider? [b]		(8) Are there any places you would not consider being injected?[b]	
	Animals	113 (64.9%)	Spinal Cord	83 (46.1%)
	Embryo/foetus	87 (49.2%)	Brain	73 (40.1%)
	Other people's	63 (35.6%)	Artery	41 (22.8%)
	Your own	24 (13.3%)	Stomach	37 (20.9%)
			Vein	32 (18.0%)
	(9) What area would you most like to improve?			
	Physical	118 (64.5%)		
	Cognitive	45 (24.6%)		
	Psychological	20 (10.9%)		
Perceived accessibility and affordability of stem cell treatments	(10) How difficult do you think it would be to locate/attend a clinic?[a]		(11) How affordable do you think it would be for you to do so?[a]	
	Unsure	64 (35.0%)	Unsure	57 (31.2%)
	Very difficult	65 (35.5%)	Not affordable	78 (42.6%)
	Difficult	36 (19.7%)	Potentially affordable	34 (18.6%)
	Easy	14 (7.65%)	Affordable	13 (7.1%)
	Very easy	4 (2.2%)	Easily affordable	1 (0.5%)
	(12) Do your family/friends want you to locate/attend a clinic?[a]		(13) Would you have stem cell treatments if they were available in Australia?	
	Unsure	54 (29.5%)		
	Very unlikely	54 (29.5%)	Yes	91 (49.7%)
	Unlikely	46 (25.1%)	No	32 (17.5%)
	Likely	23 (12.6%)	Unsure	60 (32.8%)
	Very likely	6 (3.3%)		
Main concerns regarding stem cell treatments	(14) What would be your main concern?			
	Side effects / complications	88 (48.2%)		
	Cost	46 (25.1%)		
	No improvement	28 (15.3%)		
	Traveling overseas	16 (8.7%)		
	No concerns	5 (2.7%)		

Notes: a: Item scores used to calculate stem cell treatment attitude score. b: Multiple responses permitted (percentage totals > 100%).

n = number.

Other Self-Report Measures

A range of additional self-report measures were included in order to help identify the demographic, medical, cognitive and psychological variables that may influence the likelihood that a stroke survivor would consider SC treatments.

Demographic variables

Respondents' age, sex and year of stroke were all surveyed, as was information pertaining to their type of stroke (ischaemic/haemorrhagic), residential location, relationship status (married/partnered/single), work status (employed/unemployed due to health or age), years of education, and recruitment source.

Medical & cognitive variables

Physical independence was assessed using the Nottingham Extended Activities of Daily Living Scale (Nouri & Lincoln, 1987), which measures the level of assistance required to perform 22 tasks in the past 2 weeks (4-point Likert scale: lower scores = greater physical dependence; range: 0 - 66). Health-related quality of life was evaluated using the pain, sleep and communication items from the Assessment of Quality of Life questionnaire (Version 4-D) (Hawthorne, Richardson, & Osborne, 1999). Item scores range from 1 to 4; with lower total scores indicating poorer health-related quality of life (range: 3 - 12). The memory/thinking subscale of the Stroke Impact Scale (Duncan et al., 1999) was used to evaluate cognitive functioning. Using a 5-point Likert scale, participants reported how difficult it was in the past week to perform seven tasks that required concentration, immediate and delayed recall, or executive functioning (lower scores = poorer cognition; range: 7 - 35).

Psychological Variables

Treatment satisfaction was assessed using the Patients' Satisfaction with Stroke Services Questionnaire (Pound, Gompertz, & Ebrahim, 1994) in which respondents rate 14

areas using a 4-point Likert scale (lower scores = less satisfaction; range: 14 - 56). Social support was evaluated using the 12-item Multidimensional Scale of Perceived Social Support (7-point Likert scale, lower scores = less social support; range: 12 - 84) (Zimet et al., 1988). Anxiety and depression were measured using the Hospital Anxiety and Depression Scale (Snaith, 2003), which assesses how frequently (0 to 3) respondents experienced 14 symptoms during the past week (higher scores = greater anxiety/depression; range: 0 - 21; scores ≥ 4 = clinically-significant levels of symptoms [Sagen et al., 2009]). Participants who had an unpaid/informal caregiver (spouse/family/friend) additionally completed the 10-item Self-Perceived Burden Scale (Cousineau, McDowell, & Hotz, 2003); reporting how often (scale of 1 - 5) they felt guilty/worried/concerned about the demands placed on their carer (higher scores = greater concern; range: 10 - 50).

Attitudinal Variables

Participant attitudes toward experimental SC treatments were measured using a five-item scale that was designed for this study. Specifically, respondents were asked to rate (5-point Likert scale) how safe, effective, accessible and affordable they perceived SC treatments to be, and how likely it was that their family/friends would want them to have SC injections (see table 1, items 5, 6, 10-12). Item scores were summed to create a composite score, with higher scores indicating more positive attitudes regarding experimental SC treatments (range: 5 - 25).

Data Preparation & Statistical Analysis

Summary results (means [*SDs*], frequencies [percentages]) were calculated, both for the full sample and for two subgroups (Considering SC treatment: Yes and No/Unsure). In the small number of cases ($n = 6$) where respondents were 'unsure' of the timing of their stroke, the mean duration of 5.3 years was imputed (average of observed values) (Horton & Kleinman, 2007). Where missing questionnaire items were identified, multiple imputations

were performed for each respondent based on their mean item score. This method was selected because the number of respondents with missing data was less than 10% (Eekhout et al., 2014).

A multivariate logistic regression was conducted, using the Purposeful Selection of Covariates approach (Hosmer, Lemeshow, & Sturdivant, 2013), to identify which demographic, medical, cognitive, psychological and attitudinal variables increased the probability that a stroke survivor would consider experimental SC treatments (model 1). This analysis was repeated for respondents who had an unpaid/informal caregiver (n = 106) in order to determine whether perceived caregiver burden was an additional predictor (model 2). Exploratory univariate analyses were conducted (nominal/categorical data: Pearson χ^2 tests; continuous and ordinal data: Mann Whitney *U*-tests) to identify significant differences (p < .20) between those who were considering SC treatments and those who were not or were unsure.

Odds ratios (ORs) and confidence intervals (95% CIs) were calculated for each of the predictors that were included in the final models (p < .05). An OR > 1 represented the increased odds of a patient considering SC treatment per unit increase in score on the corresponding measure (higher scores = higher odds) and an OR < 1 represented the decreased odds of a patient considering SC treatment per one unit increase in score (lower scores = higher odds) (Hosmer et al., 2013). Bootstrapped beta-coefficient standard errors (*SEs*) and p values were used when assessing the statistical significance of the predictors (based on 1,000 samples) to establish the internal validity of the respective models (Steyerberg & Harrell, 2016). Likelihood ratio and Hosmer and Lemeshow tests assessed the quality and 'fit' of the final models; respectively (Hosmer et al., 2013).

As an additional step, the ORs were also converted to predicted probabilities using the marginal standardisation method (predicted probabilities summed to calculate a weighted

average), so that the cumulative probability of an individual considering experimental SC treatments could be estimated based on their scores across multiple variables (Muller & MacLehose, 2014). Probabilities ≥ 80%, ≥ 60% and < 60% were categorised as likely, possible and unlikely to consider experimental SC treatments, respectively (Zipkin et al., 2014); consistent with guidelines for the identification of 'at risk' patients in clinical practice (Muller & MacLehose, 2014). Statistical power was evaluated when the final N was known, based on a minimum event-to-predictor ratio of 5:1 (Vittinghoff & McCulloch, 2007). All analyses were performed using IBM SPSS Statistics for Windows (version 24.0) (IBM Corp, 2012).

5.6 Results

Demographic and Clinical Details

A total of 183 stroke survivors, aged between 26 and 96, completed the survey (see table 2 for summary details). The post-stroke interval ranged from 1 to 36 years. Similar numbers of women and men responded, the majority of whom were married/partnered, had completed high school, and were unemployed/retired due to age or health. Ischaemic strokes were more common than haemorrhagic strokes. Most respondents had received medical treatment and rehabilitation after their stroke, although only around one in three had also accessed psychological and/or psychiatric care.

Table 5-2. *Demographic and Clinical Characteristics of the Full Sample and Stem Cell Treatment Subgroups*

				Considering Stem Cell Treatment				
				Yes ($n = 46$)		No/ Unsure ($n = 137$)		p
Demographic variables		M	*SD*	M	*SD*	M	*SD*	
Age, years[a]		60.2	16.9	55.4	16.5	61.9	16.5	0.025[b]
Time post-stroke, years[a]		5.7	5.4	7.3	6.4	5.1	4.9	0.190[b]
Education, years		13.4	4.6	13.9	4.6	13.2	4.6	0.177[b]
		n	%	*n*	%	*n*	%	
Sex, male[a]		95	51.9	28	60.9	67	48.9	0.160[c]
Relationship status:								.025[c]
Married/partnered		127	69.4	38	82.6	89	65.0	
Single/separated/divorced/widowed		56	30.6	8	17.4	48	35.0	
Employment status:								0.271[c]
Not employed/retired due to age		73	39.9	14	30.4	59	43.1	
Not employed/retired due to health		56	30.6	15	32.6	41	29.9	
Employed		54	29.5	17	37.0	37	27.0	
Stroke type:								0.588[c]
Ischemic		109	59.6	30	65.2	79	57.7	
Hemorrhagic		42	23.0	10	21.7	32	23.3	
Unsure		32	17.4	6	13.0	26	19.0	
Treatments received post-stroke:								
Medical		142	77.6	34	73.9	108	78.8	0.686[c]
Physio-/Occupational-/Speech-therapy		139	80.0	42	91.3	97	70.8	0.012[c]
Psychological/Psychiatry		67	36.6	22	47.8	45	32.8	0.096[c]
Medical and cognitive variables	Score range	M	*SD*	M	*SD*	M	*SD*	
Physical independence[a]	0–66	49.1	17.5	39.8	15.0	52.3	17.1	0.000[b]
Health-related quality of life[a]	3–12	9.9	1.8	9.6	2.0	10.0	1.7	0.304[b]
Cognition[a]	7–35	29.3	6.5	29.4	6.6	29.3	6.4	0.983[b]
Psychological variables								
Treatment and service satisfaction[a]	14–56	42.4	8.7	40.8	7.9	43.0	8.9	0.124[b]
Social support[a]	12–84	65.7	14.7	65.8	13.5	65.6	15.1	0.862[b]
Anxiety[a]	0–21	6.6	4.5	6.9	4.3	6.4	4.5	0.542[b]
Depression[a]	0–21	6.2	4.3	7.7	4.0	5.7	4.3	0.002[b]
Perceived burden[a, d]	10–50	25.1	9.3	27.9	9.7	23.7	8.8	0.025[b]
Attitudinal variables								
Stem cell treatment attitude score[a]	5–25	13.1	2.9	13.9	3.1	12.9	2.7	0.050[b]

Notes: a: variables included in multivariate logistic regression analyses. Tests performed to compare Considering Stem Cell Treatment: Yes and No/Unsure groups. b: Mann–Whitney U-test. c: Pearson χ^2. d: Subgroup of respondents with caregiver (n = 106): Considering Stem Cell Treatment: Yes: n=35, No/Unsure: n = 71)

M = mean; *n* = number; *SD* = standard deviation; *p* = *p* values.

On average, scores on the measures of physical independence (Nottingham Extended Activities of Daily Living Scale), health-related quality of life (Assessment of Quality of Life Scale) and cognition (Stroke Impact Scale) fell within the moderate-to-good range, as did the treatment satisfaction (Patient Satisfaction with Stroke Services Questionnaire) and social support (Multidimensional Scale of Perceived Social Support) scores. Conversely, 70% and 66% of the sample reported levels of anxiety and depression; respectively, that exceeded stroke guidelines (Hospital Anxiety and Depression subscale scores ≥ 4 [Sagen et al., 2009]). Self-Perceived Burden Scale scores fell in the moderate range for those with a carer (*n* = 106).

SC Treatment Survey Responses

Summary findings from the SC treatment survey are provided in table 1. In total, 46 respondents (25.1%) indicated that they were considering experimental SC treatments, the remainder were not (*n* = 97, 53.0%) or were unsure (*n* = 40, 21.9%). Most respondents (57.4%) indicated that they knew nothing about such treatments. The media was the main source of people's information (48.1%); far less had discussed SC treatments with doctors/nurses (3.8%) or read empirical research (3.8%), and only 18 respondents had previously accessed a patient information booklet. Most respondents were unsure about the risks (62.3%) and benefits (67.2%) associated with experimental SC treatments.

A large proportion said they would not consider treatments that used SCs from animals (64.9%) and human embryos/foetuses (49.2%), or involved injections to the spinal cord (46.1%) and brain (40.1%). In terms of preferred treatment outcomes, the majority wanted physical (64.5%), rather than cognitive (24.6%) or psychological (10.9%), improvements. Most indicated that it would be difficult/very difficult to locate/attend a SC clinic (55.2%) or to afford treatment (42.6%), and few indicated that their family and friends would want them to do so (15.9%). However, 91 respondents (49.7%) indicated that they

would consider SC treatments if they were available domestically. Respondents were mainly concerned about treatment-related side-effects (48.2%) and treatment costs (25.1%). The mean score for SC treatment attitude fell in the mid-range (see table 2), suggesting a high level of uncertainty and ambivalence about the safety, effectiveness, accessibility and affordability of these experimental stroke treatments.

Characteristics of those Considering Experimental SC Treatments

The sample of 183 respondents was divided into two groups to enable a comparison of those who were considering experimental SC treatments (Yes: $n = 46$) with those who were not or were unsure (No/Unsure: $n = 137$). Descriptive statistics and the results of the univariate analyses are provided in table 2. The multivariate regression models are provided in table 3. Model 1 (full sample) indicates that stroke survivors who had positive attitudes towards SC treatments (OR: 1.22, $p = .008$), longer post-stroke intervals (OR: 1.08, $p = .024$), lower levels of physical independence (OR: 0.95, $p = .001$) and younger ages (OR: 0.96, $p = .003$) were more likely to be considering experimental SC treatments. In model 2 (respondents with carers; $n = 106$), stroke survivors with positive attitudes towards SC treatments (OR: 1.30, p=.004), longer post-stroke intervals (OR: 1.10, $p = .010$) and greater self-perceived caregiver burden (OR: 1.07, $p = .029$) were more likely to be considering experimental SC treatments. Likelihood ratio tests confirmed that the final models were better than intercept-only/null models at predicting which respondents were at risk of considering experimental SC treatments. Hosmer and Lemeshow tests indicated the 'goodness-of-fit' for each model was fair (see table 3). Each model predicted 70% to 80% of overall cases correctly; although both models identified those who were not considering experimental SC treatments with greater accuracy (see table 3). Residual plots were inspected for potential outliers and influential cases, but all data were retained (Sarkar, Midi, & Rana, 2011).

Table 5-3. *Multivariate Logistic Regression Analysis Identifying Potential Predictors for Stroke Survivors considering Experimental Stem Cell Treatments*

Model 1: Total sample ($N = 183$)	β	*SE*[a]	Wald's x^2	*df*	*p*[b]	OR (95% CI)
Stem cell treatment attitude score	0.202	0.10	7.92	1	0.008	1.22 (1.06–1.41)
Time post-stroke	0.075	0.03	5.82	1	0.024	1.08 (1.01–1.15)
Physical independence	−0.051	0.01	19.74	1	0.001	0.95 (0.93–0.97)
Age	−0.043	0.01	12.35	1	0.003	0.96 (0.94–0.98)
Constant	0.626	1.30	0.23	1	0.673	n/a
Model 1 evaluation			x^2	*df*	*p*	
Likelihood ratio test			40.62	4	0.000	
Hosmer and Lemeshow test			7.61	8	0.472	
	Predicted					
Observed frequencies	Yes	No/Unsure	% Correct			
Yes	18	28	39.1	False positive rate (%) = 35.7		
No/Unsure	10	127	92.8	False negative rate (%) = 18.1		
Overall % correct			79.2			
Model 2: Subgroup with caregivers ($n = 106$)	β	*SE*[a]	Wald's x^2	*df*	*p*[a]	OR (95% CI)
Stem cell treatment attitude score	0.262	0.09	8.42	1	0.004	1.30 (1.09–1.55)
Time post-stroke	0.092	0.04	5.38	1	0.010	1.10 (1.01–1.19)
Perceived burden	0.066	0.03	6.82	1	0.019	1.07 (1.02–1.12)
Constant	−6.434	1.62	15.74	1	0.001	n/a
Model 2 evaluation			x^2	*df*	*p*	
Likelihood ratio test			17.23	3	0.001	
Hosmer and Lemeshow test			4.68	8	0.791	
	Predicted					
Observed frequencies	Yes	No/Unsure	% Correct			
Yes	12	23	34.3	False positive rate (%) = 36.8		
No/Unsure	7	64	90.1	False negative rate (%) = 26.4		
Overall % correct			71.7			

Note: a: Values based on 1000 bootstrapped samples.

CI: confidence interval; *df*: degrees of freedom; *N*: number; OR: odds ratio; *p*: *p* values; *SE*: standard error. Predicted frequencies were calculated using 0.50 cutoff.

The probability that a stroke survivor would be likely, possibly, or unlikely (≥ 80%, ≥ 60%, < 60%; respectively) to consider experimental SC treatments was calculated, based on the variables identified by models 1 and 2. As shown in Figure 5-1, the cumulative probability estimates for model 1 indicate that stroke survivors who: had high scores (i.e. positive responses) on the questions relating to SC treatment attitudes, were ≥ 10 years post-stroke, had low levels of physical independence (e.g., were unable to walk around outside, feed themselves, socialise without assistance; Nottingham Extended Activities of Daily Living Scale scores ≤ 10), and were aged ≤ 40 years, were more likely to consider experimental SC treatments. If they had a carer, model 2 indicates that stroke survivors who: had high scores (i.e. positive responses) on the questions relating to SC treatment attitudes, were ≥ 10 years post-stroke, and had strong concerns regarding their burden on caregivers (Self-Perceived Burden Scale scores ≥ 50) were more likely to consider experimental SC treatments (see Figure 5-2).

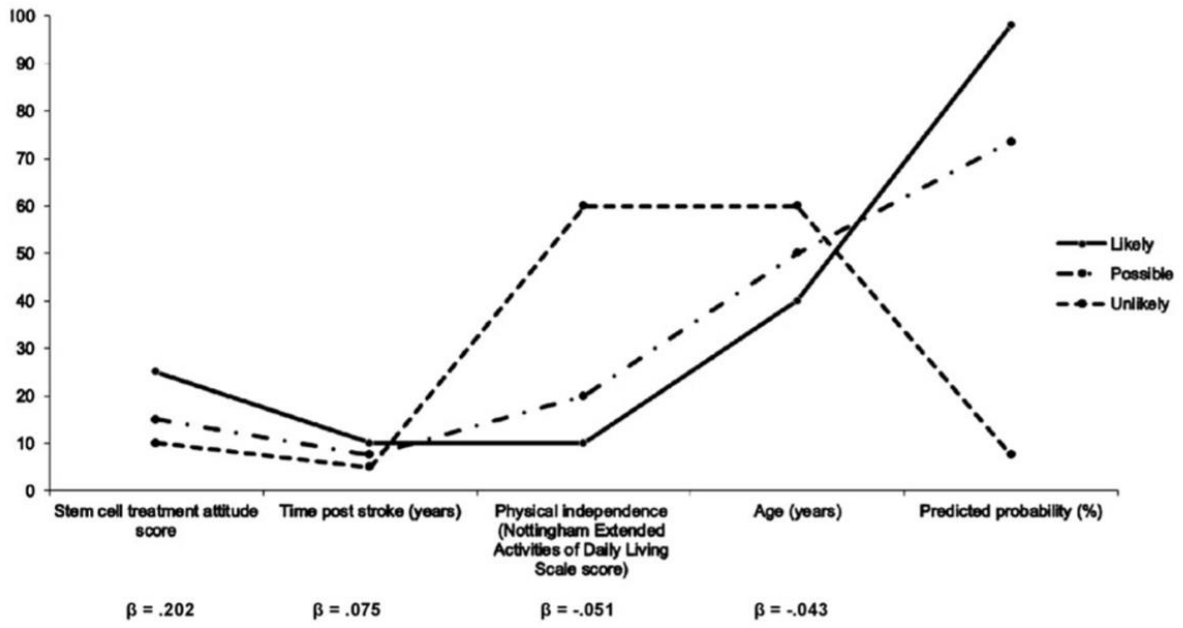


Figure 5-1. Cumulative predicted probabilities of stroke survivors considering experimental stem cell treatments (model 1, *N* = 183).

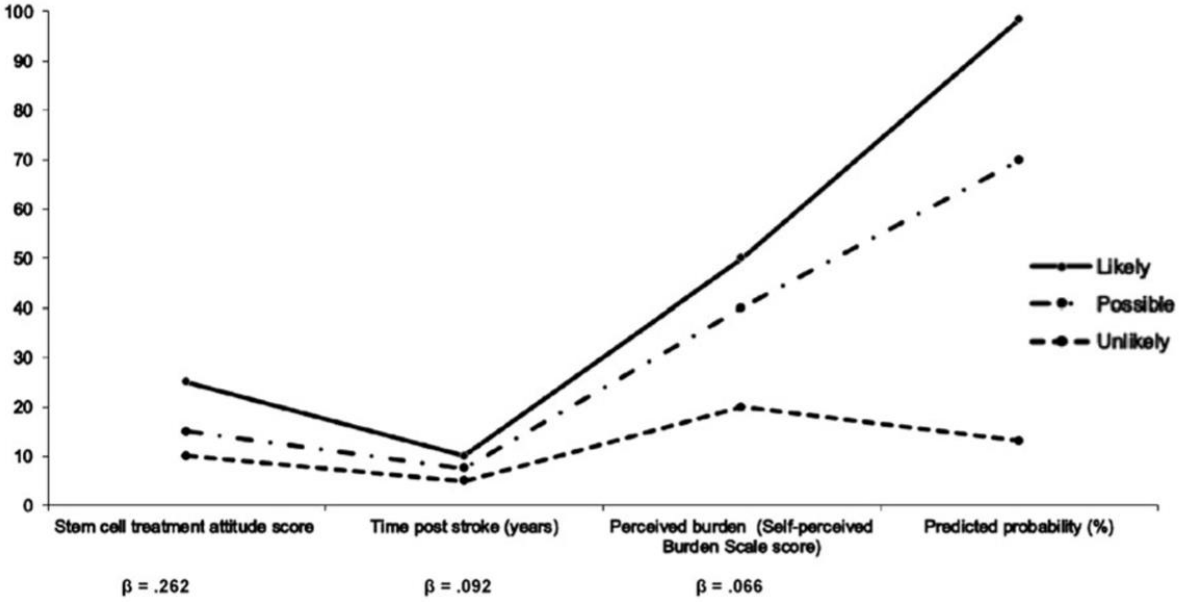


Figure 5-2. Cumulative predicted probabilities of stroke survivors with caregivers considering experimental stem cell treatments (model 2, *n* = 106).

5.7 Discussion

This study surveyed Australian stroke survivors to examine their level of interest in, and attitudes toward, experimental SC treatments. Overall, one in four (25%) respondents reported that they were considering experimental SC treatments, which is lower than a previous South Korean study (46%) (Kim et al., 2012), possibly reflecting cultural differences in attitudes and/or knowledge relating to experimental medical treatments (Yu & Ko, 2012). Consistent with studies conducted in Asia (Kim et al., 2012) and Europe (Aked et al., 207), this study found that the majority of respondents knew very little about the risks and benefits of SC treatments, with most relying on the media (e.g. radio, newspapers, internet) for health information. This is concerning, given that media reports – particularly those found online – tend to exaggerate the positive aspects of experimental SC treatments, without disclosing financial interests (Petersen et al., 2017).

Most respondents also indicated that they would not consider treatments that involve injections into the spine (intrathecal) or brain (intracranial), but would consider having SC injections into a vein (intravenous) or the abdomen (subcutaneous). These findings are noteworthy because the only large-scale clinical trial currently being conducted for patients in the chronic phase of stroke involves intracranial injections (NCT02448641), which suggests that stroke survivors may be reluctant to have this form of treatment even if it were to become publically available. Moreover, the findings also suggest that stroke survivors may be more likely to seek out SC treatments that they perceive to be safer (i.e. intravenous, subcutaneous injections), despite evidence suggesting that the cells do not reach damaged brain tissue and are, therefore, less effective (Nagpal et al., 2017; Unsworth et al., 2017). In addition, approximately one third of respondents reported that, if they were to have SC injections, they would prefer improvements in their cognitive (i.e. executive functioning, memory) or psychological functioning (i.e. reduced depression and anxiety), rather than their

physical capabilities; however, neither of these areas have been routinely examined by current SC research (Unsworth et al., 2016; 2017).

Lastly, although most respondents thought it would be difficult to locate or attend a private clinic and to afford SC treatments, approximately half indicated that they would consider having them if they were available domestically. This is concerning because private clinics that offer adipose (stomach fat, bone marrow) SC injections, primarily for chronic pain conditions (i.e. osteo/rheumatoid arthritis), have begun operating throughout the United States, United Kingdom and Australia, without the need for regulatory approval (Knoepfler, 2018; Tanner, Petersen, & Munsie, 2017). Given chronic pain is also common after a stroke, the findings from this study suggest patients may seek treatment from these clinics, despite only two Phase I clinical trials currently being conducted to establish the safety and tolerability of adipose SC injections for stroke (NCT03570450; NCT02813512 [clinicaltrials.gov]).

The present study also highlighted a number of potential factors that may influence whether a stroke survivor considers experimental SC treatments. In each of the groups that were examined (i.e. full sample and respondents with carers), positive attitudes regarding the safety, effectiveness, accessibility and affordability of SC treatments appeared to be the most important factors. These findings are consistent with the Theory of Planned Behaviour (Ajzen, 1991) and suggest that educational interventions – which are designed to improve stroke survivors and their family/friends' understanding of the risks and benefits associated with experimental SC treatments – may assist in reducing the likelihood that patients will consider having these treatments. However, given that only 10% of respondents indicated that they had previously read an online patient-information booklet, more appealing and interactive methods of communicating this information (e.g. telephone advice lines, stroke community events) may prove to be more effective (Tanner et al., 2017). Moreover, the responses provided in the current study suggest that, by making it more difficult to access

private clinics (i.e. via increased local and international regulation), fewer stroke survivors may consider undergoing experimental treatments. However, it is also important to note, that, despite ongoing attempts to regulate and/or close SC clinics that offer non-evidence based treatments, progress has been slow, particularly in non-Westernised countries (Sipp et al., 2017; Tanner et al., 2017).

The findings from this study also suggest that the longer an individual continues to live with stroke-related disabilities, the more likely they may be to consider SC treatment. This result was particularly evident amongst respondents who were also physically dependent on others; a source of frustration for many patients (Kim et al., 2012; Petersen et al., 2014). Younger stroke survivors may also be more likely to consider experimental SC treatments, possibly reflecting lower risk aversion and a greater focus on treatment benefits, rather than risks (Sparrow & Spaniol, 2016). Lastly, stroke survivors who are concerned about the burden they place on their caregivers (i.e. a spouse or family member) may be more likely to consider experimental SC treatments. Rehabilitation interventions to help patients and their caregivers prepare for, and adjust to, the physical, cognitive, and emotional difficulties that commonly arise after a stroke are important in this respect (Kneebone, 2016; Winson, Wilson, & Bateman, 2016). Lastly, the cumulative probability estimates provide valuable information for treatment planning, by highlighting the importance of inter-disciplinary interventions that aim to maximise physical functionality, in addition to supporting patient mental wellbeing, and patient-caregiver communication.

Study Limitations

There are a number of limitations that should be taken into account when considering these results. First, the current study was underpowered, having a 4:1 event-to-variable ratio (i.e. 46 'Considering SC treatment: Yes' cases; 12 predictors) (Vittinghoff & McCulloch, 2007). Although a reduction in the number of variables would have improved this ratio, one

of the main aims of the study was to identify a list of potentially useful predictors. Therefore, all variables were examined using conservative statistical techniques (e.g., 95% CIs, bootstrapped beta-weight *SEs* and *p* values). Second, the generalisability of these findings to other countries remains to be determined, due to demographic, clinical, treatment (Thrift et al., 2014) and attitudinal differences (Yu & Ko, 2012). Third, stroke survivors who were considering SC treatments may have been more likely to participate in the online survey (i.e. selection bias) and, those who were not, may have been less likely to have responded to the mailed surveys (i.e. non-response bias). Consequently, stroke survivors who were considering SC treatments may be over-represented in the current sample. Fourth, some of the variables that were identified (physical dependence, age) only marginally increased the odds of considering SC treatment, therefore predictions based upon multiple variables were provided. Lastly, the current study did not collect specific information about the respondents' strokes (e.g. size and location of the infarct), premorbid health/functioning, or current health (e.g. blood pressure, blood gas levels), nor did it examine a number of potentially important variables (e.g. history of overseas travel, prior experimental treatments for other conditions). Future research should examine these additional variables, preferably within the context of a large international study, in order to extend the current findings.

Conclusions

The current survey suggests that some stroke survivors may consider having experimental SC treatments, despite the significant costs and ongoing uncertainty regarding the medical risks and benefits. Having to travel overseas for treatment may have previously acted as a deterrent; however, clinics offering adipose SC treatments for related conditions (e.g. chronic pain) are now operating in the United States, United Kingdom, and Australia, which may result in more patients undergoing these experimental treatments for their ongoing stroke-related symptoms. Consequently; health professionals working with stroke survivors need to be aware of the factors that may potentially increase the likelihood of patients

considering these risky, experimental and unproven treatments. By clarifying misconceptions regarding the safety and effectiveness of experimental SC treatments, and educating families to help them prepare for, and adjust to the difficulties arising after a stroke, treating clinicians may help stroke survivors and their caregivers to make more informed decisions regarding their treatment options.

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Chapter 6. Study 4

Are Patient Educational Resources Effective at Deterring Stroke Survivors from Considering Experimental Stem Cell Treatments? A Randomised Controlled Trial

This chapter presents a paper currently under peer review, the details of which are:

Unsworth, D. J., Mathias, J. L., Dorstyn, D. S., & Koblar, S. A. Are patient educational resources effective at deterring stroke survivors from considering experimental stem cell treatments? A randomised controlled trial. *Patient Education and Counselling, 103, 1373-1381*. doi: 10.1016/j.pec.2020.02.012

Please note:

- Tables and figures are formatted in line with the Journal's requirements.
- Australian/British English spelling was used.

6.1 Preface

In response to the concerning level of interest in 'stem cell tourism' that was identified in Study 3 (Chapter 5), a prospective parallel-group RCT (Study 4) was conducted to evaluate whether two existing online resources influenced stroke survivors' attitudes about the safety and effectiveness of experimental SC treatments (objective 5). Patient education is part of a widespread effort by SC research and patient advocacy groups to decrease growing participating in these risky, unproven treatments (Weiss, Turner, Levine, & Ikonomou, 2018). Although a wide range of educational resources are currently available, it is unclear whether existing online resources, which were primarily developed for patients with neurodegenerative disorders, can effectively dissuade stoke survivors from undergoing experimental SC treatments.

To evaluate the effectiveness of the pre-existing patient education resources, 112 stroke survivors, recruited from international support groups, were randomly allocated to one of three groups: 37 received a downloaded version of an online resource ('booklet') developed by the International Society for Stem Cell Research (2015), 38 accessed a brief video provided by the Stem Cell Network (2014), and 37 were assigned to a waitlist control group. The SC treatment attitudes of each group were evaluated before, immediately after and 30-days post-intervention, using a purposely designed measure based on the Theory of Planned Behaviour (Ajzen, 1991). The SC resources were additionally examined in terms of how interesting, informative, intelligible and comprehensive they were in order to determine their suitability for use in a stroke setting.

6.2 Statement of Authorship

Title of Paper	Are patient educational resources effective at deterring stroke survivors from considering experimental stem cell treatments? A randomized controlled trial
Publication Status	☐ Published ☐ Accepted for Publication ☑ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Unsworth, D. J., Mathias, J. L., Dorstyn, D. S., & Koblar, S. A. Are patient educational resources effective at deterring stroke survivors from considering experimental stem cell treatments? A randomized controlled trial. Patient Education and Counseling (under review).

Principal Author

Name of Principal Author (Candidate)	Mr. David J. Unsworth
Contribution to the Paper	Study design, preparation of ethics application, participant recruitment, data collection and analysis, manuscript preparation.
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 30/09/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Prof. Jane L. Mathias
Contribution to the Paper	Study design, preparation of ethics application, data interpretation, editing manuscript.
Signature	Date: 1/10/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Dr. Diana S. Dorstyn
Contribution to the Paper	Study design, preparation of ethics application, data interpretation, editing manuscript.
Signature	Date: 30/9/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Prof. Simon A. Koblar
Contribution to the Paper	Study design, preparation of ethics application, data interpretation, editing manuscript.
Signature	Date: 9/10/2019

6.3 Abstract

Objective: To evaluate whether online resources developed to educate people about the risks associated with experimental stem cell (SC) treatments influence stroke survivors' attitudes about the safety and effectiveness of these treatments. **Methods:** Adult stroke survivors who had not previously received SC treatments (N= 112) were recruited from international stroke advocacy/support groups for a prospective, parallel-group randomised controlled trial. Participants indicated whether they were considering SC treatments (yes/no) prior to, immediately following, and 30-days after reading/viewing the International Society for Stem Cell Research booklet or Stem Cell Network video. Participant attitudes regarding the safety, effectiveness, accessibility and affordability of SC treatments were examined on each occasion, and compared to those of a waitlist control group. **Results:** Significantly fewer participants were considering SC treatments immediately after reading the SC research booklet ($p = .031$), although neither intervention had any impact after 30-days ($p > .05$). Waitlist and intervention groups reported positive attitudes toward SC treatments at each assessment. **Conclusions:** Stroke survivor attitudes toward SC treatments were initially influenced by the patient booklet, however these changes were not maintained. **Practical Implications:** Clinicians are encouraged to initiate discussions about experimental SC treatments during inpatient rehabilitation and to reinforce the risks throughout subsequent care.

6.4 Introduction

Globally, an estimated 33 million people live with stroke-related disabilities (Thrift et al., 2014). Although recent advances in emergency treatments (Nogueira et al., 2018) and rehabilitation (Bernhardt et al., 2017) have improved the prognosis for many patients, only 10% fully recover (Mozaffarian et al., 2016). Preliminary research suggests that certain intracranial and intrathecal stem cell (SC) injections may improve functional recovery following a stroke (Boltze et al., 2015; Nagpal et al., 2017; Unsworth, Mathias, & Dorstyn, 2016; 2017; Xue, Wang, & Yan, 2018). To date, however, few large-scale placebo-controlled clinical trials have been conducted (Sarmah et al., 2018). Therefore, despite some promising initial results (Savitz et al., 2019; Steinberg et al., 2018; Wang & Wang 2017), the risks and benefits associated with these SC treatments remain poorly understood (Boltze et al., 2015; Nagpal et al., 2017; Unsworth et al., 2016; 2017; Xue, Wang, & Yan, 2018).

Undeterred by this, private clinics throughout Asia, Russia and South America offer non-evidence based, experimental SC treatments for a range of neurological conditions, including stroke (Weiss, Turner, Levine, & Ikonomou, 2018), some of which have resulted in patients developing brain tumours and spinal lesions (Amariglio et al., 2009; Berkowitz et al., 2016; Hurst et al., 2013). Although the number of patients who are undergoing experimental SC treatments is unclear, a recent study found that up to 25% of stroke survivors were considering them after seeing media reports and online advertisements promoting the benefits of SC injections (Unsworth, Mathias, Dorstyn, & Koblar, 2019).

Recognising the significant medical and financial risks associated with experimental SC treatments, government bodies have sought to restrict the availability of these treatments by tightening international regulatory guidelines (Sipp, 2018; Sipp & Okano, 2018). SC research and advocacy organisations have additionally developed a range of patient resources to raise awareness about the risks associated with private, unregulated SC treatments (Sipp et

al., 2017; Weiss et al., 2018). However, whether these educational resources effectively deter stroke patients from considering risky SC treatments is unknown. Indeed, previous research conducted with patients diagnosed with either multiple sclerosis or Parkinson's disease suggest that they may not (Rachul, 2011; Seear, Petersen, Munsie, & Skinner, 2010; Tanner, Petersen, & Munsie, 2017). Given that the available resources were designed for use with patients who have a range of medical conditions, it is possible that the content, level of detail, and design of the materials may not be optimal for stroke survivors who frequently experience cognitive, language, communication and/or visual problems (Du, Ma, & Li, 2016; Hackett, Köhler, & O'Brien, 2014).

This study examined whether two online patient educational resources, one in a booklet and the other in a video format: (1) deterred stroke survivors from considering experimental SC treatments, and (2) influenced stroke survivors' attitudes regarding the safety, effectiveness, accessibility and affordability of these treatments, relative to a waitlist control group. Participants indicated whether they were considering SC treatments prior to (pre-intervention), immediately after (post-intervention), and 30-days (follow-up) after reading or viewing their allocated resource. The booklet and video resources were additionally examined in terms of how interesting, informative, intelligible and comprehensive they were in order to determine their suitability for use in a stroke setting.

6.5 Methodology

This prospective, parallel group, randomised controlled trial was approved by the University of Adelaide Human Research Ethics Committee (Approval Number: H-2018-128) and was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12618001094268). This project adhered to the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Moher et al., 2010) (see Appendix K for CONSORT checklist).

Resource Evaluation and Selection, Participant Recruitment, and Trial Design

An online search, using the Google search engine, was undertaken to identify patient education resources that were produced by key SC research and advocacy organisations (i.e. International Society for Stem Cell Research, Stem Cell Network, Stem Cells Australia, Euro Stem Cell, International Society of Cell and Gene Therapy). For the purposes of the current study, preference was given to resources with an explicit focus on providing evidence-based patient information regarding experimental SC treatments, as opposed to SC fact sheets, presentation slides, or cartoons/comics. Resources that required participants to access large amounts of information from external links were excluded so as to standardize the patient education interventions. Four booklets and two videos were located that met these broad criteria (refer to Appendix L for details). These resources were independently evaluated by the first author (DJU) and a clinical psychologist specializing in stroke rehabilitation using the Patient Education Materials Assessment Tool (PEMAT; Shoemaker, Wolf, & Brach, 2014) for printed materials (26-items, score range: 0-26) or audio-visual materials (25-items, score range: 0-25).

Two of the six resources received the highest PEMAT score from both raters and were subsequently selected for this study (refer to Appendix L for summary scores). The *"Nine Things to know about Stem Cell Treatments"* booklet (International Society for Stem Cell Research, 2015) outlines the different SC types, associated medical (short/long-term effects, potential ineligibility for future evidence-based SC treatments) and financial risks, distinguishes between treatments provided by private clinics and those offered by clinical trials, and flags the potential misleading nature of online marketing and patient testimonials. The *"What Is Stem Cell Tourism?"* video (Stem Cell Network, 2014) identifies the promise associated with SC treatments, summarizes the lengthy bench-to-bedside translational process, and warns against travelling overseas before understanding the potential risks

(medical and financial). Written permission was subsequently obtained from the relevant organisations to use both educational materials in this study.

English-speaking adult (≥18 years) stroke survivors were recruited from two international stroke advocacy organisations and 18 online brain injury support groups between June and December 2018. Participants were screened to ensure that they had not previously received SC treatments for their stroke, stroke-related symptoms (e.g. chronic pain), or any other medical or neurological disorder. Eligible participants were randomised (1:1:1) to a booklet intervention (Group 1), video intervention (Group 2), or waitlist control (Group 3) group by the first author (DJU) using computer-generated number sets (Urbaniak & Plous, 2013). Those who reported having visual and/or hearing difficulties upon screening were allocated to either the waitlist control group or most appropriate intervention group (Group 1 booklet: if hearing difficulties; Group 2 video: if visual difficulties). Participants in the waitlist control group received both educational resources at the conclusion of the study. Informed consent was obtained prior to participation.

Patient Education Intervention

Demographic (age, sex, relationship status, country of residence), stroke (type, year) and outcome data were collected from each participant upon study enrolment. Specifically, physical independence was assessed using the Nottingham Extended Activities of Daily Living Scale (NEADL; Nouri & Lincoln, 1987), which measures the level of assistance required to perform 22 tasks in the preceding 2 weeks (4-point Likert scale: lower scores = greater physical dependence; range: 0-66). Health-related quality of life was evaluated using the pain, sleep and communication items from the Assessment of Quality of Life questionnaire (AQoL; Version 4-D; Hawthorne, Richardson, & Osborne, 1999), with lower total scores indicating poorer quality of life (individual item scores: 1-4, total score range: 3-12). Cognition was assessed using the Stroke Impact Scale (SIS) (Duncan et al., 1999);

participants reported how difficult it was in the past week to perform seven memory, concentration and/or executive functioning tasks using a 5-point Likert scale, (lower scores = poorer cognition; range: 7-35). Depression and anxiety symptomatology were measured using the Hospital Anxiety and Depression Scales (HADS; Snaith, 2003), which assessed how frequently (0 to 3) participants experienced 14 symptoms (e.g. anhedonia, excessive worry) during the past week (higher scores = greater anxiety/depression; range: 0-21; scores ≥ 8 indicate clinically-significant levels of symptoms).

Participants from Groups 1 (booklet intervention), 2 (video intervention) and 3 (waitlist control), were then asked to rate their knowledge of SC treatments (1 item: nothing, a little, quite a bit, a lot) and to identify their main source of information about SC treatments (e.g. media, doctor/nurse, friends/family) - for descriptive purposes and to identify group differences that could confound intervention effects (see Table 1 for individual items). Attitudes toward SC treatments were also measured at that time (see Table 1), using four items from a previously trialled survey (Unsworth et al., 2019). Specifically, participants were asked to rate using how safe, effective, accessible and affordable they thought SC treatments were on a 5-point Likert scale (see Table 1 for individual items); item scores were summed to create a composite score (range: 4-20; negative attitude: scores 4-8, neutral attitude: scores 9-10; positive attitude: scores > 10-20). The Cronbach's alpha of the composite score indicated acceptable internal consistency ($\alpha > 0.70$) (Tabachnick & Fidell, 2019).

Table 6-1. *Pre-intervention Stem Cell (SC) Treatment Survey and the Proportion of Participants who were considering SC Treatments at Different Time Intervals*

		Group 1 Booklet (n = 37)		Group 2 Video (n = 38)		Group 3 Waitlist Control (n = 37)		
Pre-intervention knowledge about SC treatments	**(1) How much do you know about SC therapies for stroke?**							
		n	**%**	***n***	**%**	***n***	**%**	***p***
	Nothing	10	27.0	15	39.5	15	40.5	.699 †
	A little	23	62.2	16	42.1	15	40.5	
	Quite a bit	4	10.8	6	15.8	6	16.2	
	A lot	0	0	1	2.6	1	2.7	
	(2) From what source have you heard about them most?							
		n	**%**	***n***	**%**	***n***	**%**	***p***
	Media	29	78.4	27	71.2	19	51.4	.347 †
	No source	5	13.5	7	18.4	14	37.8	
	Empirical research	1	2.7	1	2.6	2	5.4	
	Family / Friends	2	5.4	1	2.6	1	2.7	
	Doctors/Nurses	0	0.0	2	5.2	1	2.7	
SC treatment attitude scores per item at each time interval #	**(3) How safe do you think experimental SC treatments are?**							
		M	***SD***	**M**	***SD***	**M**	***SD***	***p***
	Pre-intervention:	3.3	0.8	3.5	0.9	3.4	0.8	.456 ɔ
	Post-intervention:	3.3	0.9	2.9	1.0	-	-	.117 ɔ
	30-day follow up	3.4	0.8	3.2	0.8	3.4	0.8	.529 ɔ
	(4) How effective do you think experimental SC treatments are?							
	Pre-intervention:	3.5	1.0	3.0	0.9	3.1	0.7	.071 ɔ
	Post-intervention:	3.2	1.3	2.7	1.0	-	-	.094 ɔ
	30-day follow up	3.5	1.1	3.3	0.9	3.3	0.9	.300 ɔ
	(5) If you wanted SC treatments now, how difficult do you think it would be to locate and attend an overseas clinic?							
	Pre-intervention:	2.1	1.1	2.2	1.2	2.4	1.2	.576 ɔ
	Post-intervention:	2.5	1.2	2.7	1.4	-	-	.636 ɔ
	30-day follow up	2.3	1.2	2.4	1.2	2.4	1.2	.738 ɔ
	(6) How affordable do you think it would be for you to do so?							
	Pre-intervention:	1.9	0.9	1.7	0.8	2.0	1.1	.719 ɔ
	Post-intervention:	1.9	0.8	1.4	0.7	-	-	.004 ɔ
	30-day follow up	1.8	0.9	1.6	0.8	1.8	0.9	.014 ɔ
Proportion of respondents who were considering SC treatments at each time interval	**(7) Are you considering SC treatments?**							
		n	**%**	***n***	**%**			***p***
	Pre-intervention: Yes	18	48.6	17	44.7			.728 π
	Pre-intervention: No	19	51.4	21	55.3			
	Post-intervention: Yes	12	32.4	15	39.5			
	Post-intervention: No	25	67.6	23	60.5			
	p	.031 ¥		.500 ¥				
	30-day follow up: Yes	17	45.9	17	44.7			
	30-day follow up: No	20	54.1	21	55.3			
	p	.076 €		.717 €				

Notes: † = Pearson's χ^2 test; # = Score range per item: 1 – 5; higher scores = more safe, effective, accessible and affordable. ɔ = Kruskal-Wallis test; π = proportion difference test, ¥ = McNemar test comparing proportion of participants considering SC treatments from each intervention group pre-intervention and post-intervention; € = Cochran's-*Q* test proportion of participants considering SC treatments from each intervention group at each of the three assessed time intervals.

n = number of participants, *p* = *p* value, SC = stem cell.

Participants in Groups 1 and 2 were asked to read/watch their allocated resource. Groups 1 and 2 indicated whether they were considering having SC treatments (yes/no) prior to (i.e. the pre-intervention time interval), and immediately after reading/watching their allocated resource (i.e. the post-intervention time interval), as well as re-doing the SC attitude survey following the educational intervention. In addition, participants in both groups were asked to rate whether the booklet or video resource: was interesting (yes, no), informative (yes, no), easily understood (5-point Likert scale: very easy-very difficult), and answered any questions they had about SC treatments (yes, no; see Table 2 for individual items). Finally, participants in Groups 1, 2 and 3 were contacted after 30-days and, once again, asked whether or not they were considering SC treatments at that time, and how safe, effective, accessible and affordable they thought these treatments were.

Table 6-2. *Intervention Group Ratings of the Stem Cell Treatment Educational Resources*

		Group 1 Booklet (n = 37)		Group 2 Video (n = 38)		
(1) Did you find the resource interesting?						
		n	**%**	***n***	**%**	***p***
	Yes	35	94.6	36	94.7	.978 †
	No	2	5.4	2	5.3	
(2) Did you find the resource informative?						
		n	**%**	***n***	**%**	***p***
	Yes	34	91.9	35	92.1	.973 †
	No	3	8.1	3	7.9	
(3) How easy was the information for you to understand?						
		n	**%**	***n***	**%**	***p***
	Very easy	8	21.6	25	65.8	.002 †
	Easy	21	56.8	12	31.6	
	Unsure	5	13.5	1	2.6	
	Difficult	3	8.1	0	0.0	
	Very difficult	0	0.0	0	0.0	
(4) Did it answer any questions about SC treatments that you may have had?						
		n	**%**	***n***	**%**	***p***
	Yes	32	86.5	23	60.5	.011 †
	No	5	13.5	15	39.5	

Notes: † = Pearson's χ^2 test used to compare groups.

n = number of participants, *p* = *p* value, SC = stem cell.

Statistical Analysis

Summary demographic, stroke and outcome data (means [*SD*s], frequencies) were calculated for the three groups. Non-parametric tests were used to compare the clinical characteristics (e.g. stroke type, year of stroke, stroke outcomes) of the three groups due to skewed data (nominal data: Pearson χ^2 tests; continuous/ordinal data: Mann Whitney *U*-tests; $p < .05$). Summary data regarding participants' knowledge about SC treatments and their main sources of information were calculated (%) and then compared using Pearson's χ^2 tests (Table 1: items 1-2; $p < .05$). Kruskal-Wallis tests were used to compare group responses to individual SC treatment attitude items (Table 1: items 3-6; $p < .05$). McNemar and Cochran's Q tests were used to determine whether the education resources deterred participants from seeking out experimental SC treatments by comparing the proportion of people in Groups 1 (booklet intervention) and 2 (video intervention) who were considering having them at the three time intervals (pre- and post-intervention, 30-day follow-up, see Table 1: item 7; $p < .05$).

A 3 (group: booklet, video, waitlist control) x 3 (time: pre-intervention, post-intervention, 30-day follow-up) mixed Analysis of Variance (ANOVA) was conducted to compare the average SC treatment attitude composite scores (dependent variable) for each group at each time interval (Group 3: waitlist controls were not assessed post-intervention, pre-intervention scores were instead used). Normality (histogram), homogeneity of variance (Box's M: $p > .001$) and sphericity (Mauchley's test: $p > .05$) assumptions were assessed. Between-group, within-group and interaction effects were examined using *F* ratios ($p < .05$), and partial-eta squared ($\boldsymbol{\eta}_p^2$) effect sizes were calculated to determine the magnitude of the effects (≤ 0.05 = small effect; 0.06 to 0.13 = medium effect; ≥ 0.14 = large effect) (Tabachnick & Fidell, 2019).

A power analysis indicated that a minimum of 108 participants (36 per group) would be required to detect moderate differences in SC attitude scores between the three groups across the three time intervals ($F \geq 0.25$) with 80% chance and at an alpha level of .05 (Tabachnick & Fidell, 2019). Those who were lost to follow-up at any point during the study were excluded from the final analysis (as per the study analysis protocol) because adequate statistical power was dependent on data from all three assessments (Ranganathan, Pramesh, & Aggarwal, 2016). The ratings obtained from Groups 1 and 2 were compared using Pearson χ^2 tests to identify any perceived strengths or weaknesses in the resources. All analyses were performed using SPSS version 24.0 (IBM Corp, 2016).

6.6 Results

Participant Recruitment

Of the 225 prospective participants initially screened for eligibility, three had previously had SC injections and were therefore ineligible (see Figure 6.1). The remaining 222 participants were randomly assigned to the booklet, video or waitlist control group. Between 50% and 60% of the participants who were allocated to each group provided pre-intervention data, most of whom completed the post-intervention (Groups 1 & 2 only: 100%) and 30-day follow-up (Groups 1, 2, 3: 86% to 95%) assessments.

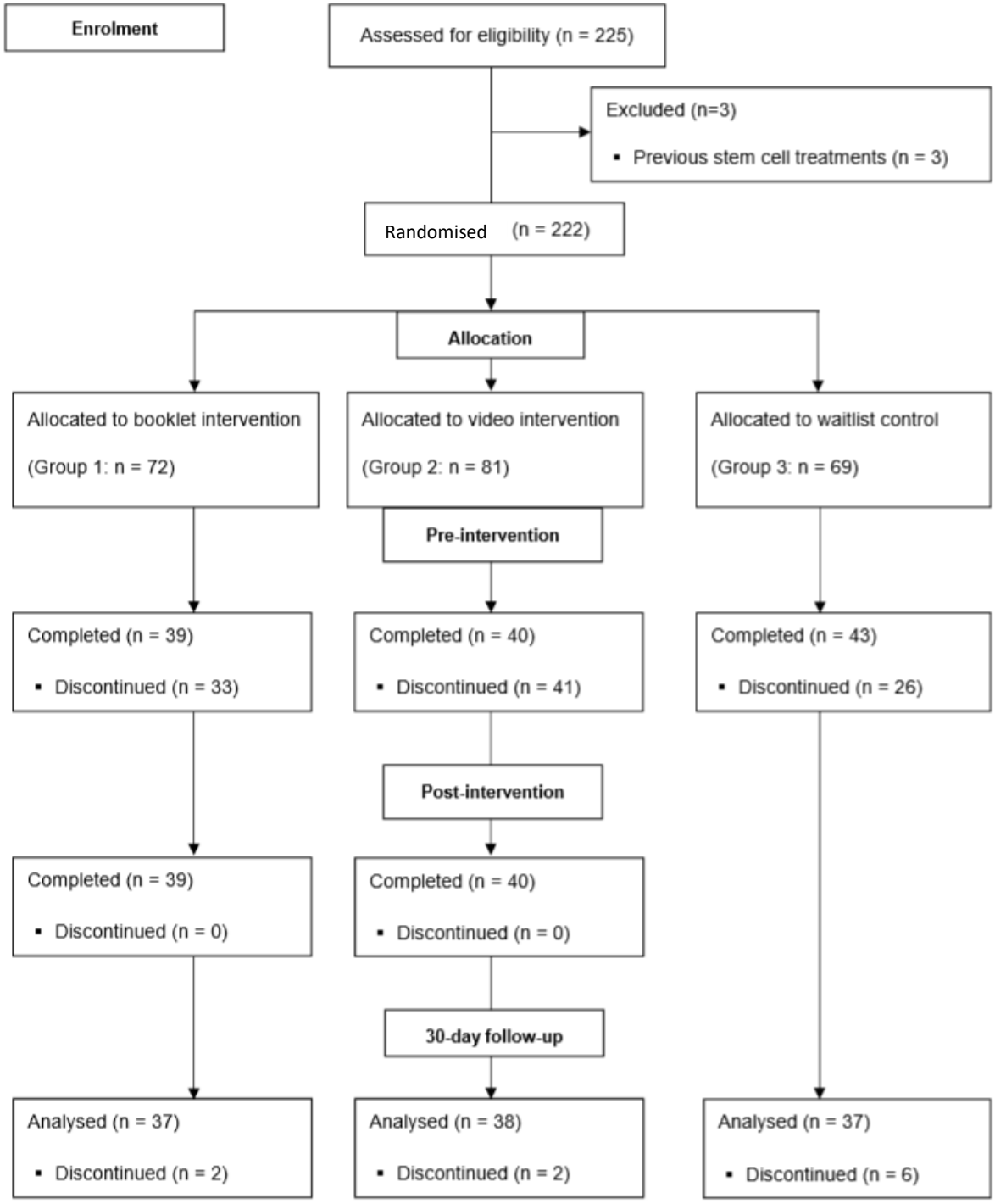

Figure 6-1. CONSORT flow diagram of the participant enrollment process.

Summary Demographic and Clinical Details

Table 6.3 provides summary data for the three groups. Participants in Group 1 (booklet) were significantly younger than those in Group 3 (waitlist control), but not Group 2 (video). The length of time-post stroke was similar across the three groups. There were more females than males in each group, and the majority were married/partnered. Ischaemic strokes were more common than haemorrhagic strokes. Most of the participants lived in the United States and Australia, with the remaining from the United Kingdom, Canada, Europe, Africa, and New Zealand or Tonga ('Other'). There were no significant group differences in physical independence (NEADL), health-related quality of life (AQoL) or cognition (SIS). Group depression (HADS-D) and anxiety (HADS-A) scores were also similar, as was the number of participants who screened positive for clinically-significant levels of depression and/or anxiety (standard subscale cut-offs ≥ 8) (Snaith, 2003).

Table 6-3. *Demographic, Stroke and Outcome Data for Intervention and Waitlist Control Groups*

		Group 1: Booklet (*n* = 37)		Group 2: Video (*n* = 38)		Group 3: Waitlist Control (*n* = 37)		*p*-values (Comparing Groups 1, 2, and 3)		
Demographic variables		**M**	***SD***	**M**	***SD***	**M**	***SD***	**1 vs 2**	**2 vs 3**	**1 vs 3**
Age (years) *		48.0	12.9	50.9	15.8	54.1	13.0	.370	.219	.023 †
Time post-stroke, years		4.8	4.3	5.6	5.4	5.0	4.0	.557	.849	.324 †
		n	**%**	***n***	**%**	***n***	**%**			
Sex, female		23	62.2	25	66.0	21	56.8	.744	.422	.636 π
Relationship status:								.193	.725	.344 π
Married/partnered		24	65.0	19	50.0	20	54.1			
Single/separated/widowed		13	35.0	19	50.0	17	45.9			
Stroke type:								.328	.077	.577 π
Ischaemic		19	51.4	20	52.6	21	56.8			
Haemorrhagic		15	40.5	11	29.0	15	40.5			
Unsure		3	8.1	7	18.4	1	2.7			
Visual difficulties *		0	0.0	11	28.9	20	54.1	.002	.027	>.001 π
Hearing difficulties *		4	10.8	0	0.0	9	24.3	.157	.006	.127 π
Country of residence:								.556	.109	.692 π
U.S.A		16	43.2	16	42.1	14	37.8			
Australia		13	35.1	11	28.9	16	43.3			
U.K.		4	10.8	1	2.6	5	13.5			
Canada		2	5.5	5	13.2	1	2.7			
Europe		1	2.7	2	5.3	0	0.0			
Africa		1	2.7	2	5.3	0	0.0			
Other		0	0.0	1	2.6	1	2.7			
Medical & cognitive outcomes	**Scale (range)**	**M**	***SD***	**M**	***SD***	**M**	***SD***	**1 vs 2**	**2 vs 3**	**1 vs 3**
Physical independence	NEADL (0-66)	47.4	16.5	52.1	12.3	48.9	14.4	.286	.369	.803 †
Health-related quality of life	AQoL (3-12)	9.4	1.7	9.4	1.7	9.2	1.8	.970	.694	.583 †
Cognition	SIS (7-35)	26.2	7.1	29.3	5.2	28.2	6.1	.074	.537	.218 †
Psychological outcomes		**M**	***SD***	**M**	***SD***	**M**	***SD***	**1 vs 2**	**2 vs 3**	**1 vs 3**
Depression	HADS-D (0-21)	8.7	5.0	6.7	4.4	8.2	4.1	.084	.126	.749 †
Anxiety	HADS-A (0-21)	8.1	5.1	6.9	4.1	8.1	4.2	.416	.274	.803 †
		n	**%**	***n***	**%**	***n***	**%**			
Depression	HADS-D (≥ 8)	22	59.5	17	44.7	22	59.5	.202	.202	1.00 π
Anxiety	HADS-A (≥ 8)	17	45.9	17	44.7	20	54.1	.916	.420	.485 π

Note: * = Mean age of Group 1 significantly lower than Group 3 ($p < .05$); participants with visual or hearing difficulties were not allocated to Group 1 or Group 2, due to the reading and listening required to participate in the respective interventions. Tests performed to compare groups: † = Mann Whitney *U*-test; π = Pearson's χ^2 test.

AQoL = Assessment of Quality of Life questionnaire; HADS-A = Hospital Anxiety & Depression Scale – anxiety subscale; HADS-D = Hospital Anxiety & Depression Scale – depression subscale M = mean; *n* = number of participants; NEADL = Nottingham Extended Activities of Daily Living Scale; SIS = Stroke Impact Scale; *SD* = standard deviation; U.K. = United Kingdom; U.S.A = United States of America.

Effectiveness of the Intervention

The results of the patient educational interventions are displayed in Table 6-1. On enrolment, most of the participants in each of the three groups indicated that they knew either 'a little' or 'nothing' about SC treatments (item 1), and confirmed that the media (internet, newspapers, radio) was their main source of information (item 2). No significant between-group differences in perceived safety, effectiveness, and accessibility of SC treatments were noted at any time interval (items 2-5). However, Group 2 (video) reported significantly lower mean scores in relation to perceived affordability (item 6; i.e. SC treatments less affordable) compared to Group 1 (booklet) at post-intervention and 30-day follow up, and to Group 3 (waitlist control) at 30-day follow up. Comparable numbers of participants from Groups 1 (booklet) and 2 (video) were considering having SC treatments at the pre-intervention time interval (p = .728). Post-intervention, there was a significant reduction in the number of participants who were considering SC treatments in Group 1 (booklet) (p = .031), but not in Group 2 (video) (p = .500). However, 30-days later, the number of participants in Groups 1 and 2 who were considering SC treatments was similar to that seen pre-intervention (Group 1: Q[2] = 5.17, p = .076; Group 2: Q[2] = .67, p = .717). Thus, although the booklet resource initially acted as a deterrent, this effect was not maintained (see item 7).

The results of the 3 (group) x 3 (time interval) mixed ANOVA, which compared the SC attitude scores of each group during the study, are displayed in Figure 2 (scores > 10 = positive attitude range). Normality, homogeneity of variance (p > .001) and sphericity (p > .05) assumptions were met. No significant main effect was observed between the groups' SC attitude scores (F [2, 109] = 1.56, p = .214, η_p^2= .028), which were all in the positive range (Group 1: M = 10.88, SE = .35; Group 2: M = 10.19, SE = .34; Group 3: M = 10.98, SE = .35). A significant main effect was observed for time (F [2, 218] = 3.15, p = .045, η_p^2= .028), with post-hoc tests indicating that the mean overall attitude scores were less positive after the

educational intervention (M = 10.46, SD = 2.59) than they were 30-days later (M = 11.32, SD = 2.19, p = .011). Despite this, no significant interaction was observed when comparing the mean attitude scores of the groups at any of the assessed time intervals (F [4, 218] = 1.20, p = .313, $\boldsymbol{\eta}_p^2$= .021). Further analysis revealed that the mean scores for Group 2 were lower than Group 1 (Mean difference range: -0.58 to -1.18) and Group 3 (Mean difference range: -0.87 to -1.10) at each time interval, although these differences were not significant (p = .108 to .160).

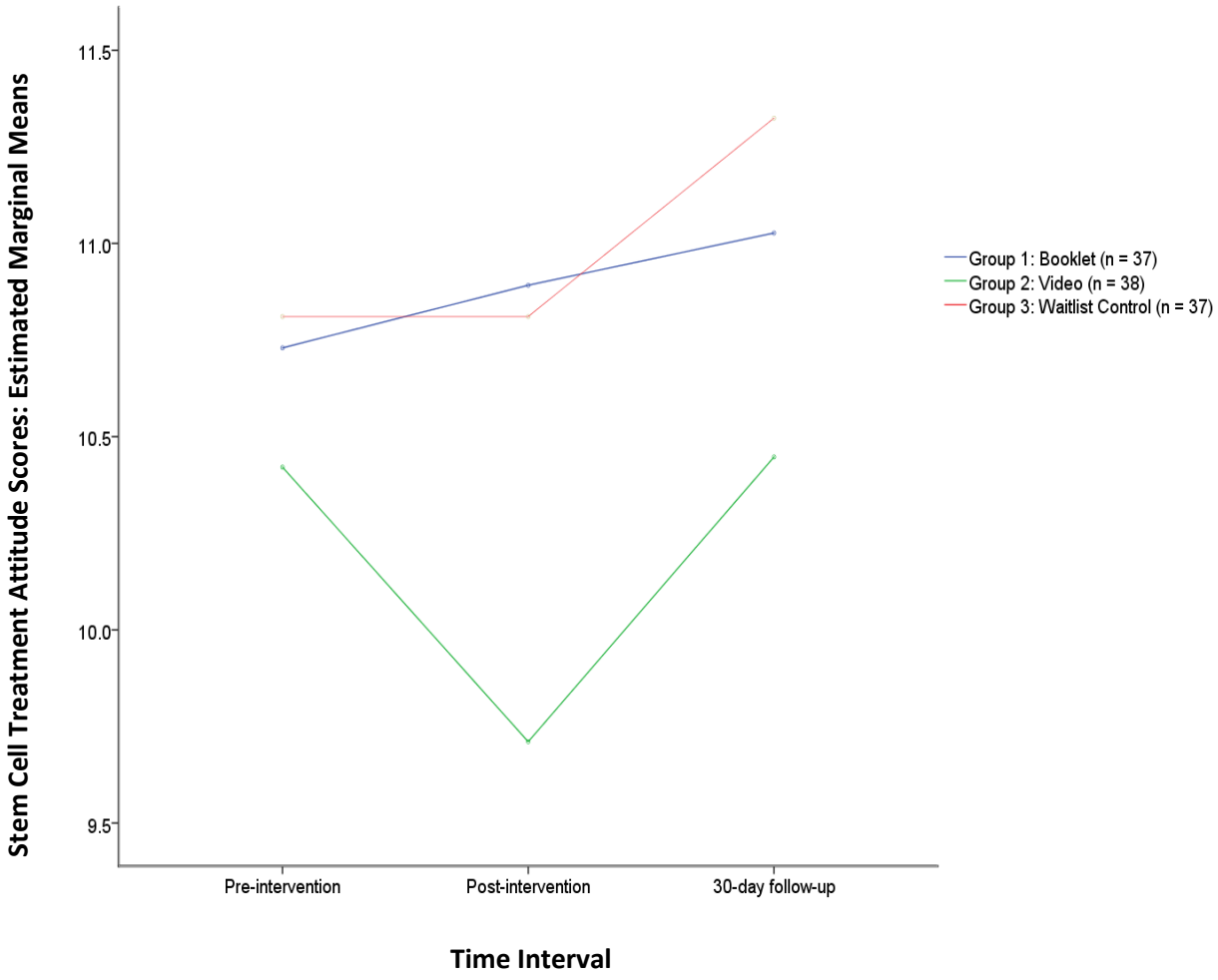

Figure 6-2. Mean differences in stem cell treatment attitude scores for the intervention and waitlist control groups at each time interval.

When asked to rate the educational materials, participants in Groups 1 (booklet) and 2 (video) mostly reported that they found the resources interesting and informative (Table 2, items 1 & 2). A significantly larger proportion of participants in the video intervention group indicated that their resource was 'very easy' to understand (p = .002), although more in the booklet group reported that the resource adequately addressed their questions (p = .011) (items 3 & 4).

6.7 Discussion

This prospective parallel-group randomised controlled trial examined whether two online patient education resources (booklet, video) deterred stroke survivors from considering experimental SC treatments and influenced their attitudes regarding the safety, effectiveness, accessibility and affordability of these treatments. At the commencement of the study, a large proportion (45% to 49%) of the stroke survivors indicated that they were considering SC treatment, despite most reporting that they knew 'little' or 'nothing' about these treatments. Although more specific details were not sought from participants regarding what they knew about SCs, the majority listed their primary source of information to be the media (internet, radio, newspapers); a source known to preference public opinion over empirical data (Du, Rachul, Guo, & Caulfield, 2016; Kamenova, Reshef, & Caulfield, 2014). These findings are consistent with other stroke studies (Aked et al., 2017; Kim et al., 2012; Unsworth et al., 2019) and highlight the need to for up-to-date evidence-based educational resources that are both patient- and media-friendly in order to provide accurate and balanced information about SC treatments to the general public (Benjaminy & Illes, 2016; Kamenova et al., 2014; Tanner et al., 2017).

The number of stroke survivors who were considering experimental SC treatments was found to initially decrease after reading the International Society for Stem Cell Research patient booklet, but not after viewing the Stem Cell Network video. Differences in the

content and level of detail provided by each of the resources may account for these findings (Du et al., 2016). Although participants from both groups found the resources interesting, informative and easy to understand, nearly 40% indicated that the video did not answer all of their questions: a significantly higher number than for the booklet (14%). Unlike the video, the booklet contained additional information regarding the potential for biased patient testimonials and the fact that recipients of experimental treatments may be ineligible for future proven SC treatments; information which may have influenced their subsequent ratings. However, given that both groups maintained a broadly positive attitude after reading or viewing their respective resources, it appears that SC safety and efficacy need to be better communicated in order to safe-guard this group.

Although the booklet intervention was initially effective at reducing the number of people who were considering experimental SC treatments, this figure returned to pre-intervention levels 30 days after reading the educational materials. Poorer memory, concentration and executive functioning may have contributed to this finding (Du et al., 2016), with the cognition scores being lowest for the booklet group. It was not possible to determine the features and characteristics of those who were most likely to change their mind at each assessment, due to sample size limitations. However, previous research suggests that stroke patients who are physically dependent and/or feel like they are 'burdening' family members and friends may be more likely to consider experimental treatments, despite knowing the risks (Unsworth et al., 2019).

The results also suggest that treating clinicians may need to be proactive in promoting these resources in order to raise awareness about the risks associated with experimental SC treatments. Indeed, the provision of more balanced, evidence-based information regarding the multifaceted risks associated with experimental SC treatments may be one of the most effective means of counteracting the highly persuasive effect that online patient testimonials appear to have (Hawke et al., 2019). Clinicians may also wish to consider warning patients

about the growing number of 'patient-funded' SC trials listed on clinicaltrials.gov, which effectively require patients to pay for the opportunity to receive experimental SC treatments for research purposes (Turner, 2017; Wagner et al., 2018).

Patient and caregiver education on these matters may need to occur during rehabilitation (Faux et al., 2018; Forster et al., 2012), given that community-based stroke survivors have been shown to have less contact with health-professionals (Pindus et al., 2018) and are, therefore, more reliant on informal sources of information (e.g. the media) about SC treatments (Unsworth et al., 2019). Stroke advocacy organisations and support groups may also want to consider providing regular information sessions to ensure that the effectiveness of their educational materials does not diminish over time (Forster et al., 2012). In addition, it has been suggested that dedicated SC information centres be established to allow patients and caregivers to seek advice via telephone, email or video conferencing at times convenient to them (Tanner et al., 2017).

Limitations

The following limitations should be considered when interpreting these findings. First, the current study measured SC treatment attitudes in terms of the perceived risks, benefits, accessibility and affordability. However, the results suggest that additional factors – such as the potentially misleading nature of online patient testimonials and the fact that recipients may be ineligible for future evidence-based SC treatments – may also influence patient attitudes. Second, the effects of the selected educational interventions may be overestimated given that approximately 50% of participants did not complete all of the assessments and were, therefore, excluded from the final results (per-protocol analysis). An intention-to-treat analysis that included data from all of the enrolled participants was considered but not undertaken because statistical power was estimated assuming participant data from three time intervals (Ranganathan et al., 2016). Third, the waitlist control group

were, inadvertently, not asked about considerations of stem cell treatments upon study enrolment; information that would be valuable in future research. Fourth, detailed information regarding the stroke type and location, and the nature and severity of the visual, hearing, or any additional communication difficulties (i.e. aphasia) was not obtained, hence the potential impact of these variables on the interventions could not be assessed. Fifth, the brief cognitive screening tool was based on self-report (Duncan et al., 1999) and may, as such, have lacked the sensitivity needed to accurately identify any cognitive or communication deficits that could influence the long-term effectiveness of the resources (Lonie, Tierney, & Ebmeier, 2009; Tsoi et al., 2015). Lastly, future research might consider a longer-term (i.e. 6-12 month) follow-up interval with additional questions about whether experimental SC treatments were accessed, in order to determine the overall effectiveness of the educational interventions in this regard.

Conclusions

The patient education resources employed in the current study did not deter stroke survivors from considering experimental SC treatments. Thus, the findings highlight the need for stroke-specific, SC educational resources to be created for use by patients, preferably in both written and audio-visual formats due to the visual and auditory problems experienced by many stroke survivors (Du et al., 2016). These resources should highlight the significant medical and financial risks, the potential to be misled by biased testimonials, and the likelihood of being deemed ineligible for future approved SC trials and treatments, should they become available. In addition, educational materials could be improved by including patient and media-friendly summaries of current research findings, and by updating the stroke community about emerging SC treatments and when they may become available through government regulated health services. Having done this, the resources will need to be actively promoted via the media (e.g. newspapers, radio, television) and online platforms in

order to challenge the claims that are made by less credible sources (Datta, 2018; Du et al., 2016; Kamenova et al., 2014).

Practical Implications

Clinicians should consider initiating discussions about experimental SC treatments with stroke survivors and their significant others early during inpatient rehabilitation (Faux et al., 2018; Forster et al., 2012). Stroke advocacy organisations should also consider providing regular SC information sessions, particularly given that community-dwelling stroke survivors may have limited ongoing contact with health professionals (Pindus et al., 2018) and, as such, may be more reliant on the media for information about SC treatments (Unsworth et al., 2019). Lastly, dedicated SC centres which provide information via telephone, email or video conferencing may prove useful to patients and their families (Tanner et al., 2017) to address their specific questions, and to ensure that the deterrent effects from previous educational interventions do not diminish over time.

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APPENDIX K

Study 4: Consolidated Standards of Reporting Trials (CONSORT) guidelines checklist

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	167
	1b	Structured summary of trial design, methods, results, and conclusions	170
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	171
	2b	Specific objectives or hypotheses	172
Methods			
Trial design	3a	Description of trial design including allocation ratio	172-4
	3b	Important changes to methods after trial commencement, with reasons	Not applicable
Participants	4a	Eligibility criteria for participants	174
	4b	Settings and locations where the data were collected	174
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	175-8
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	179
	6b	Any changes to trial outcomes after the trial commenced, with reasons	Not applicable
Sample size	7a	How sample size was determined	180
	7b	When applicable, explanation of any interim analyses	Not applicable
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	174
	8b	Type of randomisation; details of any restriction	174
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence, describing any steps taken to conceal the sequence until interventions were assigned	174
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	174
Blinding	11a	If done, who was blinded after assignment to interventions and how	Not applicable
	11b	If relevant, description of the similarity of interventions	Not applicable
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	180
	12b	Methods for additional analyses	180
Results			
Participant flow	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 6-1

	13b	For each group, losses and exclusions after randomisation	182
Recruitment	14a	Dates defining the periods of recruitment and follow-up	182
	14b	Why the trial ended or was stopped	n/a
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 6-3
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Figure 6-1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	184-7
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Not applicable
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	184-5
Harms	19	All important harms or unintended effects in each group	Not applicable
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, and imprecision	189-91
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	189-91
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	188-91
Other information			
Registration	23	Registration number and name of trial registry	172
Protocol	24	Where the full trial protocol can be accessed, if available	Not applicable
Funding	25	Sources of funding and other support, role of funders	See publication

APPENDIX L

Study 4: Summary Results for Assessment and Selection of Patient Education Materials

	Booklet: PEMAT-P Score				Video: PEMAT-AV Score	
	Resource 1	**Resource 2**	**Resource 3**	**Resource 4**	**Resource 5**	**Resource 6**
Understandability (%)	12 / 17 (71%)	16 / 17 (94%)	10 / 13 (77%)	12 / 13 (92%)	9 / 12 (75%)	8 / 10 (80%)
Actionability (%)	5 / 6 (83%)	3 / 6 (50%)	4 / 6 (67%)	3 / 5 (60%)	2 / 3 (67%)	0 / 3 (0%)
Total Score (%)	**17 / 23 (74%)**	**19 / 23 (82%)**	**14 / 19 (74%)**	**15 / 18 (83%)**	**11 / 15 (73%)**	**8 / 13 (62%)**

Notes: Resource 1: National Stem Cell Foundation of Australia. What you should know about stem cell therapies: Now and in the future 2015. Accessed from: http://www.stemcellsaustralia.edu.au/AboutUs/GetFile.axd?oid=B832BE26-D103-418D-98B4-DEAAA1049172 on 1st February 2018. Resource 2: Stem Cell Network. What you need to know about stem cell therapies 2015. Accessed from: https://oirm.ca/wp-content/uploads/2018/07/sc_patient_booklet_feb_2014.pdf on 1st February 2018. Resource 3: International Society for Stem Cell Research. Patient handbook on stem cell therapies 2008. Accessed from: http://www.isscr.org/docs/default-source/patient-handbook/isscrpatienthandbook.pdf on 1st February 2018. Resource 4: International Society for Stem Cell Research. Nine things to know about stem cells 2015. Accessed from: https://www.closerlookatstemcells.org/stem-cells-medicine/nine-things-to-know-about-stem-cell-treatments/ on 1st February 2018. Resource 5: Stem Cell Network. What is stem cell tourism? 2014. Accessed from: https://www.youtube.com/watch?v=WZ4KETVYYX8 on 1st February 2018. Resource 6: National Stem Cell Foundation of Australia. Stem cell tourism. Accessed from: https://www.youtube.com/watch?v=ZjYfQUm1_6g on 1st February 2018.

PEMAT-AV: Patient Education Materials Assessment Tool for Audio-visual Materials; PEMAT-P: Assessment Tool for Printable Materials

Chapter 7: Discussion

7.1 Thesis Overview

This thesis outlined the findings of four studies that were designed to examine the safety and efficacy of SC therapies, and investigate stroke survivors' level of interest in 'stem cell tourism'. The main objectives were to: (1) review and evaluate clinical research examining the safety and efficacy of SC therapy for stroke; (2) identify the extent to which psychological factors have been considered by existing SC researchers, (3) provide an estimate of the number of stroke survivors who are considering having experimental SC treatments; (4) clarify some of the biopsychosocial and attitudinal factors that increase the likelihood that stroke survivors will consider having experimental SC treatments; and (5) examine whether existing patient education resources deter stroke survivors from 'stem cell tourism'.

Two meta-analyses were conducted to evaluate the potential risks and benefits associated with SC therapies administered in the hyper-acute, acute or sub-acute (Study 1) and chronic (Study 2) phases of stroke (objective 1). As part of these analyses, the number of studies that screened for psychological disorders pre-treatment or evaluated psychological wellbeing pre-/post treatment were also identified (objective 2). Next, a cross-sectional survey of Australian stroke survivors was conducted (Study 3) to ascertain the number of patients who were considering undergoing experimental SC treatments (objective 3), and to identify some of the biopsychosocial, attitudinal and demographic factors that increased the likelihood of them doing so (objective 4). Lastly, a RCT trial (Study 4) was conducted to evaluate whether an online educational booklet and video altered stroke survivor attitudes toward experimental SC treatments (objective 5).

This chapter summarises the key findings from these four studies, discusses the clinical implications, and outlines the methodological strengths and limitations. The chapter concludes with a statement outlining how the findings fulfilled the overall aims of the thesis.

7.2 Summary of Findings

Safety and Efficacy of SC Therapies administered in the Hyper-Acute, Acute, Sub-Acute and Chronic stages after Stroke.

The first meta-analysis (Study 1, Chapter 3) reviewed and summarised existing data relating to the safety and/or efficacy of SC therapy throughout the hyper-acute, acute and sub-acute stages of stroke. Extending on earlier meta-analyses (Boncoraglio et al., 2010; Cao & Li, 2015; Fan et al., 2015; Jeong et al., 2014; Lalu et al., 2012; Wang et al., 2016), this study included data from RCTs and non-RCT/observational studies. SC therapies were also stratified based upon the delivery route and cell type, due to proposed differences in the mechanisms of action (Rodríguez-Frutos et al., 2016). Eleven different SC therapies were evaluated based on data from 1,485 stroke survivors. When evaluating the safety of these therapies, considerable variability was observed in the types and frequency of treatment-related serious adverse effects. Five therapies resulted in serious complications, such as death, tumours, strokes and seizures, while the remaining six therapies reported no adverse effects. However, many of the studies that reported no safety concerns had small participant numbers and generally only monitored patients for a period of six months. This suggests further research and longer-follow up is required to more adequately establish the risks.

Moreover, when assessing efficacy, most of the hyper-acute, acute and sub-acute SC therapies failed to produce neurological, functional and/or radiological improvements that exceeded standard care. This is likely due to the fact that most studies were designed to test the safety of specific dosages among small groups of stroke survivors, rather than to assess the effectiveness of the treatment. Notwithstanding this, few studies examined patients on

more than one occasion to establish stable neurological baselines, or incorporated placebo/sham treatment conditions into the trials, both of which are of critical importance when evaluating the effectiveness of SC therapy, relative to standard care (Savitz, 2014).

Of the six SC therapies that resulted in better outcomes than standard care, neural SC injections delivered via lumbar puncture into the spinal canal (intrathecal injections) were most effective for haemorrhagic stroke, and a combination of subcutaneous G-CSF and intravenous bone-marrow mesenchymal SC injections were most effective for ischaemic stroke. Although the specific mechanisms underpinning the improvements were not identified, neural SCs delivered via lumbar puncture are thought to migrate to the lesion site via the cerebral ventricles, where they secrete protective growth factors, thereby reducing the amount of damage caused by a stroke (Janowski et al., 2015). G-CSF is also understood to migrate to the stroke site where it protects against further damage, and bone-marrow mesenchymal SCs are thought to travel through the permeable blood-brain-barrier to the lesion where they stimulate and support natural repair processes (dela-Peña & Borlongan, 2015).

Overall, the reporting quality of the included studies was found to be high, with information regarding the research objectives, baseline demographics and key results consistently provided. However, contrary to research guidelines, no studies screened for psychological comorbidities (e.g. depression) as part of their inclusion/exclusion criteria. It should be acknowledged that it is difficult to assess mood symptoms throughout the hyper-acute and acute stages of stroke due to the cognitive and communication difficulties experienced by many patients (Hankey, 2017). However, a failure to screen for mood symptoms in the sub-acute studies may have impacted on the results because of the established link between depression and poorer stroke outcomes (Dar et al., 2017; Savitz et al., 2014). Similarly, changes in psychological outcomes pre-/post treatment were not evaluated. Consequently, the broader impact of SC therapies on patients' wellbeing (positive

or negative) throughout the early stages of stroke remains to be determined. Whether psychological support was routinely provided as part of the standard care rehabilitation process was also unclear; a concerning finding given the prevalence of post-stroke depression (Dar et al., 2017). Taken together, these results suggest that little is currently known about the potential impact of psychological symptoms, including depression and anxiety, on hyper-acute, acute or sub-acute SC therapy outcomes, and the impact of these treatments on the psychological wellbeing of patients' remains poorly understood.

The second meta-analysis (Study 2, Chapter 4) extended on these findings, by reviewing data pertaining to the safety and efficacy of SC therapy throughout the chronic phase (> 90 days) of stroke. For this study, 17 different SC therapies (stratified by administration route and SC type) were analysed, based upon data from 23 independent studies (RCT's and non-RCTs/observational) and 461 stroke survivors. The safety risks were again found to vary considerably. For instance, whilst 14 therapies reported nil adverse effects, the remaining three, all of which involved SC injections into the brain (intracerebral administration), reported a mix of deaths, tumours, strokes and seizures. Although these results appear to suggest that the alternate administration routes (i.e. intrathecal, intravenous, subcutaneous) are relatively safe, it was not possible to determine this with any certainty due to the small number of patients who received these treatments and the short-follow up periods that were utilised.

In terms of efficacy, only four of the 17 SC therapies administered in the chronic phase of stroke improved neurological or functional outcomes more than standard care. Of these, neural SC injections into the spinal canal (intrathecal) were again found to be most efficacious - for both ischaemic and haemorrhagic strokes - followed by combined subcutaneous G-CSF and intracerebral peripheral blood SC injections (ischaemic stroke only). As the blood-brain-barrier is no longer permeable during the chronic phase of stroke, administering SCs into the certebral ventricles via the spinal canal, or by direct injection into

the brain are thought to be preferable (Savitz, 2018). Once present, neural SCs have the potential to regenerate damaged brain tissue (Janowski et al., 2015), while G-CSF, in combination with peripheral blood SCs, promotes and supports the brain's naturally occurring (endogenous) repair processes (dela Peña & Borlongan, 2015; Solaroglu et al., 2015).

As with Study 1, the reporting quality of the studies that were examined in Study 2 was high, with details regarding the study objectives, participants, interventions, key results and funding sources frequently provided. However, once again, few studies were found to have incorporated placebo/sham-treatment conditions. Utilising sham treatment conditions may be particularly important when evaluating SC therapies that involve injections into the brain or spine, as invasive medical procedures more broadly are thought to produce larger placebo effects (Schedlowski, Enck, Rief, & Bingel, 2015). To this end, one of the included studies, a Phase 2 clinical trial, was found to have gained regulatory approval for the inclusion of a sham-treatment condition for intracerebral SC injections (Steinberg et al., 2018). In the absence of a placebo/sham-treatment group, which may be controversial from an ethical standpoint, the inclusion of a waitlist control group that receives standard care may provide a secondary option (Savitz, 2014).

In addition, only a small number of studies labelled and tracked the implanted cells to identify the mechanisms of action. This remains a challenging process throughout each phase of stroke (Gavins & Smith, 2015) and it is likely that most researchers were unable to access reliable and affordable means of doing so. In light of this, the routine labelling and tracking of implanted SCs remains an aspirational goal for many SC researchers. This is expected to change in the coming years as a range of safe and cost-effective ways of performing these tasks are currently being developed, which will further aid our understanding of how individual SCs improve stroke outcomes (Jasmin, Louzada, Rosado-de-Castro, Mendez-Otero, & de Carvalho, 2017; Lu et al., 2018).

More encouragingly, around one quarter of the studies in Study 2 screened for the presence of serious psychological disorders prior to SC treatment, including major depression, anxiety, psychosis, and schizophrenia. Although, this step was necessary to ensure patient safety and to control for the confounding effects of these conditions, future SC therapy trials may need to incorporate patients with depression and/or anxiety to determine the safety and efficacy of the treatment among more representative stroke samples (Savitz, 2014). That said, the inclusion of patients with psychosis or schizophrenia remains a far more difficult proposition, given the potential severity of the symptoms and the level of consent required to participate in clinical trials (Weissinger & Ulrich, 2019).

Two studies also measured the severity of depression and/or anxiety symptoms before and after treatment. Depression increased 12 months after intracranial bone-marrow mesenchymal SC injections (Suarez-Monteagudo et al., 2009), while depression and anxiety symptoms were stable 6 months after intracerebral NT2/D1 cell injections (Kondziolka et al., 2005). Interestingly, when comparing the results of the studies, it was found that the stroke survivors with higher levels of depression post-treatment (Suarez-Monteagudo et al., 2009) had experienced less neurological and functional benefits compared to those who experienced stable mood (Kondziolka et al., 2005). Overall, these findings provide important preliminary data regarding the potential psychological impact of receiving SC therapies with unknown long-term benefits. More specifically, they suggest that patients may be at risk of experiencing depression following ineffective treatments (Mancuso et al., 2018), particularly those that involve invasive medical procedures (Ghoneheim & O'Hara, 2016).

Stroke Survivor Attitudes Toward, and Motivations for, Considering Experimental SC Treatments

The findings from Studies 1 and 2 highlighted ongoing uncertainty in relation to the safety and efficacy of SC therapies throughout each phase of stroke. Previous studies have

identified growing interest in the experimental SC treatments offered by private clinics among patients with neurological conditions, such as multiple sclerosis and Parkinson's disease (Rachul, 2011; Rai et al., 2019; Tanner et al., 2017). However, far less is currently known about the level of interest among stroke survivors. The survey described in Chapter 5 (Study 3) was designed to provide these much needed data (objective 3), in addition to identifying possible biopsychosocial, attitudinal and demographic factors that may increase the likelihood of a stroke survivor seeking out experimental SC treatments (objective 4).

One-hundred and eighty three Australian stroke survivors were surveyed, of which 25% indicated that they were considering undergoing experimental SC treatments. Those with positive attitudes toward SC treatments were found to be more likely to be considering having them. This observation is consistent with the Theory of Planned Behaviour (Ajzen, 1991) and supports previous data concerning the important role that patient attitudes toward the perceived safety, efficacy, accessibility, and affordability of an intervention play when weighing up 'medical tourism' options (Seow et al., 2017). Although the contribution of specific attitudes to this result were not evaluated, around one third of those surveyed indicated that they thought SC therapies were safe and effective to some degree, which is at odds with the risks that were identified in Studies 1 and 2. Moreover, participants also identified the media (i.e. television, radio, newspapers, internet) as their main source of SC information. Similar findings have been reported from SC studies in Asia (Kim et al., 2012) and Europe (Aked et al., 207), which further highlights the need for clinicians to initiate discussions about SC treatments with patients in order to clarify any misconceptions.

From a biopsychosocial standpoint, stroke survivors who viewed themselves as being a burden on family and friends were also more likely to be considering undergoing experimental SC treatments. This is noteworthy given the high level of caregiver burnout among this cohort (Oliva-Moreno et al., 2018) and the possibility that stroke survivors may undergo risky and expensive SC treatments in response to caregiver distress. Patients who

were more physically dependent on others were also identified as being at an increased risk of considering experimental SC treatments, as were individuals who had longer post-stroke intervals, and who were younger in age. While each of these medical and demographic variables have been flagged previously as potential risk factors (Kim et al., 2013), the cumulative effect of multiple biopsychosocial and attitudinal factors presented by Study 3 appears to present a more comprehensive explination as to why stroke survivors may consider 'stem cell tourism'. Moreover, based on the identified factors, a role for psychologists has emerged to help mitigate some of the risks. One such example would be by working with stroke suvivors to reduce their level of perceived burden and to assist caregivers to improve self-care.

Participants were also asked to nominate a range of SC treatment preferences. Interestingly, the majority of stroke survivors indicated that they would not consider undergoing treatments that involved injections into the spinal cord (intrathecal) or brain (intracranial), despite these methods being the focus of most chronic-phase SC therapy research trials (Marei et al., 2018; Wechsler et al., 2018). Approximately half of those surveyed also indicated that they would not consider treatments that used SCs derived from human embryos/foetuses. This highlights strong ethical concerns regarding particular types of SCs, even those that have the greatest potential to regenerate stroke-damaged brain tissue (Janowski et al., 2015). Lastly, around one in three respondents indicated that, if they were to have SC injections, they would prefer to experience improved psychological or cognitive, rather than physical functioning. This finding is, perhaps, not surprising given the extent of cognitive and psychological problems following a stroke, although the fact that neither area are currently being routinely investigated by SC researchers is significant (see Study 1 & 2; Chapters 3 & 4).

Effectiveness of Online Patient Educational Resources at deterring Stroke Survivors from considering Experimental SC Treatments

The findings from Study 3 identified a concerning level of interest among stroke survivors in experimental SC treatments, and also highlighted the need for evidence-based patient resources that clarify potential misperceptions about the efficacy and safety of such treatments (Petersen et al., 2017). Study 4 therefore evaluated the effectiveness of two pre-existing online resources designed to inform and deter patients with a range of neurological disorders from undergoing experimental SC treatments (objective 5). One hundred and twelve stroke survivors were recruited, primarily from online stroke support groups, to participate in a RCT. Participants were allocated to one of two intervention groups (booklet, video) or a wait-list control group.

At the commencement of the study, 45% of the full sample indicated that they were considering undergoing experimental SC treatments. This number was significantly higher than that reported by the previous Australian sample (25%; Study 3), but is equal to that observed in an earlier South Korean study (45%) (Kim et al., 2012). The reason for the difference is unclear, but may reflect variability in the quality or accessibility of stroke treatments between countries (Thrift et al., 2017), a growing awareness of SC treatments throughout the global stroke comunity, and/or broader cultural differences in attitudes toward experimental medical treatments (Yu & Ko, 2012). A large number of the Study 4 participants were also located in closer proximity to countries more renowned for 'stem cell tourism' (i.e. South America, Russia), thereby making accessibility easier, which Study 3 identified as an important attitudinal factor (refer Chapter 5).

Following the educational interventions, the number of stroke survivors who were considering SC treatments decreased among the group that read the booklet, but not among the group who viewed the video. The contrasting results may be attributable to the level of

detail and type of content provided by each of the resources (Du et al., 2016). For instance, the booklet was more comprehensive (six pages of online written content vs 1.18 minute video) and, unlike the video, warned readers about the potential for biased patient testimonials. Furthermore, the booklet highlighted the fact that patients who receive experimental SC treatments may be ineligible for future proven SC treatments, which may have also served as a strong initial deterrant.

Despite the promising initial results, the intervention effects observed within the booklet group were not maintained at the 30-day assessment point. Although, a clear reason for the increasing interest in 'stem cell tourism' was not identified, written information is thought to be more difficult for stroke survivors to recall than information provided in a video format, due to the level of detail incorporated in some texts, and the involvement of additional neural pathways when information is received using multimedia formats (Denny, Vahidy, Vu, Sharrief, & Savitz, 2017). Educational interventions are also based on the assumption that patients rationally weigh up information regarding the associated risks and benefits, however research suggests that frustration with existing treatment approaches, in addition to hope, also factor into decision-making regarding SC treatments (Master et al., 2014). Furthermore, the attitudes of both intervention groups toward experimental SC treatments remained positive during the course of this study. This is again consistent with the findings of Study 3, which also found that personal attitudes toward SC treatments were the strongest single predictor of whether a stroke survivor would consider such treatments. Lastly, the majority of respondents once again confirmed that the media (television, internet, radio) was their main source of information about SC treatments, reinforcing the need for more widespread promotion of stroke-specific SC resources.

7.3 Clinical Implications and Opportunities for Future Research

The results of the four studies have a number of important clinical implications. The combined findings of Studies 1 and 2 suggested that some SC therapies may improve the neurological, functional and/or radiological outcomes of stroke survivors beyond that of current treatments. Preliminary results, such as these, are highly encouraging and, as a result are often promoted throughout the media (Petersen et al., 2017). However, by emphasising the positive results, other important aspects, such as the need for further research to establish the associated risks, tends to be overlooked. Experimental SC clinics have also been criticised for their misleading use of observational research data, which typically emphasise the benefits without acknowledging the potential role of natural recovery, standard care, and/or placebo effects (Petersen et al., 2017). The ongoing proliferation of inaccurate information about SC treatments remains a significant challenge for SC researchers, media outlets, patients and caregivers. However, it also presents an opportunity for doctors, psychologists and other allied-health staff, who could potentially reduce the future risk of stroke survivors undergoing experimental treatments by educating them about the risks throughout the various phases of care.

Incorporating psychological assessments within future clinical trials will also be important given that undetected symptomology may impact upon the overall safety and efficacy of the SC therapies being investigated (Dar et al., 2017; Savitz, 2014). Routine screening of this kind may be achieved using a range of tools validated for stroke survivors (e.g. Hospital Anxiety and Depression Scale [Snaith, 2003]), including patients with aphasia (e.g. Depression Intensity Scale Circles [Turner-Stokes, Kalmus, Hirani, & Clegg, 2005]), followed by a more comprehensive diagnostic interview, where indicated (e.g. Structured Clinical Interview for DSM-5; First, 2014). As an extension to routine screening, reserchers may wish to incorporate stroke survivors who present with quantifiable mood symptoms into

future trials, rather than excluding them, in order to investigate the risks and benefits of the therapy among more representative stroke samples.

Similar routine screening practices should also be considered to monitor the psychological wellbeing of participants throughout clinical trials and beyond. Indeed, performing routine psychological screening arguably forms part of the researchers' duty of care, given that participants enter into SC trials with little certainty regarding the potential risks and benefits which places them at an increased risk of future depression and anxiety (Ghoneheim & O'Hara, 2016; Mancuso et al., 2018). The timeframe for mood symptoms to develop may also vary given that they may result from treatment-related complications during the acute, sub-acute or chronic phase of recovery, and/or due to a lack of treatment efficacy over the longer term. In light of this, regular psychological follow-up throughout the months and years following treatment appears to be the most appropriate strategy for future trials.

Concerningly, Studies 3 and 4 suggest that between one quarter and one half of stroke survivors may be considering 'stem cell tourism' options. Given that published case studies have documented brain and spinal tumours as a result of SC treatments, these findings highlight an ongoing need for governments, regulatory bodies, advocacy organisations, and clinicians to deter stroke survivors from doing so (Amariglio et al., 2009; Berkowitz et al., 2016; Nakamura et al., 2016). Educational interventions targeting higher-risk groups, namely patients with positive attitudes toward SC treatments, longer post-stroke intervals, poorer physical functioning, younger age, and greater perceived caregiver burden may be an important first step toward this goal (Study 3). Psychological support may also need to be extended to the caregivers of stroke survivors, in order to help counteract perceived caregiver burden. This might include CBT for depression and anxiety, or problem-solving therapy (Panzeri, Ferrario, & Vidotto, 2019; Swartz & Collins, 2019).

Further confirmation of the factors identified in Study 3 using an international sample would help to confirm the current findings. Additionally, identifiying whether the likelihood of considering experimental SC treatments is higher among patients who have travelled overseas previously, have had prior experimental treatments for other conditions, or who live in close proximity to countries where 'stem cell tourism' is more common, would also be of interest. Following this, longitudinal research to confirm the number of stroke survivors who were not only considering undergoing experimental SC treatments, but actually proceeded to do so would provide important insight into the differences between those patients who did and did not follow through with treatment.

The findings from Study 3 also suggest that the current focus of SC therapy research, which is designed to improve patients' physical and functional outcomes primarily via intracerebral transplantation, may in fact differ from patient treatment preferences. Indeed, a large proportion of stroke survivors indicated that they would prefer less invasive approaches that improve psychological and cognitive outcomes. Therefore, whether SC therapy may reduce the severity of stroke-related mood disorders (Aizenstein et al., 2016) and cognitive problems may be an avenue for future research. However, for this to occur, it is likely that methods of SC labelling and tracking will have to improve, in order to enable a clearer understanding of whether the changes are the result of structural brain changes or are secondary to improved physical or functional outcomes (Gavins & Smith, 2015).

The results of Study 4 provide further insight into the issue of 'stem cell tourism', indicating that existing online educational resources, may not be an effective means of deterring stroke survivors from considering experimental SC treatments. Rather, the findings suggest that stroke-specific SC resources that individually cater for the broad the range of physical (i.e. vision, hearing), functional (i.e. difficulties accessing materials), cognitive (i.e. attention, executive functioning, memory) and psychological (depression, anxiety) problems experienced by stroke survivors are required (Du et al., 2016). To this end, it should be noted

that information presented in an audio-visual format has been found to be easier for stroke survivors to recall (Denny et al., 2017). Moreover, psychological research in the field of decision making suggests that patients who are interested in an area and are motivated to make a decision are more likely to be influenced by text/audio-based information (i.e. direct marketing), whereas those who are not are more likely to be influenced by non-verbal/imagery-based information (indirect marketing) (Boudewyns & Williams, 2016).

New methods of disseminating information about the risks associated with experimental SC treatments may also be required. Methods that allow for updated information to be provided should be considered given that the effectiveness of the educational booklet was found to have diminished over time. Previous studies have promoted telephone advice lines as one means of achieving this (Tanner et al., 2017). Regular community events run by stroke-advocacy groups may be another means. In addition, more aggressive promotion of evidence-based SC information via the media and internet, may be warranted given that both mediums were consistently found to be the main source of information regarding SC therapies (Studies 3 and 4). As younger stroke survivors were found to be more likely to consider having experimental SC treatments, increased promotion via social media – a platform used to great effect by private SC clinics – may be a priority (Kamenova, Reshef, & Caulfield, 2014).

Lastly, the combined results of Study 3 and 4 strongly suggest that clinicians, such as doctors and psychologists, may need to be more proactive in initiating discussions about SC treatments when consulting with patients, particularly where frustration with current treatment approaches is detected. Warning patients about the risks associated with experimental SC treatments during the sub-acute rehabilitation program may be one way of achieving this (Faux et al., 2018; Forster et al., 2012). However, given that a longer length of time post-stroke was identified as a potential risk factor for considering experimental SC

treatments, ongoing discussions with patients throughout the chronic phase of stroke also appear to be required.

7.4 Methodological Strengths and Limitations

The four studies in this thesis endeavoured to address some of the research and knowledge gaps identified in Chapters 1 and 2 by incorporating a number of novel methodological and theoretical approaches. The meta-analyses outlined in Studies 1 and 2 included non-English publications and extracted data from charts/plots, thereby resulting in more data than previous meta-analyses (Boncoraglio et al., 2010; Cao & Li, 2015; Fan et al., 2015; Jeong et al., 2014; Lalu et al., 2012; Wang et al., 2016). The safety and effectiveness of individual SC therapies that were stratified by SC type and delivery route were also provided to help identify the optimal approaches for the different phases of stroke. Moreover, safety and efficacy estimates were provided for single and combined SC therapies, which highlighted the potential benefits associated with G-CSF and SC injections.

In addition, acute/sub-acute and chronic data were analysed separately, which had not been done previously, despite differences in the medical stability of patients and differences in blood-brain-barrier permeability. By doing so, progress was made toward understanding the optimal cell types and administration routes for each phase of stroke. RCT and non-RCT/observational data were also included, with the latter compared to control group data pooled from the RCTs. As a result of these comparisons, the benefits suggested by some observational data were no longer statistically significant, relative to standard care/normal recovery. Lastly, a checklist was created based upon recommended SC research guidelines, which was used to identify a number of important gaps in the literature, some of which were highly pertinent from a psychological perspective.

The logistic regression models created for Study 3 were constructed using the Purposeful Selection of Covariates approach (Hosmer, Lemeshow, & Sturdivant, 2013),

which is considered to be more accurate than standard logistic regression techniques (Zhang, 2016). The predictors identified by the final logistic regression models were also combined to create cumulative predictive probabilities based on multiple, rather than single, variables (Hosmer et al., 2013). A further strength relates to the theoretical framework used in Study 3. This included a biopsychosocial model, which focused on the dynamic interaction between biological (e.g. level of physical functioning), psychological and social factors (e.g. health-related quality of life, depression, anxiety, cognitive functioning, social support, perceived caregiver burden) - many of which have not been investigated in SC research (Wade & Halligan, 2017). Moreover, stroke survivor attitudes were assessed using the Theory of Planned Behaviour (Ajzen, 1991); a framework which correctly predicted that patients who perceive SC injections to be safe, effective, accessible, affordable, and who felt that their family/friends also want them to have them, were more likely to be considering undergoing them (Sheeran et al., 2016).

In Study 4, a mixed-ANOVA was used to compare the effectiveness of the two educational interventions with a wait-list control group across three assessment time intervals (Tabachnick & Fidell, 2019). This was achieved by comparing the attitudes of participants regarding experimental SC treatments before, immediately following and 30-days after the interventions, in addition to comparing the proportion of stroke survivors who responded 'yes' or 'no' when asked if they were considering these treatments at those time points. Study 4 also used an international sample, thereby expanding on the Australian findings in Study 3, and providing greater insight into global 'stem cell tourism' trends.

Nonetheless, there were also a number of study design and data quality limitations that could not be overcome and, should therefore be acknowledged. First, despite the comprehensive literature search undertaken in Studies 1 and 2, much of the safety and efficacy data for the meta-analyses were based on small samples and short-term (< 1 year) findings. Therefore, rather than providing conclusions about the safety and efficacy of

individual therapies, the current thesis summarised and evaluated the preliminary data from a broad range of treatments. Second, stroke survivors with an interest in experimental SC treatments may have been more likely to respond to the mailed survey in Study 3 (response bias) or the online survey in Study 4 (selection bias), which may have lead to an overestimation in the the number of stroke survivors who were reportedly considering SC treatments. Third, Studies 3 and 4 were predominantly conducted via mail and online, which necessitated the use of self-report data when assessing a range of key areas (e.g. functional independence, depression, anxiety). Although, valid measures were selected, the reliance on self-report data may have impacted on the reliability of some results, due to memory errors (recall bias) or a desire to represent themselves in a certain manner (social desirability bias; Althubaiti, 2016). Fourth, brief measures were used to minimise the time commitment for respondents and increase the likelihood of their participation. Although, a more comprehensive measure of cognition may have been more appropriate for Study 4, given that unidentified attention, memory or executive functioning deficits may have influenced the long-term effectiveness of the educational resources (Stolwyk, 2016; Stolwyk, O'Neill, McKay, & Wong, 2014; Tsoi et al., 2015). Fifth, although the tool used to select the patient education resources (Shoemaker, Wolf, & Brach, 2014) in Study 4 had been previously used to evaluate stroke-related materials (Du, Ma, & Li, 2016), other studies have incorporated more specific questions about the SC information provided, rather than focussing on the 'understandability' and 'actionability' of the resource, which allowed for more in-depth comparisons of the tools (Master et al., 2014). Sixth, the online patient education resources that were evaluated in Study 4 contained addresses and links for patients to obtain further information about experimental SC treatments, which suggests they may not have been intended for use as stand-alone resources. Whether participants accessed this additional information was not recorded, therefore the level of standardisation that was achieved throughout the interventions may have varied between participants. Seventh, a range of

potentially useful medical information was not requested and could not be accessed directly via medical records, including specific stroke details (e.g., size and location of the infarct), premorbid health and functioning, and current health (e.g., blood pressure). Lastly, around half of the participants in Study 4 failed to complete all of the assessments and were, therefore, excluded from the final results. While adequate statistical power was still achieved, excluding data in this manner can impact on the reliability of the intervention effects (Ranganathan, Pramesh, & Aggarwal, 2016).

7.5 Conclusion

The four studies detailed in this thesis highlight the promise and risks associated with both regulated (i.e. clinical trials) and unregulated ('stem cell tourism') forms of treatment. The results of Studies 1 and 2 suggest that a small number of different SC therapies may be safe and effective for use throughout the various phases of stroke, but also highlight the need for further testing of these therapies via large-scale, placebo-controlled RCTs. Both studies also identified a distinct lack of research concerning a range of potentially significant psychological factors. Most importantly, major depression was not routinely screened for prior to participating in SC therapy trials, which may have confounded the results due to the established link between depression and poorer stroke outcomes (Dar et al., 2017; Savitz et al., 2014). There was also preliminary evidence to suggest that participants in SC therapy trials may be at increased risk of developing long-term depression due to the uncertain treatment outcomes.

The findings from Studies 3 and 4 suggest that a significant proportion of stroke survivors may be considering experimental SC treatments and that a range of biopsychosocial (e.g. poorer physical functioning, younger age, greater perceived caregiver burden) and attitudinal characteristics increase the likelihood of patients considering experimental SC treatments. Lastly, the findings of Study 4 suggest that pre-existing patient educational

resources, particularly when delivered in booklet form, may help deter stroke survivors from considering experimental SC treatments in the short-term. However, they also suggest that regular updates by clinicians and stroke-advocacy organisations via the media and internet are necessary to maintain the change in attitudes over the longer term.

From a clinical perspective, the findings highlight the promise associated with SC therapies for stroke, but also the need for further research to improve our understanding of the medical and psychological risks and benefits. Given the strong level of interest in 'stem cell tourism' that was reported by both Australian and international stroke survivors, there is a need for clinicians to work with patients and their caregivers to reduce the likelihood that they will undergo these risky treatments. One way to achieve this goal may be for clinicians to work with SC advocacy groups to develop stroke-specific educational resources that are appropriate for use with this complex cohort of patients.

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